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Human exposure to endotoxins and fecal indicators originating from water features

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ABSTRACT

Exposure to contaminated aerosols and water originating from water features may pose public health risks. Endotoxins in air and water and fecal bacteria in water of water features were measured as markers for exposure to microbial cell debris and enteric pathogens, respectively. Information was collected about wind direction, wind force, distance to the water feature, the height of the water feature and the tangibility of water spray. The mean concentration of endotoxins in air nearby and in water of 31 water features was 10 endotoxin units (EU)/m^3 (Geometric Mean (GM), range 0–85.5 EU/m^3 air) and 773 EU/mL (GM, range 9–18,170 EU/mL water), respectively. Such mean concentrations may be associated with respiratory health effects. The water quality of 26 of 88 water features was poor when compared to requirements for recreational water in the Bathing Water Directive 2006/7/EC. Concentrations greater than 1000 colony forming units (cfu) *Escherichia coli* per 100 mL and greater than 400 cfu intestinal enterococci per 100 mL increase the probability of acquiring gastrointestinal health complaints. Regression analyses showed that the endotoxin concentration in air was significantly influenced by the concentration of endotoxin in water, the distance to the water feature and the tangibility of water spray. Exposure to air and water near water features was shown to lead to exposure to endotoxins and fecal bacteria. The potential health risks resulting from such exposure to water features may be estimated by a quantitative microbial risk assessment (QMRA), however, such QMRA would require quantitative data on pathogen concentrations, exposure volumes and dose–response relationships. The present study provides estimates for aerosolisation ratios that can be used as input for QMRA to quantify exposure and to determine infection risks from exposure to water features.

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1. Introduction

Water features may include decorative fountains, ornamental features and interactive fountains. These water features are often located in public areas such as shopping areas, hospitals, ponds, canals, parks or roundabouts. People may come into contact with water from the water features, which create aerosols to which people may be exposed e.g. by inhalation or ingestion.

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Source water to fill water features may include local surface water, groundwater, rainwater or tap water determining the initial water quality. Subsequent contamination may occur through fecal bird droppings, runoff from paved surfaces (including e.g. dog feces), growth of micro-organisms in water (*Legionella* and algae), and in some cases discharges of combined sewer overflows (Schets et al., 2008). These contaminants may include a variety of chemical and biological contaminants, including pathogens and microbial cell debris, such as endotoxins.

A water feature sprays water, including its contaminants, into the air in the form of droplets and smaller water based aerosols. These aerosols may contaminate air, depending on feature specific factors such as water quality, flow rate, aerosolisation ratio (the fraction of the sprayed water that becomes an aerosol) and plume height (Environmental Protection Agency, 1982) and climatic factors such as temperature, rainfall, wind velocity and wind direction (Hunter, 2003). Contaminated aerosols potentially may have negative health effects for people who are exposed through contact, ingestion or inhalation (Carducci et al., 2000). Inhalation of aerosols will be the most likely route of exposure at water features if the spray device at the water feature produces aerosols within the respirable size range. At water features where produced aerosols are not in the respirable size range, ingestion of water may occur, whether intended (by swallowing mouthfuls of water) or unintended (through ingestion of aerosols or water droplets or through hand-mouth contact).

Exposure to contaminated aerosols has been discussed in the context of many studies in agricultural and industrial environments, and is potentially associated with adverse health effects (Health Council of the Netherlands, 2010). For instance, exposure through inhalation of endotoxins causes an increased prevalence of (work-related) airway and flu-like symptoms (Pillai and Ricke, 2002), and gastrointestinal and neurological complaints and joint pain in sewage workers (Laitinen et al., 1994; Lundholm and Rylander, 1983). Furthermore, exposure through ingestion of aerosols with fecal pathogens can cause gastrointestinal diseases (Uhrbrand et al., 2011).

To be able to indicate possible public health risks from exposure to water features we measured endotoxins in air and water as well as fecal indicator bacteria in water. Endotoxins and fecal indicators were measured to provide insight into exposure through inhalation and ingestion of water(spray) from water features. Since, exposures near water features may be influenced by several climatic and feature-specific factors (i.e. wind direction, distance to the fountain, height of the plume of the fountain etc.), these factors were measured as well.

