

## Inactivation of indicator organisms on different surfaces after urban floods

Scoullos, Iosif Marios; Adhikari, Sabita; Lopez Vazquez, Carlos M.; van de Vossenberg, Jack; Brdjanovic, Damir

**DOI**

[10.1016/j.scitotenv.2019.135456](https://doi.org/10.1016/j.scitotenv.2019.135456)

**Publication date**

2020

**Document Version**

Final published version

**Published in**

Science of the Total Environment

**Citation (APA)**

Scoullos, I. M., Adhikari, S., Lopez Vazquez, C. M., van de Vossenberg, J., & Brdjanovic, D. (2020). Inactivation of indicator organisms on different surfaces after urban floods. *Science of the Total Environment*, 704, Article 135456. <https://doi.org/10.1016/j.scitotenv.2019.135456>

**Important note**

To cite this publication, please use the final published version (if applicable).  
Please check the document version above.

**Copyright**

Other than for strictly personal use, it is not permitted to download, forward or distribute the text or part of it, without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license such as Creative Commons.

**Takedown policy**

Please contact us and provide details if you believe this document breaches copyrights.  
We will remove access to the work immediately and investigate your claim.



## Inactivation of indicator organisms on different surfaces after urban floods

Iosif Marios Scoullou<sup>a,b,1,\*</sup>, Sabita Adhikari<sup>a,1</sup>, Carlos M. Lopez Vazquez<sup>a</sup>, Jack van de Vossenberg<sup>a</sup>, Damir Brdjanovic<sup>a,b</sup>

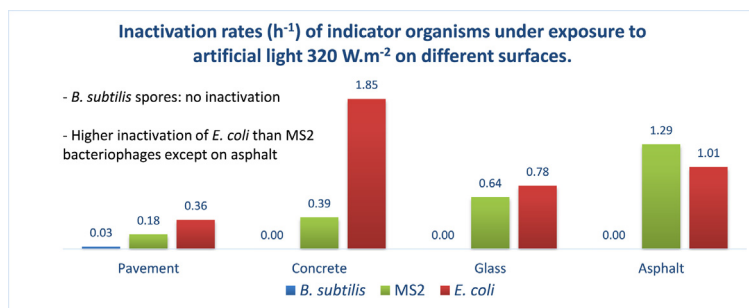
<sup>a</sup> Environmental Engineering & Water Technology Department, IHE Delft Institute for Water Education, P.O. Box 3015, Delft 2601 DA, The Netherlands

<sup>b</sup> Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, Delft 2629 HZ, The Netherlands

### HIGHLIGHTS

- Different surfaces were tested for inactivation of indicator organisms ( $25 \pm 5$  °C).
- Dark conditions (18 h): practically no inactivation for any organism and surface.
- *B. subtilis* spores: no significant inactivation under exposure to light for 6 h.
- MS2 bacteriophages: highest inactivation under exposure to light (6 h) on asphalt.
- *E. coli* was inactivated faster than MS2 on all surfaces except asphalt.

### GRAPHICAL ABSTRACT



### ARTICLE INFO

#### Article history:

Received 21 August 2019

Received in revised form 12 October 2019

Accepted 7 November 2019

Available online 20 November 2019

Editor: Patricia Holden

#### Keywords:

Solar inactivation  
Urban floods  
Waterborne diseases  
Surfaces

### ABSTRACT

The high frequency and intensity of urban floods caused by climate change, urbanisation and infrastructure failures increase public health risks when the flood water contaminated from combined sewer overflows (CSOs) or other sources of faecal contamination remains on urban surfaces. This study contributes to a better understanding of the effects of urban and recreational surfaces on the occurrence of waterborne pathogens. The inactivation of selected indicator organisms was studied under controlled exposure to artificial sunlight for 6 h followed by 18 h in dark conditions. Concrete, asphalt, pavement blocks and glass as control were inoculated with artificial floodwater containing, as indicator organisms, *Escherichia coli* bacteria, which are common faecal indicator bacteria (FIB) for water quality assessment, *Bacillus subtilis* spores chosen as surrogates for *Cryptosporidium parvum* oocysts and *Giardia* cysts, and bacteriophages MS2 as indicators for viral contamination. On practically all the surfaces in this study, *E. coli* had the highest inactivation under light conditions followed by MS2 and *B. subtilis*, except asphalt where MS2 was inactivated faster. The highest inactivation under light conditions was seen with *E. coli* on a concrete surface (pH 9.6) with an inactivation rate of  $1.85 \text{ h}^{-1}$ . However, the pH of the surfaces (varying between 7.0 and 9.6) did not have any influence on inactivation rates under dark conditions. MS2 bacteriophage had the highest inactivation under light conditions on asphalt with a rate of  $1.29 \text{ h}^{-1}$ . No die-off of *B. subtilis* spores was observed on any of the surfaces during the experiment, neither in light nor in dark conditions. This study underpins the need to use different indicator organisms to test their inactivation after flooding. It also suggests that given the

**Abbreviations:** AWI, air-water interface; CFU, colony forming units; CSOs, combined sewer overflows; FIB, faecal indicator bacteria; QMRA, Quantitative Microbial Risk Assessment; TPB, triple-phase boundary; UV, ultraviolet.

\* Corresponding author at: Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, Delft 2629 HZ, The Netherlands.

E-mail address: [I.M.Scoullou@tudelft.nl](mailto:I.M.Scoullou@tudelft.nl) (I.M. Scoullou).

<sup>1</sup> These authors contributed equally to this work.

<https://doi.org/10.1016/j.scitotenv.2019.135456>

0048-9697/© 2019 The Author(s). Published by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

sunlight conditions, concentration of indicator organisms and type of surface, the fate of waterborne pathogens after a flood could be estimated.

© 2019 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

The occurrence of extreme rainfalls, their impacts, and phenomena like urban flooding are predicted to be more frequent because of climate change, rapid urbanization, high population density and due to the failure and aging of the sewage infrastructure (Arnell and Gosling, 2016; ten Veldhuis et al., 2010). Along with physical damage, there is always a high chance of disease, when people are exposed to contaminated flood water.

Depending on the source of contamination, urban flood water quality varies. Flood water flowing as a result of high surface runoff in heavy rainfall may contain suspended and dissolved particles, as well as faeces from animals and birds (de Man et al., 2014). In addition, several urban surfaces such as streets and pathways get contaminated with human faeces from CSOs during extreme events (ten Veldhuis et al., 2010). As a result, numerous human and animal pathogens present in faeces may also be present in urban flood water. Urban and recreational surfaces have a possibility to act as a reservoir of FIB after urban floods. After the Elbe river flood in 2002 in Germany (Abraham and Wenderoth, 2005), high numbers of pathogenic bacteria were detected in the mud, streets, playgrounds and in the basement of flooded houses. In Toronto, Ontario, Canada, FIB have been detected in beach sand and in sand at playgrounds and sandboxes of urban settings and they were found to be detectable for several months (Staley et al., 2016).

Several outbreaks of waterborne diseases have been recorded after urban floods across the globe (Andrade et al., 2018). Presence of pathogens like norovirus, rotavirus, enterovirus, *Giardia* oocyst, *Cryptosporidium*, *Campylobacter*, and *Salmonella* was reported in the canals and recreational lakes of Amsterdam, contaminated from CSOs (Schets et al., 2008). These are the most common pathogens causing gastrointestinal problems, even in the developed world (de Wit et al., 2001). In a water plaza in The Netherlands (serving with the dual function of recreational area in dry seasons and of storm water retention during extreme events), Sales-Ortells and Medema (2015) found a high concentration of *Campylobacter*. The risk corresponding to recreational exposure appeared to be higher than the Dutch national incidence of *Campylobacter* disease. Also, a strong correlation between cholera outbreaks and flooding was reported in the urban area of Dhaka, Bangladesh (Reiner et al., 2012).

