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Cover image: © Dr. William Veerbeek – Bellamyplein, Rotterdam, NL, a water square combining recreational and storm water detention functions, where faecal contamination has been detected in the flood water, posing public health risks after rain events.

ASSESSMENT OF THE FATE OF SURROGATES FOR ENTERIC PATHOGENS RESULTING FROM THE SURCHARGING OF COMBINED SEWER SYSTEMS

DISSERTATION

Submitted in fulfillment of the requirements of
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and
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by

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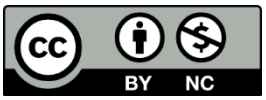

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καὶ ἐὰν εἰδῶ τὰ μυστήρια πάντα καὶ πᾶσαν τὴν γνῶσιν, ἀγάπην δὲ μὴ ἔχω, οὐδέν εἰμι

To my parents

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As a last remark, my thought goes to the millions of people affected by floods worldwide, unprotected and unprepared against the increasing consequences of climate change. May we never forget that we are all part of the same world!

SUMMARY

In the last decade (2009-2019), flooding has caused the death of over 48,000 people, and affected over 697 million people globally. This is expected to increase as a result of climate change, increased populations and urbanisation. Floods can cause infections due to the release of water-borne pathogenic microorganisms from surcharged combined sewers and other sources of faecal contamination. Pathogens can occur after the surcharge of sewer networks on surfaces commonly found in the urban environment such as concrete, asphalt, pavement blocks and grass.

The goal of this research was to contribute to a better understanding of how the concentration of water-borne pathogens on different urban and recreational surfaces is affected by different environmental conditions after urban floods. Also, in order to assess the concentration of water-borne indicator organisms on flood-prone urban surfaces it is necessary to identify the most reliable method for the recovery of these organisms.

The inactivation of faecal indicator bacteria *Escherichia coli* in the water phase was studied in an open stirred reactor, under controlled exposure to simulated sunlight, mimicking the effect of different latitudes and seasons, and different concentrations of total suspended solids (TSS) corresponding to different levels of dilution and runoff. While attachment of bacteria on the solid particles did not take place, the inactivation rate coefficient, k (d^{-1}), was found to depend on light intensity, I ($W.m^{-2}$), and duration of exposure to sunlight, T (h d^{-1}), in a linear way ($k=k_D+0.03 \cdot I$ and $k=k_D+0.65 \cdot T$, respectively) and on the concentration of TSS ($mg.L^{-1}$), in an inversely proportional exponential way ($k=k_D+14.57e^{-0.02 \cdot [TSS]}$). The first order inactivation rate coefficient in dark conditions, k_D=0.37 d^{-1}, represents the effect of stresses other than light.

Four different sampling methods were compared for retrieving samples from concrete, asphalt, pavement blocks and grass: swabbing, direct agar contact, stamping and adhesive tape-lifting. The surfaces were inoculated with known amounts of *E. coli*. A glass surface was used as control. Contact plating had the highest log recovery ratio, 96.1% on glass, for concentrations up to 10^3 CFU.100 cm^{-2} of *E. coli*, but this method has a limited range of bacterial numbers because it is not possible to dilute or concentrate the samples. Swabbing was the most reliable technique because it could be used for a wide range of concentrations with high recovery ratios of up to 96.2% for 10^5 CFU.100 cm^{-2} of *E. coli*. Comparatively, the indirect methods of stamping and tape had no additional advantages.

Further experiments using the swabbing technique revealed that the water accumulated on rougher surfaces affected the swabbing recovery ratios when the samples got diluted. Swabbing any amount of sample higher than what the swab heads could absorb (0.15 mL) reduced the recovery ratio. Furthermore, swabbing was more efficient without the use of a detergent (Tween 80) in wetting solution and eluent. After the recession of an artificial

flood, swabbing and contact plating were confirmed as reliable methods to sample and enumerate the presence of *E. coli* on different urban surfaces, with the log recoveries ranging from 21.0% to 59.0%, depending not only on the sampling method, but also on the actual amount of bacteria on the surfaces.

The inactivation of faecal indicator *E. coli* was studied under controlled exposure to simulated sunlight on a range of different surfaces found in urban environments: gravel, sand, asphalt, pavement blocks, concrete, playground rubber tiles and grass, using glass as control. The surfaces were inoculated with artificial flooding water containing 10^5 $CFU.mL^{-1}$ of *E. coli* and sampled periodically using the sterile cotton swab technique, after lowering the water level. The results show that inactivation in dark conditions was not statistically significant for any surface, suggesting that chemical composition and pH (varying from 6.5 to 9.2) did not affect significantly the inactivation rates in the short term. The highest light-induced inactivation rates for *E. coli* after the floodwater recess, observed on rubber (>3.46 h^{-1}) and asphalt (2.7 h^{-1}), were attributed to thermal stress and loss of surface moisture.

The inactivation of *E. coli*, *Bacillus subtilis* spores, and bacteriophage MS2, that all are surrogates for different groups of pathogens, was studied under controlled exposure to simulated sunlight. Concrete, asphalt and pavement blocks were inoculated with artificial floodwater containing these organisms. The research took into account the pH of the water that is exposed to these surfaces and its role on the survival of the organisms. The results showed that inactivation in dark conditions was not statistically significant for any organism and surface, suggesting that pH alone (varying from 7.0 to 9.6) did not affect significantly the inactivation rates in the short term. The highest light inactivation was seen on *E. coli* on a concrete surface (pH 9.6) with an inactivation rate of 1.85 h^{-1}. MS2 phage had the highest light inactivation on asphalt with a rate of 1.3 h^{-1}. No inactivation of *B. subtilis* spores was observed on any of the surfaces on both light and dark conditions. In general, the light inactivation on all surfaces followed the ascending order of *B. subtilis* spores < MS2 phage < *E. coli* except on asphalt, where the light inactivation of *E. coli* and MS2 was found to be same and that of *B. subtilis* spores was the least.

This study suggests that given the sunlight conditions after an urban flood, the concentration of indicator organisms, TSS and the type of flooded surfaces it is possible to estimate the fate of selected water-borne pathogens. The observations and results presented in this study, in combination with Quantitative Microbial Risk Assessment (QMRA) and mapping of urban surfaces, can be used to develop policy-making tools for rapid implementation of appropriate measures to mitigate public health risks after flooding.

SAMENVATTING

In het afgelopen decennium (2009-2019) hebben overstromingen geleid tot de dood van meer dan 48.000 mensen, en hebben ze wereldwijd de levens van meer dan 697 miljoen mensen beïnvloed. Het is de verwachting, dat deze aantallen nog zullen toenemen als gevolg van klimaatverandering, toenemende bevolkingsgroei en verstedelijking. Overstromingen kunnen infecties veroorzaken door het vrijkomen van uit water afkomstige pathogene (ziekteverwekkende) micro-organismen uit overgelopen riolen en andere bronnen van fecale besmetting. Pathogenen kunnen worden achtergelaten nadat rioolwater oppervlakten heeft overstroomd in de stedelijke omgeving, zoals beton, asfalt, plaveisel en gras.

Het doel van het onderzoek was het bijdragen aan een betere verklaring van hoe de concentratie van uit water afkomstige pathogenen op verschillende stedelijke en recreatieve oppervlakken beïnvloed wordt door verschillende omgevingsfactoren na stedelijke overstromingen. Daarom diende een betrouwbare methode ontwikkeld te worden om uit water afkomstige indicator-organismen te isoleren van de overstroomde stedelijke oppervlakten.

De inactivatie van fecale indicatorbacterie *Escherichia coli* in de waterfase werd onderzocht in een open geroerde reactor, waarmee het effect van verschillende breedtegraden en seizoenen nagebootst konden worden. Dat werd gedaan met gecontroleerde blootstelling aan kunstmatig zonlicht. Er werd getest bij verschillende concentraties van totale hoeveelheid deeltjes in suspensie (total suspended solids, TSS) die overeenkomen met verschillende niveaus van verdunning en afvoer. Er vond geen hechting plaats van bacteriën aan de vaste deeltjes, en het werd duidelijk dat de snelheids-coëfficiënt voor inactivatie, k (d^{-1}), lineair afhankelijk was van lichtsterkte, I ($W.m^{-2}$), en aan de tijdsduur van blootstelling aan zonlicht, T ($h\ d^{-1}$), waarbij respectievelijk gold $k=k_D+0.03 \cdot I$ en $k=k_D+0.65 \cdot T$. De invloed van de concentratie van TSS ($mg.L^{-1}$) op de inactivatie coefficient was omgekeerd evenredig exponentieel ($k=k_D+14.57e^{-0.02 \cdot [TSS]}$). Het effect van andere factoren dan licht, weergegeven met een eerste orde snelheids-coëfficiënt voor inactivatie onder donkere omstandigheden, was k_D=0.37 d^{-1}.

Vier verschillende bemonsteringsmethodes voor het verzamelen van monsters van beton, asfalt, straattegels en gras werden met elkaar vergeleken: het afnemen van swabs met steriele wattenstaafjes, direct agar contact (contactplaten), afstempelen en het gebruik van zelfklevende tape. De test-oppervlakten werden geïnoculeerd met vastgestelde hoeveelheden *E. coli*. Een glazen oppervlak werd gebruikt als controle. Contactplaten gaven de hoogste terugwinning van de geïnoculeerde bacteriën, 96.1% op glas, voor concentraties tot 10^3 CFU.100 cm^{-2} *E. coli*. Deze methode heeft echter een beperkt meetbereik voor mogelijke concentraties van bacteriën, omdat het niet mogelijk is om de

monsters te verdunnen of te concentreren. Het afnemen van swabs bleek daarna de meest betrouwbare techniek te zijn, omdat het gebruikt kon worden over een groot concentratie-bereik met hoge efficiëntie, tot 96.2% voor 10^5 CFU.100 cm^{-2} *E. coli*. In vergelijking met de andere twee methoden hadden de indirecte stempelmethode en het gebruik van tape geen toegevoegde waarde.

Verdere experimenten met de swab techniek toonden aan dat water op ruwere oppervlakten de efficiëntie van de swab techniek nadelig beïnvloedde zodra de monsters werden verdund. En zodra de swabs werden gebruikt voor een groter volume dan het wattenstaafje kon absorberen (0.15 mL) verminderde de terugwinning. Toevoeging een detergens (Tween 80) in de oplossingen om het wattenstaafje van de swab te bevochtigen en om de bacteriën van de swab te elueren, bleek minder efficiënt dan zonder de toevoeging. Na het verdwijnen van een kunstmatige overstroming bleken de swab methode en contactplaten opnieuw de meest betrouwbare methodes om de aanwezigheid van *E. coli* op verschillende stedelijke oppervlakten te analyseren. De log terugwinning varieerde van 21.0% tot 59.0%, en was afhankelijk van de bemonsteringsmethode en de oorspronkelijke hoeveelheid bacteriën op de oppervlakten.

Bij gecontroleerde blootstelling aan kunstmatig zonlicht werd de inactivatie van de fecale indicator *E. coli* onderzocht op een reeks verschillende oppervlakten uit stedelijke omgevingen: grind, zand, asfalt, straattegels, beton, en rubberen tegels die gebruikt worden op speelplaatsen, met gras als controle. De oppervlakten werden geïnoculeerd door kunstmatige overstromingen met water wat 10^5 CFU.mL^{-1} *E. coli* bevatte, en werden na het verlagen van het waterniveau regelmatig bemonsterd met de steriele watten-staafjestechniek. Uit de resultaten bleek dat inactivatie in het donker statistisch gezien niet significant was, voor alle organismen en oppervlaktes, met de aanname dat chemische samenstelling en pH (variërend van 6.5 tot 9.2) de inactivatiesnelheid op korte termijn niet noemenswaardig beïnvloedden. Door licht geïnduceerde snelheden van inactivatie, na het verdwijnen van het overstromingswater, waren echter wel significant, en de snelste inactivatie was voor *E. coli*, op rubber (>3.46 h^{-1}) en asfalt (2.7 h^{-1}). Deze afnames werden toegeschreven aan temperatuurstress en uitdroging van het oppervlak.

De inactivaties van *E. coli, Bacillus subtilis* sporen, en bacteriofaag MS2, tevens surrogaten voor verschillende groepen pathogenen, werden onderzocht onder gecontroleerde blootstelling aan gestimuleerd zonlicht. Beton, asfalt en straattegels werden geïnoculeerd met kunstmatig overstromingswater met deze organismen. Het onderzoek hield rekening met de pH van het water dat blootgesteld werd aan deze oppervlakten en de rol van de pH van het water wat betreft de overleving van de organismen. De resultaten toonden opnieuw aan dat donkere inactivatie niet significant was voor alle organismen en oppervlakten, er van uitgaande dat pH alleen (variërend van 7.0 tot 9.6) de inactivatiesnelheid op korte termijn niet noemenswaardig beïnvloedde. De hoogste licht-geïnduceerde inactivatie was zichtbaar bij *E. coli* op een oppervlakte van beton (pH 9.6) met een inactivatiesnelheid van 1.85 h^{-1}. Faag MS2 had de hoogste licht-geïnduceerde inactivatie op asfalt met een snelheid van 1.3 h^{-1}. Inactivatie van *B. subtilis*

sporen werd niet waargenomen, zowel onder lichte als onder donkere omstandigheden. Over het algemeen was op alle oppervlakten de volgorde van de licht-geïnduceerde inactivatie van langzaam naar snel: *B. subtilis* sporen < MS2 phage < *E. coli*. De uitzondering was asfalt, waar licht-geïnduceerde inactivatie van *E. coli* en MS2 hetzelfde was en waar de inactivatie van *B. subtilis* sporen het traagst was.

Het onderzoek in dit proefschrift laat zien dat het mogelijk is om na een stedelijke overstroming, met behulp van de zonlichtomstandigheden, de concentratie van indicator-organismen, TSS en de typen overstroomde oppervlakten, in te schatten wat gebeurt met een aantal categorieën uit water afkomstige pathogenen. De waarnemingen en resultaten gepresenteerd in dit onderzoek zouden, gecombineerd met Quantitative Microbial Risk Assessment (QMRA) en het in kaart brengen van stedelijke oppervlakten, gebruikt kunnen worden om beleid te ontwikkelen voor een snelle uitvoering van geschikte maatregelen om na een overstroming volksgezondheidsrisico's te beperken.

ΠΕΡΙΛΗΨΗ

Την τελευταία δεκαετία (2009-2019), πλημμυρικά φαινόμενα προκάλεσαν τουλάχιστον 48.000 θανάτους και επηρέασαν πάνω από 697 εκατομμύρια ανθρώπους παγκοσμίως. Οι αριθμοί αυτοί αναμένεται να αυξηθούν ως συνέπεια της κλιματικής αλλαγής, της αύξησης του πληθυσμού και της αστικοποίησης. Οι πλημμύρες δύνανται να προκαλέσουν λοιμώξεις λόγω της απελευθέρωσης παθογόνων μικροοργανισμών που μεταδίδονται μέσω του νερού από υπερφορτωμένα παντορροϊκά αποχετευτικά δίκτυα και άλλες πηγές κοπρανώδους μολύνσεως. Παθογόνοι μικροοργανισμοί μπορούν να προκύψουν σε επιφάνειες που απαντώνται συχνά στο αστικό περιβάλλον, όπως σκυρόδεμα, άσφαλτος, πλάκες πεζοδρομίου και γρασίδι.

Σκοπός της παρούσας έρευνας ήταν η βαθύτερη κατανόηση της επίδρασης διαφορετικών περιβαλλοντικών συνθηκών στη συγκέντρωση παθογόνων μικροοργανισμών που μεταδίδονται μέσω του νερού σε διαφορετικές αστικές επιφάνειες και επιφάνειες αναψυχής ύστερα από αστικές πλημμύρες. Επίσης, για την αξιολόγηση της συγκέντρωσης οργανισμών-δεικτών που μεταδίδονται μέσω του νερού σε αστικές επιφάνειες εκτεθειμένες σε πλημμύρες είναι απαραίτητη η ταυτοποίηση της πιο αξιόπιστης μεθόδου για την ανάκτηση αυτών των οργανισμών.

Η μελέτη της φυσικής αδρανοποίησης του βακτηρίου-δείκτη κοπρανώδους μολύνσεως *Escherichia coli* στην υδατική φάση έλαβε χώρα σε ανοιχτό αναδευόμενο αντιδραστήρα, υπό ελεγχώμενη έκθεση σε προσομοιωμένη ηλιακή ακτινοβολία, μιμούμενη την επίδραση διαφορετικών γεωγραφικών πλατών και εποχών, και με διαφορετικές συγκεντρώσεις ολικών αiωρούμενων στερεών (TSS), αντιστοιχούντων σε διαφορετικά επίπεδα αραίωσης και απορροών μετά από πλημμύρες. Ο συντελεστής ρυθμού αδρανοποίησης, k (d^{-1}), βρέθηκε να αυξάνεται γραμμικά με την αύξηση της έντασης της φωτεινής ακτινοβολίας, I ($W.m^{-2}$), και της διάρκειας έκθεσης στο φως, T ($h\ d^{-1}$), ($k=k_D+0.03 \cdot I$ και $k=k_D+0.65 \cdot T$, αντιστοίχως) και με τη μείωση της συγκέντρωσης των TSS ($mg.L^{-1}$), με εκθετική σχέση ($k=k_D+14.57e^{-0.02 \cdot [TSS]}$), χωρίς να έχει λάβει χώρα σύνδεση των βακτηρίων στα στερεά σωματίδια. Ο συντελεστής ρυθμού αδρανοποίησης πρώτου βαθμού σε σκοτεινές συνθήκες, k_D=0.37 d^{-1}, εκφράζει την επίδραση καταπονήσεων εκτός του φωτός.

