

# Abschlussbericht TransMit

## Teil B

### B 3.4

# Microbiological water quality and derived health risks from exposure to water from blue elements during recreational activities in an inner courtyard in the Südstadt district in the city of Hannover

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#### **Kurzbeschreibung des Einzelkapitels**

*In diesem Kapitel befassen wir uns mit der Qualität der Wasserelement im Innenhof in der Südstadt. Neben den Zisternen, in die das Regenwasser direkt fließt, wurden die beiden Teiche beprobt. Es wurden die Konzentration der fäkalen Markermikroorganismen E. coli und Enterokokken sowie P. aeruginosa und Salmonella non-typhoid bestimmt. Die Mikroorganismen Vibrio cholera, Listeria monocytogenes und Campylobacter spp. waren nicht nachweisbar. Für Salmonella non-typhoid und Enterokokken besteht ein Risiko der Infektion, während für E. coli und P. aeruginosa keine Gefahr besteht.*

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## 1. Introduction

Water scarcity is becoming a growing issue to be addressed worldwide; reduced access to this resource is being tackled in many countries, like Germany, by giving subsidies to develop strategies and encourage the use of roof-harvested rainwater (RHRW) for potable and non-potable uses (Ahmed, Vieritz, Goonetilleke, & Gardner, 2010b). However, while RHRW can be used as an alternative water source, it is exposed to several chemical and microbiological contaminants during its travel through the atmosphere and the harvesting process (Hamilton et al., 2019).

In general, quality of RHRW varies greatly depending on the characteristics of the catchment i.e., urban or rural catchment, its location and weather conditions, as well as the properties of the system collecting and storing the water for further use (Zdeb, Zamorska, Papciak, & Słyś, 2019). RHRW quality can be greatly affected by the roofing materials (Lee, Bak, & Han, 2012; Mendez et al., 2011); (Lee et al., 2012; Mendez et al., 2011). Several studies have reported presence of potentially pathogenic bacteria in RHRW such as *E. coli*, Enterococci, *Salmonella spp.*, *P. aeruginosa*, *Listeria monocytogenes*, *Campylobacter spp.* and *Vibrio spp.*, most of which cause water-borne diseases (Ahmed, Hodgers, Sidhu, & Toze, 2012; Denissen, Reyneke, Waso, Khan, & Khan, 2021; Man et al., 2014b).

Regulations, such as the ones presented by the USEPA, are designed to protect public drinking water systems; nevertheless, they do not apply to privately owned wells or any other individual water system, such as rainwater collection systems. As a result, owners of private water systems are responsible for ensuring that their water is safe in terms of physicochemical and microbiological pollutants. (CDC, 2014)

Given the great variability regarding RHRW quality and the health risks that could be derived from its use, both for potable and non-potable purposes, are of general concern (Denissen et al., 2021). Evaluating the health risks derived from RHRW use can be particularly challenging when it is to be used for non-potable applications, such as garden hosing, car washing, laundry, sanitary flushing and so on, where the main exposure routes are inhalation of aerosols, contact with the skin and unintentional ingestion, as these activities involve many uncertainties, making it difficult to estimate the health risks (BCCDC, 2011).

Given that outbreaks of water borne diseases coming from private households utilizing RHRW are not generally reported for epidemiological studies, the importance of this study lies in identifying the specific health risks derived from recreational interaction with water from ornamental blue elements in a inner courtyard located in the city of Hannover.

Therefore, the aims of the present study were three-fold (i) determine the presence of potentially pathogenic bacteria in the blue elements fed with RHRW, (ii) perform a QMRA of the studied locations for the reference bacteria identified and (iii) evaluate and compare the health risk implications for the children exposed within this scenario.

## 2. Results and discussion

### 2.1 Hazard identification

To determine the water quality of the cisterns and ponds, the studied bacteria were *E. coli*, Enterococci, *Salmonella non-typhoid*, *Vibrio cholerae*, *Listeria monocytogenes*, *Campylobacter spp.* and *P. aeruginosa*. However, concentrations of *Vibrio cholerae*, *Listeria monocytogenes*, *Campylobacter spp.* were always below the limit of detection (LOD) and thus, not further considered for the risk analysis. Concentrations of the bacteria found in the samples were highly variable at each location during the monitoring (Figure 3).

Table 1 shows the mean, geometric mean (GM) and 95-percentile of the concentration data collected in each pond and cistern for each bacterium considered for the QMRA.