Although monitoring of microbial quality of flood water and of surface and recreational waters (e.g. in beaches and lakes) and the assessment of related public health risks from exposure to these waters is performed routinely on the basis of local and national guidelines (de Man et al., 2014; Mark et al., 2018; Sales-Ortells and Medema, 2015; ten Veldhuis et al., 2010), standard procedures and guidelines for FIB monitoring on recreational surfaces (e.g. beach sand) are almost inexistent (Staley et al., 2016). Moreover, although sunlight-mediated inactivation is considered as the main cause of disinfection in waste stabilisation ponds, with mechanisms depending on pH, salinity and dissolved oxygen (DO) (Dias et al., 2017), research on the survival of pathogens and indicator organisms on urban surfaces to quantify the associated public health risks is limited.

Previous studies showed that *E. coli* is not a suitable indicator for environmentally stable human viral, spore and oocyst forming pathogens (Niemiński et al., 2010; Harwood et al., 2005;

Bonadonna et al., 2002; Medema et al., 1997). Aerobic spores such as *B. subtilis* spores have been proposed as suitable surrogates of *Cryptosporidium* because of their higher persistence (Stelma, 2018; Bradford et al., 2016; Headd and Bradford, 2016; Mazoua and Chauveheid, 2005; Rice et al., 1996) and bacteriophages have been proposed as indicators of viral pathogens (Dias et al., 2018).

To better understand and quantify the effects of different surface types on the inactivation of different indicator organisms after urban floods, the inactivation of *E. coli*, *B. subtilis* spores and MS2 bacteriophages was studied. The study was done under controlled exposure to simulated sunlight on artificially flooded concrete, asphalt, pavement blocks and glass as control. This study contributes to a better estimation of the fate of waterborne pathogens, so that in combination with Quantitative Microbial Risk Assessment (QMRA) and mapping of urban surfaces, policy-making tools can be developed for the implementation of measures to mitigate public health risks after flooding.

## 2. Materials and methods

### 2.1. Experimental setup

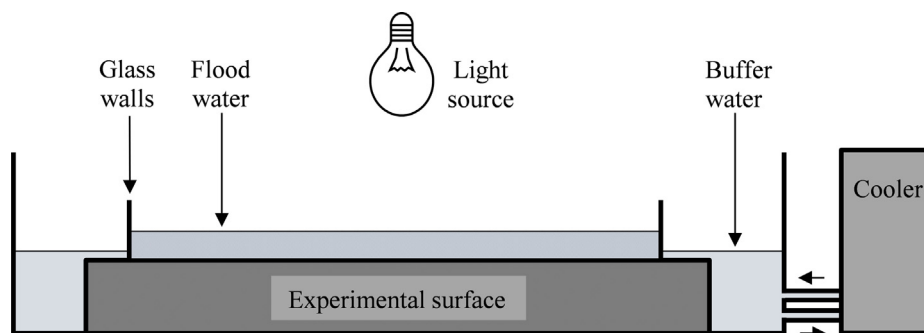
The experimental setup consisted of two open batch reactors in which different surfaces were tested (Fig. 1). Samples of asphalt and concrete and pavement blocks were collected from ongoing construction sites in Delft, The Netherlands. A glass panel, being nonporous and pH neutral was used as control. The reactors contained the tested materials submerged in demineralised water, and tubing around the inner side of the walls, connected to a cooler to maintain a stable temperature on the surfaces (at  $25 \pm 5$  °C). Sampling areas were defined on each surface in triplicate by glass walls, creating water wells of 100 cm<sup>2</sup> area each. The glass walls were glued watertight with aquarium silicone sealant that was tested for absence of biocidal activity before use. The sampling wells were filled with 100 mL (equivalent to 1 cm of water height in each well) of demineralised water to mimic rain, spiked with the initial concentration of indicator organisms according to each phase. This was the minimum amount of water necessary to keep the surfaces moist throughout the experiments because it has been shown by Scoullos et al., 2019b that loss of surface moisture leads to high *E. coli* inactivation rates. Similar conditions can be met in all types of shallow water bodies in urban and sub-urban open areas, including multifunctional storm water retention and detention basins often used as sport facilities or playgrounds during dry weather. The pH of the samples was recorded.

### 2.2. Indicator organisms

The indicator organisms studied were *Escherichia coli* (ATCC 25922), a common FIB for water quality assessment, *Bacillus subtilis* (12.01.31, GAP Lab, Canada) chosen as a non-pathogenic surrogate for *C. parvum* oocysts and *Giardia* cysts, and *E. coli* bacteriophage MS2 (ATCC 15597-B1) as an indicator for viral contamination.

#### 2.2.1. *E. coli*

Before each batch experiment *E. coli* was incubated in 1.3% w/v sterile Oxoid CM0001 Nutrient Broth (Oxoid Ltd., Basingstoke, UK)



**Fig. 1.** Schematic representation of a reactor exposed to artificial light. Two such reactors were used in parallel. In the experiments under real sunlight the cooler was not used.

solution in Erlenmeyer flasks for 24 h at 37 °C. After incubation, the concentration of the inoculum was around  $3 \cdot 10^9$  colony forming units (CFU) per mL. The initial concentration of *E. coli* for the experiment was chosen to be around  $10^5$  CFU.mL<sup>-1</sup> based on the maximum event mean concentration of *E. coli* and enterococci in urban storm water runoff of first flush (Mark et al., 2018; Hathaway and Hunt, 2010). The enumeration of *E. coli* in the samples was performed by counting the number of CFU on chromocult coliform agar (CCA) (Merck KGaA, Darmstadt, Germany) plates after 24 h of incubation at 37 °C. Appropriate 10-fold dilution steps in 0.1% peptone physiological salt solution were used where needed. The plates were spread in triplicate.

#### 2.2.2. *B. subtilis*

The *B. subtilis* spores were enumerated with the spread plate method. The spores' propagation was adapted from USEPA (2006), UV (ultraviolet) Disinfection Guidance Manual For the Final Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). The resulting culture was stained (with malachite green as primary stain and safranin as counter stain) and examined under the microscope to confirm the presence of free spores without the vegetative cells (Hussey and Zayaitz, 2007; Chang et al., 1985). The *B. subtilis* spore suspension was assayed in triplicate by spreading it over plate count (PC) agar plates. Appropriate dilutions of the samples were made with 1 mM phosphate buffered saline (PBS). They were plated and spread uniformly on PC plates and incubated for 24 h at 37 °C.

#### 2.2.3. MS2 bacteriophages

The enumeration of MS2 bacteriophages was carried out by double layer plaque assay following the ISO 10705-1:1995 procedure. *E. coli* (ATCC 15597) was used as a host organism. The host culture was grown to exponential phase (concentration  $10^8$  CFU.mL<sup>-1</sup>) and mixed with the diluted sample. The solution was mixed and poured uniformly over the surface of tryptone yeast glucose agar (TYGA) plates and incubated for  $18 \text{ h} \pm 2 \text{ h}$  at 37 °C. Sterile filter pipette tips were used in all steps to avoid contamination.