Πραγματοποιήθηκε σύγκριση τεσσάρων διαφορετικών μεθόδων δειγματοληψίας για την ανάκτηση μικροβιακών δειγμάτων από σκυρόδεμα, άσφαλτο, πλάκες πεζοδρομίου και γρασίδι: λήψη επιχρισμάτων, άμεση επαφή άγαρ, συμπίεση και χρήση κολλητικής ταινίας. Οι επιφάνειες εμβολιάστηκαν με δεδομένες συγκεντρώσεις *E. coli*, ενώ γυάλινη επιφάνεια χρησιμοποιήθηκε ως επιφάνεια ελέγχου. Η μέθοδος άμεσης επαφής άγαρ παρουσίασε το υψηλότερο λογαριθμικό ποσοστό ανάκτησης, 96,1% στο γυαλί, για συγκεντρώσεις *E. coli* έως 10^3 $CFU.100\ cm^{-2}$, αλλά αυτή η μέθοδος ανιχνεύει

περιορισμένο εύρος βακτηριακών συγκεντρώσεων επειδή δεν είναι δυνατή η αραίωση ή συμπύκνωση των δειγμάτων. Η λήψη επιχρισμάτων ήταν η πιο αξιόπιστη μέθοδος επειδή ανιχνεύει μεγάλο εύρος συγκεντρώσεων με υψηλά λογαριθμικά ποσοστά ανάκτησης, έως 96,2% για συγκεντρώσεις *E. coli* 10^5 CFU.100 cm^{-2}. Οι έμμεσες μέθοδοι με συμπίεση και χρήση κολλητικής ταινίας δεν εμφάνισαν συγκριτικά πλεονεκτήματα.

Περαιτέρω πειράματα με λήψη επιχρισμάτων έδειξαν πως νερό συσσωρευμένο σε τραχείες επιφάνειες επηρέασε τα ποσοτά ανάκτησης λόγω της αραίωσης των δειγμάτων. Η λήψη επιχρισμάτων δειγμάτων μεγαλύτερου όγκου απ' ό,τι μπορεί να απορροφήσει η κεφαλή (0,15 mL) παρουσίασε μειωμένα ποσοστά ανάκτησης. Επίσης, η λήψη επιχρισμάτων ήταν πιο αποδοτική χωρίς τη χρήση απορρυπαντικού (Tween 80) στα διαλύματα διαβροχής και εκλούσεως. Μετά την ύφεση τεχνητής πλημμύρας, η χρήση επιχρισμάτων και η άμεση επαφή άγαρ επαληθεύτηκαν ως αξιόπιστες μέθοδοι δειγματοληψίας και απαρίθμισης *E. coli* σε διαφορετικές αστικές επιφάνειες, με λογαριθμικά ποσοστά ανάκτισης μεταξύ 21,0% και 59,0%, αναλόγως όχι μόνο της μεθόδου δειγματοληψίας, αλλά και της συγκέντρωσης βακτηρίων στις επιφάνειες.

Η αδρανοποίηση του βακτηρίου-δείκτη κοπρανώδους μολύνσεως *E. coli* μελετήθηκε υπό ελεγχόμενη έκθεση σε προσομοιωμένη ηλιακή ακτινοβολία σε διαφορετικές επιφάνειες που απαντώνται στο αστικό περιβάλλον: χαλίκι, άμμο, άσφαλτο, πλάκες πεζοδρομίου, σκυρόδεμα, ελαστικές πλάκες παιδικής χαράς και γρασίδι, με τη χρήση γυαλιού ως επιφάνειας ελέγχου. Οι επιφάνειες εμβολιάστηκαν με τεχνητά πλημμυρικά ύδατα περιέχοντα *E. coli* σε συγκέντρωση 10^5 CFU.mL^{-1}. Περιοδική δειγματοληψία έλαβε χώρα με τη λήψη επιχρισμάτων μετά τη μείωση του επιπέδου του νερού. Τα αποτελέσματα έδειξαν πως η αδρανοποίηση σε σκοτεινές συνθήκες δεν ήταν στατιστικώς σημαντική για καμία επιφάνεια και συνάγεται ότι η χημική σύνθεση και το pH (κυμαινόμενο από 6,5 έως 9,2) δεν επηρέασαν σημαντικά το ρυθμό αδρανοποίησης βραχυπρόθεσμα. Οι υψηλότεροι ρυθμοί αδρανοποίησης *E. coli* υπό την επίδραση φωτός μετά την ύφεση των πλημμυρικών υδάτων, οι οποίοι παρατηρήθηκαν στις ελαστικές πλάκες (>3,46 h^{-1}) και την άσφαλτο (2,7 h^{-1}), αποδόθηκαν στο θερμικό σοκ και στην απώλεια της υγρασίας των επιφανειών.

Η αδρανοποίηση *E. coli*, σπόρων *Bacillus subtilis* και βακτηριοφάγων MS2, οι οποίοι όλοι είναι υποκατάστατα διαφορετικών ομάδων παθογόνων, μελετήθηκε υπό ελεγχόμενη έκθεση σε προσομοιωμένη ηλιακή ακτινοβολία. Σκυρόδεμα, άσφαλτος και πλάκες πεζοδρομίου εμβολιάστηκαν με τεχνητά πλημμυρικά ύδατα περιέχοντα αυτούς τους οργανισμούς. Η έρευνα έλαβε υπ' όψιν το pH του νερού στις διαφορετικές επιφάνειες και το ρόλο του pH στην αδρανοποίηση των οργανισμών. Τα αποτελέσματα έδειξαν πως η αδρανοποίηση σε σκοτεινές συνθήκες δεν ήταν στατιστικώς σημαντική για κανένα οργανισμό και καμία επιφάνεια και συνάγεται ότι το pH (κυμαινόμενο από 7,0 έως 9,6) από μόνο του δεν επηρέασε σημαντικά το ρυθμό αδρανοποίησης βραχυπρόθεσμα. Ο υψηλότερος ρυθμός αδρανοποίησης (1,85 h^{-1}) υπό την επίδραση φωτός παρατηρήθηκε στο *E. coli* σε επιφάνεια σκυροδέματος (pH 9,6). Ο βακτηριοφάγος MS2 παρουσίασε τον υψηλότερο ρυθμό αδρανοποίησης υπό την επίδραση φωτός στην άσφαλτο (1,3 h^{-1}). Δεν

παρατηρήθηκε αδρανοποίηση των σπόρων *B. subtilis* σε καμία από τις επιφάνειες, τόσο σε σκοτεινές όσο και σε φωτεινές συνθήκες. Γενικά, η αδρανοποίηση υπό την επίδραση φωτός ακολούθησε την αύξουσα σειρά σπόροι *B. subtilis* < βακτηριοφάγοι MS2 < *E. coli* σε όλες τις επιφάνειες εκτός από την άσφαλτο, όπου το *E. coli* και ο MS2 αδρανοποιήθηκαν με ίδιο ρυθμό.

Από την έρευνα προκύπτει πως γνωρίζοντας τις συνθήκες ηλιακής ακτινοβολίας ύστερα από αστικές πλημμύρες, τη συγκέντρωση οργανισμών-δεικτών και TSS, και το είδος των πλημμυρισμένων επιφανειών, είναι δυνατόν να εκτιμηθεί η τύχη επιλεγμένων παθογόνων που μεταδίδονται μέσω του νερού. Οι παρατηρήσεις και τα αποτελέσματα που παρουσιάζονται στην παρούσα μελέτη, σε συνδυασμό με Ποσοτική Εκτίμηση Μικροβιολογικής Επικινδυνότητας (QMRA) και χαρτογράφηση των αστικών επιφανειών, μπορούν να χρησιμοποιηθούν για την ανάπτυξη εργαλείων χάραξης πολιτικής για την ταχεία υλοποίηση κατάλληλων μέτρων για την άμβλυνση κινδύνων δημόσιας υγείας ύστερα από πλημμύρες.

CONTENTS

1

INTRODUCTION

The present thesis is a contribution in addressing one of the most acute health problems of our days, namely the spreading of water borne diseases through floods, by studying the inactivation of indicator organisms in characteristic background surfaces of various urban environments. Considering the close relationship between floods and climate change, this work contributes also to the adaptation to climate change.

1.1 URBAN FLOODS AND PUBLIC HEALTH

Floods worldwide pose a range of threats to human life, health and livelihoods. In the last ten years (2009-2019), flooding has caused the death of more than 48,000 people, and affected over 697 million people. In 2010 alone, reported flood disasters killed over 8,000 people directly (EM-DAT, 2019). The occurrence of extreme rainfalls, their impacts, and phenomena like urban flooding are predicted to become more frequent and intense 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). For instance, it has been projected that in Europe, 250,000-400,000 additional people will be affected per year by river flooding by the 2080s, if no effective measures are taken, which is more than double the numbers in the period 1961–1990 (Menne and Murray, 2013). In many parts of the world and in Africa in particular, most flood-related fatalities occur mainly due to enhanced vulnerability to disasters and lack of, or poor, flood management schemes. In developing countries the majority of flood-related deaths are caused by diarrhoea and other water-borne diseases, or from drowning and snake bites (Jonkman and Kelman, 2005; Jha et al., 2012).

Floods can be classified according to their (i) cause (high rainfall, tidal extremes, storm waves, lack of drains or drains blocked by waste and debris, land use changes like urban expansion or engineering works upstream, waste dumping, structural failure, etc.), (ii) nature (regularity, speed of onset, velocity and depth of water, spatial and temporal scale, etc.) and (iii) health outcomes (Ahern et al., 2005). The health impacts of floods (physical, chemical or biological) can be either short-term, with disease symptoms or mortality appearing during or immediately after the flood, or long-term, that usually appear later as a result of the damages caused to infrastructure (Alderman et al., 2012).

The deaths which are the most easily monitored and connected with floods are those related to physical health effects, which occur from drowning (two-thirds of direct deaths) or trauma in flash and coastal floods. Nonfatal injuries are, together with the exacerbation of chronic illness, the main cause of mortality among affected populations shortly after the flood. However, the identification and attribution of deaths to a single cause is often particularly difficult because of coexistence of many hazards. After hurricane Katrina/Rita in Greater New Orleans, 7,500 incidents of nonfatal “injuries” were recorded among residents and relief workers. It is believed that many more were affected. In general, little information is available on the occurrence of nonfatal injuries during floods because they are not always reported or related to floods. Although the international Emergency Events Database (EM-DAT) records such injuries, this data is much less robust than reports of fatalities (Ahern et al., 2005; Alderman et al., 2012). If handling of “injuries” and the data related to them is difficult in a developed country like the USA, it is easily understood that the situation in developing countries under flood crisis is in many cases totally out of control.

In terms of chemical health effects, the causal relationships between floods, pollution from pesticides and other agricultural and industrial wastes (dioxin, heavy metals, etc.) released during flooding, contamination from sewage and health outcomes are still inconclusive. Apart from diarrhoea, exposure to such contaminants is linked to cancer, cardiovascular, gastrointestinal, kidney, liver and neurological diseases (Euripidou and Murray, 2004). Chronic illness and related conditions such as cardiovascular disease, cancer, chronic lung diseases and diabetes can also be worsened by disasters, increasing a person's vulnerability to synergetic adverse health outcomes following floods (Alderman et al., 2012).

The research presented in this work focuses on water-borne pathogens. However, it is worth mentioning that, in parallel, vector-borne diseases also increase during floods because of increased exposure to vectors (mosquitoes), which breed in or close to stagnant or slow-moving, usually polluted waters and rodents transmitting pathogens, changes in their habitat and compromise in vector control programs during floods (Ahern et al., 2005; Alderman et al., 2012). There have been developments in mapping the current and potential future distribution of important disease vector species (Liu-Helmersson et al., 2019). Australia, Europe and North America are projected to have the largest increases in human exposure to vector mosquitoes due to climate change (Monaghan et al., 2018). For instance, in Europe the spread of vector mosquitoes may extend from the southern part eastward and northward due to climate change (Fischer et al., 2011; Roiz et al., 2011; Caminade et al., 2012).

Although flood-related mortality has been studied in both developed and developing countries, evidence about the effects of floods in public health is limited. Detailed data is limited because rigorous epidemiological studies of flooding are difficult to carry out, especially in developing countries where most of the affected populations live, and because it is difficult to quantify the true burden of ill health due to flood events, especially when most cases are not adequately investigated, classified and monitored (Alderman et al., 2012; Jha et al., 2012)

1.2 WATER-BORNE DISEASES RELATED TO FLOODS

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 combined sewer overflows (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. Although immediately after floods outbreaks rarely occur, despite the high risks of transmission of communicable diseases

(WHO, 2019), flooding events are statistically associated with disease, and one of the potential sources of this link is combined sewer overflows (CSOs) (Curriero et al., 2001).

Evidence about risks from climate change with respect to infectious diseases is still limited (Semenza and Menne, 2009; Randelph and Rogers, 2010; Semenza et al, 2012; Kovats et al., 2014) and the surveillance of health effects of disasters remains inadequate (Fewtrell and Bartram, 2001; Kovats et al., 2014). Case studies from Africa and Asia show that floods and other events linked to climate change may exacerbate the risk of water-borne infectious diseases (Cissé, 2019). Diarrhoeal diseases have been found to increase during higher than average rainfall and associated flooding in Kenya (Okaka and Odhiambo, 2018). In high-income countries like Norway, the United States, former Czechoslovakia, the risk of faecal-oral diseases is mainly associated with cases of residing in flooded dwellings and contact with flood waters (Ahern et al., 2005). The risks are high when infrastructure, water supply systems and drinking water facilities are seriously damaged and when people have to leave their dwellings. Contamination from the handling of bodies of diseased people and animals can also be a risk if correct precautions are not taken. In general, the extent of transmission of diseases and the risk of epidemics after floods depend on population density and displacement, as well as the extent to which the natural environment has been altered or disrupted. Common diseases resulting from water contamination include cholera, diarrheal disease, hepatitis A and E, leptospirosis, parasitic diseases, rotavirus, shigellosis and typhoid fever. Deaths related with many of these diseases can occur during a relatively long period following the reported flood and are not always recorded in disaster databases (Alderman et al., 2012; Jha et al., 2012).

Pathogens can gain access to the human body through the gastrointestinal tract, the respiratory tract, or the skin, through wounds and abrasions (Bitton, 1999). Direct contact and ingestion of contaminated water (either flood water or contaminated tap water) is very common during severe flood events. During disasters, electricity cut offs often take place. Most of the drinking water supply systems are pressurized and even in the gravity systems, the pipes are pressurized. However, when there is no electricity, the pumps are not functioning, which results into intrusion of contaminated ground and flood water in the water supply systems, which are often not appropriately flushed and disinfected before being used again. Inappropriate cross-connections between sewage and water supply networks can also take place (Laine et al., 2011). The faecal-oral transmission cycle of pathogens is presented in Fig. 1.1 and the most common flood-borne pathogens and related human diseases transmitted by water are presented in Table 1.1.

Wound infections, dermatitis, conjunctivitis and ear, nose and throat infections are the most usual water-borne diseases related to floods (Alderman et al., 2012). During and after floods there is a higher risk for gastrointestinal diseases like diarrheal disease, cholera and norovirus-based gastroenteritis. The main reasons for this are poor hygiene and sanitation conditions, inadequate provision of clean drinking water, over-crowding and resettlement, contact with flood water and consumption of crops grown on soil which

was contaminated during floods with wastewater from municipal and livestock operations (Wakuma Abaya et al., 2009). For some diseases such as hepatitis A and E it is known that they are transmitted through the faecal-oral route by ingestion of contaminated food or water. Person-to-person transmission is rare. Outbreaks of hepatitis E after floods, which often affect large populations, is frequent in areas where the virus is endemic and the spread of the disease is mainly attributed to the contamination of water sources (Watson et al., 2007; Alderman et al., 2012).

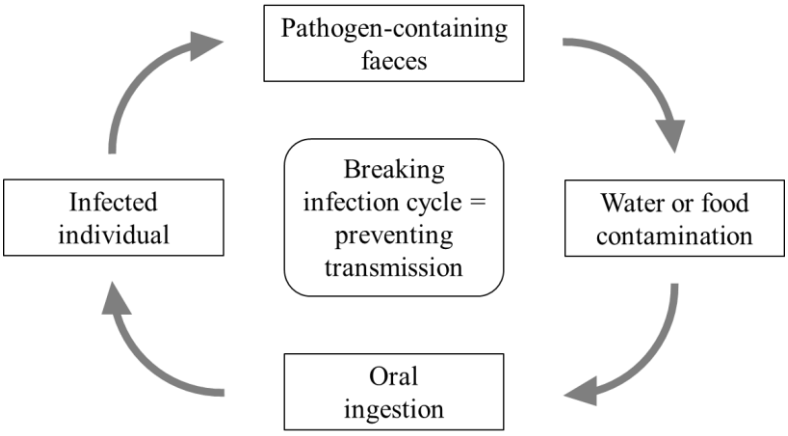


Figure 1.1 Faecal-oral transmission cycle of pathogens (Strande et al., 2014).