2. Materials and methods

2.1. Selection of markers to quantify exposure originating from water features

Endotoxin was measured as a marker for human exposure through inhalation of contaminated aerosols near water features. Endotoxins are lipopolysaccharides present in the outer membrane of gram-negative bacteria and some algae (Anderson et al., 2002) and were often regarded as a marker of exposure through inhalation to gram-negative bacteria (Douwes et al., 2003). Furthermore, endotoxins are easy to measure, which is an advantage as compared with the detection of living micro-organisms in air, such as pathogens and algae. Concentrations of endotoxin were measured in water and in air.

The fecal indicator bacteria *Escherichia coli* and intestinal enterococci were measured in water to indicate the presence of enteric pathogens (World Health Organization, 2011). It should be noted that there is no correlation between these fecal indicator bacteria and endotoxins (Anderson et al., 2002), and that endotoxins do not equate to gram negative enteric pathogens.

2.2. Sampling sites

From March until June 2010, water samples were taken at 57 water features to determine the concentration of endotoxins, *E. coli* and intestinal enterococci. Based on the concentrations of endotoxin in water, the minimum duration to take air samples was predicted using a dilution factor from water to air of 10^8 (Stellacci et al., 2010). Subsequently, from June until November 2010, air and water samples were taken at 31 water features in the cities of Utrecht, Nijmegen, Rotterdam and Groningen. Samples were taken at locations where people potentially could be exposed to water from decorative and interactive water features. We installed a tripod at multiple wind directions from individual water features where possible. The sampling sites included 13 ornamental water features, 13 fountains in a pond or a canal and 5 interactive features. An interactive fountain was defined as a fountain where children were encouraged to play with water.

2.3. Sampling procedure

Air samples were collected using Gilian GilAir5 portable pumps at a flow of 3.5 L per minute in combination with GSP inhalable dust sampling heads (JS Holdings) with 37 mm glass fiber filters (Whatman GF/A). The sampling device was placed on a tripod (height 1.5 m) at the nearest location to the fountain where people potentially could be exposed through inhalation. The measurement equipment was installed and collected on average 4.1 h later (SD 1.1 h, range 3.1–8 h). If possible, the measurement equipment was installed at two sides of each fountain: one downwind and the other upwind of the location. On each sampling day, the equipment contained a control filter which was handled in the same way as the other samples, except for the active sampling. Information was collected about the height of the fountain, the distance from the fountain to the measurement equipment, the water and outside temperatures, and precipitation. Furthermore, the sample taker determined if water spray was tangible at the location of the tripod. The tangibility of the water spray was defined as water spray on skin of the sample taker during installation of the measurement equipment. Subsequently, it was recorded whether the fountain was sheltered from the wind or was influenced by the wind, causing aerosols to be entrained in a certain direction. Furthermore, information

was collected on the volume of the water system, whether it was a closed system and whether the water was disinfected by some kind of treatment.

2.4. Analytical procedures of water samples

Samples were collected directly into individual sterile 500 mL bottles and transported to the laboratory in a chilled cold box. Samples were analyzed for endotoxins as described previously by Spaan et al. (2008). Samples of 50 mL were centrifuged at 1000 g and the supernatant was stored at −20 °C. After collection of all samples, samples were analyzed by the quantitative kinetic chromogenic Limulus amebocyte lysate (LAL) method (Cambrex, Verviers, Belgium; lot no. lysate 1L676S, lot no. standard 2L 20090 (RSE/CSE ratio 11.5 EU/ng)), in which the assay solution was pyrogen-free water +0.05% Tween-20. Samples were assayed at an initial dilution of 1:500 and, when the measured concentration was above the detection limit of the assay, retested at higher dilutions (up to 1:10,000). Additionally, water samples of 40 mL, 10 mL and 0.1 mL were analyzed within 24 h of sampling for fecal indicator bacteria *E. coli* and intestinal enterococci. *E. coli* was enumerated using the Rapid Test on Tryptone Soy Agar (996292, Oxoid, Wesel, Germany) and Tryptone Bile Agar (806567, Oxoid) according to ISO9308-1 (Anonymous, 2000a). Colonies were confirmed with James Reagens (BioMerieux, Marcy ll'Etiole, France) according to the manufacturer's instructions. Intestinal enterococci were enumerated according to ISO7899-2 (Anonymous, 2000b) on Slanetz and Barley Agar (1005125, Oxoid) and Bile Esculin Azide Agar (726007, Remel).