#### 2.3. Light source and parameters

Simulated sunlight was produced using an OSRAM HQI-BT 400 W/D PRO (OSRAM GmbH, Munich, Germany) metal halide lamp with built-in UV filter that blocks wavelengths shorter than 320 nm. The cut-off filter was used because lower wavelengths are highly attenuated by the terrestrial atmosphere, especially in a cloudy sky (Calbó et al., 2005). The same lamps were used by Scoullos et al., 2019b, 2019a and a similar cut-off filter was used in a solar disinfection study on poliovirus and *Acanthamoeba polyphaga* cysts by Heaselgrave et al. (2006). These metal halide lamps

were used because their spectrum is continuous, like sunlight, with a few peaks in the area of visible light, the highest one being at 540 nm (Calin and Parasca, 2008). The light intensity was fixed at  $320 \text{ W.m}^{-2}$ , approximating the extreme conditions of minimal daily direct solar irradiance at a latitude of 60° N, observed at winter solstice (Arabali et al., 2017), as very few cities are located at higher latitudes. The photon flux, in  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ , was measured with a LI-250A Light Meter equipped with an underwater quantum sensor (LI-COR Biosciences, Inc., Lincoln, Nebraska, USA). This sensor has a uniform sensitivity in the range of wavelengths between 400 and 700 nm, limitation taken into account when calculating the lamp irradiance spectrum. Light intensity was calculated considering the spectral power distribution of the lamp, as done by Scoullos et al., 2019a using the same lamp, who calculated that the UV-A band wavelengths account for 5.1% of the total irradiance. In the tests performed with natural sunlight, the light intensity was calculated in a similar manner, taking into account the spectral power distribution of solar light from reference spectra (Air Mass 1.5) (ASTM, 2012), which has 5.9% UV-A and 0.2% UV-B. The average intensity was calculated as a weighted average of the measured values.

#### 2.4. Experimental design

##### 2.4.1. Inactivation of *E. coli*, *B. subtilis* spores and MS2 bacteriophages on surfaces under artificial light and dark conditions

This phase was carried out in two batches. In the first batch, the inactivation of *E. coli* and *B. subtilis* was measured in the water wells set up on the pavement blocks, concrete, asphalt, and glass. The inactivation was studied on a light phase of 6 h followed by 18 h of dark phase, representing the minimal daylight duration at a latitude of 60° N and 60° S, observed at winter and summer solstice, respectively. In addition, the inactivation of *E. coli* was studied separately on the concrete surface for 24 h in dark conditions to test whether the high pH of concrete affects inactivation in these conditions. The experiments with MS2 were carried out separately (second batch) under the same conditions because high concentrations of bacteria other than the host for MS2 could hinder plaque enumeration. The effect of temperature on the inactivation of all three indicator organisms was studied separately, by keeping all the organisms on a glass surface at a constant temperature of 33 °C for 24 h in dark conditions, in the incubator.

##### 2.4.2. Inactivation of *E. coli*, *B. subtilis* spores and MS2 bacteriophages on pavement under natural sunlight and dark conditions

The inactivation of *E. coli*, *B. subtilis*, and MS2 was studied outdoors on pavement, under natural sunlight, in two open batch reactors, in duplicate. Pavement was tested in real conditions to evaluate whether the real sunlight would accelerate inactivation,

because the lowest, most critical inactivation under artificial light was observed on pavement. Each reactor contained two pavement blocks of the same type as in the previous experiment and water around the tiles was kept as a buffer to minimize the heating effect from sunlight, but without the use of a cooler. The pavement blocks in the first reactor were inoculated with *E. coli* and *B. subtilis* together, while in the second reactor they were inoculated with MS2. During the night, the reactors were covered using a transparent glass cover to be protected from wind and other interferences. The experiment was carried out on February 26, 2019, a dry day with continuous sunlight (apparent sunrise at 7:36 and sunset at 18:16 (NOAA, 2019)). As “light phase” was considered a period of 8 h, from 9:00 to 17:00, when the setup was exposed to direct sunlight. The following 16 h were considered as part of the “dark phase”.

### 2.5. Sampling and physicochemical parameters

Samples of 2.5 mL were taken from each water well on the surfaces. During the light phase, hourly samples were taken for the enumeration of *E. coli* and *B. subtilis*, while for MS2, as well as for all organisms under dark conditions, samples were taken every two hours. Samples were taken from the centre of each water well after mixing the water with the help of a pipette by drawing up and releasing the sample from the pipette tip for around 3–5 times. The temperature of the surfaces was measured by using an infrared thermometer (FERM, ITM1001, The Netherlands). pH was measured with a handheld pH meter (WTW pH 323, WTW GmbH, Weilheim, Germany) and with pH paper (Fisherbrand pH indicator sticks). The data for each test sample was taken in triplicate for the first part while for the rest it was taken in duplicate.

### 2.6. Data analysis

The inactivation of indicator organisms was calculated based on the Chick–Watson first-order exponential decay equation (Eq. (1)).

$$\frac{C_t}{C_0} = e^{-kt} \quad (1)$$

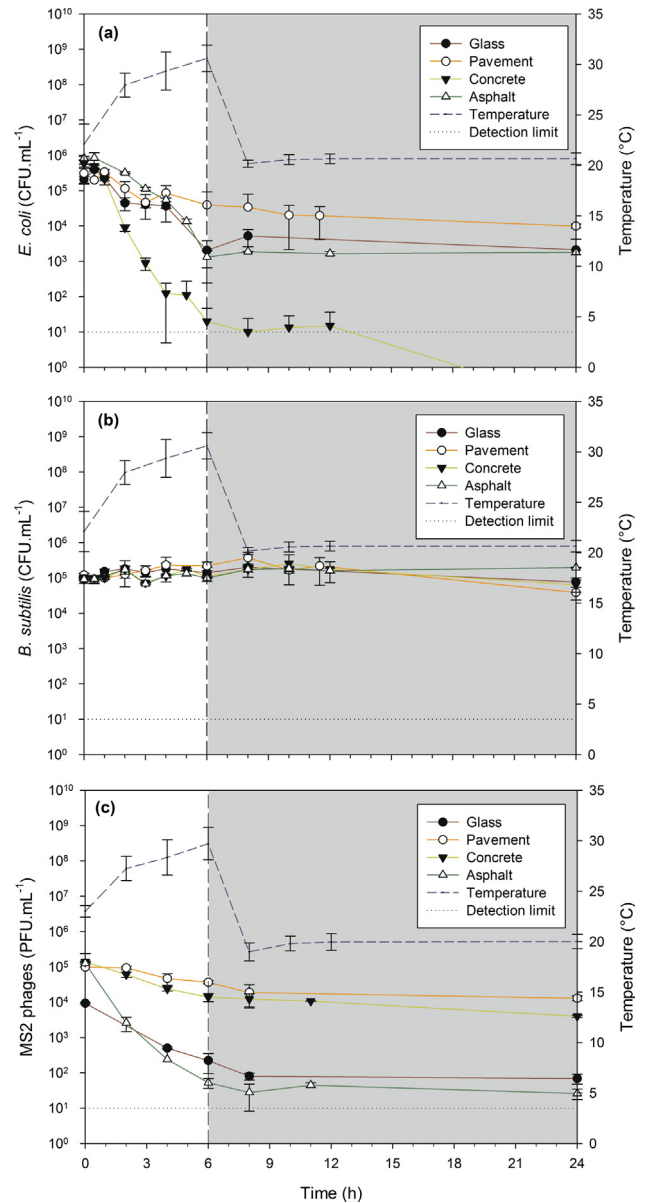
Where  $t$  is the time (d),  $C_t$  is the concentration of microorganisms (CFU.mL<sup>-1</sup> for bacteria or PFU.mL<sup>-1</sup> for bacteriophages) in time  $t$ ,  $C_0$  is the initial concentration of the microorganisms and  $k$  (d<sup>-1</sup>) is the first order inactivation rate constant. The sample for initial concentration of the indicator organisms was taken immediately after the inoculum was added to the water well on the surfaces. The log survival rate of indicator organism  $\ln(C_t/C_0)$  was plotted against time ( $t$ ) and the inactivation rate was calculated for each experiment as the value corresponding to the slope of linear regression.

