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Inactivation of indicator organisms on different surfaces after urban floods

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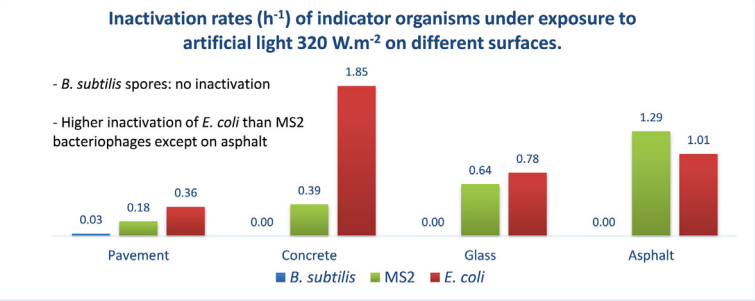
HIGHLIGHTS

- Different surfaces were tested for inactivation of indicator organisms (25 ± 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



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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 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 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.

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

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 *Campylobacte*r. 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 (Nieminski 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 ± 5 °C). Sampling areas were defined on each surface in triplicate by glass walls, creating water wells of 100 cm^2 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)

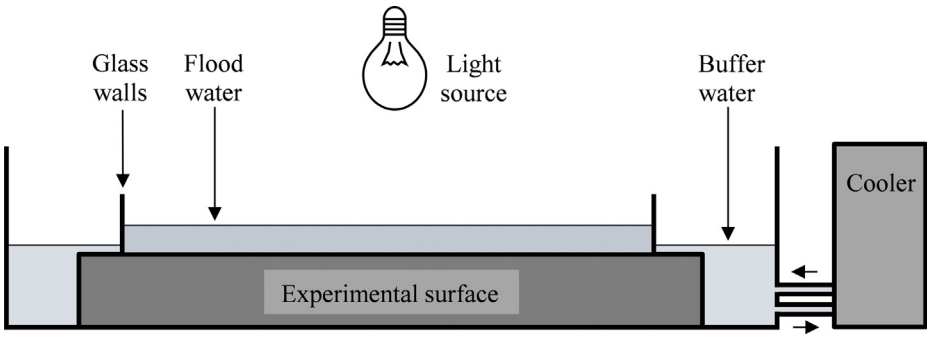

Fig. 1. Schematic representation of a reactor exposed to artificial light. Two such reactors were used in parallel. In the experiments under real sunlight the cooler was not used.

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^{-1} 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^{-1}) 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 h ± 2 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 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 μmol.m^{-2}.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)).

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

Where t is the time (d), C_t is the concentration of microorganisms (CFU.mL^{-1} for bacteria or PFU.mL^{-1} for bacteriophages) in time t, C_0 is the initial concentration of the microorganisms and k (d^{-1}) 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