2.5. Analytical procedures of air samples

Air filters were stored at −20 °C until all samples were collected. For extraction of endotoxin, each filter was immersed in 5 mL of pyrogen-free water with 0.05% Tween-20 in a glass tube and rocked vigorously for 1 h at room temperature on a horizontal shaker. After 15 min of centrifugation at 1000 g, 1 mL aqueous supernatant per sample was collected and vortexed. The endotoxin concentration in the extract was assayed with the kinetic chromogenic Limulus Amoebocyte Lysate (LAL) method. Samples were assayed at an initial dilution of 1:50, and if the concentration was below detection limit, retested at lower dilutions (up to 1:25).

2.6. Computational and statistical methods

Data were analyzed with SPSS statistical software version 16. The concentration of endotoxins in air (EU/m^3) was calculated by dividing the number of endotoxin units (EU) on the filter by Q*t, in which Q was the flow rate of the filtered air (m^3/min) and t was the sampling duration in minutes. Descriptive statistics for endotoxins, *E. coli* and intestinal enterococci were given as arithmetic mean (AM), standard deviation of AM (SD), geometric mean (GM), geometric standard deviation (GSD) and minimum and maximum per type of fountain. Because all measured concentrations were assumed to be log-normally distributed (Spaan et al., 2007), all calculations were performed with log-transformed concentrations to the base e. Regression analysis was used to explore which factors were associated with exposure, with exp(β) representing the factor by which the estimated exposure was changed per unit change. For dummy variables, exp(β) represented the difference in estimated exposure when the characteristic was present versus absent. In addition, a logistic regression analysis was conducted, because a high number of observations were below the detection limit. With a logistic regression analysis, an odds ratio (OR) was obtained that can be interpreted as the likelihood of observations above the detection limit when a certain factor was present compared to the likelihood of observations above the detection limit when a certain factor was absent. The significance of this association was shown by the *p*-value, where a *p*-value lower than 0.05 was assumed to be significant.

3. Results

3.1. Air measurements

Endotoxin concentrations in air were measured at 31 locations, described in Table 1, yielding 79 air samples. Of these samples, 6 samples were excluded due to failure of equipment, 34 samples (taken at 19 locations) exhibited endotoxin concentrations above the detection limit of 0.8 EU/m^3 and the other 39 samples exhibited endotoxin concentrations below this detection limit.

The average distance between the fountain and the air measurement equipment was 8 m (GM, range 0.5–49 m) with 18 measurements performed within a distance of 2 m, 16 measurements performed at a distance between 2 and 10 m and 39 measurements performed at a distance greater than 10 m. According to the Royal Netherlands Meteorological Institute, all measurements took place at a wind speed below three Beaufort (5.5 m/s). Furthermore, all measurements took place on dry, cloudy days with temperatures below 20 °C. Thirty measurements (41%) were carried out at locations where the water spray was tangible by the sample taker during installation of the measuring equipment. Twenty-two measurements (30%) were performed downwind, and during the other 51 measurements (70%) no main wind direction was observed. Forty-five measurements (62%) were executed at locations that were sheltered from wind; during the other 28 measurements (38%), the fountain was influenced by the wind causing aerosols to be entrained in a certain direction.

The concentrations of endotoxin in air ranged up to 85.5 EU/m^3, the average concentration (GM) was 10 EU/m^3 (Table 1). Statistical analysis showed that there were no differences between concentrations of endotoxin in air and type of fountain, such as fountains in a pond or a canal, ornamental features or interactive features (*T*-test: $p > 0.2$). The concentration of endotoxin in air at water features was dependent on the water source from which water features were filled. The concentration of endotoxin in air at sites fed by surface water contaminated by a combined sewer overflow (CSO) was significantly higher than locations which received surface water or tap water as its source water (*T*-test: $p < 0.05$). No statistical significant difference was found between the concentrations of endotoxin in air measured at water features fed by surface water as compared with tap water (*T*-test: $p > 0.2$).

Table 1 – Description of 31 locations with water features where air measurements were performed and overview of measurement results.