The statistical significance of the data in all the experiments was analysed using paired two-sample  $t$ -tests for means with a significance level of  $\alpha = 0.05$ . In the case of *E. coli* and MS2 phase, the  $t$ -test was performed only for dark conditions. However, for *B. subtilis* spores, the test was carried out between the initial concentration at the beginning of the light phase (0 h) and the final concentration at the end of the dark phase (24 h). To compare inactivation rates between *E. coli* and MS2, two-factor ANOVA tests with replication were used ( $\alpha = 0.05$ ).

## 3. Results

### 3.1. Inactivation of *E. coli* under artificial light and dark conditions

The inactivation profiles of *E. coli* on all the tested surfaces is shown in Fig. 2a. The graph shows that there was no significant



**Fig. 2.** Inactivation of *E. coli* (a), *B. subtilis* (b) and MS2 bacteriophages (c) on different surfaces. The white area represents the light phase and the grey area represents the dark phase.

inactivation under dark conditions on any of the surfaces over the measurement period. The  $t$ -test for the dark phase confirmed that there was no significant difference in the concentration of *E. coli* before and after the dark phase. In the case of concrete the last point (24 h) was under the detection limit, but a repetition of the experiment in dark conditions confirmed that the inactivation was not statistically significant. Under exposure to light, the inactivation was significant on all surfaces: the highest one took place on concrete with an inactivation rate of 1.85 h<sup>-1</sup> followed by asphalt (1.01 h<sup>-1</sup>), glass (0.78 h<sup>-1</sup>) and the lowest was on pavement (0.36 h<sup>-1</sup>). The summary can be seen in Table 1.

The inactivation of all three organisms was statistically insignificant at 33 °C for 24 h in dark conditions on glass ( $p = 0.19$  and sample size  $n = 8$  for *E. coli*,  $p = 0.43$  and  $n = 8$  for *B. subtilis*,  $p = 0.78$  and  $n = 5$  for MS2, data not shown). Therefore, since in dark conditions there was no inactivation at any of the tested temperatures, the increase of temperature alone did not affect inactivation.

**Table 1**

Summary of all decay rates under artificial light, sunlight and dark conditions for all indicator organisms. NSS is “Not Statistically Significant” ( $\alpha = 0.05$ , log values). The number of samples ( $n$ ) is indicated.

Surfaces	Average pH	<i>E. coli</i>		<i>B. subtilis</i> spores		MS2 bacteriophages			
		Light		Dark		Light		Dark	
		Decay rate ( $\text{h}^{-1}$ )	R <sup>2</sup>	Decay rate ( $\text{h}^{-1}$ )	Overall	Decay rate ( $\text{h}^{-1}$ )	R <sup>2</sup>	Decay rate ( $\text{h}^{-1}$ )	
<i>Artificial light (320 W.m<sup>-2</sup> for 6 h)</i>									
Glass	6.9	0.78 ( $n = 7$ )	0.90	NSS ( $p = 0.59$ , $n = 3$ )	NSS ( $p = 0.47$ , $n = 9$ )	0.64 ( $n = 4$ )	0.99	NSS ( $p = 0.12$ , $n = 3$ )	
Pavement	8.1	0.36 ( $n = 7$ )	0.78	NSS ( $p = 0.27$ , $n = 5$ )	0.03 ( $p = 0.03$ , $n = 11$ )	0.18 ( $n = 4$ )	0.91	NSS ( $p = 0.51$ , $n = 3$ )	
Concrete	9.6	1.85 ( $n = 8$ )	0.96	NSS ( $p = 0.19$ , $n = 4$ )	NSS ( $p = 0.17$ , $n = 12$ )	0.39 ( $n = 4$ )	0.99	NSS ( $p = 0.07$ , $n = 4$ )	
Asphalt	7.6	1.01 ( $n = 7$ )	0.93	NSS ( $p = 0.65$ , $n = 4$ )	NSS ( $p = 0.19$ , $n = 11$ )	1.29 ( $n = 4$ )	0.96	0.03 ( $p = 0.01$ , $n = 4$ )	
<i>Natural sunlight (512 W.m<sup>-2</sup> for 8 h)</i>									
Pavement	8.0	1.03 ( $n = 5$ )	0.84	NSS ( $p = 0.97$ , $n = 2$ )	NSS ( $p = 0.93$ , $n = 6$ )	0.11 ( $n = 4$ )	0.91	NSS ( $p = 0.50$ , $n = 2$ )	

### 3.2. Inactivation of *B. subtilis* spores under artificial light and dark conditions

No significant inactivation under light or dark conditions was observed for *B. subtilis* spores on any of the surfaces in this study (Fig. 2b). The concentration of *B. subtilis* spores was constant, regardless of the different surfaces used. Statistical  $t$ -tests conducted for *B. subtilis* spores confirmed for all surfaces except pavement that any differences between the concentrations at 0 h and 24 h were not statistically significant, implying that no inactivation was induced on the spores. In the case of pavement, a minor overall inactivation rate of  $0.03 \text{ h}^{-1}$  was observed ( $p = 0.03$ ). However,  $t$ -tests performed separately on pavement for the data of the light phase (0 h and 6 h) and for the data from the beginning of the dark phase (6 h) until 12 h, showed no statistical significance (with  $p = 0.052$  and  $p = 0.77$ , respectively). Therefore, the only statistical difference is caused by the last experimental point at 24 h, and can be considered to be an outlier.

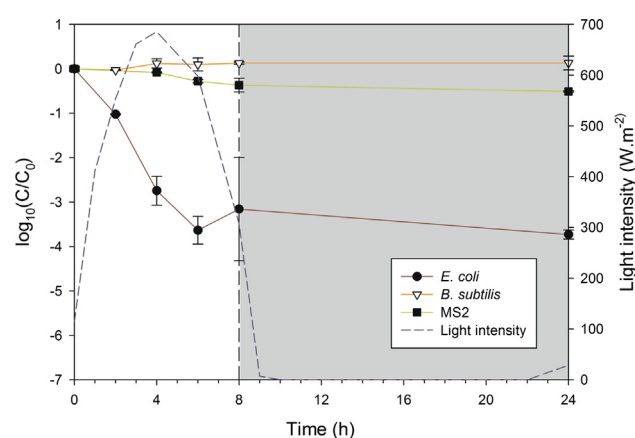
### 3.3. Inactivation of MS2 bacteriophages under artificial light and dark conditions

The inactivation of MS2 bacteriophages on all the surfaces is depicted in Fig. 2c. The  $t$ -tests performed for MS2 bacteriophages in dark conditions, for all surfaces except asphalt, indicated that inactivation was statistically insignificant. In the case of asphalt the inactivation was statistically significant ( $p = 0.01$ ), but the rate ( $0.03 \text{ h}^{-1}$ ) is an order of magnitude lower than the inactivation under light conditions, making it negligible in practice. On the other hand, under light conditions the highest inactivation was observed on asphalt, followed by glass, concrete and the lowest one was on pavement blocks, all being statistically significant. The summary of all the inactivation rates can be seen in Table 1.