Table 1 Mean, geometric mean and 95th-percentile of bacterial concentrations in water from the blue elements

Parameter	<i>E. coli</i> <sup>a</sup>	Enterococci <sup>a</sup>	<i>Salmonella</i> <sup>b*</sup>	<i>P. aeruginosa</i> <sup>a</sup>	
n	13				
Flat pond	Mean	$3.5 \times 10^2$	$2.5 \times 10^2$	$6.7 \times 10^3$	$3.1 \times 10^2$
	GM	$1.1 \times 10^2$	$6.3 \times 10^1$	$1.7 \times 10^3$	$1.4 \times 10^1$
	95%	$1.1 \times 10^3$	$9.8 \times 10^2$	$2.4 \times 10^4$	$1.0 \times 10^3$
n	14				
Deep pond	Mean	$5.2 \times 10^2$	$2.7 \times 10^2$	$6.7 \times 10^3$	$4.6 \times 10^2$
	GM	$1.1 \times 10^2$	$8.0 \times 10^1$	$2.0 \times 10^3$	$2.8 \times 10^1$
	95%	$1.8 \times 10^3$	$9.8 \times 10^2$	$2.3 \times 10^4$	$1.5 \times 10^3$
n	13				
Cistern shadow	Mean	$3.9 \times 10^0$	$1.3 \times 10^2$	$7.9 \times 10^3$	$1.1 \times 10^3$
	GM	$2.2 \times 10^0$	$8.4 \times 10^0$	$2.2 \times 10^3$	$2.1 \times 10^2$
	95%	$1.4 \times 10^1$	$6.8 \times 10^2$	$3.1 \times 10^4$	$2.4 \times 10^3$
n	14				
Cistern sun	Mean	$6.8 \times 10^0$	$5.5 \times 10^1$	$2.4 \times 10^4$	$9.3 \times 10^2$
	GM	$2.3 \times 10^0$	$4.0 \times 10^0$	$5.7 \times 10^3$	$1.8 \times 10^2$
	95%	$2.8 \times 10^1$	$3.0 \times 10^2$	$1.1 \times 10^5$	$2.4 \times 10^3$

a: MPN/ 100 mL, b: CFU/ 100 mL, \*non-thyphoid, MPN: Most Probable Number. CFU: Colony Forming Units

The USEPA recommends in the RWQC 2012 that the GM of a water body should not exceed  $1.26 \times 10^2$  CFU/ 100 mL for *E. coli* and  $3.5 \times 10^1$  CFU/ 100 mL for Enterococci in any 30-day interval, these concentrations correspond to an estimated illness rate of 36 NGI per 1000 primary contact recreators. Based on these criteria, the GM of both ponds and cisterns are below the recommended concentration for *E. coli*, as well as both cisterns are below the threshold for Enterococci; however, both flat and deep pond exceed the suggested GM for Enterococci, this could be explained by surface runoff of areas surrounding the ponds that can be directly discharged in the ponds. Additionally, birds, which were observed swimming in the ponds during the sampling campaigns, and plant debris from neighboring trees can also bring this bacterial pollution to the water (Boehm & Sassoubre, 2014).

Our results for mean concentrations of *E. coli* in the flat and deep ponds, which were  $3.50 \times 10^2$  and  $5.50 \times 10^2$  MPN/ 100 mL, respectively (Table 3), are consistent with those found by Hamilton et al. (2017), who assessed the correlations between opportunistic pathogens and FIB in RHRW tanks in Australia. Mean concentrations for this bacterium in the cisterns were

found to be  $3.92 \times 10^0$  and  $6.82 \times 10^0$  MPN/ 100 mL at the cisterns in the shadow and the sun, respectively; these results indicate that the primary source of microbial pollution is likely to be feces from birds and other animals accessing the ponds directly, as the mean concentrations of *E. coli* at the cisterns were rather low and comparable with the results from Lee et al. (2010) who found concentrations in the range of 0 -  $6.0 \times 10^1$  CFU/ 100 mL in fresh rainwater.

Regarding Enterococci, the mean concentrations found in both cisterns lie within the range identified by Zdeb et al. (2019) for this bacterium (0 – 150 CFU/ 100 mL) in a study where they investigated the quality of rainwater collected from outlet pipes from roofs with different materials; meaning that the presence of these bacteria in the cisterns could be linked to bird droppings and accumulation of fecal matter from other small mammals on the roof. Moreover, mean concentrations of Enterococci in the ponds were observed within range of 240 – 270 MPN/ 100 mL, similar concentrations were found by Ahmed et al. (2010a), where 14 % of the 100 RHRW samples taken from residential houses had a concentration in the range from 101 – 500 CFU/ 100 mL; however, the mean concentrations in our study are higher than the ones found in the study from Ahmed et al. (2008), this could be explained by different weather and catchment conditions as well as differences in the design of the collection system in both investigations.