Kind of fountain[a]	Source water	Closed circulating system	Disinfection	Concentration fecal indicators in water		Endotoxin concentration in water	n/N[d]	Concentration of endotoxin in air	Distance[e]	Height[f]
				E.C.[b]	I.E.[c]			Mean (range)	M	m
		Yes/No	Yes/No	cfu/100 mL	cfu/100 mL	EU/mL		EU/m^3		
A	Surface water + CSO[i]	No	No	2000	800	18,170	4/4	61.8 (33.1–85.5)	15	7
A	Surface water	No	No	300	80	3000	2/2	20.0 (6.4–34.1)	15	3
A	Surface water	No	No	80	550	1370	3/3	12.7 (3.0–17.8)	6–20	6
A	Surface water	No	No	800	70	916	1/1	5.9	5	2
A	Surface water	No	No	60	90	1103	1/4	6.3	10–20	3
A	Surface water	No	No	50	20	1017	0/2	–	30	7
A	Surface water	No	No	450	100	9029	0/2	–	33	10
A	Surface water	No	No	180	40	6976	0/2	–	23	6
A	n.a.[g]	n.a.	n.a.	100	23	3063	1/2	16.8	10	2
A	n.a.	n.a.	n.a.	40	50	211	0/2	–	8	7
A	n.a.	n.a.	n.a.	6000	500	2385	2/2	2.6 (2.5–2.7)	22	7
A	Surface water	No	No	90	35	5092	2/3	3.2 (2.9–3.5)	32	9
A	Surface water	No	No	1700	500	1603	1/2	2.9	33	6
B	Surface water	Yes	No	190	260	404	1/2	6.0	7	3
B	Tap water	Yes	Yes SF[h] and UV	<1	4	1332	1/2	10.6	0.5	1
B	Tap water	Yes	Yes Chlorine	<1	<1	669	1/3	7.8	13–24	18
B	n.a.[7]	n.a.	n.a.	1	10	75	0/2	–	2	1
B	n.a.	n.a.	n.a.	<1	<1	54	0/1	–	1	1
B	n.a.	n.a.	n.a.	640	150	161	0/2	–	2	1
B	Surface water	Yes	No	300	80	2490	3/4	11.0 (3.7–25.5)	45	20
B	n.a.	n.a.	n.a.	30	20	1415	2/2	5.8 (5.0–6.6)	8 5	3
B	n.a.	n.a.	n.a.	6	2	929	2/3	7.1 (5.3–8.9)	0.5–1	0.5
B	Tap water	Yes	Yes Chlorine	<1	<1	47	0/3	–	0.5–8	9
B	n.a.	n.a.	n.a.	200	3	1540	2/3	16.7 (13.5–19.9)	4	6
B	Surface water	Yes	No	240	60	3639	2/4	20.0 (18–22)	22	20
B	n.a.	n.a.	n.a.	10	20	1619	1/2	–	17	5
C	Tap water	Yes	Yes SF and UV	40	32	9	0/2	–	1	1
C	Tap water	Yes	Yes SF and UV	12	26	11	0/2	–	1	0.5
C	Surface water	Yes	Yes Chlorine	3,000,000	1,000,000	2799	1/1	19.0	4	2
C	Tap water	Yes	No	8	200	67	0/2	–	0.5	1
C	Tap water	Yes	Yes Chlorine	1	60	923	2/2	8.5 (7.2–9.8)	0.5	2

[a] Kind of fountain is subdivided in Fountain in a pond or a canal (A), Ornamental fountain (B)and Interactive fountain (C).
[b] *E. coli*.
[c] Intestinal Enterococci.
[d] n/N represents the number of positive air samples divided by the number of total air samples.
[e] Distance of air sampling device to fountain in meters.
[f] Height of the plume of the fountain in meters.
[g] n.a. = not available; h SF = Sand Filtration UV = Ultraviolet.
[i] Combined Sewer Overflow.

3.2. Water measurements

Endotoxin and fecal indicator bacteria concentrations were measured at 88 locations. All water samples contained detectable concentrations of endotoxin, with the majority containing both *E. coli* and intestinal enterococci (72%): The arithmetic means, geometric means (GM) and concentration ranges in all samples are shown in Table 2, organized to the origin of water of the fountain. The water samples showed concentrations of endotoxin ranging between 9 and 18,170 EU/mL, the geometric mean concentration being 773 EU/mL. Furthermore, at 26 of the 88 locations, the concentration of *E. coli* and/or intestinal enterococci in water exceeded the standards for fecal indicators in recreational waters according to European Bathing Water Directive 2006/7/EC. The mean temperature of all water samples taken from 88 water features was 18.1 °C (range 15.1 °C–19.7 °C; data not shown). The highest concentration of endotoxin, *E. coli* and intestinal enterococci in water has been detected in a pond that was contaminated by a discharge of a combined sewer overflow. Furthermore, one interactive fountain displayed very high concentrations of fecal indicator bacteria on the sampling day (Table 1).