The trends followed by the inactivation rate of all three indicator organisms under light conditions were the same on all surfaces (glass, pavement, concrete), except on asphalt, *E. coli* being the most sensitive organism, followed by MS2 and *B. subtilis* spores. In the case of asphalt, the inactivation under light conditions of *E. coli* and MS2 were similar followed by the inactivation of *B. subtilis* spores.

### 3.4. Inactivation of *E. coli*, *B. subtilis* spores and MS2 bacteriophages on pavement under natural sunlight and dark conditions

The results of the study performed under natural sunlight on pavement can be seen in Fig. 3. The average pH measured on the water well on the pavement was 8.2. The weighted average light



**Fig. 3.** Inactivation of *E. coli*, *B. subtilis* and MS2 on pavement under natural sunlight (white area) and dark (grey area).

intensity during the light phase was calculated to be  $515 \text{ W.m}^{-2}$ . Also, the average temperature during the light and dark phase was  $15.5 \text{ }^{\circ}\text{C}$  and  $4.5 \text{ }^{\circ}\text{C}$ , respectively.

The results show that there was insignificant inactivation of all three indicator organisms under dark conditions. On the other hand, under light conditions, *E. coli* had an inactivation rate of  $1.03 \text{ h}^{-1}$  and the MS2 bacteriophage showed a very low inactivation ( $0.11 \text{ h}^{-1}$ ), both statistically significant. There was no significant change in the concentration of *B. subtilis* spores even after a second day of exposure (data shown only for the first 24 h). Table 1 summarises all the inactivation rates of the three indicator organisms for the experiment conducted under natural sunlight and dark conditions.

## 4. Discussion

### 4.1. *E. coli*

The highest inactivation under light conditions was observed on *E. coli*. Biological weighting functions of *E. coli* show that the inactivation effect of wavelengths higher than 400 nm is negligible (Nelson et al., 2018). Therefore, as the lamp has a cut-off filter at 320 nm, the total inactivation under artificial light was caused by UV-A radiation (Scoullou et al., 2019b) which can cause damage to *E. coli* cells when the photons are absorbed by sensitizer molecules and induce the formation of photo-reactive intermediates, within or outside the cells (Nelson et al., 2018). Nevertheless,

photosynthetically active radiation (400–700 nm) can still be important for *E. coli* inactivation (Dias and von Sperling, 2018), especially in deeper waters or higher turbidity levels where UV is attenuated (Dias and von Sperling, 2017).

On concrete the highest inactivation under light conditions of *E. coli* observed may be explained due to the combined effect of UV from the light source and pH of concrete (average pH 9.6). This was interpreted based on the result that all the surfaces, except concrete, had neutral pH ranging between 6.5 and 8.0 and the same light intensity was applied to all surfaces. On the other hand, *E. coli* did not die-off when kept for 24 h in dark, therefore the pH of concrete alone did not lead to the inactivation of *E. coli*. Further research is needed on the combined effect of concrete samples with different properties (with varying composition and wearing) and light intensity.

The inactivation of *E. coli* seen on asphalt could be explained due to the combined effect of light and the presence of complex aromatic hydrocarbons, extracts of crude oils, in asphalt (Xia et al., 2019) that may be detrimental to *E. coli*. However, the composition of asphalt does not have a significant impact on inactivation on its own, as the inactivation under dark conditions was negligible. Furthermore, there is rather limited literature regarding the inactivation effects of *E. coli* on asphalt. Hence, the effects of the composition of asphalt on the inactivation of *E. coli* (and other organisms) under light conditions can be the starting point for future studies.

The inactivation of *E. coli* was lower on pavement ( $0.36 \text{ h}^{-1}$ ) than on the glass control ( $0.78 \text{ h}^{-1}$ ). This may be attributed to the fact that the glass surface was shiny and acted as a reflector, increasing the exposure to light, in a similar way to solar mirrors and reflectors used in solar photo-reactors (Nalwanga et al., 2014). The neutral role of the surface of the pavement blocks on *E. coli* die-off is also supported by the fact that the inactivation rate on pavement is relatively similar to the rates measured in a previous study in clear artificial flood water under exposure to the same lamp and light intensity for a duration of 6 h per day ( $0.21 \text{ h}^{-1}$ ) or 12 h per day ( $0.37 \text{ h}^{-1}$ ) (Scoullou et al., 2019a).

#### 4.2. *B. subtilis* spores

*B. subtilis* spores had a constant concentration on all surfaces (except a very small die-off on pavement) regardless the light or dark conditions, including the effects of natural sunlight on pavement. These results reflect that *B. subtilis* spores are much more resistant to environmental stresses such as UV light and pH as compared to *E. coli* and MS2 viruses. Several studies confirm the endurance of *B. subtilis* spores against various environmental stresses. For instance, Chang et al. (1985) found that *B. subtilis* spores were more resistant to UV doses than vegetative bacteria like *E. coli*, *Staphylococcus aureus* and *Shigella sonnei*. Nicholson et al. (2000) also concluded that the decrease in concentration of *B. subtilis* spores could be barely seen even after exposure to solar heating (but protected from UV primary effects) at a temperature higher than  $70 \text{ }^\circ\text{C}$ . The high resistance of *B. subtilis* spores has been attributed by Nicholson et al. (2000) to different factors like presence of spore coat and low relative permeability of spore coat, among other characteristics and features. The layers present within the spore coat were found to be responsible for the resistance of spores in exposure to UV for both sunlight and artificial radiation (Mamane et al., 2007; Riesenman and Nicholson, 2000). This makes *B. subtilis* spores suitable surrogates for persistent organisms like *Cryptosporidium* (Headd and Bradford, 2016).

#### 4.3. MS2 bacteriophages

During the dark phase, the concentration of MS2 remained constant on all surfaces, with a negligible inactivation on asphalt

( $0.03 \text{ h}^{-1}$ ), therefore the composition of the materials alone did not affect MS2.

The inactivation rate of MS2 under light conditions increased from pavement to glass and concrete, reaching the highest with asphalt. Furthermore, although on concrete and glass under light conditions MS2 was more resistant than *E. coli* ( $F = 38.4$ ,  $p = 0.004$  and  $F = 110.2$ ,  $p = 0.001$ , respectively), and there was no statistically significant difference on pavement ( $F = 0.68$ ,  $p = 0.46$ ) the inactivation rate of MS2 was slightly higher than the one of *E. coli* on asphalt,  $1.29 \text{ h}^{-1}$  and  $1.01 \text{ h}^{-1}$ , respectively ( $F = 128.8$ ,  $p = 0.0003$ ). However, the possibility of a substantial attachment of MS2 viruses on the hydrophobic surface of asphalt (Farkas et al., 2015; Dika et al., 2013; Hefer et al., 2006) was ruled out because of the absence of significant decrease of MS2 concentration in the water phase in dark conditions. The same applies to the other indicator organisms as well because in all cases the samples were taken from the bulk water phase and any organisms attached would not be included in the sample. Moreover, the phenomenon of aggregation of bacteriophages, which would lead to fewer plaque counts than the actual viruses, did not take place because the pH of the samples on all the surfaces was higher than the isoelectric point of MS2 bacteriophages (pH 3.9) at which this phenomenon occurs (Furiga et al., 2011; Gassilloud and Gantzer, 2005; Langlet et al., 2007).

Bacteriophage inactivation has also been attributed to the exposure of bacteriophages to interfaces like air–water interface (AWI) or gas, liquid and solid interface known as triple-phase boundary (TPB), due to the lethal unfolding of the hydrophobic part of the viral capsid on the interface, an effect which is stronger on hydrophobic surfaces (Furiga et al., 2011; Thompson et al., 1998; Thompson and Yates, 1999; Trouwborst et al., 1974). However, in the experimental setup no inactivation caused by exposure to an AWI or TPB interface was observed in dark conditions, therefore the above is likely not a significant factor.