Several studies have suggested Enterococci as a better indicator of fecal contamination than *E. coli* in RHRW, as it has shown higher prevalence in samples where *E. coli* was not identified (Ahmed et al., 2008; Ahmed et al., 2010a; Spinks, Phillips, Robinson, & van Buynder, 2006). Also, Enterococci has been found to persist longer in water and to resist better in drying periods compared with *E. coli* (Ahmed et al., 2010a; Chidamba & Korsten, 2015).

Ahmed et al. (2010) found a concentration range of *Salmonella spp.* of  $6.5 \times 10^0$  to  $3.8 \times 10^1$  per 100 mL in roof-harvested rainwater; conversely, in our study, this range was exceeded in 2-log unit in the ponds and up to 3-log units in both cisterns, with a higher mean value identified in the cistern receiving sun (Table 3). Our results also showed higher concentrations than the ones found by Ahmed et al. (2012), who identified one from 24 samples to be positive for *Salmonella spp.* with a mean concentration of  $7.3 \times 10^3$  bacterial cells/ 100 mL; in the same study, they isolated *Salmonella spp.* from bird and possum fecal samples and found concentrations between  $6.3 \times 10^2$  and  $1.8 \times 10^3$  bacterial cells/ g of bird feces; this could explain that high concentrations found in our study could be mostly related to bird droppings, not only on the roofs but throughout the inner courtyard, which has trees and vegetation attracting birds.

Regarding the opportunistic pathogen *P. aeruginosa*, mean concentrations from the ponds were lower than the mean concentrations found in the cisterns; these concentrations are consistent with the range found by Nawaz et al. (2014) during the wet season of their study (200 – 1800 CFU/ 100 mL) while researching variation of *P. aeruginosa* from different water catchments and storage conditions. Our results are also comparable with the ones from a pilot-scale study in American Samoa from Kirs et al. (2017) which compared different water sources that are used for potable purposes. The presence of this bacterium in RHRW could suggest stagnation of water and presence of organic matter which could leachate from the roofs (Sánchez, Cohim, & Kalid, 2015). From the review made by Roser et al. (2015),  $1 \times 10^6$  CFU/ 100 mL is considered the minimum concentration to constitute a hazard for skin infections, which is much higher than the concentration we found in the studied blue elements (Table 2).