3.3. Factors associated with exposure to endotoxin originating from water features

Table 3 shows the results of the multivariate logistic regression. Endotoxins in the air were detected more often when high concentrations of endotoxin were present in water. For each natural log increase of endotoxins in water, endotoxins in air were detected 3.0 times more frequently and for each meter further away from the water feature, the likelihood to detect endotoxins decreased 0.9 times. The tangibility of water spray and the height of the plume of the water feature were also associated with the presence of endotoxin in air, however, this association was not significant. Other characteristics, such as the presence of a closed water system and use of disinfection procedures, were not included in regression models because this information was unavailable for too many water features. Also the wind direction was not included because it was impossible to observe the main wind direction for the majority of the measurements. Results from the multi-variate linear regression analyses are presented in Table 3. Significant associations were also checked by use of scatter plots (not shown). For the concentration of endotoxins in water, the regression coefficient amounted to 0.47 per increase of a natural log unit in water, which implied that for the mean concentration of 773 EU/mL in water features, the concentration in air was 22.7 EU/m^3 higher than when the concentration in water was 0 EU/m^3. For the distance of the fountain to the measurement location, the regression coefficient amounted −0.05, which implied that for a distance of 10 m to the fountain, the concentration in air was 0.9 EU/m^3 lower than at the location of the water feature. For the tangibility of water spray, the regression coefficient amounted to 0.8, which implied that when the aerosols were tangible, the concentration in air was 2.2 EU/m^3 higher.

Table 2 – Concentrations of endotoxin, *E. coli* and intestinal enterococci in water of water features.

		Tap water	Surface water
Endotoxin in water (EU/mL)	n/N[a]	20/88	54/88
	AM[b]	$6.9*10^2$	$1.1*10^3$
	SD[c]	$7.9*10^2$	$1.7*10^3$
	GM[d]	$2.6*10^2$	$3.6*10^2$
	Min[e]	$9.0*10^0$	$1.3*10^1$
	Max[f]	$3.1*10^3$	$1.8*10^4$
E. coli in water (cfu/100 mL)	n/N	11/88	40/88
	AM	$9.8*10^1$	$7.6*10^4$
	SD	$1.9*10^2$	$4.7*10^5$
	GM	$3.5*10^1$	$3.4*10^2$
	Min	$2.0*10^0$	$2.0*10^0$
	Max	$6.2*10^3$	$3.0*10^6$
Intestinal Enterococci in water (cfu/100 mL)	n/N	14/88	34/88
	AM	$4.9*10^2$	$3.0*10^4$
	SD	$1.6*10^3$	$1.7*10^5$
	GM	$2.5*10^1$	$7.2*10^1$
	Min	$1.0*10^0$	$6.0*10^0$
	Max	$6.4*10^2$	$1.0*10^6$

[a] Number of positive samples/number of total samples.
[b] Arithmetic Mean.
[c] Standard Deviation of AM.
[d] Geometric Mean.
[e] Minimum
[f] Maximum.

4. Discussion

4.1. Air quality

In this study, we explored whether human exposure to microbial markers can occur from inhalation near water features. The geometric mean of all air samples of 10 EU/m^3 air (GSD = 2.1, range 0–85.5 EU/m^3 air) was high compared to the study of Mueller-Annelling et al. (2004) who reported a weekly average with a geometric mean of 0.44 EU/m^3 in outdoor air (GSD 3.1, range 0.03–5.5 EU/m^3 air). Since similar data on endotoxin levels in outdoor air were limited (Health Council of the Netherlands, 2010), further comparisons cannot be made. Endotoxin concentrations in air have also been measured in situations where risks of exposure to endotoxins were expected, such as around livestock facilities and wastewater treatment plants. In a major survey in air near and at Dutch wastewater treatment plants, endotoxin concentrations were measured with a geometric mean of 27–64 EU/m^3, which were associated with work-related health effects in sewage workers (Spaan et al., 2008; Smit et al., 2005). Furthermore, in the USA, endotoxin concentrations of 30 EU/m^3 air were measured downwind of swine livestock facilities (Thorne et al., 2009). Such exposure levels in air were associated with respiratory health effects for neighboring residents (Radon et al., 2007; Schinasi et al., 2011). It should however be noted that, in our study, for 12 of the 31 water features (39%), the endotoxin measurements were below the detection limit, indicating that respiratory health effects were unlikely.