From all these observations, it can be concluded that a detailed quantification of the contribution of each process (inactivation, adhesion, exposure to TPB, etc.) on the occurrence of MS2 bacteriophage is complex (Dika et al., 2013), as is for bacteria. Hence, subsequent studies can include further analysis at a microscopic level, including the nature of the viruses (such as surface charge and hydrophobicity, among others), properties of the surfaces where viruses were exposed to (like roughness, hydrophobic/hydrophilic nature), properties of the medium and interactions of viruses with particles and with other organisms (Verbyla and Mihelcic, 2015).

#### 4.4. Inactivation of *E. coli*, *B. subtilis* spores and MS2 bacteriophages on pavement under natural sunlight and dark conditions

The die-off of *E. coli* on pavement was almost triple under natural sunlight than in the tests exposed to the artificial light source. This can be explained owing to the fact that the weighted average sunlight intensity measured was  $512 \text{ W}\cdot\text{m}^{-2}$ , which was around 1.5 times higher than the light intensity used in the setup with artificial light source (i.e.  $320 \text{ W}\cdot\text{m}^{-2}$ ). Also, the duration of the light phase was 8 h under sunlight while it was only 6 h for artificial light. Thus, both the intensity and exposure time were higher in the experiment carried out under natural sunlight. In addition, even though sunlight comprises only small amounts (0.2% of reference spectrum) of UV-B radiation that was cut-off in the lamp, UV-B can have detectable effects on survival of microorganisms (Nelson et al., 2018; Dias et al., 2017; USEPA, 2010). The inactivation of MS2 under exposure to natural sunlight was similar to the inactivation under artificial light. The effect of UV-B on MS2 was little compared to *E. coli*, as was also observed by Lian et al. (2018) when both were exposed to

4.5 W.m<sup>-2</sup> of UV-B radiation, probably because of the larger physical size and amount of genetic material in *E. coli* compared to MS2.

#### 4.5. General observations

*E. coli* and MS2 followed similar trends of inactivation under light conditions on the different surfaces tested (with the exception of asphalt), *E. coli* having the highest inactivation, followed by MS2, while *B. subtilis* practically did not show any inactivation. In the case of asphalt, the inactivation rates of *E. coli* and MS2 under light conditions were similar and that of *B. subtilis* spores was the lowest. A similar trend was observed for UV disinfection by Chang et al. (1985) where viruses (polio and rota virus), spores (*B. subtilis*) and cysts (*Acanthamoeba castellanii*) were found to be 3–4, 9 and 15 times more resistant than vegetative bacterial cells (*E. coli*, *S. aureus* and *S. sonnei*). This again brings up an important conclusion that in order to assess the public health risk after urban floods, it is necessary to monitor viral and spore forming organisms that can act as surrogate for environmentally stable pathogens capable of surviving as spores or oocysts (Dias et al., 2018; Headd and Bradford, 2016). For this reason, it is also necessary to develop low cost, fast and reliable detection techniques for these indicators.

Although this study provides a better understanding of the inactivation of indicators for bacterial and viral contamination and parasite oocysts after urban floods, further research can be undertaken with different kinds of indicator organisms, in particular helminth eggs because they are very resistant (Nelson, 2003), cysts and oocysts, or with a combination of selected indicator organisms, in real environmental conditions, with different levels of turbidity and/or in a wider variety of surfaces found in the urban environment with different levels of hydrophobicity and pH, as well as different weathering conditions. This research, in combination with QMRA and mapping of urban surfaces, and taking into consideration the influence of the geographical location and time of the year on surface solar irradiance, can be used to develop policy-making tools for the implementation of measures to mitigate public health risks after flooding. Based on the behaviour and survival of the indicator organisms on those surfaces, and with the help of GIS maps, precautionary and public awareness measures can be taken. This can be a valuable input to plan immediate actions regarding the microbial risk and safety, to concerned authorities, after urban flooding.

## 5. Conclusions

After testing different surfaces and sunlight exposures to *E. coli*, *B. subtilis* and MS2, the following conclusions can be made:

- *E. coli* had the highest inactivation under artificial light exposure followed by MS2 bacteriophages, while *B. subtilis* spores were very stable, practically not inactivated on any of the tested surfaces, with the exception of asphalt. On asphalt the inactivation of *E. coli* and MS2 under light conditions was similar, due to much faster inactivation of MS2.
- The highest inactivation under light conditions was that of *E. coli* on concrete, with an inactivation rate of 1.85 h<sup>-1</sup>, attributed to the synergetic effect of light and high pH (pH 9.6) of concrete.
- MS2 bacteriophages had the highest inactivation under light conditions on asphalt with a rate of 1.29 h<sup>-1</sup>.
- No inactivation of *B. subtilis* spores was observed in any of the experiments conducted, indicating the resistance of *B. subtilis* spores to the conditions applied.

- No inactivation under dark conditions of *E. coli*, *B. subtilis*, and MS2 bacteriophages was observed, concluding that the sole effect of pH and other properties of the surfaces (pavement, concrete and asphalt) was not significant.
- *E. coli* cannot be used as a suitable indicator for human viral, spore forming and protozoan pathogens. Hence, it is always necessary to monitor a combination of indicator organisms that resemble more to environmentally stable pathogens, like those surviving as spores or oocysts, in order to assess the public health risk after urban floods.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

Mr. Scoullos would like to gratefully acknowledge the Academy of Athens for his Scholarship and the Alexander S. Onassis Public Benefit Foundation for a Research Grant, thanks to which the present work was realised. Ms. Adhikari would like to gratefully acknowledge the Netherlands Fellowship Programme (NFP) for its financial support. Thanks are due to the staff of the IHE Delft laboratories for their support and Dr. Arjan van Dijk for his scientific advice on action spectra.