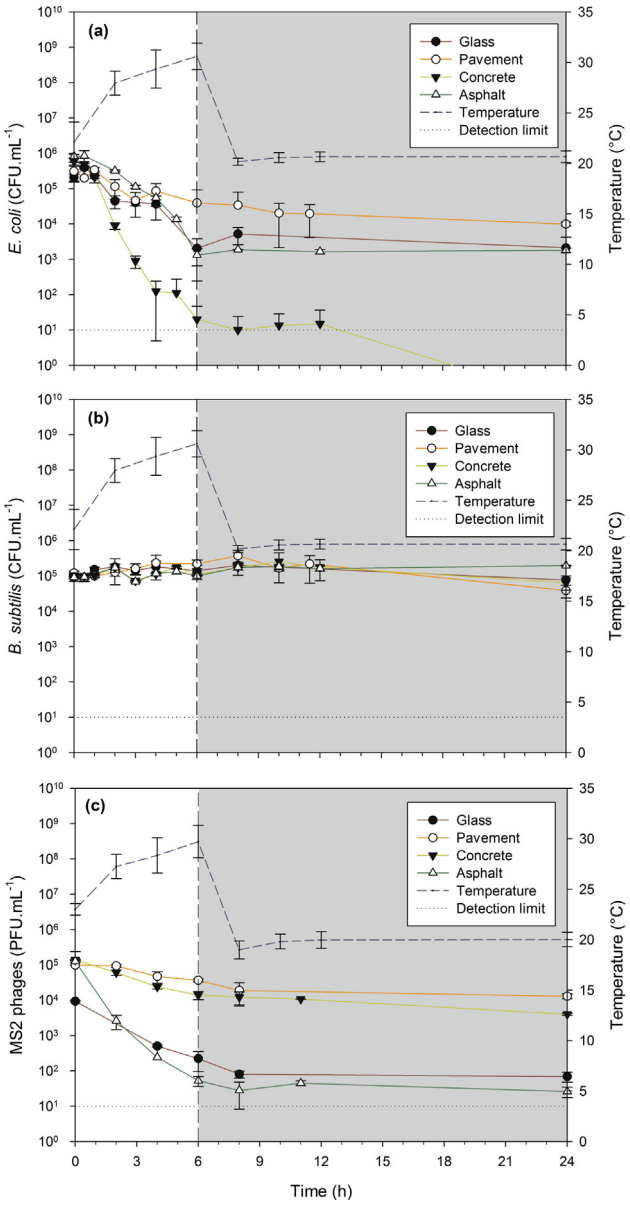

Fig. 2. Inactivation of *E. coli* (a), *B. subtilis* (b) and MS2 bacteriophages (c) on different surfaces. The white area represents the light phase and the grey area represents the dark phase.

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^{-1} followed by asphalt (1.01 h^{-1}), glass (0.78 h^{-1}) and the lowest was on pavement (0.36 h^{-1}). 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" (α = 0.05, log values). The number of samples (n) is indicated.

Surfaces	Average pH	*E. coli*			*B. subtilis* spores	MS2 bacteriophages		
		Light		Dark	Overall	Light		Dark
		Decay rate (h^{-1})	R^2	Decay rate (h^{-1})		Decay rate (h^{-1})	R^2	Decay rate (h^{-1})
Artificial light (320 W.m^{-2} for 6 h)								
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)
Natural sunlight (512 W.m^{-2} for 8 h)								
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 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 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 intensity during the light phase was calculated to be 515 W.m^{-2}. Also, the average temperature during the light and dark phase was 15.5 °C and 4.5 °C, respectively.

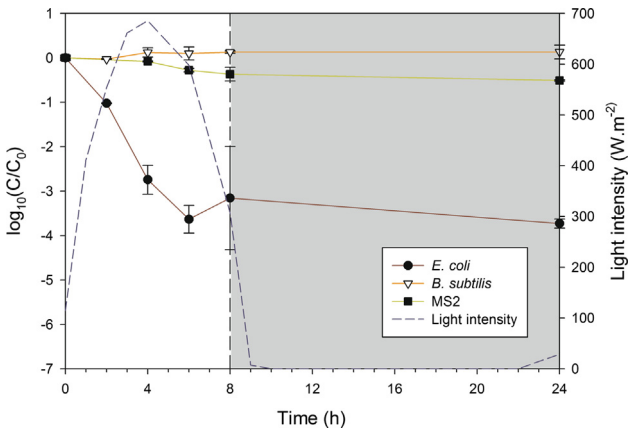

Fig. 3. Inactivation of *E. coli*, *B. subtilis* and MS2 on pavement under natural sunlight (white area) and dark (grey area).

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 h^{-1} and the MS2 bacteriophage showed a very low inactivation (0.11 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 (Scoullos 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 h^{-1}) than on the glass control (0.78 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 h^{-1}) or 12 h per day (0.37 h^{-1}) (Scoullos 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 °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 core, 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 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 h^{-1} and 1.01 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 $W.m^{-2}$, which was around 1.5 times higher than the light intensity used in the setup with artificial light source (i.e. 320 $W.m^{-2}$). 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^{-2} 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^{-1}, 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^{-1}.
- 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.

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