The maximum measured exposure concentration of 85.5 EU/m^3 air in this study approaches the exposure limit set

Table 3 – Results of multi-variate logistic and linear regression analysis.

Monitoring parameters	Logistic regression					Linear regression		
	OR[b]	95%CI			P-value	Regression coefficient ß[c]	S.E.	P-value
Concentration of endotoxin in water (EU/mL water) (lognormal transformed)	3.0	2.2	–	4	<0.01	0.47	0.12	<0.01
Distance of fountain to measurement location (m)	0.9	0.84	–	0.93	<0.01	−0.05	0.02	<0.01
Height of the plume of the fountain (m)	1.1	1.1	–	1.2	0.21	0 02	0.02	0.41
Tangibility of water spray (0/1)[a]	1.6	0.8	–	3.0	0.84	0.8	0.24	<0.01
Downstream wind (0/1)	1.2	0.5	–	5.7	0.52	0.07	0.15	0.65

[a] (0/1) dummy variable: present versus absent.
[b] Odd Ratios can be interpreted as the likelihood of the presence of endotoxin in air compared to a unit change or the presence or absence of a monitoring parameter.
[c] exp(β) represented the factor by which the concentration of endotoxin (EU/m^3 air) was changed per unit change or the presence or absence of a monitoring parameter.

by the Health Council of The Netherlands for the work environment of 90 EU/m^3 air over an 8-h period (Health Council of the Netherlands, 2010). Exposure time at water features may range from several seconds/minutes to several hours, but is unlikely to reach an 8-h period. However, the measured exposure concentration near water features may be a health risk for people with atopic diseases including rhinitis and asthma, since they have an increased susceptibility to endotoxins (Smit et al., 2009), which may require shorter exposure times.

4.2. *Water quality*

No requirements for water quality of water features exist. However, at one-third of the water features, the concentrations *E. coli* and intestinal enterococci in water exceeded the standards for fecal indicator bacteria according to the European Bathing Water Directive, 2006, indicating that pathogens might be present. Such pathogens may originate from specific sources, such as feces of animals (e.g. birds) or people (in case of combined sewer overflows). Ingestion of such pathogens may cause gastrointestinal infections. Ingestion may occur due to ingestion of aerosols or due to ingestion of water through hand-mouth contact, through ingestion of water droplets during splashing or through drinking of mouthfuls of water. Therefore, especially at interactive water features, infection risks through ingestion may be larger than risks through inhalation.

Pathogens may also originate from the environment (such as *Legionella* spp.). Several outbreaks are known to have been caused by *Legionella* spp. at water features (Haupt et al., 2012; Hlady et al., 1993; O'Loughlin et al., 2007; Palmore et al., 2009; Correia et al., 2001). This, together with the fact that for many cases of infection with *Legionella* spp. the source of the pathogen is unknown, indicates a possible role for water features as a transmission route/source of this pathogen (Schalk et al., 2012). Furthermore, *Legionella* is gram-negative and, as a result, a source of endotoxins. Therefore, exposure to pathogens such as *Legionella*, as well as to endotoxin near water features cannot be excluded as posing a potential health risk.

The concentrations of endotoxins found in water samples in this study are in line with those found in other studies (Anderson et al., 2002; Watson et al., 1977), except for the concentration of endotoxin in waters influenced by discharges of combined sewer overflows. As reviewed by Anderson et al. (2002), the concentration of endotoxins in raw untreated surface water ranged from >10 to 10,500 EU/mL, with concentrations in general below 500 EU/mL water. These concentrations were determined at locations including those where a combined sewer overflow (CSO) discharged on surface water. Furthermore, a study near showers and humidifiers (Anderson et al., 2007) indicated that humidifiers filled with drinking water with concentrations of endotoxin above 1000 EU/mL were likely to induce symptoms like chills and fever, often referred to as Organic Dust Toxic Syndrome (Basinas et al., 2012). In this study, the endotoxin concentrations ranged up to 18,170 EU/mL in water of water features that were influenced by a combined sewer overflow. Therefore, water features in surface water influenced by a combined sewer overflow should be avoided to prevent human exposure to contaminated aerosols.