A common type of infectious diseases occurring after floods including upper respiratory tract infections with flu-like symptoms (throat infections, coughs and general symptoms, earache, skin rashes, inflammatory dermatoses and infectious skin conditions) common among inhabitants and construction workers are attributed to damp buildings (Noe et al., 2007; Carroll et al., 2010), due to growth of indoor mould, although their role in this process is not well understood. Conditions found in urban environments and surfaces, such as those of flooded buildings of various types, particularly under dark and poorly aerated conditions can support the existence and growth of mould, bacteria, protozoa and algae. Microbial contaminants grow, persist and produce toxins in these places. In addition, indoor humidity and temperature affect the transmission of aerolised respiratory viruses (Taylor et al., 2011). Different species of mould can impact public health either through direct respiratory infection, generation of a harmful immune response or severe reactions when mycotoxins are ingested. Measurements of the change in the ratio of indoor microbial levels before and after floods show that the 2009 flood in Taiwan resulted in an important increase in the concentrations of indoor fungi, some of which, e.g. *Aspergillus versicolor* are associated with negative health outcomes (Hsu et al., 2011; Alderman et al., 2012).

Table 1.1. Potential flood-related pathogens and associated human diseases in parentheses below the respective pathogens (Pond, 2013; Taylor et al., 2011; Gloyna, 1971).

Bacteria	Protozoa	Viruses	Fungi	Helminths
Aeromonas spp.	*Acanthamoeba* spp.	Adenovirus	*Candida albicans*	*Ascaris*
Campylobacter spp.	*Cryptosporidium*	Coxsackievirus	*Candida parapsilosis*	*Fasciolopsis buski* (fasciolopsiasis)
Enterococcus spp.	*Entamoeba* spp. (amoebic dysentery)	Echovirus (aseptic meningitis)	*Torulopsis glabrata*	*Schistosoma* spp. (schistosomiasis)
Escherichia coli (gastroenteritis)	*Giardia duodenalis*	Enterovirus		
Helicobacter pylori	*Giardia lamblia*	Hepatitis A virus		
Legionella spp. (legionnaires disease)	*Naegleria fowleri*	Norovirus		
Leptospira spp. (leptospirosis)		Parvovirus		
Listeria monocytogenes (listeriosis)		Rotavirus		
Mycobacterium spp.				
Pseudomonas spp.				
Salmonella spp. (typhoid fever)				
Shigella spp. (shigellosis)				
Vibrio spp. (cholera)				
Yersinia enterolitica (enteric yersiniosis)				

Leptospirosis, an acute febrile illness, is contracted through direct contact of human skin with areas contaminated with the urine of infected rodents. It is the only flood-related water-borne disease proven to be epidemic by the World Health Organization. Outbreaks of leptospirosis after flood events have been observed globally in urban and rural areas, especially in Latin America and Asia and with higher risks in highly populated areas with suboptimal drainage, low-lying areas and small island states. An example was the outbreak of leptospirosis in the Philippines after the devastating cyclones of 2009, with 2,158 confirmed cases and 167 deaths reported. Timely diagnosis and appropriate

antibiotic therapy are necessary to avoid the progression of disease, system failure and fatalities (Gaynor et al., 2007; Jha et al., 2012).

One of the major sources of pathogens related to water-borne diseases, along with open defecation and contamination of water supply sources with faecal sludge, is urban wastewater. Wastewater is a mixture of natural and man-made organic and inorganic substances discharged into the sewers from households (domestic/sanitary wastewater, also called sewage), from industries (industrial/trade wastewater), from roads and roofs, as well as groundwater infiltrated into sewers. The quantity and quality of wastewater in sewers depends on the time of day and the season of the year, the infiltration and surface run-off, as well as the per capita water usage in an area, local habits and diet. In average, 99.9% of the volume of wastewater is water. The remaining part (0.1%) consists of faeces, food particles, grease, oils, soap, salts, metals, detergents, plastics, sand and grit, which can be converted by wastewater treatment into a manageable sludge, while leaving only a small portion in the final effluent (0.003% dry solids) disposed of in the environment (Gray, 1989). Wastewater needs to be treated for a series of reasons. First of all, the inactivation of pathogens is crucial for the protection of public health and hygiene. Also, the removal (and recovery) of water and waste materials present in the wastewaters can prevent the appearance of adverse ecological effects. The disposal of treated wastewater should also happen without nuisance or offence to the society and the environment. Therefore, it is important to recycle and recover nutrients and other valuable components of wastewater. The purpose of modern treatment plants is to achieve all the above mentioned goals in an economically feasible and sustainable way, according to legal environmental and public health standards (Gray, 1989) and in accordance to the sustainable development goals (SDGs) (UN, 2015). In most of the developing countries sewage is still discharged “raw”, without any treatment or after often inadequate pre-treatment, while 2.4 billion people have no access to improved sanitation facilities (UNICEF and WHO, 2015), despite the progress made in the efforts to achieve the Millennium Development Goals (MDGs) and the ongoing ones to achieve the relevant SDGs.

Sewer networks can be either combined or separate. Combined sewers collect sanitary drainage from households, industrial sewage and surface runoff waters in a single pipe (or channel) system. In this case, during wet weather conditions, if the amount of wastewater exceeds the hydraulic capacity of the wastewater treatment plant (WWTP), wastewater (as a mixture of urban/industrial drainage and precipitation runoff) is either stored temporarily within the catchment or is directly discharged into the receiving water as CSO without any treatment, bypassing the WWTP (Rauch et al., 2002). Although urban runoff dilutes the pollutants included in the household and industrial drainage, CSOs contain significant loads of pathogens, next to various pollutants, as toxic substances and persistent materials (heavy metals, polycyclic aromatic hydrocarbons (PAHs), etc.). In addition, runoff water often contains pollutants and pathogens that have

been accumulated on catchment surfaces and/or small pools during dry weather and washed off during rainfall events (Rauch et al., 2002). A study has shown that the probability of developing gastrointestinal diseases from incidentally ingesting water near CSOs has a range of 0.14-0.70 over the course of a year for people coming in contact with it, associated with the presence of faecal indicators *Streptococcus* and *Enterococcus* (Donovan et al., 2008).

A significant volume of the flow is also likely to emerge on urban surfaces such as streets, pavement tiles, soil and playgrounds, through surface discharge (surcharge) of wastewater out of manholes. Thus, urban and recreational surfaces can present a potential reservoir/source of pathogens (Shah et al., 2011). Urban areas have been identified as one of the main sources of faecal indicator organisms, with the highest values occurring for high-flow conditions during or after rainfall (Kay et al., 2008). Flood water mixed with sediments exhibited an increased abundance of putative pathogens after floods caused by Hurricane Harvey in Houston, USA, in both residential areas and public parks (Yu et al., 2018). After the Elbe river flood in 2002 in Germany, high numbers of pathogenic bacteria were detected in the mud, streets, playgrounds, and in the basement of flooded houses (Abraham and Wenderoth, 2005). In Toronto, Ontario, Canada, faecal indicator bacteria (FIB) have been reported 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). Concentrations of pathogens are often measured in samples from sewer flooding incidents. For instance, *E. coli*, intestinal enterococci and *Campylobacter* were found in samples from three sewer flooding incidents in the Netherlands (ten Veldhuis et al., 2010). Presence of pathogens like norovirus, rotavirus, enterovirus, *Giardia* oocyst, *Cryptosporidium*, *Campylobacter*, and *Salmonella* was reported in the canal 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 (Wit et al., 2001). The estimated risk of infection in canals and recreational lakes in Amsterdam, polluted by combined sewers, raw sewage from houseboats and dog and bird faeces per exposure event has been measured around 0.0002%-0.007% with *Cryptosporidium* and 0.04%-0.2% with *Giardia* for occupational divers professionally exposed to canal water (Schets et al., 2008). Furthermore, possible associations between the occurrence of gastroenteritis and environmental strains of human enteric viruses in sewage in Tunisia was found using molecular detection and characterisation techniques (Sdiri-Loulizi et al., 2010). Sales-Ortells and Medema (2015) found a high concentration of *Campylobacter* at the Bellamyplein water square in Rotterdam, The Netherlands (cover image), serving with the dual function of recreational area in dry seasons and of storm water detention during extreme events. Rain simulation events with drinking water showed that the mean *Campylobacter* disease risk for children playing in the water square is $2.5 \cdot 10^{-3}$ in the presence of animal faeces contamination and $4.5 \cdot 10^{-2}$ per person per event in the presence of human faecal contamination, 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).

An important knowledge gap in the vast majority of cases is related to the lack of data about the time and conditions of the inactivation of pathogens in the environment, particularly on urban surfaces. This has triggered a major part of the research presented in this work.

1.3 INDICATOR AND SURROGATE ORGANISMS

Since the detection of individual pathogenic agents in wastewater is difficult, costly and time-consuming, indicator organisms are used to indicate the presence of faecal contamination and the potential presence of pathogens. Indicators are used to define the limits of pathogen concentrations above which a particular environment is susceptible to increased risk and contamination. Surrogates are organisms used as substitutes or models, imitating the growth and persistence of pathogens of interest in processes like disinfection, processing or other procedures (Busta et al., 2003).

Traditionally, microbial quality of water resources was assessed with the use of FIB only, mainly faecal coliforms and enterococci (Ashbolt et al., 2001). The indicator microorganism for which bacteriological analyses of water are most commonly performed is *E. coli*. *E. coli* can be found in the intestinal tract of humans, has been studied thoroughly and can grow easily under laboratory conditions (Gloyna, 1971). Prototype strains of *E. coli* (ATCC 25922, used in this study) and *Enterococcus faecalis* (UMRL 1053) are usually selected as representative FIBs (Fujioka and Yoneyama, 2002).

However, after the documentation of some outbreaks of cryptosporidiosis in waters which did meet the standards based on bacterial indicators (Ashbolt et al., 2001), it is assumed 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 the *B. subtilis* spores used in Chapter 5 of this study have been proposed as suitable surrogates of *Cryptosporidium* because of their higher persistence to environmental extremes (Stelma, 2018; Bradford et al., 2016; Headd et al., 2016; Mazoua and Chauveheid, 2005; Rice et al., 1996). Furthermore, *B. subtilis* spores have been recommended as a surrogate for *Cryptosporidium* because they are safe, relatively cheap, can be measured easily and with high reproducibility and the recovery of spores from spiked water samples is acceptable. Also, they have similar shape with oocysts, although a bit smaller (US EPA, 2010).

Human enteric sewage-borne viruses, such as poliovirus, echovirus and coxsackievirus have been detected even in marine waters, causing diarrheal diseases among swimmers (Fujioka and Yoneyama, 2002). Faecal bacteriophages are not suitable as indicators of the presence of human enteric viruses because they are also present in animal faeces, and

because human enteric viruses have been detected also in water samples that do not contain bacteriophages (Brookes et al., 2004). However, bacteriophages can be used as surrogates of the behaviour of human enteric viruses, because of their similar size and morphology, and the low cost, the ease and the higher speed of detection compared to human enteric virus assays (Brookes et al., 2004; Dias et al., 2018). There is evidence that bacteriophage fate and transport in ambient waters may resemble that of viral pathogens more closely than FIB, suggesting that the former may be suitable surrogates under some environmental conditions (McMinn et al., 2017). Most studies until now have focused on coliphage systems and a range of bacterial hosts. The ideal host bacteria would be bacteria originating from human faeces only and lysed by phages that do not replicate in another host or the environment, such as *Bacteroides fragilis*, although the latter have low phage numbers for general use (Brookes et al., 2004). The MS2 bacteriophages used in this study (Chapter 5) are RNA coliphages infecting *E. coli* by attaching to the F-pili plasmids of the bacterial cells. They have been used in disinfection studies to model properties of human pathogens, especially noroviruses (Dawson et al., 2005).

It is important to note that 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 urban and recreational surfaces (e.g. beach sand) are almost inexistent (Staley et al., 2016).

Finally, it is critical to have knowledge of the specific system of the site studied instead of relying on universal rules. In any case, the sampling must be coupled with deep understanding of the biological, physical and chemical factors which influence the processes of transport, distribution and inactivation of the pathogens (Brookes et al., 2004).

In this study, the inactivation of *E. coli*, the most common FIB, *B. subtilis* spores, chosen as a non-pathogenic surrogate for *C. parvum* oocysts and *Giardia* cysts, and *E. coli* bacteriophage MS2 as a surrogate for viral contamination was studied.

1.4 FACTORS AFFECTING THE SURVIVAL OF PATHOGENS

The spread of pathogenic microorganisms by contaminated flood waters is a serious global problem connected with the spread of diseases. The initial presence or absence of pathogens in flood waters and on surfaces after the recession of the waters does not provide a reliable direct indication of the risks to human health. The inactivation of the pathogens appear to play a crucial role in defining public health risks since most of the

outbreaks of diseases occur within different time intervals after flooding events (Alderman et al., 2012; Du et al., 2010; Taylor et al., 2011).

1.4.1 Survival conditions in water

The inactivation of pathogens depends mainly on temperature, solar radiation and predation, and to a smaller extent on salinity and pressure. In order to understand the fate of pathogens and predict public health risks and safe conditions, it is necessary to link the timescales of hydrodynamic events with the timescales of pathogen inactivation (Brookes et al., 2004).

In a typical aqueous environment, enteric pathogens are not able to replicate, with the exception of some bacteria. Viruses and parasitic protozoa do not replicate because they rely on a specific animal or human host for replication. Infected hosts, with or without symptoms of illness, excrete these pathogens in large numbers. After entering the aqueous environment the pathogens are slowly being inactivated. Pathogen concentrations can increase when the water flow rate increases, due to the resuspension of settled pathogens. Sedimentation of the protozoa during periods of low flow rates is stronger than settling of viruses because protozoa are much larger than viruses. This means that during rainfall the protozoa that are settled in the sewer system get mobilised in addition to those coming from the direct discharge of raw wastewater (Schijven and de Roda Husman, 2005).

Cryptosporidium parvum is capable of surviving for long periods of time in water. The inactivation of *C. parvum* in natural river water follows first-order kinetics with rates ranging from 0.013 to 0.039 $\log_{10}$ per day. Inactivation in natural river water has been found higher than in synthetic hard water (Weber and Rutala, 2001). Retention time and contamination by water flow were considered to be the major factors influencing these rates (Brookes et al., 2004).

Suspended matter in water allows the encasement (adsorption/absorption) of microorganisms, offering protection from environmental stressors, such as grazing, adverse hydrodynamic conditions and irradiance, by offering shading (refraction, reflection and/or scattering). It can also offer increased access to nutrients of the particles or enhance settling (Walters et al., 2014). Faecal indicator bacteria have shown affinity for fine particle attachment, which increases their survival and deposition rate. Around 30-55% of faecal indicator organisms can be found attached to particles in storm water runoffs (Characklis et al., 2005). The fraction of microbes associated with settleable particles varies according to the microorganism types. The percentage is around 40% for faecal coliforms, *E. coli* and enterococci, 65% for *Clostridium perfringens* spores and 13% for total coliphages (Krometis et al., 2007). Bacterial mortality due to predation or environmental exposure has been shown to be reduced when cells attach to particles, as particles settle faster (Fries et al., 2008).

Also it is important to note that microorganisms can survive in nutrient-rich sediments for long periods of time. The ability of microorganisms to survive in aquatic sediments implies that faecal coliforms detected in the water column may not always indicate recent contamination but may be the result of sediment resuspension (Davies et al., 1995).

The present section elaborates on the inactivation of indicator organisms in the water column, without any sediment, but only with suspended solids in order to assess light attenuation and inactivation. The level of attachment to the suspended solids is therefore also tested.

1.4.2 Survival conditions on different urban surfaces

Water-borne outbreaks are an acute aftermath of flood disasters, mainly as a result of contaminated drinking water supply and poor access to waste management and healthcare services (Brown and Murray, 2013), but little is known about the concentration of pathogens on different surfaces after the recession of floodwater (Hellberg and Chu, 2016). Several studies have shown that water-borne pathogens from human and animal sources are present on flooded surfaces (Andrade et al., 2018; Rui et al., 2018; Sales-Ortells and Medema, 2015; ten Veldhuis et al., 2010) in concentrations higher than prior to flooding (Eccles et al., 2017). For example, a five-fold increase in the levels of *E. coli* was observed in the Salinas Valley, California, USA during months with a high water flow and frequent flooding downstream (Cooley et al., 2007).

Despite the obvious importance of the issue, most studies until now were based on case-specific events, making it very difficult to have a broader understanding of the mechanisms of inactivation of pathogens. Research on rainwater harvesting showed that the microbial communities collected from different roofing materials such as concrete tiles, acrylic-surfaced bituminous membrane, grass, steel, and asphalt fiberglass shingle were not the same as in ambient rainwater and varied depending on the material used (Bae et al., 2019). The effect of pH is presented separately below, but it is important to note that the age of concrete surfaces affects their pH. Carbonation, the process of hydroxide anions of calcium hydroxide being replaced over time by carbonate anions results in the formation of calcium carbonate and decrease of pH (Pade and Guimaraes, 2007).