## References

- Abraham, W.-R., Wenderoth, D.F., 2005. Fate of facultative pathogenic microorganisms during and after the flood of the Elbe and Mulde rivers in August 2002. *Acta Hydrochim. Hydrobiol.* 33, 449–454. <https://doi.org/10.1002/abeh.200400587>.
- Andrade, L., O'Dwyer, J., O'Neill, E., Hynds, P., 2018. Surface water flooding, groundwater contamination, and enteric disease in developed countries: A scoping review of connections and consequences. *Environ. Pollut.* 236, 540–549. <https://doi.org/10.1016/j.envpol.2018.01.104>.
- Arabali, A., Ghofrani, M., Bassett, J.B., Pham, M., Moeini-Aghataei, M., 2017. Chapter 7 – Optimum sizing and siting of renewable-energy-based DG units in distribution systems. In: Erdinc, O. (Ed.), *Optimization in Renewable Energy Systems*. Butterworth-Heinemann, Boston, pp. 233–277. <https://doi.org/10.1016/B978-0-08-101041-9.00007-7>.
- Arnell, N.W., Gosling, S.N., 2016. The impacts of climate change on river flood risk at the global scale. *Clim. Change* 134, 387–401. <https://doi.org/10.1007/s10584-014-1084-5>.
- ASTM, 2012. ASTM G173-03(2012), Standard Tables for Reference Solar Spectral Irradiances: Direct Normal and Hemispherical on 37° Tilted Surface. ASTM International, West Conshohocken, PA. <https://doi.org/10.1520/G0173-03R12>.
- Bonadonna, L., Briancesco, R., Ottaviani, M., Veschetti, E., 2002. Occurrence of *Cryptosporidium* oocysts in sewage effluents and correlation with microbial, chemical and physical water variables. *Environ. Monit. Assess.* 75, 241–252. <https://doi.org/10.1023/A:1014852201424>.
- Bradford, S.A., Kim, H., Headd, B., Torkzaban, S., 2016. Evaluating the transport of *Bacillus subtilis* spores as a potential surrogate for *Cryptosporidium parvum* oocysts. *Environ. Sci. Technol.* 50, 1295–1303. <https://doi.org/10.1021/acs.est.5b05296>.
- Calbó, J., Pagès, D., González, J.-A., 2005. Empirical studies of cloud effects on UV radiation: a review. *Rev. Geophys.* 43, RG2002. <https://doi.org/10.1029/2004RG000155>.
- Calin, M.A., Parasca, S.V., 2008. Light sources for photodynamic inactivation of bacteria. *Lasers Med. Sci.* 24, 453–460. <https://doi.org/10.1007/s10103-008-0588-5>.
- Chang, J.C.H., Ossoff, S.F., Lobe, D.C., Dorfman, M., Dumais, C.M., Qualls, R., Johnson, J. D., 1985. UV inactivation of pathogenic and indicator organisms. *Appl. Environ. Microbiol.* 49, 1361–1365.
- de Man, H., van den Berg, H.H.J.L., Leenen, E.J.T.M., Schijven, J.F., Schets, F.M., van der Vliet, J.C., van Knapen, F., de Roda Husman, A.M., 2014. Quantitative assessment of infection risk from exposure to waterborne pathogens in urban floodwater. *Water Res.* 48, 90–99. <https://doi.org/10.1016/j.watres.2013.09.022>.
- de Wit, M.A., Koopmans, M.P., Kortbeek, L.M., van Leeuwen, N.J., Vinjé, J., van Duynhoven, Y.T., 2001. Etiology of gastroenteritis in sentinel general practices in The Netherlands. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* 33, 280–288. <https://doi.org/10.1086/321875>.