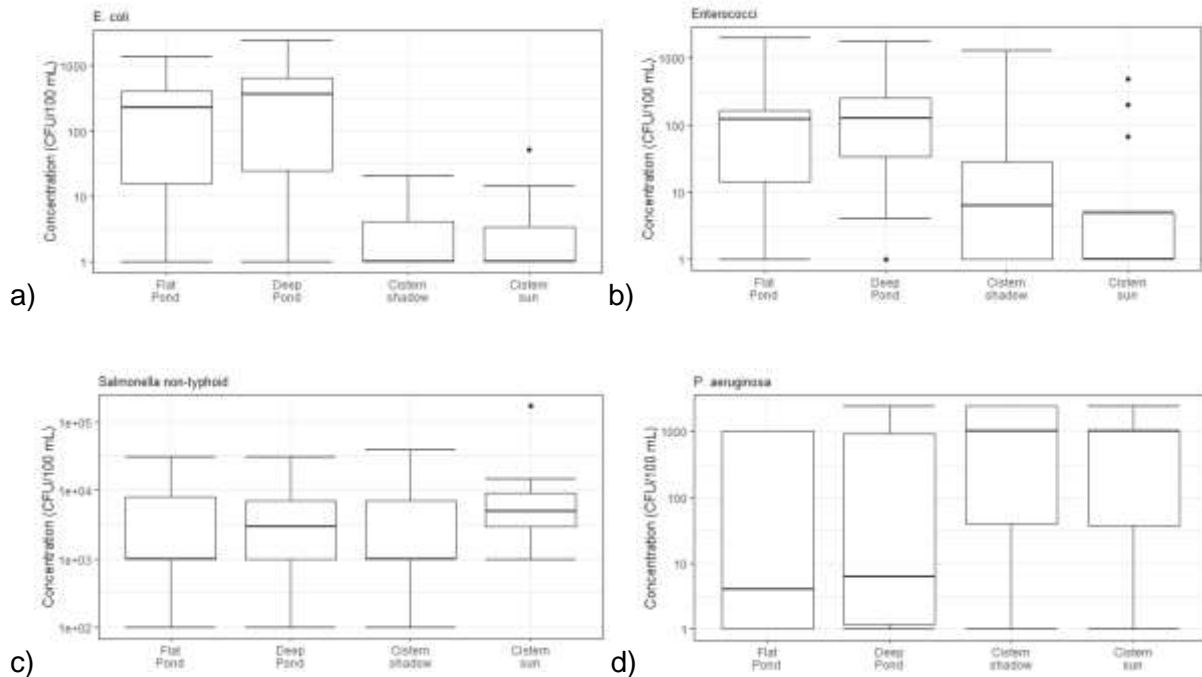


Figure 1: Box plots of the bacterial concentrations at each location. Line inside the box represents the median value, box represents the interquartile range (25–75 percentiles), black dots outside box represent the outliers, and whiskers show the maximum and minimum values.

In Figure 1a, it can be identified that the deep pond was the location with the highest median concentration, as well as the one with more variability during the sampling regarding *E. coli*. The same tendency can be observed from Figure 1b, where the highest median concentration of Enterococci was found in the deep pond. Moreover, the highest median concentration for *Salmonella non-typhoid* was found in the cistern under the sun, although this was the location with less variability during sampling (Figure 1c). Meanwhile, Figure 1d shows that the highest median concentration for *P. aeruginosa* was observed in the cistern under the shadow and the highest concentration variability for this bacterium was found in the ponds.

## 2.2 Exposure assessment

In order to perform a QMRA, considering the distribution of bacteria in the water is one of the key points. Bacteria are discrete variables with concentrations that can vary on each sampling event; therefore, bacterial statistics must be characterized to acknowledge the risks differences from diverse concentration exposures (Haas et al., 2014).

The bacterial concentration results obtained from the lab analysis were used to identify the probability distribution that was a best fit to each bacterial concentration, the selected distribution was the one that returned the highest Loglikelihood and the lowest AIC values, suggesting the best fit. As a result, the concentration of *E. coli* in the ponds was described by a Beta probability distribution; and, in the case of the cisterns the concentration was described by a log-normal probability distribution. Regarding Enterococci, three of the locations fitted a Beta distribution, and for the cistern under the sun a log-normal was the best fit. For

*Salmonella non-typhoid* and *P. aeruginosa* concentrations at the four locations the best fit was a Beta probability distribution (Table 2).

Table 2: Best fit probability distributions and parameters used for bacterial concentration

	Parameters	<i>E. coli</i>	Enterococci	<i>Salmonella</i> *	<i>P. aeruginosa</i>
	samples	14			
Flat Pond	probability distribution	Beta	Beta	Beta	Beta
	parameters	$\alpha = 0.258$ $\beta = 0.559$	$\alpha = 0.211$ $\beta = 0.577$	$\alpha = 0.147$ $\beta = 0.418$	$\alpha = 0.101$ $\beta = 0.180$
	samples	13			
Deep Pond	probability distribution	Beta	Beta	Beta	Beta:
	parameters	$\alpha = 0.198$ $\beta = 0.502$	$\alpha = 0.239$ $\beta = 0.626$	$\alpha = 0.152$ $\beta = 0.428$	$\alpha = 0.125$ $\beta = 0.405$
	samples	14			
Cistern shadow	probability distribution	Lognormal	Beta	Beta	Beta
	parameters	$\mu = -6.085$ $\sigma = 4.208$	$\alpha = 0.109$ $\beta = 0.430$	$\alpha = 0.192$ $\beta = 0.477$	$\alpha = 0.145$ $\beta = 0.182$
	samples	14			
Cistern sun	probability distribution	Lognormal	Lognormal	Beta	Beta
	parameters	$\mu = -7.664$ $\sigma = 4.364$	$\mu = -8.835$ $\sigma = 5.149$	$\alpha = 0.128$ $\beta = 0.457$	$\alpha = 0.157$ $\beta = 0.234$

\*non-typhoid

## 2.3 Risk assessment

Generally, a point estimate calculation of the risk of illness/ infection is a widely used approach in QMRA. Our study uses a probabilistic approach as it allows us to consider the variability and the uncertainty within each input parameter. Therefore, for the dose-response model of each bacterium, the probability distributions of bacterial concentration and exposure rates were used, considering a mean exposure time of 3.5 min.