Literature about survival of pathogens on surfaces other than agricultural soils or food industry and hospital environments is very limited. For example, a suspension of *C. parvum* oocysts on glass slides and air-dried at room temperature showed a 97% loss of viability after 2 hours and full inactivation after 4 hours (Robertson et al., 1992). More prolonged survival, up to 72 hours, has been reported when *Cryptosporidium* in a diarrheal stool was applied on a wooden surface (Weber and Rutala, 2001). In order to obtain a more realistic account of the overall health risks, it is necessary to assess the critical factors influencing the viability of pathogenic microorganisms in the urban environment, such as different environmental conditions and the properties of different

surfaces. The scope of this thesis is exactly to focus on this research gap. Chapters 3, 4 and 5 elaborate on this topic.

1.4.3 Effect of temperature

The survival of pathogens such as *E. coli*, that may grow in natural waters, outside the animal host, is affected by temperature fluctuations in two ways: first by the growth rate, and secondly, by the rate of inactivation. When temperature is increased, growth is favoured and thermal and light inactivation as well. This double effect explains why water temperature variation between 12 ˚C and 40 ˚C does not seem to be important in the inactivation of bacteria, although inactivation does happen at higher temperatures (Wegelin et al., 1994). In contrast, the inactivation of viruses has been reported to increase steadily over a range from 20 ˚C to 50 ˚C (Reed, 2004). At the same time, there is more complication due to the lower solubility of oxygen at higher temperatures; this leads to less than expected light inactivation in increased temperatures (Giannakis et al., 2014).

The survival of pathogens that do not grow in natural waters when present outside of the animal host, such as *C. parvum*, is reduced as temperature increases, by a higher/faster inactivation. The effect of temperature on *Cryptosporidium* infectivity and/or viability can be described by a first-order inactivation function:

$$C(t) = C_0 e^{-kt} \qquad (1.1)$$

$$k = 10^{-2.68} 10^{0.058T} \qquad (1.2)$$

where t is time in days, C is the concentration of viable oocysts, k is the inactivation rate (dimensionless) and T is the temperature in Celsius degrees (Brookes et al., 2004).

The ability of oocysts to initiate an infection is related to finite carbohydrate energy reserves in the form of amylopectin, which is metabolised in direct response to the temperature. Inactivation at higher temperatures is a result of increased oocyst metabolic activity, with a close relationship between infectivity and the level of ATP in the oocyst (Peng et al., 2008).

Data suggests that temperature affects the rate of *C. parvum* inactivation regardless of whether the oocysts are freely suspended or bound within a matrix of faecal or organic material (Brookes et al., 2004). However, in faeces and soil the degradation can be more rapid because of microbial antagonism and/or predation (Brookes et al., 2004).

In the case of viruses, inactivation is observed at temperatures higher than 50 ˚C (Bertrand, 2012). According to WHO (2004), most of the virus proteins get destroyed when heated above 60 ˚C, except the lyophilized ones, which can endure up to 80 ˚C.

The behaviour of microorganisms in soil is much more complex than in water, due to the simultaneous presence of gaseous, water and solid phases, numerous chemicals, microorganisms, macrofauna and plants. Various physical properties which can vary over small distances are also important factors affecting the behaviours of microorganisms. For these reasons the inactivation rates of pathogens determined for different soils show a wide variation with respect to temperature (Peng et al., 2008).

1.4.4 Effect of pH

Most microorganisms show stability and optimal growth near neutral pH (7.0) (Sinclair et al., 2012). *E. coli* cannot grow at alkaline pH and it gets inactivated above pH 9.3 (Parhad and Rao, 1974). High pH induces bacterial inactivation either by enhancing the generation of reactive forms of oxygen (hydrogen peroxide, superoxide and hydroxyl radicle) that are lethal to bacteria or by reducing their ability to resist the sunlight effects (Curtis et al., 1992) or disruption of the internal pH by weak bases like ammonia.

To describe the effect of pH on the maximum specific growth rate $k_{max}(pH)$ (h^{-1}) the Cardinal pH Model equation (CPM, Eq.2, Rosso et al., 1995) can be used (Eq. 1.3).

$$k_{max}(pH) = k_{opt} \cdot \gamma_{pH}(pH) = k_{opt} \cdot \frac{(pH - pH_{min}) \cdot (pH - pH_{max})}{(pH - pH_{min}) \cdot (pH - pH_{max}) - (pH - pH_{opt})^2} \qquad (1.3)$$

where $\gamma_{pH}(pH)$ (-) is the reduction of the maximum specific growth rate due to a deviation from the optimal pH (pH_{opt}) at which the maximum specific growth rate is equal to the optimal growth rate k_{opt} (h^{-1}). pH_{min} is the minimum pH below which growth is not possible and pH_{max} is the maximum pH beyond which growth is not possible.

For most enteric viruses, the optimal pH is between 3 and 9 (Melnick et al., 1980).

1.4.5 Effect of light

Sunlight has been identified as one of the predominant factors leading to inactivation of enteric microorganisms in surface waters (Schultz-Fademrecht et al., 2008; Burkhardt et al., 2000; Davies-Colley et al., 1999; Garvey et al., 1998). Inactivation rates of bacteria and bacteriophages under solar radiation are more than 10 times higher compared to a dark environment. The sensitivity of microorganisms, including viruses, to electromagnetic radiation depends on the wavelength and on the species of organisms and is described by biological weighting functions (Silverman and Nelson, 2016). The relative susceptibility to light, is higher for enterococci, followed by faecal coliforms, *E. coli*, somatic coliphages and F-RNA phages have the lowest inactivation (Sinton et al., 2002).

Although bacteria have greatest sensitivity to short wavelengths of light (Reed, 2004), the short wavelength light is faster attenuated in the water column having a smaller fraction of the incident radiation (Silverman and Nelson, 2016). Therefore, the lethal effect of sunlight on bacteria is more important in wavelengths higher than 320 nm (Oppezzo, 2012). These wavelengths can cause indirect (photo-chemical, particularly photo-oxidative) damage, when absorbed by photosensitiser macromolecules within microbial cells (endogenous, such as porphyrins, bilirubin or chlorophyll) or in the surrounding water (exogenous, such as dissolved organic matter or humic acids) (Maraccini et al., 2016).

Cryptosporidium oocysts are susceptible to solar inactivation and UV-B wavelengths have been experimentally identified as the most germicidal ones affecting the oocysts' infectivity in environmental waters. UV-B radiation is detrimental also to a wide range of organisms including bacteria, fungi, plants and annelids (King et al., 2008). In general, inactivation is more acute at shorter wavelengths, but the greatest inactivation occurs at full sunlight. Although the UV-B component of sunlight may directly damage biomolecules, such as DNA, UV and visible light more often cause indirect damage, when they are absorbed by photosensitiser molecules inside or around microbial cells (Reed, 2004). These molecules are raised by light into an excited state and can subsequently react with cellular biomolecules or with molecular oxygen (Reed, 2004). When reacting with oxygen, various reactive oxygen species (ROS) are produced, such as singlet oxygen, superoxide, hydroxyl radicals and hydrogen peroxide. ROS react with DNA, proteins and cell membrane components, leading to the inactivation of cells (Reed, 2004). Therefore, high levels of dissolved oxygen (DO) in water lead to increased light inactivation rates, up to 4-8 times higher compared to deoxygenated water, which shows that photo-oxidation is the principal reason for the rapid decrease in bacterial counts in water of low turbidity (Reed, 2004).

The wide range of wavelengths inactivating enterococci and F-RNA phages means that the effect of solar radiation on these microorganisms is photo-oxidative damage. On the other hand, the inactivation of faecal coliforms and somatic coliphages by a more specific range of wavelengths of UV-B shows that the effect of solar radiation on these microorganisms is photo-biological damage (Sinton et al., 2002; King et al., 2008). *E. coli* strains are slightly more resistant to death by solar radiation than other bacteria (*P. aerugenosa*, *S. flexneri*, *S. typhi*, and *S. enteritidis*). *E. coli* strains therefore serve as indicators to assess the effects of solar radiation on enteric bacteria. Past experiments showed a 3-log reduction of *E. coli* after 75 minutes of exposure under test conditions. However, experiments showed that *Str. faecalis* is slightly more resistant than *E. coli* (Wegelin et al., 1994).

The concentration of bacterial cells also affects the sensitivity to solar radiation. Very high concentrations show lower sensitivity compared to low or moderate density, because

of shielding from one cell to another (Giannakis et al., 2014). Ultraviolet (UV) radiation represents around 3-4% of incoming solar (<2,800 nm) radiation. The hourly average incoming shortwave radiation around midday, at mid-latitude, during summer is 1,000 $W.m^{-2}$. This corresponds to around 30 $W.m^{-2}$ of UV radiation and to an hourly UV dose of 360,000 $mJ.cm^{-2}$, which is much greater than typical water treatment dosages (20-120 $mJ.cm^{-2}$) (Brookes et al., 2004).

Oocyst inactivation due to UV exposure follows an exponential inactivation function similar to temperature:

$$C(t) = C_0 e^{-k_{UV} H} \qquad (1.4)$$

$$H = I_{UV} t \qquad (1.5)$$

where *C(t)* is the concentration of viable oocysts at time t, *H* is UV dosage (intensity integrated over time), I_{UV} is light intensity, k_{UV} is the inactivation coefficient of UV light, which is approximately equal to 1.2 (Brookes et al., 2004).

Particulate matter in water often results in lower inactivation rates of microorganisms due to shielding of harmful UV rays, but the degree to which particle association can impact light inactivation of enteric microorganisms in surface waters is not yet clear (Walters et al., 2014). The vertical distribution of UV light is described by an exponential function equivalent to Beer's law:

$$I_{UV(z)} = I_{UV(surface)} e^{-\mu z} \qquad (1.6)$$

where μ is the attenuation coefficient of UV light, which is closely related to dissolved organic carbon (DOC) and has been measured at values between 0.1 and 40 m^{-1}. Given the wide range of μ values, the penetration depth of UV light (the depth reached by only 1% of surface irradiation) can range considerably (e.g. between 0.1 and 46 m) (Brookes et al., 2004).

Therefore, the impact of UV light on oocyst viability is governed by a double exponential function, which means that there is a sharp transitional depth in the water column between the area where UV light has a dramatic effect and the area where its effect is insignificant. This transitional depth varies a lot from one reservoir to another, depending on DOC (Craik et al., 2001; Brookes et al., 2004).

The underwater light field is influenced by the properties of the incoming irradiance and the optical properties of the aquatic medium, which can be divided into inherent properties and apparent properties (Baffico, 2013). Inherent properties, such as the absorption coefficient, the scattering coefficient etc., depend on the nature and composition of suspended particles and dissolved compounds. The apparent properties

depend on the medium and on the geometrical structure of light and one example of such properties is the angular distribution of the underwater radiation (Baffico, 2013).

The presence of particulate matter in water reduces considerably the transmission of sunlight, either by shading (refraction, reflection or scattering of light) or by encasement. This in turn results in lower inactivation rates of the microorganisms. Many studies report a direct correlation of particle size and/or concentration on UV disinfection efficiency of secondary wastewater effluents, but more research needs to be done to understand to which degree they are correlated (Walters et al., 2013).

One of the most important parameters in characterising the attenuation (scattering and absorption) of UV light within solids is the attenuation coefficient. It represents the decline in light as a function of depth as a result of both scattering and absorption and it is defined by the following equation:

$$K = \frac{\log\left(\frac{E_1}{E_2}\right)}{z_1 - z_2} \qquad (1.7)$$

where K is the attenuation coefficient (μm^{-1}), E_1 and E_2 is the scalar irradiance at depth one and depth two, respectively ($mW.cm^{-2}$) and z_1 and z_2 is the depth at point one and point two within the particle, respectively (μm). The attenuation coefficient equals the absorption coefficient when the medium strictly absorbs. Once the absorption coefficient is determined for the particle solids, light intensity can be calculated for any given depth into the solid (Loge et al., 1999).

1.5 SAMPLING METHODS

Most methods for recovery of microorganisms from surfaces found in the literature are designed for smooth and non-porous surfaces, such as stainless steel surfaces used in the food industry (Garayoa et al., 2207; Maunula et al., 2017; Moore and Griffith, 2007), and in healthcare environments (Rawlinson et al., 2019; Claro et al., 2015). In the food industry, methods and techniques to recover microorganisms from surfaces have been developed, namely non-microbiological surface sampling, including visual assessment, ATP bioluminescence, protein and other assays, and microbiological surface sampling, i.e. indirect methods such as swabbing, sponges and wipes, direct methods such as contact plates, dip slides, petrifilm, rollers and agar sausages, and molecular methods (Griffith, 2016). However, parameters such as the diversity of experimental conditions or samples hinder the choice of the best method (Griffith, 2016). Hospital environments in Europe are usually sampled only in response to outbreaks, but guidelines on monitoring do not provide with microbiological protocols (Otter et al., 2015). Sampling becomes even more complicated for urban surfaces such as concrete, pavement and asphalt that are rough and porous and for which there is no standardised procedure in the literature.

Table 1.2. Surface sampling methods, their typical applications, their main advantages and limitations and indicative references.

Methods		Typical applications	Advantages	Limitations	References
Direct agar contact (printing)	**Contact agar plates (RODAC)**	Small, smooth, nonporous, flat or gently curved surfaces	Simple, no processing needed	Sampling area limitations, not possible to count high concentrations, colonies and aggregations	Lemmen et al. 2001 ; Tamburini et al., 2015
	Dip slides / Petrifilm				
	Roller				Lutz et al., 2013
	Agar syringe/sausages				
Pouring agar (Direct Surface Agar Plating, DSAP)		Nonporous	High recovery (no transport issues)	Fixed surfaces, contamination, coalescence of colonies, incubation temperature	
Indirect contact	**Stamp**	Polyurethane foam cylinders	Autoclavable, "dilution" of high concentrations by serial impressions		Tresner and Hayes, 1970
	Adhesive tape/sheet	Mainly to examine samples by microscopy		Difficulty to recover from tape	Urzì et al, 2001; Yamaguchi et al., 2002
Swabbing	**Rayon tipped swab**	Smooth, nonporous, or crevices and corners	Good for small areas, corners, crevices	Sampling area limitations	Moore and Griffith, 2007; Ismaïl et al., 2013; Lahou and Uyttendaele, 2014
	FLOQSwab				(Finazzi et al., 2016)
Friction/ scrubbing	**Sponges / Wipes**	Smooth, nonporous	Easier to cover larger areas.	Need of handles or sterile gloves for each sample.	
	Brushes				Ismaïl et al., 2015
Vacuum methods	**Vacuum filter sock (HEPA)**	Porous or nonporous, smooth or rough	Multiple surface types	Large volume of dust makes detection more difficult, need for power source, desiccation	
	Microvacuum cassette				
	Wet vacuum	Nonporous, smooth or rough	Direct collection, less desiccation		
Bulk sample	**Immersion/Rinsing**	Porous or nonporous, smooth or rough	Multiple surface types	Need of sterile tools, no standard method for processing, difficult interpretation of results	Boehm et al., 2009; Taylor et al., 2013; Martínez-Bastidas et al., 2014; Staley et al., 2016
	Scraping, grinding				Ismaïl et al., 2015
	Chopping grass				Cao et al., 2018
	Soil Sprinkling				
Radiometry		Bacteria tagged with radioisotopes	Sensitive and accurate with most surfaces	Not possible in the field	

Most methods referring to porous and rough surfaces include either the collection of bulk samples of the material with methods such as scrapping and grinding or collection of water samples after immersion or rinsing. For example, Taylor et al. (2013) acquired bacterial samples from a 0.1% Tween solution after immersing and stirring in brick, plaster and wood coupons. Several recovery methods and techniques exist for bacteria and fungi contained in soil or rock. Non-destructive methods such as washing, scraping, printing off of surface microorganisms, or *in situ* tests of physiological activity by applying fluorogenic substrate analogues for the study of monuments and destructive methods like rock-coring or other method for aseptic collection of samples, can be followed by fragmentation to release the microorganisms to study natural rock deterioration by microorganisms (Hirsch et al., 1995). *In situ* microscopy can also contribute to demonstrating the presence of microorganisms on stone, but this does not allow precise identification of the organisms, for which molecular biology techniques such as high throughput sequencing and metagenomics are necessary (Mihajlovski et al., 2015).

Swabbing is used for the recovery of microbial cells, especially from hard-to-reach and irregular surfaces, but although the procedure and data evaluation are standardised and inexpensive, this method is also prone to significant variations due to multiple factors such as the size and roughness of the area swabbed, the material of the swab head, the wetting solution and extraction protocol, operator contributions, ambient temperature and humidity, as well as the morphological and physiological properties of the microorganisms (Digel et al., 2018).

According to Moore and Griffith (2007), the effectiveness of the swabbing technique depends on the percentage of bacteria that the swabs can collect from the sampled surface, on how many of these bacteria can be removed from the swabs and on the cultivation of these bacteria. The authors studied separately the parameters that affect the removal of bacteria from the surface (swab type, swab absorbency, wetting solutions affecting viability, de-clumping and detachment, wet or dry surface) by applying agar on the surfaces before and after swabbing and the parameters that affect the release of bacteria from the swab (swab type, wetting solutions) by directly inoculating swabs with a pipette. They found that swabs coated with a brush-textured nylon tip in combination with non-growth enhancing wetting solution allowed the highest recovery from wet surfaces, but noted that this combination may not always be ideal.