4.3. *Factors that determine exposure near water features*

Possible health effects from exposure to micro-organisms in air are determined by the density of micro-organisms in the aerosolized liquid, the height of the plume of aerosols and the distance between an exposed person and the source of aerosolisation (Environmental Protection Agency, 1982). In addition, possible health effects from exposure to bioaerosols are likely to be affected by the particle distribution of the aerosols. The size of aerosols influences deposition efficiency in the respiratory tract (Heyder et al., 1986). Smaller aerosols (<5 μm) have a higher deposition in the alveolar tract. Larger particulates (<15 μm and >5 μm) predominantly deposit in the higher airways and will be ingested. In this study, particulates smaller than approximately 15 μm were measured by GSP inhalable dust sampling heads; those particulates can enter the respiratory tract during inhalation. Furthermore, the concentration of aerosols in air is determined by the flow rate of the water (i.e. the sprayed volume per time unit) and the aerosolisation factor (i.e. the fraction of water which becomes an aerosol) (Environmental Protection Agency, 1982). These factors differ for different types of water

features, for instance, a decorative fountain regularly aerosolizes more water than an interactive feature. The flow rate of the sprayed water and the aerosolisation factor were not taken into account in this study due to difficulties in gathering this information for all water features; however, these factors are critical to determine how closely a person may interact with a fountain. Moreover, although these factors may differ for the different types of water features, in this study no differences in concentrations of endotoxin in air were found between ornamental features, interactive features and fountains in surface waters. The concentration of endotoxin in air was found to be associated with the concentration of endotoxin in water, the distance to the water feature and the tangibility of water spray. As a result, possible health effects are mainly determined by these factors and by the susceptibility of exposed people for waterborne disease (immunocompromised or not), the level of exercise and the duration of exposure. For instance, the duration of exposure for those playing with an interactive water fountain may be longer than the duration of exposure for those walking next to a fountain. Health effects due to exposure through inhalation of endotoxins may include respiratory symptoms such as dry cough or shortness of breath, accompanied by a decrease in lung function (Health Council of the Netherlands, 2010). Exposure through inhalation of pathogens may cause infectious diseases and allergies (Douwes et al., 2003). Furthermore, exposure through ingestion of aerosols with pathogens originating from feces may cause gastrointestinal diseases (Uhrbrand et al., 2011).

4.4. *The value of this study for quantitative microbial risk assessment (QMRA)*

Public health risks associated with water features may be quantified by means of microbial risk assessment (World Health Organization, 2011). A QMRA requires data about pathogen concentrations, exposure volumes and dose–response relationships. To perform a QMRA, a characterization of spray devices (i.e. their flow rate and aerosolisation ratio) is required to transform the sprayed volume of water into the volume of water that is inhaled or ingested by people exposed to it (Environmental Protection Agency, 1982). However, in absence of these data, an aerosolisation ratio can be estimated based on the concentrations of endotoxin in air and water. In this study, the aerosolisation ratio (expressed as EU m^{-3} aerosol/EU m^{-3} water) ranged from $2.0 \cdot 10^{-10}$ to $1.1 \cdot 10^{-7}$ with on average $8.6 \cdot 10^{-9}$ for all water features. These aerosolisation ratios represent the inhalable fraction of aerosols and can be used, as was done by Stellacci et al. (2010), to calculate infection risks.

QMRA may also provide insight into possible intervention measures. To achieve this, information is required about faecal and other inputs to source water(s), the performance (effectiveness and reliability) of water treatment applied to water supplying fountains, as well as properties of spray devices. While all of this data were not collected as part of this study, our data can be used to inform preliminary risk assessments and as a basis for designing future studies to determine the most appropriate intervention measures to reduce health risks associated with water features.

5. Conclusion

The present study has demonstrated the presence of endotoxins in air and water and fecal indicator bacteria in water of water features. Exposure to microbial content near water features may give rise to respiratory and gastrointestinal complaints. The extent to which exposure to water features causes these complaints should be investigated, probably through an epidemiological study. Exposure can be minimized by precautionary measures that improve the water quality or decrease the contact with contaminated aerosols. Water quality can be improved by water filtration (to remove endotoxins and pathogens) and disinfection (to inactivate pathogens) and in case of combined sewer overflows no water features should be installed or otherwise CSO storage and treatment facilities should be built. The contact with aerosols can be minimized by choosing locations for water features well away from people or by changing the water spray to spray fewer aerosols. A quantitative microbial risk assessment (QMRA) could be used to estimate the potential risks of infection (World Health Organization, 2011) resulting from exposure to water and aerosols from water features.

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