To analyze the risk of GI illness due to ingestion of *E. coli*, Enterococci, *Salmonella non-typhoid*, as well as dermal infection due to *P. aeruginosa*, Monte Carlo simulations were ran considering the respective dose-response model of each bacterium and the results are displayed in box-and-whisker plots (Figure 2).

Considering that recreational activities do not take place every day of the year, the risk of illness and infection is measured in units per day, eliminating the dependency on the days and assuming that a person is exposed to one recreational event per day (Haas et al., 2014). For the risk assessment in this study, it was assumed that no bacterial decay happened during water transport and exposure.



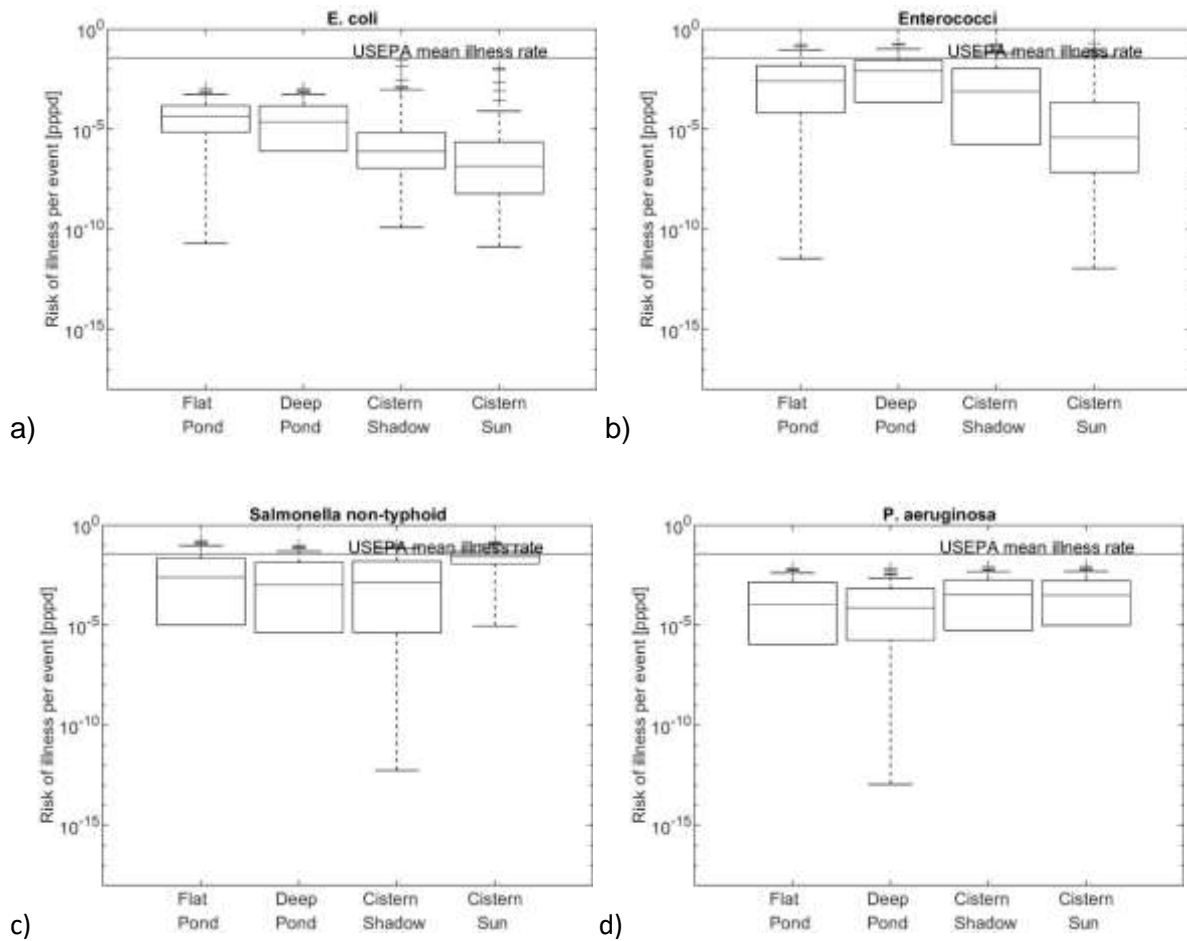


Figure 2: Box Plot for the risk of illness/infection per 1000 users per day at cisterns and ponds due to exposure to a) *E. coli*, b) *Enterococci*, c) *Salmonella non-typhoid* and d) *P. aeruginosa* obtained from the Monte Carlo simulations. Horizontal line represents USEPA mean illness rate of 36/1000 users. Line inside the boxes represents the median value, box represents the interquartile range (25–75 percentiles), crosses outside box represent the outliers, and whiskers show the minimum and maximum values.