For any method, a variety of recovery ratios may be obtained. In an optimum method, the tool, the reagents and the equipment must produce the same end result even if they are used by different operators under the same sampling conditions (Ismaïl et al., 2013).

1.6 PROBLEM STATEMENT

The spread of diseases during and after flood events is largely connected with the spread of pathogenic microorganisms from CSOs and surcharged combined sewers, especially in poorly managed systems. Even in developed countries when recreational activities take place in the storm water detention and retention basins, there is a high risk for inhabitants, especially children to be infected. The literature review has indicated that more research needs to be undertaken in areas concerning the inactivation of pathogenic organisms after urban floods because the presence or absence of pathogens immediately after floods does not provide a direct indication of risk to human health. Some of the pathogens are inactivated by the influence of sunlight, while others are protected, depending on the surface type and its characteristics. The different parts of the system, their interactions and their relationships are illustrated in Fig. 1.2.

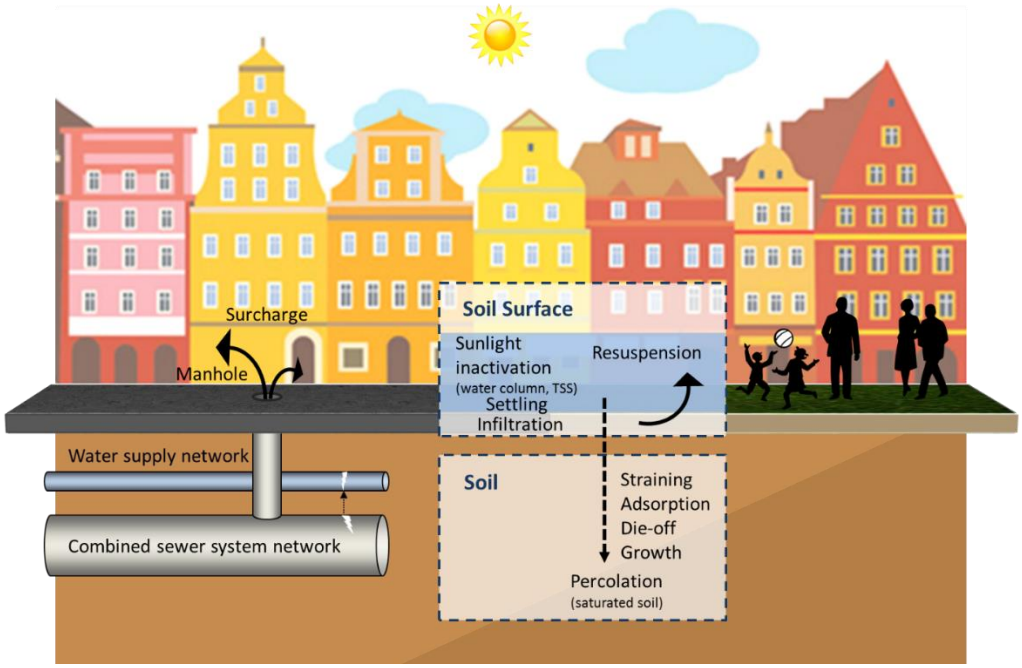


Figure 1.2. Simplified schematic of boundaries of research focus of this thesis.

Although sunlight-mediated inactivation is considered as the main cause of disinfection in waste stabilisation ponds, with mechanisms depending on the characteristics of the sunlight and its capacity to penetrate through water, as well as pH, temperature, nutrients available, salinity and DO (Dias et al., 2017), research on the survival of pathogens and indicator organisms on different urban surfaces is limited. Most studies are based on case-specific events, making it very difficult to have a broader understanding of all the complex processes taking place and to quantify the associated public health risks in the hours and days following the flooding events and the recession of flood waters.

In order to obtain a more realistic assessment of the overall health risks and to determine when access into flooded areas is safe, it is necessary to understand the critical factors influencing the inactivation of pathogens on different urban surfaces, for which literature is very limited. Laboratory experiments on different surfaces under controlled conditions of intensity and duration of sunlight and concentration of suspended solids can improve the understanding of the inactivation of indicator organisms. These results can be further applied to provide predictive information for effective public health management.

1.7 RESEARCH OBJECTIVES

1.7.1 Overall aim

The aim of this research is to contribute to the reduction of public health risks related to floods and caused by water-borne pathogens resulting from the surcharge of combined sewer systems. The main environmental factors that affect the inactivation of indicator organisms on different urban surfaces will be assessed. Since floods are increasingly connected to climate change, this research should be considered also as a contribution to climate change adaptation.

1.7.2 Research questions

The key research questions which occur based on the literature review are the following:

1. What is the most reliable method for collecting samples of faecal indicator bacteria from different urban surfaces after the recession of flood water?
2. What is the effect of solar radiation and total suspended solids on the inactivation of indicator organisms that originate from the surcharging of combined sewer systems?
3. What are the effects of solar radiation on the inactivation of different indicator organisms (originated from the surcharging of combined sewer systems) on different urban surfaces?

1.7.3 Research hypotheses

The following research hypotheses correspond to the research questions set above:

1. The most reliable method for collecting samples of faecal indicator bacteria from urban surfaces is swabbing.
2. Increased exposure to solar radiation increases inactivation of surrogate microorganisms for enteric pathogens in water. Therefore, increased suspended

solid concentrations reduce the inactivation rates due to limited penetration of light in the water column.

3. The pH of different surfaces affects solar inactivation of surrogate microorganisms for enteric pathogens on these surfaces.

1.8 RESEARCH APPROACH

In order to achieve the overall aim and to address the research questions, this thesis uses simulated laboratory experiments with certain indicator organisms to study processes that attempt to mimic natural phenomena taking place in the water column during floods and on urban surfaces after the recession of flood water. The thesis has been structured in six chapters (Fig. 1.2), including an introduction, four research phases and an outlook.

Chapter 1 is a general introduction on the background of the study, including a literature review relevant to the problem statement of this research.

In the first research phase (Chapter 2) the effect of artificial solar radiation on the inactivation of indicator bacteria *E. coli* was studied in a water column to mimic a water column occurring during a flood. This phase took place in a relatively deep vessel/unit to assess the effect of different total suspended solids' concentrations on the light attenuation and on the inactivation of the indicator organisms in the water column, corresponding to the phase when urban surfaces are still flooded.

In the second research phase (Chapter 3) the most reliable method was identified for sampling water-borne indicator *E. coli* from flood-prone urban surfaces where pathogens can occur after the surcharge of sewer networks: concrete, asphalt, pavement blocks and grass. Different sampling methods were compared: swabbing, direct agar contact, stamping and adhesive tape-lifting. The swabbing method was selected as the most appropriate one for the collection and enumeration of *E. coli* in samples from different surfaces in the next research phase.

In the third research phase (Chapter 4) the inactivation of indicator bacteria *E. coli* on different urban surfaces was studied. These were typical surfaces which are found in urban and sub-urban open areas and storm water detention and retention basins: asphalt, concrete, pavement blocks, gravel, sand, rubber tiles and grass, with glass as control. The experiments took place on the surfaces after the recession of artificial flood waters, mimicking the post-flood phase when the surfaces start drying after urban floods and people start using them for transport, recreational purposes etc. This allowed to better understand the effects of pH and other surface characteristics on bacterial inactivation.

The fourth research phase (Chapter 5) studied the inactivation of *E. coli, B. subtilis* spores, and bacteriophages MS2 under controlled exposure to simulated sunlight and to real sunlight. Concrete, asphalt and pavement blocks, and glass as control, were inoculated

with artificial flood water of 1cm height to control the temperature and to keep the surfaces saturated, compared to the previous phase when drying was studied.

The outlook (Chapter 6) summarises and draws conclusions on the knowledge gained from the above studies. It also gives recommendations and prospects for future research.

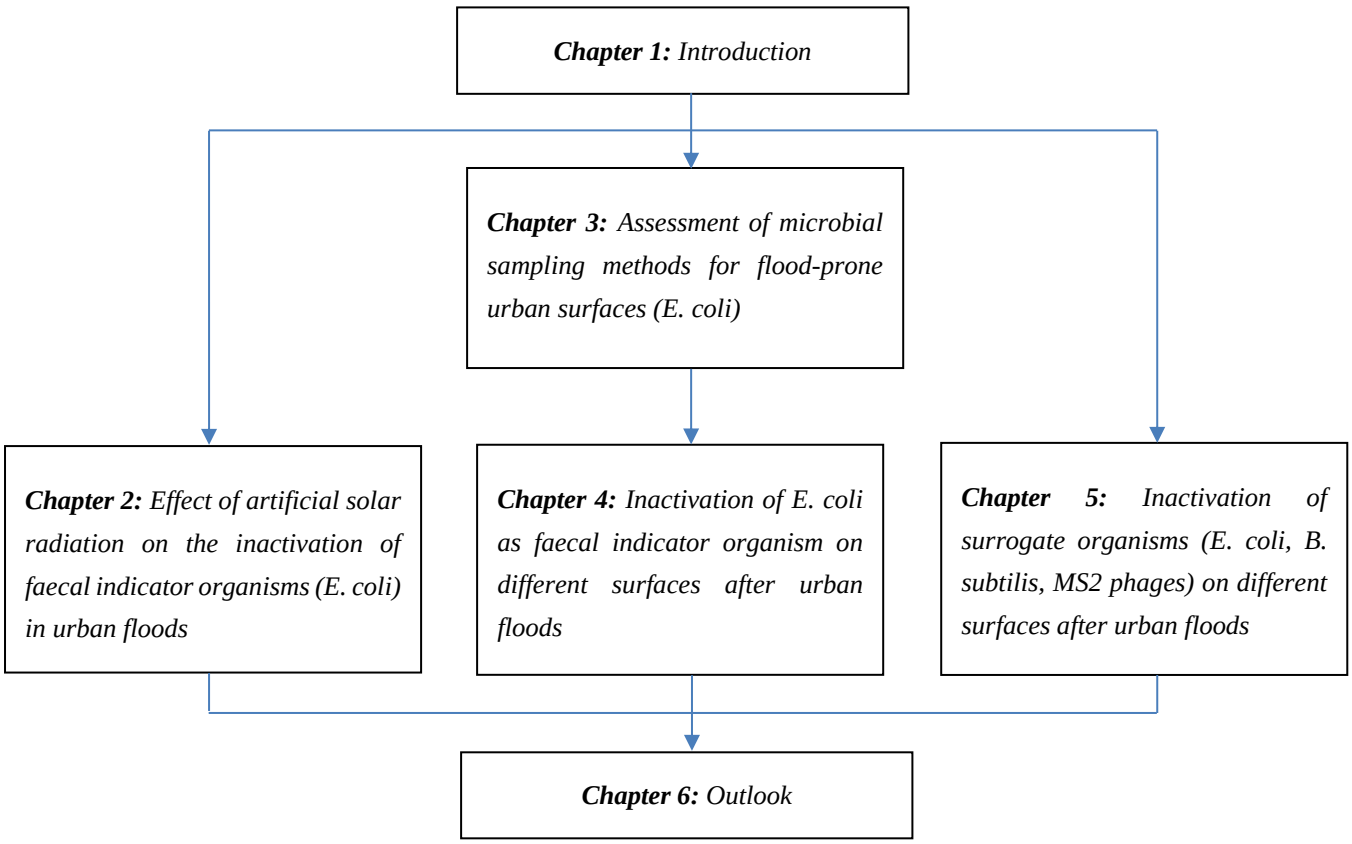


Figure 1.3 Structure of this PhD thesis.

2

EFFECT OF ARTIFICIAL SOLAR RADIATION ON FAECAL INDICATOR BACTERIA AFTER URBAN FLOODS

This research contributes to a better understanding of how the concentration of water-borne pathogens in contaminated shallow water bodies is affected by different environmental conditions. The inactivation of faecal indicator bacteria *E. coli* was studied in an open stirred reactor, under controlled exposure to simulated sunlight, mimicking the effect of different latitudes and seasons, and different concentrations of total suspended solids (TSS) corresponding to different levels of dilution and runoff. While attachment of bacteria on the solid particles did not take place, the inactivation rate coefficient, k (d^{-1}), was found to depend on light intensity, I ($W.m^{-2}$), and duration of exposure to sunlight, T ($h.d^{-1}$), in a linear way ($k=k_D+0.03 \cdot I$ and $k=k_D+0.65 \cdot T$, respectively) and on the concentration of TSS ($mg.L^{-1}$), in an inversely proportional exponential way ($k=k_D+14.57e^{-0.02 \cdot [TSS]}$). The first order inactivation rate coefficient in dark conditions, k_D=0.37 d^{-1}, represents the effect of stresses other than light. This study suggests that given the sunlight conditions during an urban flood, and the concentration of indicator organisms and TSS, the above equations can give an estimate of the fate of selected pathogens, allowing rapid implementation of appropriate measures to mitigate public health risks.

This chapter is based on: Scoullos, I.M., Lopez Vazquez, C.M., van de Vossenberg, J., Hammond, M. and Brdjanovic, D.: Effect of artificial solar radiation on the inactivation of pathogen indicator organisms in urban floods. Int. J. Environ. Res. 13, 107-116, doi:10.1007/s41742-018-0160-5, 2019. (IF=1.5)

2.1 INTRODUCTION

In this chapter, the effects on the inactivation of *E. coli* under various light conditions and in the presence of different concentrations of suspended solids were examined in an open reactor using an artificial light source with a spectrum similar to solar light. The experimental setting allowed to simulate different solar light intensities and durations that can occur at different times of the year and at different places around the globe. These conditions can be applied to all kinds of shallow water bodies, including multifunctional urban flood retention and detention basins often used as sport facilities or playgrounds during dry weather. Contamination could originate from wastewater from surcharged combined sewers, pit latrines, cross connections, open defecation, animal litter, or municipal and livestock operations. The selection of the light intensity and duration parameters was based on the conditions at two different latitudes: the equator and 60°. The equator zone was selected because it gets the maximum light intensity at a stable daytime length of 12 h throughout the year. 60° N and S were selected because of the daylight duration of 6 and 18 h respectively in winter and summer solstice in the North and the opposite in the South.

2.2 MATERIALS AND METHODS

2.2.1 Experimental reactor

Batch experiments took place in an open cylindrical stirred reactor of 40 cm internal diameter and 80 cm height. For each batch the reactor was filled with 75 L of demineralised water (up to around 60 cm); suspended solids and *E. coli* were added, according to each experimental phase. Continuous stirring at 95 rpm (IKA® dual-speed overhead stirrer, Staufen, Germany) was used to avoid settling. For maintaining the temperature of the reactor adequately constant at around 25 ˚C (fluctuating between 20 and 28 ˚C) and to neutralise the heating effect of the lamp, the reactor was placed in a larger tank containing water as a buffer while a ventilator was also employed. The experimental setup can be seen in Fig. 2.1. Each batch experiment lasted around one week, depending on the inactivation rate and until the microorganisms were not fully detected.

Figure 2.1. The experimental setup used.

2.2.2 Indicator organisms

E. coli ATCC 25922 was chosen as the faecal indicator bacterium of study, because it has been studied thoroughly and can be grown easily under laboratory conditions. Before each batch experiment *E. coli* was incubated in 1.3% *w/v* Oxoid CM0001 Nutrient Broth (Oxoid Ltd., Basingstoke, United Kingdom) solution in Erlenmeyer flasks for 24 h at 37 ˚C. After incubation the concentration of the inoculum was around $3 \cdot 10^9$ CFU.mL^{-1}. The inoculation took place in the reactor by adding 280 mL of inoculum in demineralised water, to achieve an initial concentration of *E. coli* of around 10^7 CFU.mL^{-1}. The initial concentration of each batch was measured by sampling 15 minutes after the inoculation to allow full mixing. The selection of the initial concentration of 10^7 CFU.mL^{-1} was based on the typical high concentrations of *E. coli* in raw municipal wastewater of around 10^6 CFU.mL^{-1} according to Mark et al., (2015) or $5 \cdot 10^6$ CFU.mL^{-1} when taking into account minor contributions of industrial wastewater (Henze and Comeau, 2008).

The enumeration of *E. coli* in the samples was performed by counting the number of colony forming units (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. The plates were spread in triplicate.

2.2.3 Suspended solids

To study the effects of different total suspended solids (TSS) concentrations, soil was used obtained from a previously flooded excavation site in Delft, The Netherlands. The soil was dried, ground and sieved. The particles selected for the experiment after sieving varied between sizes of 38 and 106 μm (silt and fine sand). This fraction corresponds to frequently found particle sizes in urban environments, for example around 28% of the particle size distribution that was measured in a parking lot and at a residential basin (Selbig et al., 2016) and to around 50% of particles measured in asphalt road runoff (Charters et al., 2015). Subsequently, the solids were heated in a muffle furnace at 520 ˚C for 6h in order to remove all organic compounds. The solids were added in the reactor in each batch experiment prior to the inoculation. The studied TSS concentrations varied from 0 to 200 $mg.L^{-1}$, because 200 $mg.L^{-1}$ is the reference value of US EPA for event mean concentration of TSS for urban runoff, while the similar ones according to NURP and to USGS and NPDES are lower, at 174 and 78.4 $mg.L^{-1}$, respectively (Acharya et al., 2010). The measurement of TSS was performed in triplicate with vacuum filtration using Whatman® Grade 1 (GE Healthcare, Little Chalfont, UK) filter papers (11 μm pore size), so that solid particles with attached bacterial cells are retained while non-attached bacteria can pass through. Prior and after the filtration the filter papers were dried at 105 ˚C for 2 hours and the weight was determined.