- Dias, D.F.C., Passos, R.G., von Sperling, M., 2017. A review of bacterial indicator disinfection mechanisms in waste stabilisation ponds. *Rev. Environ. Sci. Biotechnol.* 16, 517–539. <https://doi.org/10.1007/s11157-017-9433-2>.
- Dias, D.F.C., von Sperling, M., 2018. Vertical profiling and modelling of *Escherichia coli* decay in a shallow maturation pond operating in a tropical climate. *Environ. Technol.* 39, 759–769. <https://doi.org/10.1080/09593330.2017.1310936>.
- Dias, D.F.C., von Sperling, M., 2017. Solar radiation (PAR, UV-A, UV-B) penetration in a shallow maturation pond operating in a tropical climate. *Water Sci. Technol.* 76, 182–191. <https://doi.org/10.2166/wst.2017.203>.
- Dias, E., Ebdon, J., Taylor, H., 2018. The application of bacteriophages as novel indicators of viral pathogens in wastewater treatment systems. *Water Res.* 129, 172–179. <https://doi.org/10.1016/j.watres.2017.11.022>.
- Dika, C., Ly-Chatain, M.H., Francius, G., Duval, J.F.L., Gantzer, C., 2013. Non-DLVO adhesion of F-specific RNA bacteriophages to abiotic surfaces: importance of surface roughness, hydrophobic and electrostatic interactions. *Colloids Surf. Physicochem. Eng. Asp.* 435, 178–187. <https://doi.org/10.1016/j.colsurfa.2013.02.045>.
- Farkas, K., Varsani, A., Pang, L., 2015. Adsorption of rotavirus, MS2 bacteriophage and surface-modified silica nanoparticles to hydrophobic matter. *Food Environ. Virol.* 7, 261–268. <https://doi.org/10.1007/s12560-014-9171-3>.
- Furiga, A., Pierre, G., Glories, M., Aïmar, P., Roques, C., Causserand, C., Berge, M., 2011. Effects of ionic strength on bacteriophage MS2 behavior and their implications for the assessment of virus retention by ultrafiltration membranes. *Appl. Environ. Microbiol.* 77, 229–236. <https://doi.org/10.1128/AEM.01075-10>.
- Gassilloud, B., Gantzer, C., 2005. Adhesion-aggregation and inactivation of *Poliovirus 1* in groundwater stored in a hydrophobic container. *Appl. Environ. Microbiol.* 71, 912–920. <https://doi.org/10.1128/AEM.71.2.912-920.2005>.
- Harwood, V.J., Levine, A.D., Scott, T.M., Chivukula, V., Lukasik, J., Farrah, S.R., Rose, J.B., 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Appl. Environ. Microbiol.* 71, 3163–3170. <https://doi.org/10.1128/AEM.71.6.3163-3170.2005>.
- Hathaway, J.M., Hunt, W.F., 2010. Evaluation of first flush for indicator bacteria and total suspended solids in urban stormwater runoff. *Water Air Soil Pollut.* 217, 135–147. <https://doi.org/10.1007/s11270-010-0574-y>.
- Headd, B., Bradford, S.A., 2016. Use of aerobic spores as a surrogate for *Cryptosporidium* oocysts in drinking water supplies. *Water Res.* 90, 185–202. <https://doi.org/10.1016/j.watres.2015.12.024>.
- Heaselgrave, W., Patel, N., Kilvington, S., Kehoe, S.C., McGuigan, K.G., 2006. Solar disinfection of poliovirus and *Acanthamoeba polyphaga* cysts in water – a laboratory study using simulated sunlight. *Lett. Appl. Microbiol.* 43, 125–130. <https://doi.org/10.1111/j.1472-765X.2006.01940.x>.
- Hefer, A.W., Bhasin, A., Little, D.N., 2006. Bitumen surface energy characterization using a contact angle approach. *J. Mater. Civ. Eng.* 18, 759–767. [https://doi.org/10.1061/\(ASCE\)0899-1561\(2006\)18:6\(759\)](https://doi.org/10.1061/(ASCE)0899-1561(2006)18:6(759)).
- Hussey M.A., Zayaitz A., 2007. Endospore Stain Protocol [WWW Document]. URL <https://www.asmscience.org/content/education/protocol/protocol.3112> (accessed 25.7.19).
- Langlet, J., Gaboriaud, F., Gantzer, C., 2007. Effects of pH on plaque forming unit counts and aggregation of MS2 bacteriophage. *J. Appl. Microbiol.* 103, 1632–1638. <https://doi.org/10.1111/j.1365-2672.2007.03396.x>.
- Lian, Y., Mai, L., Cromar, N., Buchanan, N., Fallowfield, H., Li, X., 2018. MS2 coliphage and *E. coli* UVB inactivation rates in optically clear water: dose, dose rate and temperature dependence. *Water Sci. Technol.* 78 (10), 2228–2238. <https://doi.org/10.2166/wst.2018.509>.
- Mamane, H., Shemer, H., Linden, K.G., 2007. Inactivation of *E. coli*, *B. subtilis* spores, and MS2, T4, and T7 phage using UV/H<sub>2</sub>O<sub>2</sub> advanced oxidation. *J. Hazard. Mater.* 146, 479–486. <https://doi.org/10.1016/j.jhazmat.2007.04.050>.
- Mark, O., Jørgensen, C., Hammond, M., Khan, D., Tjener, R., Erichsen, A., Helwigh, B., 2018. A new methodology for modelling of health risk from urban flooding exemplified by cholera – case Dhaka, Bangladesh. *J. Flood Risk Manag.* 11, S28–S42. <https://doi.org/10.1111/jfr3.12182>.
- Mazoua, S., Chauveheid, E., 2005. Aerobic spore-forming bacteria for assessing quality of drinking water produced from surface water. *Water Res.* 39, 5186–5198. <https://doi.org/10.1016/j.watres.2005.09.027>.
- Medema, G.J., Bahar, M., Schets, F.M., 1997. Survival of *Cryptosporidium parvum*, *Escherichia coli*, faecal enterococci and *Clostridium perfringens* in river water: influence of temperature and autochthonous microorganisms. *Water Sci. Technol.* 35, 249–252. <https://doi.org/10.2166/wst.1997.0742>.
- Nalwanga, R., Quilty, B., Muyanja, C., Fernandez-Ibañez, P., McGuigan, K.G., 2014. Evaluation of solar disinfection of *E. coli* under Sub-Saharan field conditions using a 25L borosilicate glass batch reactor fitted with a compound parabolic collector. *Sol. Energy* 100, 195–202. <https://doi.org/10.1016/j.solener.2013.12.011>.
- Nelson, K.L., Boehm, A.B., Davies-Colley, R.J., Dodd, M.C., Kohn, T., Linden, K.G., Liu, Y., Maraccini, P.A., McNeill, K., Mitch, W.A., Nguyen, T.H., Parker, K.M., Rodriguez, S., Sassoubre, L.M., Silverman, A.I., Wigginton, K.R., Zepp, R.G., 2018. Sunlight-mediated inactivation of health-relevant microorganisms in water: a review of mechanisms and modeling approaches. *Environ. Sci. Process. Impacts* 20, 1089–1122. <https://doi.org/10.1039/C8EM00047F>.
- Nelson, K.L., 2003. Concentrations and inactivation of *Ascaris* eggs and pathogen indicator organisms in wastewater stabilization pond sludge. *Water Sci. Technol.* 48, 89–95. <https://doi.org/10.2166/wst.2003.0093>.
- Nicholson, W.L., Munakata, N., Horneck, G., Melosh, H.J., Setlow, P., 2000. Resistance of *Bacillus* endospores to extreme terrestrial and extraterrestrial environments. *Microbiol. Mol. Biol. Rev.* 64, 548–572. <https://doi.org/10.1128/mmb.64.3.548-572.2000>.
- Nieminski, E., Durrant, G.C., Hoyt, M.B., Owens, M.E., Peterson, L., Peterson, S., Tanner, W.D., Rosen, J., Clancy, J.L., 2010. Is *E. coli* an appropriate surrogate for *Cryptosporidium* occurrence in water? *J. Am. Water Works Assn.* 102, 65–78. <https://doi.org/10.1002/j.1551-8833.2010.tb10073.x>.
- NOAA (National Oceanic and Atmospheric Administration), 2019. NOAA Solar Calculator. Earth System Research Laboratory, Global Monitoring Division, Global Radiation Group [WWW Document]. URL <https://www.esrl.noaa.gov/gmd/grad/solcalc/> (accessed 29.7.19).
- Reiner, R.C., King, A.A., Emch, M., Yunus, M., Faruque, A.S.G., Pascual, M., 2012. Highly localized sensitivity to climate forcing drives endemic cholera in a megacity. *Proc. Natl. Acad. Sci. U. S. A.* 109, 2033–2036. <https://doi.org/10.1073/pnas.1108438109>.
- Rice, E.W., Fox, K.R., Miltner, R.J., Lytle, D.A., Johnson, C.H., 1996. Evaluating plant performance with endospores. *J. Am. Water Works Assn.* 88, 122–130.
- Riesenman, P.J., Nicholson, W.L., 2000. Role of the spore coat layers in *Bacillus subtilis* spore resistance to hydrogen peroxide, artificial UV-C, UV-B, and solar UV radiation. *Appl. Environ. Microbiol.* 66, 620–626. <https://doi.org/10.1128/aem.66.2.620-626.2000>.
- Sales-Ortells, H., Medema, G., 2015. Microbial health risks associated with exposure to stormwater in a water plaza. *Water Res.* 74, 34–46. <https://doi.org/10.1016/j.watres.2015.01.044>.
- Schets, F.M., van Wijnen, J.H., Schijven, J.F., Schoon, H., de Roda Husman, A.M., 2008. Monitoring of waterborne pathogens in surface waters in Amsterdam, The Netherlands, and the potential health risk associated with exposure to *Cryptosporidium* and *Giardia* in these waters. *Appl. Environ. Microbiol.* 74, 2069–2078. <https://doi.org/10.1128/AEM.01609-07>.
- Scoullou, I.M., Lopez Vazquez, C.M., van de Vossenberg, J., Brdjanovic, D., 2019a. Die-off of *E. coli* as fecal indicator organism on different surfaces after urban floods. *J. Environ. Manage.* 250, <https://doi.org/10.1016/j.jenvman.2019.109516>.
- Scoullou, I.M., Lopez Vazquez, C.M., van de Vossenberg, J., Hammond, M., Brdjanovic, D., 2019b. Effect of artificial solar radiation on the die-off of pathogen indicator organisms in urban floods. *Int. J. Environ. Res.* 13, 107–116. <https://doi.org/10.1007/s41742-018-0160-5>.
- Staley, Z.R., Robinson, C., Edge, T.A., 2016. Comparison of the occurrence and survival of fecal indicator bacteria in recreational sand between urban beach, playground and sandbox settings in Toronto, Ontario. *Sci. Total Environ.* 541, 520–527. <https://doi.org/10.1016/j.scitotenv.2015.09.088>.
- Stelma, G.N., 2018. Use of bacterial spores in monitoring water quality and treatment. *J. Water Health* 16, 491–500. <https://doi.org/10.2166/wh.2018.013>.
- ten Veldhuis, J.A.E., Clemens, F.H.L.R., Sterk, G., Berends, B.R., 2010. Microbial risks associated with exposure to pathogens in contaminated urban flood water. *Water Res.* 44, 2910–2918. <https://doi.org/10.1016/j.watres.2010.02.009>.
- Thompson, S.S., Flury, M., Yates, M.V., Jury, W.A., 1998. Role of the air-water-solid interface in bacteriophage sorption experiments. *Appl. Environ. Microbiol.* 64, 304–309.
- Thompson, S.S., Yates, M.V., 1999. Bacteriophage inactivation at the air-water-solid interface in dynamic batch systems. *Appl. Environ. Microbiol.* 65, 1186–1190.
- Trouwborst, T., Kuyper, S., de Jong, J.C., Plantinga, A.D., 1974. Inactivation of some bacterial and animal viruses by exposure to liquid-air interfaces. *J. Gen. Virol.* 24, 155–165. <https://doi.org/10.1099/0022-1317-24-1-155>.
- USEPA (US Environmental Protection Agency), 2006. Ultraviolet Disinfection Guidance Manual for the Final Long Term 2 Enhanced Surface Water Treatment Rule. EPA 815-R-06-007, USEPA Office of Water (4601), Washington, DC, USA.
- USEPA (US Environmental Protection Agency), 2010. Long Term 2 Enhanced Surface Water Treatment Rule Toolbox Guidance Manual. EPA 815-R-0e16, USEPA, Washington, DC, USA.
- Verbyla, M.E., Mihelcic, J.R., 2015. A review of virus removal in wastewater treatment pond systems. *Water Res.* 71, 107–124. <https://doi.org/10.1016/j.watres.2014.12.031>.
- Xia, W., Xu, T., Wang, H., 2019. Thermal behaviors and harmful volatile constituents released from asphalt components at high temperature. *J. Hazard. Mater.* 373, 741–752. <https://doi.org/10.1016/j.jhazmat.2019.04.004>.