In general, the results from the risk assessment presented in Figure 4 show that the USEPA mean illness rate could be exceeded due to exposure to *Enterococci* and *Salmonella non-typhoid* in the blue elements located in the inner courtyard.

In Figure 2a, the highest GI illness risk from exposure to water contaminated with *E. coli* could be found through interaction with water from the ponds; however, exposure to this bacterium did not exceed the considered health benchmark in any of the four sampled locations. Conversely, Denissen et al. (2021) studied the annual probability of infection from exposure to *E. coli* present in untreated rainwater for different ingestion scenarios and found that this prob of infection for accidental consumption exceeded the WHO benchmark of  $1 \times 10^{-4}$ . It should be noted that both studies consider the risk of different final responses, use different dose – response parameters and exposure rates.

In Figure 2b, the probability of GI illnesses related to *Enterococci* at the inner courtyard exceeded the USEPA mean illness rate when the simulation values were above the 75th percentile. This results are consistent with those identified by Chidamba and Korsten (2018),



they found that concentrations between 100 – 1000 CFU/ 100 mL entailed high risks ratings. As *E. coli* has been observed to live shorter than Enterococci when stored in rainwater tanks, the presence of this bacterium might indicate recent contamination; while, Enterococci tends to accumulate, high concentrations could indicate accumulated contamination in the tanks (Chidamba & Korsten, 2018).

Figure 2c presents the results regarding risk of GI illness from *Salmonella non-typhoid* due to ingestion of water from hand to mouth contact and ingestion of water droplets; here, exposure to water from the cistern under sun presented the highest risk of GI illnesses and was the only one exceeding the USEPA mean illness rate. Our results differ from the ones obtained by Ahmed et al. (2010b), they analyzed rainwater samples from collection tanks in Australia and performed a QMRA for *Salmonella spp.* and other pathogens considering exposure in different scenarios such as aerosol ingestion via hosing, and concluded that very low risks of infection could be expected from this bacterium in that exposure scenario due to extremely low volumes of ingestion. This could be explained by higher concentrations of *Salmonella non-typhoid* observed in this location and larger exposure volumes in the scenario considered in our study.

Regarding *P. aeruginosa*, from Figure 2d it can be concluded that the risks of getting a dermal infection due to this opportunistic bacterium through contact with the water from the blue elements in the inner courtyard is below the benchmark. The highest risks, however, were found at the cisterns, which could be explained by higher concentrations of this bacterium present in these locations. Our results are comparable to those of Roser et al. (2015), who reported that concentrations of  $10^4$  CFU/ mL of *P. aeruginosa* could cause an outbreak to a very low extent and a minimum geometric mean of  $1.8 \times 10^7$  CFU/ mL is needed for all the exposed population to get folliculitis.

### 3. Conclusion

This study presented the risks of illness/infection due to interaction with roof harvested rainwater present in blue elements built with ornamental purposes in an inner courtyard. RHRW microbiological quality varies greatly depending on weather conditions, catchment characteristics and design of the collection system.

From the results of our study, it can be concluded that the risks of illness/infection are relatively low at this specific location and for the studied exposure scenario; however, further analysis should be carried out in case the collected RHRW is considered for other purposes such as garden hosing, toilet flushing or other non-potable uses.

In general, microbiological quality of RHRW is rather poor, which is why appropriate treatment prior to use is recommended. Operation and maintenance of the collection system, cisterns and ponds can affect the water quality greatly, thus, it is suggested that ponds and cisterns are emptied and cleaned frequently, especially when high sediment accumulation is identified. A first flush diverter is also recommended to reduce the amount of microbial contamination.

Currently, water quality guidelines for rainwater use exist mainly for potable purposes, however, a generalized framework and quality criteria for non-potable uses should be developed to optimize public health promotion for roof-harvested rainwater. The present study intends to highlight the need for such guidelines in private areas.

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