2.2.4 Light source and parameters

Simulated sunlight was produced using an OSRAM HQI-BT 400 W/D PRO metal halide lamp with built-in UV filter at around 320 nm. Apart from a few peaks in the area of visible light (with the highest one at 540 nm, see Fig. 2.2) the spectrum of these metal halide lamps is continuous (Calin and Parasca, 2008). The light intensity was regulated by changing the distance of the light source from the water surface. The duration of light/darkness hours was set with a timer. The photon flux, in $\mu mol\ m^{-2}.s^{-1}$, 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; this was taken into account when calculating the lamp irradiance spectrum. The measurements were performed in duplicate, in different sides of the reactor.

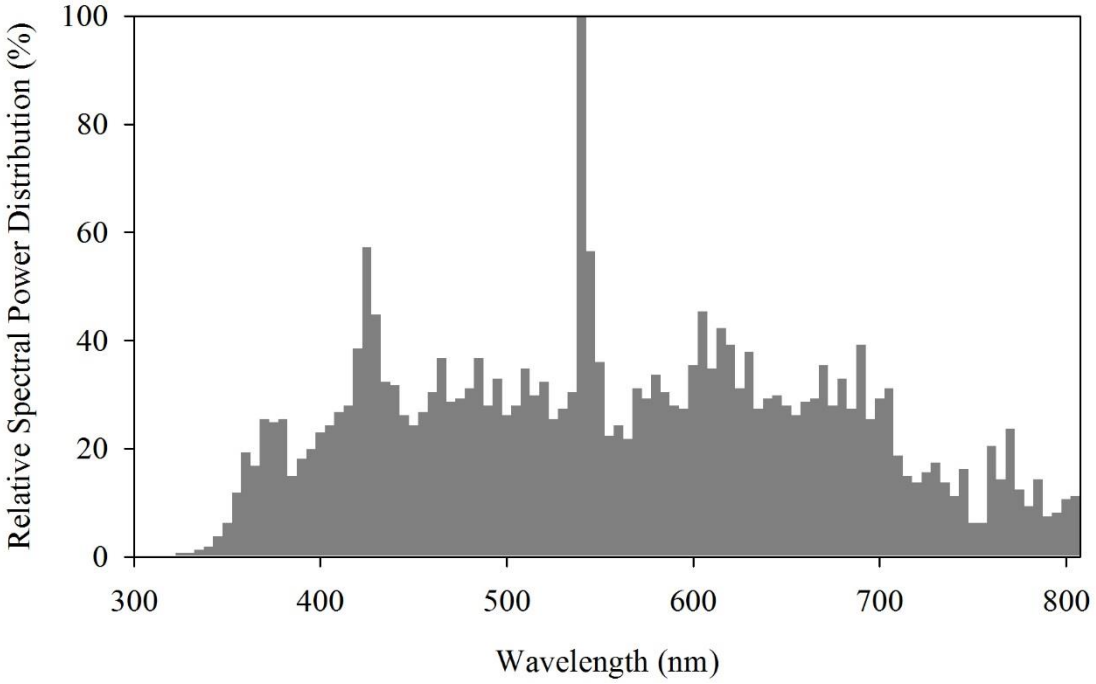


Figure 2.2 Relative spectral power distribution of the metal halide lamp used (OSRAM GmbH, Munich, Germany).

The total light intensity, I (W.m^{-2}), in the area of wavelengths (320-805 nm) for which the spectral power distribution of the lamp is provided (Fig. 2.2), at any location where the sensor is placed, was calculated with the Planck relation, taking into account the spectral power distribution of the lamp, using Eq. 2.1, adapted from Silverman and Nelson (2016):

$$I = \sum_{\lambda=320}^{805} I(\lambda) = \varphi \cdot h \cdot c \cdot N_A \cdot 10^3 \cdot \frac{\sum_{\lambda=320}^{805} M(\lambda)}{\sum_{\lambda=400}^{700} [M(\lambda) \cdot \lambda]} \qquad (2.1)$$

where φ is the photon flux (μmol m^{-2}.s^{-1}) measured by the sensor in the area of 400-700 nm (presented in Fig. 2.3), h is Planck's constant ($6.63 \cdot 10^{-34}$ J.s), c is the speed of light ($3 \cdot 10^{8}$ m.s^{-1}), N_A is Avogadro's constant ($6.022 \cdot 10^{23}$ mol^{-1}), λ is wavelength (nm), $I(\lambda)$ (W.m^{-2}) is light intensity at a specific wavelength and $M(\lambda)$ (%) is the relative power of the lamp at a specific wavelength as provided by the relative spectral power distribution.

2.2.5 Experimental design

Assessment of light attenuation

In order to address the phenomenon of light attenuation and calculate the light attenuation coefficient in the different conditions of the experiments, the photon flux was measured at different depths, with increments of 5 cm, in the reactor filled with demineralised water inoculated with *E. coli* and with the addition of soil particles of different concentrations (0, 25, 50, 100, 150 and 200 mg $TSS.L^{-1}$).

Effect of light intensity, duration of exposure to light and different concentrations of TSS on the viability of E. coli

The effects of different light intensities (190 and 320 $W.m^{-2}$, for 12 hours per day, without any solids added), periods of exposure to light (0, 6, 12, 18 and 24 hours per day, under 320 $W.m^{-2}$, without addition of any solids) and different concentrations of TSS (0, 25, 50, 100, 150 and 200 $mg.L^{-1}$, for continuous exposure to light of 320 $W.m^{-2}$ for 24 hours per day) on the inactivation of *E. coli* in demineralised water were studied. The viability of the organisms was also studied in dark conditions as a control.

Assessment E. coli attachment on the suspended particles

The attachment of *E. coli* on the suspended particles and particle-related shielding was tested in a batch experiment in Erlenmeyer flasks in an orbital shaker (150 rpm) with 200 mg $TSS.L^{-1}$ of solids in 250 mL of demineralised water by filtering samples of 25 mL and enumerating the bacteria in the filtered and unfiltered samples, in triplicate, at time zero and throughout the duration of the experiment. The soil particles used in the experiment were all selected, using sieving, to be larger than 38 μm and smaller than 106 μm. The bacteria that could have been adhered or attached to particles were retained together with the particles on the filter paper of 11 μm. A *t*-test for paired samples was performed to determine whether the concentrations of *E. coli* before and after filtration are likely to have come from distributions with equal population means. In addition to that, samples were taken from three different depths in the reactor; close to the surface (2 cm), middle (30 cm) and close to the bottom (58 cm).

Comparison of results by testing the inactivation of E. coli in artificial floodwater

The inactivation of *E. coli* was studied in artificial floodwater. Domestic wastewater was used in order to mimic flood water. It was diluted 10 times with demineralised water to simulate dilution by storm water runoff. The wastewater was collected from the influent stream of Harnaschpolder wastewater treatment plant, Den Hoorn, The Netherlands, after the first screening filters. Before dilution, the wastewater had a concentration of 29.6 mg $TSS.L^{-1}$ and $8.5 \cdot 10^4$ *E. coli* $CFU.mL^{-1}$. In order to study separately the effect of the wastewater's suspended solids, the experiment was performed by removing the wastewater's TSS with filtration using Whatman® GF/C (GE Healthcare, Little Chalfont,

UK) filters (1.2 μm pore size) and replacing the TSS with 150 $mg.L^{-1}$ of the treated soil particles, as described before. *E. coli* was added in the reactor prior to the experiment to achieve an initial concentration of 10^7 $CFU.mL^{-1}$. Wastewater quality has a high variability and the dilution factor from storm water increases the uncertainty even more. Therefore, the scope of this paper is limited in comparing demineralised water with diluted wastewater, both with the addition of soil particles. A comparison is made between reported characteristics of flooding water and the synthetic flood water used in this research (see Table 2.1.).

Table 2.1 Comparison of TSS and E. coli reported concentrations in different waters with the synthetic flood water used in this research.

Water quality	**TSS ($mg.L^{-1}$)**	***E. coli* ($CFU.ml^{-1}$)**	**References**
Raw municipal wastewater with minor contributions of industrial wastewater	250 (low) 300 (medium) 600 (high)	10^4 (low) $5 \cdot 10^6$ (high)	Henze and Comeau, 2008
Raw municipal wastewater		10^6 (high)	Mark et al., 2015
Raw influent Harnaschpolder	29.6	$8.5 \cdot 10^4$	This research
Artificial flood water	0-200	10^7	

2.2.6 Sampling and physicochemical parameters of study

Samples of 20 mL were taken from the reactor at periodic intervals; (i) approximately every 12 hours when the light was on, (ii) at the beginning and end of each dark and light phase during the experiment of light duration, (iii) every 24 hours for the dark control. The samples were taken from the top of the reactor with a 30 mL syringe. For the experiments involving suspended solids where it was important to assess stirring efficiency and sedimentation, samples were also taken from the middle and close to the bottom of the reactor by connecting a metallic tube to the syringe. The measurement of pH took place at the same intervals, with a handheld pH-meter (WTW pH 323, WTW GmbH, Weilheim, Germany). Temperature and DO were measured every hour (fluctuating between 20 and 28 ˚C and between 6.5 and 8.5 mg $O_2.L^{-1}$, respectively, data not shown) with a handheld DO-meter (WTW Oxi 3310, WTW GmbH, Weilheim, Germany).

2.2.7 Data analysis

Light attenuation due to different concentrations of suspended solids was addressed by measuring the vertical distribution of light in air and water with different concentrations of solids. This was described using an exponential function equivalent to Beer's law (Eq. 2.2), which states that the light intensity decreases exponentially as a function of depth:

$$I_{(z)}=I_{(surface)}e^{-\mu z} \quad (2.2)$$

where, z is the depth (m), μ is the light attenuation coefficient (m^{-1}), $I_{(z)}$ is light intensity ($W.m^{-2}$) at depth z and $I_{(surface)}$ is the light intensity on the surface (z=0 m) (Kirk, 1994; Morris et al., 1995).

In all the experiments where the inactivation of *E. coli* was addressed, the inactivation rates were calculated using the first order exponential inactivation "Chick-Watson" model (Eq. 1.1). In all experiments the inactivation was considered to start at time 0 with no lag period. Time 0 was considered as 15 minutes after inoculation to allow full mixing in the reactor before measuring C_0. For each batch experiment the first-order inactivation rate was assumed to be constant.

Maraccini et al. (2016) and Davies-Colley et al. (2000) used the following linear model (Eq. 2.3) to express the total inactivation under light conditions as the sum of the photo-inactivation plus the "dark" inactivation, due to stresses other than light, such as osmotic stress, predation and starvation:

$$k=k_D+k_L \cdot I \quad (2.3)$$

where k_D is the first order dark inactivation rate constant (d^{-1}), k_L is a pseudo second order photo-inactivation rate constant ($m^2\ W^{-1}.d^{-1}$) and I is light intensity ($W.m^{-2}$). In the present study, a similar expression was also presented for the relation of k to the duration of exposure to light (T). Subsequently, k_D was calculated experimentally as the mean of the values corresponding to the intercept of linear regressions, for zero intensity and exposure to light.

2.3 RESULTS AND DISCUSSION

2.3.1 Light attenuation

The vertical distribution of light intensity followed an exponential pattern, which corresponds to Beer's law, with values of R^2>0.98 for all the different TSS concentrations studied in the reactor (see Fig. 2.3).

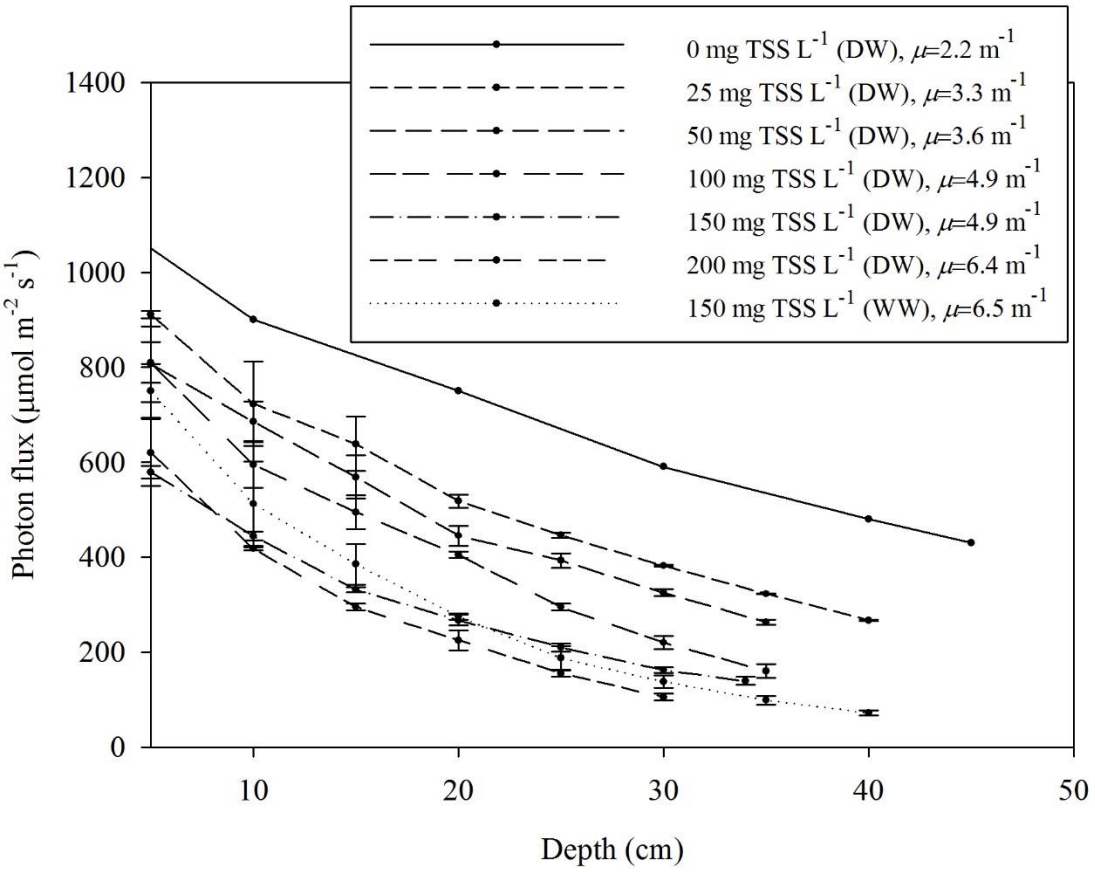


Figure 2.3 The values of photon flux measured by the sensor at different depths in the reactor filled with demineralised water (DW) or artificial flood water (FW), with E. coli and different concentrations of TSS. The light attenuation coefficients, μ, obtained from these curves are also presented.

The light attenuation coefficient was proportional to the concentration of suspended solids and Eq. 2.4 was determined from a linear regression with R^2=0.94:

$$\mu=2.59+0.02\cdot[TSS] \quad (2.4)$$

where μ is the light attenuation coefficient (m^{-1}) and *[TSS]* is the concentration of total suspended solids ($mg.L^{-1}$). The minimum theoretical value of μ, corresponding to pure water is 2.59 m^{-1}.

2.3.2 Inactivation of *E. coli* under different light intensities

The inactivation of *E. coli* was studied in the reactor under exposure to light for 12 h per day followed by 12 h in dark, without any solids added. The light intensities studied were 190 and 320 $W.m^{-2}$, with a control of 24 h in dark. The inactivation was described with exponential inactivation kinetics and the resulting inactivation rates are presented in

Table 2.2. The inactivation rate increases with an increase in light intensity and the data fit well (R^2=0.99) to the linear Equation 2.3 *with* k_D=0.19 d^{-1} and k_L=0.03 $m^2.W^{-1}.d^{-1}$.

2.3.3 Inactivation of *E. coli* under different periods of duration of exposure to light

The inactivation of *E. coli* was studied in the reactor, without any solids added, under exposure to light of 320 $W.m^{-2}$ for 0, 6, 12, 18 and 24 h per day followed by 24, 18, 12, 6 and 0 h in dark, respectively per day. The inactivation was described with exponential inactivation kinetics for the overall curves and the resulting inactivation rates are presented in Table 2.2. The inactivation rate increases with an increase in exposure time and the data fit well (R^2=0.98) to the linear Equation 2.5:

$$k=k_D+k_L'\cdot T \qquad (2.5)$$

where k_D=0.54 d^{-1} is the first order dark inactivation rate constant corresponding to 0 h of exposure to light, k_L'=0.65 h^{-1} is a pseudo second order photo-inactivation rate constant, and T is the duration of exposure to light per day ($h.d^{-1}$).

Table 2.2 Inactivation rates of E. coli in the reactor without addition of any solids, for different light intensities (with T=12 h of light per day) and for different periods of exposure to light (with I= 320 $W.m^{-2}$). Temperature fluctuated between 20 ˚C in dark conditions and 28 ˚C under light of 320 $W.m^{-2}$.

Different light intensities (*I*)		**Different periods of exposure to light (*T*)**	
***I* ($W.m^{-2}$)**	***k* (d^{-1}) (*R^2*)**	***T* (h)**	***k* (d^{-1}) (*R^2*)**
0 (dark control)	0.08 (0.76)	0 (dark control)	0.04 (0.47)
190	5.66 (0.86)	6	5.07 (0.92)
320	8.91 (0.92)	12	8.91 (0.92)
		18	10.96 (0.95)
		24 (light control)	16.59 (0.99)

2.3.4 Determination of k_D

The inactivation rate coefficient in dark conditions, k_D, represents the effect of inactivation due to stresses other than light, such as osmotic stress, predation and starvation. Its theoretical value was calculated as the average of the intercepts resulting from the previous two linear regressions, k_D=(0.19+0.54)/2=0.37 d^{-1}. Although experimentally it was measured at a lower value in the dark control experiments conducted in this research (0.08 d^{-1} and 0.04 d^{-1}, respectively), the calculated value is broadly consistent with the average values of dark inactivation rates for *E. coli*, of 0.55 d^{-1} (ranging from 0.34 to 0.79 d^{-1}) in cold season and 0.79 d^{-1} (from 0.60 to 1.10 d^{-1}) in warm season reported by Maïga et al. (2009), with those of 0.48 and 0.55 d^{-1} at average temperature of 20 ˚C reported by Craggs et al. (2004), 0.41 and 0.55 d^{-1} calculated for waste stabilisation pond and raw sewage in fresh river water, respectively, by Sinton et al. (2002) and with 0.50 d^{-1} at around 24 ˚C, pH 8.0 and 7.5 $mg.L^{-1}$ DO in environmental water reported by Gutiérrez-Cacciabue et al. (2016). Although the calculated values of k_D are around 6 times higher than the measured values, the discrepancy is small in comparison to the high values of the inactivation rates under exposure to light, which affect the coefficients of the linear Equations 2.3 and 2.5.

2.3.5 Inactivation of *E. coli* under different concentrations of TSS

In order to assess the role of suspended solids in the inactivation of *E. coli*, a series of batch experiments took place using different concentrations of TSS. The results of this experimental phase can be seen in Fig. 2.4.

Compared to the experiment in absence of suspended solids (k=16.59 d^{-1}) it is clear from Fig. 2.4 that the inactivation rate decreased with increasing TSS concentration. With absence of suspended solids the inactivation curve was $\log_{10}$-linear, which was not the case in the presence of particles. In fact, in presence of solids, especially over 25 $mg.L^{-1}$, there was a one-day slow phase, followed by more rapid inactivation in the next couple of days. For concentrations of 50 and 100 mg $TSS.L^{-1}$ the inactivation curves were almost identical, with inactivation rates of 5.21 and 5.78 d^{-1}, respectively, and with the levels of *E. coli* falling under 10^1 $CFU.mL^{-1}$ before the 4th day of the experiment. The steepest parts of these curves had inactivation rates of 9.24 d^{-1} for 50 mg $TSS.L^{-1}$ and 8.10 d^{-1} for 100 mg $TSS.L^{-1}$, which were comparable to the one without solids (16.50 d^{-1}) and to that of 25 mg $TSS.L^{-1}$ (11.03 d^{-1}). For solids' concentrations of 150 and 200 $mg.L^{-1}$ the rapid inactivation rates were lower (4.46 and 3.05 d^{-1}, respectively), followed by a plateau between day 3 and day 5, before the concentration of indicator organisms started falling more rapidly again, reaching levels below 10^1 $CFU.mL^{-1}$. The *E. coli* bacteria in the experiment came from a pure culture and the experiment was performed in separate batches for 150 and 200 mg $TSS.L^{-1}$, with sampling in triplicate; therefore, the observed plateau was obtained in a reproducible manner. Brouwer et al. (2017) also observed such

a deviation from the simple exponential inactivation, and modelled that with biphasic inactivation dynamics (fast inactivation followed by a phase of slow inactivation) attributed to population heterogeneity, hardening off (a conversion of the pathogens to a hardier phenotype through altered gene expression or other mechanisms, when exposed to the environment), viable-but-not-culturable states and/or density effects. This raised the question of whether the bacteria were eventually attached to the TSS particles. This was examined in the experiments that follow.

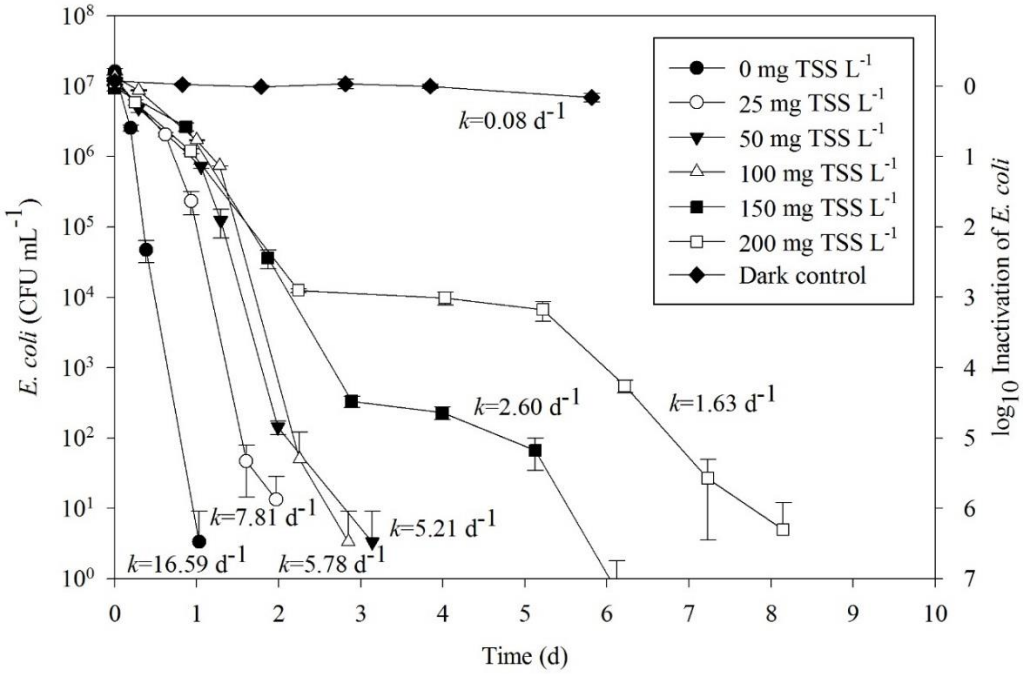


Figure 2.4 Concentration, log₁₀ inactivation and inactivation rate (k) of E. coli under exposure to artificial sunlight (320 W.m^{-2}, 24h per day) with TSS 0, 25, 50, 100, 150 and 200 mg.L^{-1}, compared to dark control (no light and no solids). These curves correspond to the samples taken at the top of the reactor prior to filtration. Temperature fluctuated between 20 ˚C at the beginning and 28 ˚C.

The inactivation rates were calculated assuming the traditional model of monophasic inactivation for the overall period of the experiment, including the lag phase and the plateau, where it exists. The correlation between TSS concentration and overall inactivation rate (*k*) can be seen in Fig. 2.5.

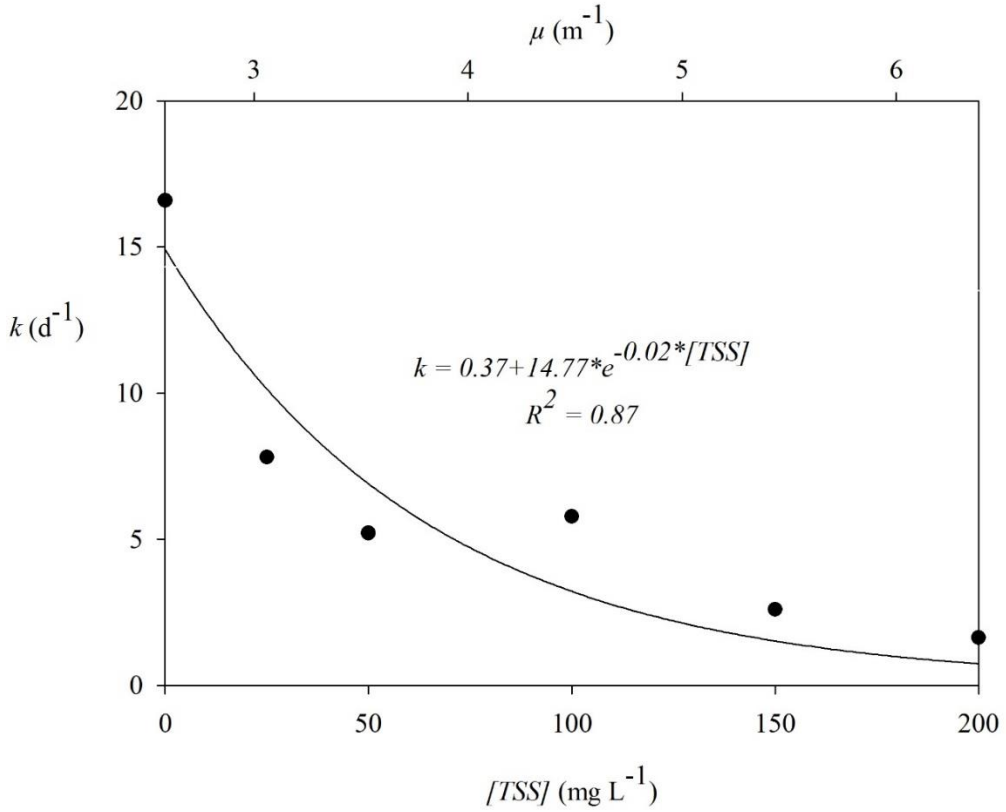


Figure 2.5 The effect of TSS concentration (mg.L^{-1}, lower horizontal axis) and of light attenuation coefficient μ (m^{-1}, upper horizontal axis) on inactivation rate k (d^{-1}) of E. coli measured in the reactor under continuous exposure to artificial sunlight (320 W.m^{-2}). The coefficient μ was calculated based on Equation 2.5.

The inactivation rate k (d^{-1}) decreases with an increase in the concentration of TSS (mg.L^{-1}) in an exponential way (R^2=0.87) as described by the Equation 2.6:

$$k=k_D+14.77e^{-0.02\cdot[TSS]} \quad (2.6)$$

It can be assumed that for very high TSS concentrations light cannot penetrate in the water, so inactivation rate is similar to that of dark conditions, therefore the k_D=0.37 d^{-1} parameter, as calculated previously, has been added to the exponential inactivation model.

The combination of this with the results of the first experiment lead to the formulation of an exponential relation (Eq. 2.7) between the inactivation rate k (d^{-1}) and the light attenuation coefficient μ (m^{-1}):

$$k=k_D+65.48e^{-0.65\cdot\mu} \quad (2.7)$$

for $\mu \geq 2.59$ m^{-1}, which is the minimum theoretical value corresponding to the light attenuation coefficient of water without any solids, as calculated previously. Again, the k_D parameter has been added because it is assumed that for very high values of μ the

inactivation rate is similar to that of dark conditions. This relation can be very useful as an indirect method for predicting the inactivation rate of *E. coli*-like pathogens in waters by measuring the vertical distribution of light, without the need of measuring the concentration of suspended solids or turbidity. More research on relevant methodologies has been conducted by Nguyen et al. (2015), Silverman and Nelson (2016) and presented in a critical review paper by Nelson et al. (2018).

At this point, it is interesting to note that although the irradiance of the UV-A band of wavelengths of this lamp accounts for 5.1% of the total irradiance, as measured from the relative spectral power distribution, biological weighting functions show that the inactivation effect of wavelengths higher than 400 nm on *E. coli* is negligible (Nelson et al., 2018). Therefore, as the lamp has a UV filter at 320 nm, we can assume that the total inactivation caused by this lamp corresponds to the UV-A part of the spectrum. The values of the average UV-A irradiance transmitted through the water column for the different TSS concentrations studied are presented in Table 2.3.

Table 2.3 Inactivation rates of E. coli in the reactor with demineralised water (DW) and artificial flood water (FW), at different concentrations of TSS. The average total irradiance, I, transmitted through the water column (calculated from Eq. 2.9, see Annex), as well as the average UV-A irradiance (320-400 nm) transmitted through the water column, $I_{UVA}=5.1\%\cdot I$, and the light attenuation coefficient, μ (measured values), are also presented.

***[TSS]* (mg.L^{-1})**	***I* (W.m^{-2})**	***I*$_{UVA}$ (W.m^{-2})**	**μ (m^{-1})**	***k* (d^{-1})**	***k* (h^{-1})**
0 (DW)	156.8	8.0	2.2	16.59	0.69
25 (DW)	125.3	6.4	3.3	7.81	0.33
50 (DW)	85.4	4.4	3.6	5.21	0.22
100 (DW)	65.7	3.4	4.9	5.78	0.24
150 (DW)	57.4	2.9	4.9	2.60	0.11
200 (DW)	37.3	1.9	6.4	1.63	0.07
150 (FW)	53.0	2.7	6.5	4.40	0.18

2.3.6 Attachment and particle-related shielding

The results can be seen in Fig. 2.6.

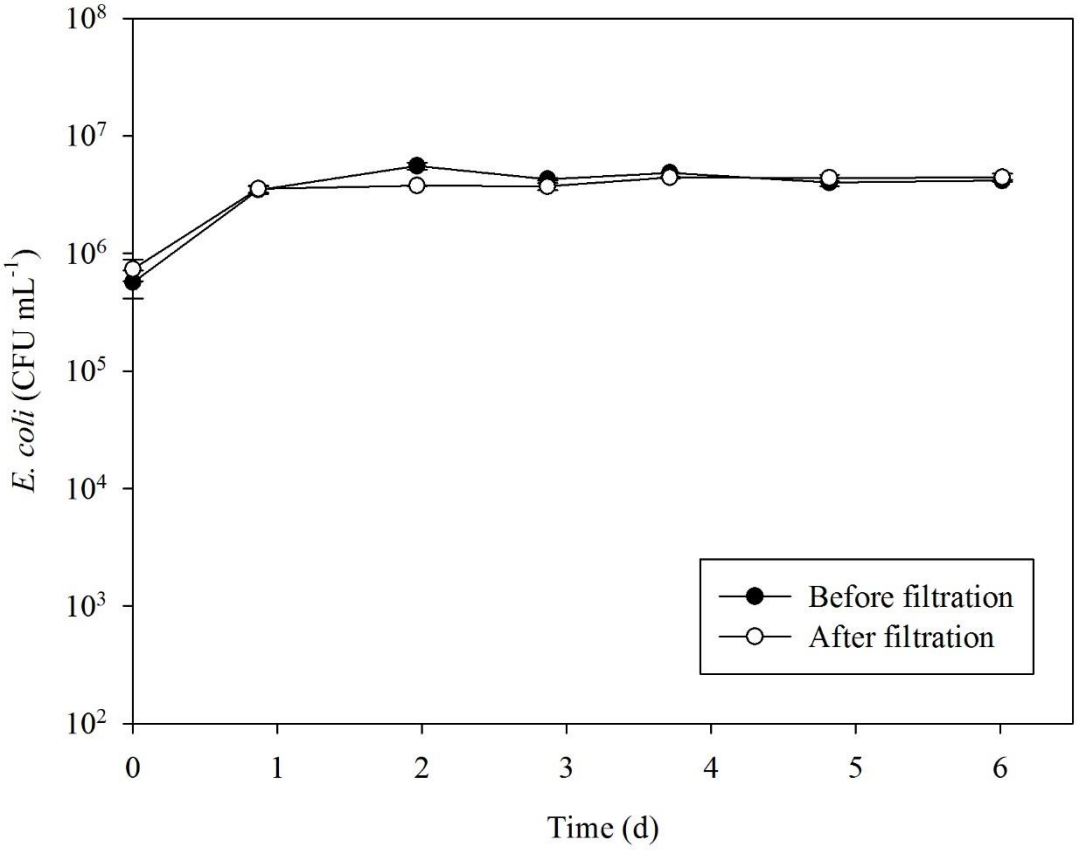


Figure 2.6 The concentration of E. coli in demineralised water with 200 mg TSS.L^{-1}, before and after filtration (11 μm) in a batch experiment in Erlenmeyer flasks.

The value of t-statistic (0.93) was lower than the critical value (2.45), so the null hypothesis (that the mean difference in concentration between filtered and unfiltered samples was zero) was accepted. Therefore, it is concluded with 95% confidence that the amount of *E. coli* associated with the suspended solids was not significant.

The concentration of suspended solids was found to be higher near the bottom of the reactor than at the middle and at the top of it because of sedimentation, with some fluctuations over time. The stirring speed was not increased, to avoid the creation of a vortex that would affect the water surface and the uniform distribution of light in the reactor. This inevitably caused the formation of a vertical TSS concentration gradient. Even so, *E. coli* was found to be uniformly distributed at different depths in the reactor (data not shown). This was observed both before and after filtration of the samples,

supporting the finding that significant attachment did not occur for any of the TSS concentrations studied.

The absence of irreversible adsorption may be attributed to short incubation time with clean particles, stirring velocity, grain characteristics (size, size uniformity and surface roughness), solution chemistry (zeta potential, ionic strength), geochemical heterogeneity and lipopolysaccharide composition in the bacterial outer membrane (Foppen and Schijven, 2006). According to Cantwell and Hofmann (2008) particle association affects the kinetics of inactivation by shielding the bacteria from exposure to light only when the number of particle-associated bacteria is significantly higher than the number of free-floating bacteria. The results indicate that also free floating bacteria could be protected for some time due to shading under high concentrations of TSS and this is a conclusion of practical importance when strategies are conducted and measures are taken for appropriate protection of population in cases of floods.

2.3.7 Comparison of results by testing the inactivation of *E. coli* in simulated floodwater

The inactivation rate of *E. coli* bacteria in the reactor filled with artificial floodwater containing 150 mg TSS.L^{-1} of treated solids and an initial *E. coli* concentration of $1.4 \cdot 10^7$ CFU.mL^{-1}, under continuous exposure to light of 320 W.m^{-2}, was k_{FW}=4.40 d^{-1}. The predicted value of the inactivation rate at 150 mg TSS.L^{-1} for the same light conditions, according to Eq. 2.7 would have been k_{DW}=1.52 d^{-1}. Part of this difference between the predicted and the measured value may be explained by the effect of factors other than light, related to the components of wastewater. Therefore, the experiment was repeated in dark conditions to identify the effect of dark inactivation. In dark conditions, the inactivation rate in artificial floodwater was $k_{D,FW}$=1.07 d^{-1}, which was higher than the one calculated in demineralised water ($k_{D,DW}$=0.37 d^{-1}). This confirms that the wastewater, although diluted, had a negative effect on the survival of *E. coli* in dark conditions. The inactivation rate solely due to light, by subtracting the effect of dark inactivation from the overall inactivation rate, was equal to $k_{L,DW}=k_{DW}-k_{D,DW}$=1.15 d^{-1} for demineralised water and $k_{L,FW}=k_{FW}-k_{D,FW}$=3.33 d^{-1} for floodwater. The results can be seen in Fig. 2.7. This means that the negative effect of light was stronger in the floodwater than in demineralised water.

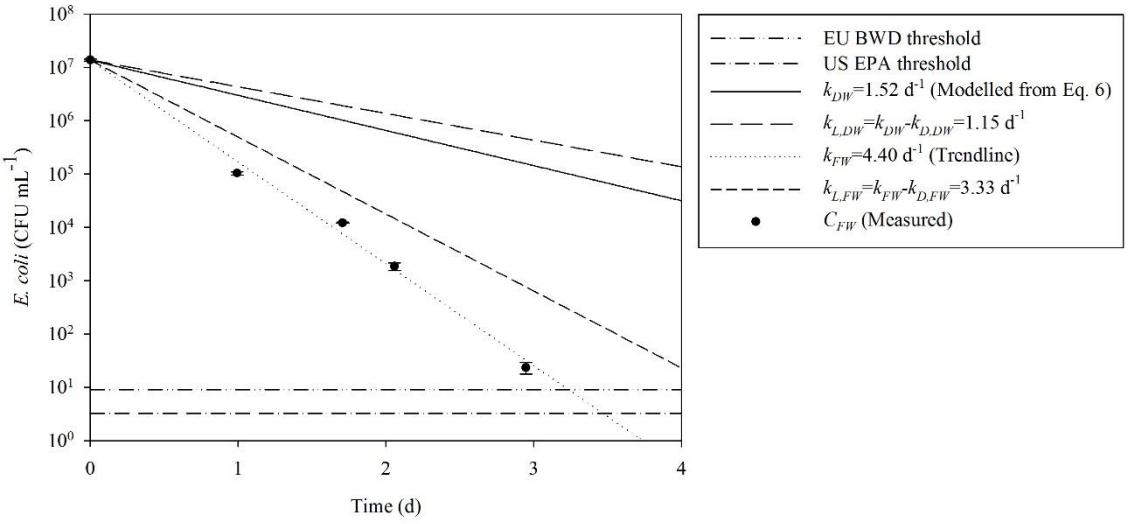


Figure 2.7 The concentration of E. coli in artificial floodwater (C_{FW}, with inactivation rate kFW) measured in the reactor, compared to the theoretical curve for demineralised water with kDW=1.52 d^{-1} calculated from Eq. 2.7 for 150 mg TSS.L^{-1}. The inactivation rates without the effect of dark inactivation for the two water qualities ($k_{L,FW}$ and $k_{L,DW}$, respectively) were calculated by subtracting the dark inactivation rate from the overall inactivation rate. The curves were plotted using Eq. 2.3. The threshold concentrations of E. coli according to US EPA Recreational Water Quality Criteria and EU Bathing Water Directive are also presented. Temperature was around 20 ˚C in dark conditions and increased up to 28 ˚C under exposure to light.

The threshold concentration of *E. coli* according to the EU Bathing Water Directive for sufficient water quality of inland waters is 9 CFU mL^{-1}, based upon a 90-percentile evaluation of the $\log_{10}$ normal probability density function of microbiological data acquired from the particular bathing water (Directive 2006/7/EC). The 2012 Recreational Water Quality Criteria by US EPA recommend a 90-percentile statistical threshold value of 3.2 CFU.mL^{-1} for *E. coli* in fresh water for primary contact recreation, including swimming, bathing, water play by children and similar water activities where a high degree of bodily contact with the water, immersion and ingestion are likely (US EPA, 2012). According to the previous results, for an initial concentration of 10^7 CFU *E. coli*.mL^{-1}, in floodwater the EU threshold would be met in 4.2 d and the US threshold would be met in 4.5 d if dark inactivation is not taken into account. In demineralised water the two thresholds will be met only after 12.1 and 13.0 d, respectively.

2.3.8 Strengths and limitations

Although this work provides a better understanding of the inactivation of faecal indicator *E. coli* in shallow water bodies, like urban retention and detention basins, further research has to be undertaken employing a wider range of water-borne pathogens or surrogate organisms and environmental conditions to be able to predict inactivation of pathogens under natural conditions. Also, the lack of replicate experiments and the fact that temperature was not constant are limitations that need to be mentioned.

2.4 CONCLUSIONS

Based on the results of the experiments, the following conclusions can be made:

- The light attenuation coefficient obtained in this study, which is proportional to the concentration of suspended solids, is: $\mu=2.59+0.02*[TSS]$, where μ is the light attenuation coefficient (m^{-1}) and *[TSS]* is the concentration of total suspended solids ($mg.L^{-1}$).
- The inactivation rate decreases exponentially with an increase in TSS concentration, which can be described in this case as $k=k_D+14.77e^{-0.02\cdot[TSS]}$, where k is the inactivation rate in d^{-1} and *[TSS]* is the concentration of total suspended solids in $mg.L^{-1}$.
- In general, it was demonstrated under lab and controlled conditions that inactivation of faecal indicators *E. coli* is higher under higher solar irradiance, longer duration of daylight and low TSS concentrations.
- The results indicate that under high TSS concentrations the bacteria, even if not attached on particles, are protected from photo-inactivation for a period of a few days.
- It is also noteworthy that the negative effect of light was stronger in the flood water experiments than in those with demineralised water.
- The results can be useful for numerical flood modelling and quantitative microbial risk assessment. Further research is needed, combining real environmental and operating conditions, like temperature and various water quality parameters, as well as with more resilient pathogens such as *Cryptosporidium*.

2.5 ANNEX - CALCULATION OF AVERAGE IRRADIANCE SPECTRA TRANSMITTED THROUGH THE WATER COLUMN

The absorbance spectra of all the different water quality solutions used $\alpha_s(\lambda)$ (cm^{-1}) were measured with LAMBDA 365 UV/Vis Spectrophotometer (PerkinElmer, Waltham, MA, USA) and are presented in Fig. 2.8.

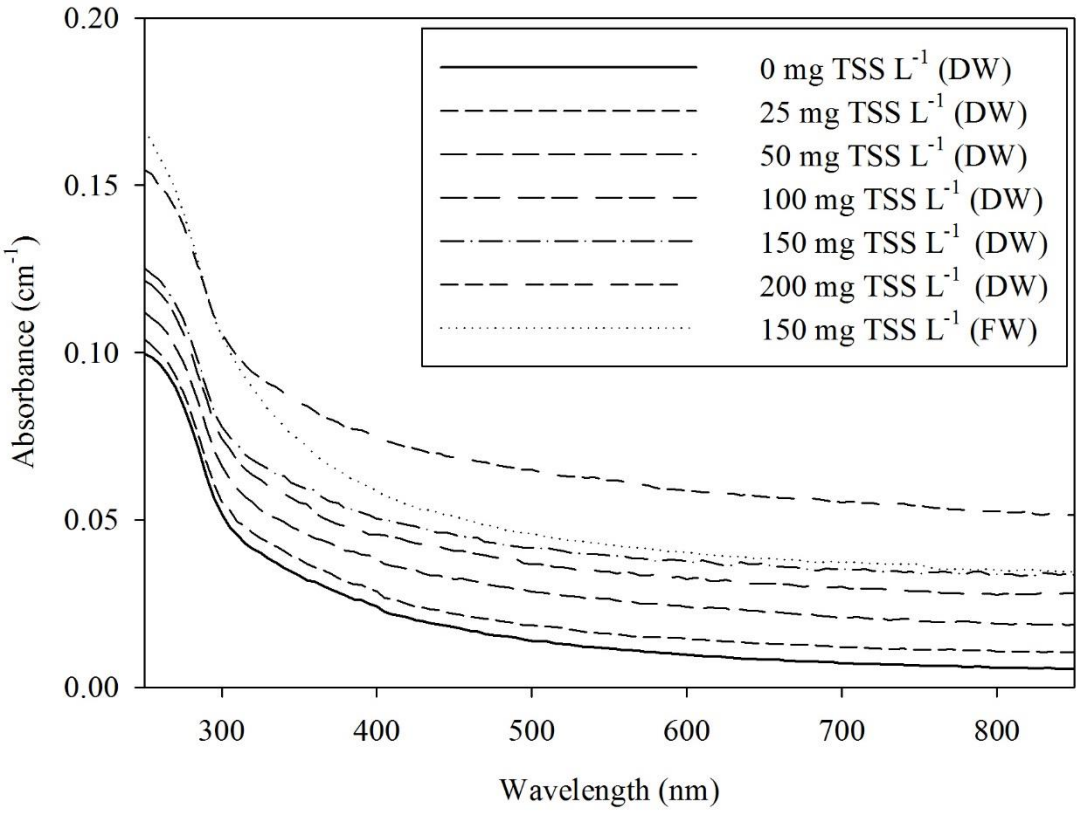


Figure 2.8 Absorbance spectra of demineralised water (DW) and artificial flood water (FW) with E. coli ($7.8 \cdot 10^6$ CFU.mL^{-1}) and different concentrations of TSS.

This data was used to calculate the average irradiance spectra transmitted through the water column, $I_0(z,\lambda)$ ($W.m^{-2}$), using the following Equation 2.8 (Silverman and Nelson, 2016):

$$I_0(z,\lambda) = I_d(0,\lambda) \cdot \left(\frac{1-10^{-\alpha_S(\lambda)\cdot z}}{2.303\cdot\alpha_S(\lambda)\cdot z}\right) \qquad (2.8)$$

where $I_d(0,\lambda)$ ($W.m^{-2}$) is the solar simulator irradiance incident spectrum on the water surface and z is the depth of the water column (cm). The results are presented in Fig. 2.9.

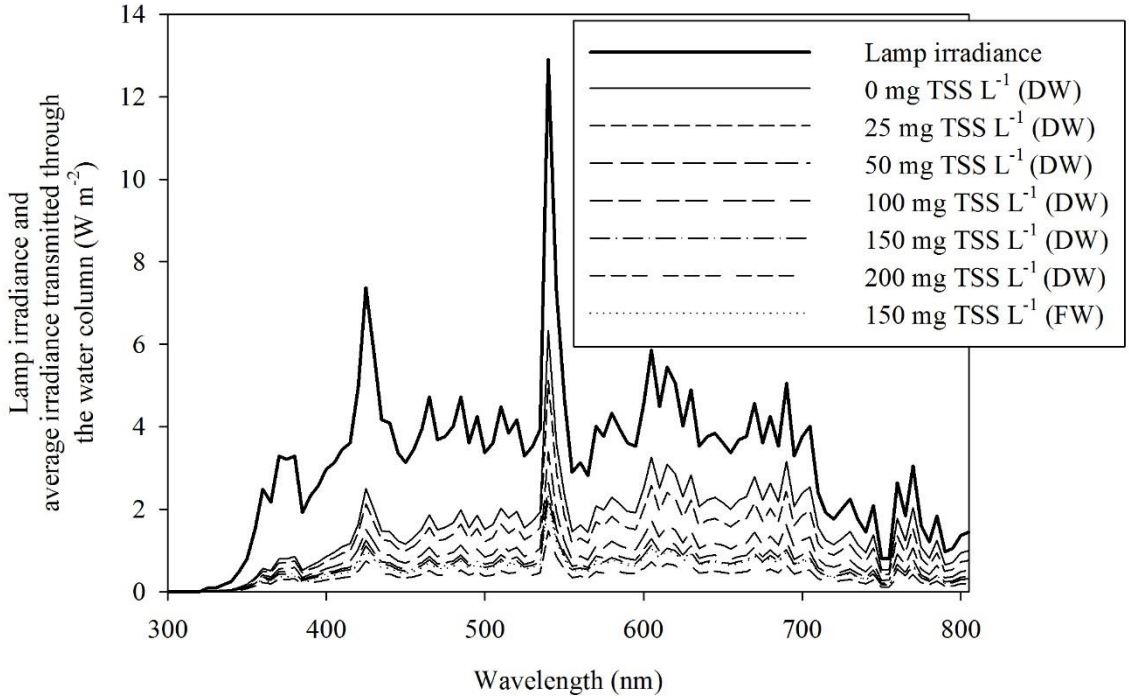


Figure 2.9 Lamp irradiance spectrum and average irradiance transmitted through the water column of demineralised water (DW) and artificial flood water (FW) with E. coli ($7.8 \cdot 10^6$ CFU.mL^{-1}) and different concentrations of TSS.

3

ASSESSMENT OF MICROBIAL SAMPLING METHODS FOR FLOOD-PRONE URBAN SURFACES

The main aim of this chapter was to identify the most reliable method for the recovery of water-borne indicator organisms from flood-prone urban surfaces. Pathogens can occur after the surcharge of sewer networks on surfaces commonly found in the urban environment such as concrete, asphalt, pavement blocks and grass. Four different sampling methods were compared: swabbing, direct agar contact, stamping and adhesive tape-lifting. The surfaces were inoculated with known amounts of *E. coli*. A glass surface was used as control. Contact plating had the highest log recovery ratio, 96.1% on glass, for up to 10^3 CFU.100 cm^{-2} of *E. coli*, but this method has a limited range of bacterial numbers because it is not possible to dilute or concentrate the samples. Swabbing was the most reliable technique because it could be used for a wide range of concentrations with high recovery ratios of up to 96.2% for 10^5 CFU.100 cm^{-2} of *E. coli*. Comparatively, the indirect methods of stamping and tape had no additional advantages. Further experiments using the swabbing technique revealed that the water accumulated on rougher surfaces affected the swabbing recovery ratios when the samples got diluted. Swabbing any amount of sample higher than what the swab heads could absorb (0.15 mL) reduced the recovery ratio. Furthermore, swabbing was more efficient without the use of a nonionic surfactant (Tween 80) in wetting solution and eluent. After the recession of an artificial flood, swabbing and contact plating were confirmed as reliable methods to sample and enumerate the presence of *E. coli* on different urban surfaces, with the log recoveries ranging from 21.0% to 59.0%, depending not only on the sampling method, but also on the actual amount of bacteria on the surface. Standardisation of sampling methods in the future will be helpful in assessing public health risks after floods.

This chapter is based on: Scoullos, I.M., van de Vossenberg, J., Lopez Vazquez, C.M. and Brdjanovic, D.: Assessment of microbial sampling methods for flood-prone urban surfaces. In preparation.

3.1 INTRODUCTION

Several studies have shown that, after the end of floods, water-borne pathogens from human and animal sources are present on the flooded surfaces (ten Veldhuis et al., 2010), a phenomenon directly linked with the amount of urbanization and density of impervious surfaces in watersheds (Olds et al., 2018). Chapter 2 showed that *E. coli* can survive in flood water for at least three days under continuous exposure to artificial solar light. Although bacterial, protozoan and viral pathogens, such as *Campylobacter*, *Salmonella*, *Cryptosporidium*, *Giardia* and adenovirus have been detected in urban storm water runoff, the quality of storm water in terms of microbial contaminants is poorly understood and it is imperative to determine the sources of contamination (Ahmed et al., 2019). In order to reliably assess the concentration of water-borne pathogens on urban surfaces after the recession of flood water and to obtain a more realistic assessment of potential health risks of floods, it is necessary to have reliable sampling methods for recovering indicator organisms from these surfaces in a quantitative way. Most methods for recovery of microorganisms from surfaces found in the literature are designed for smooth and non-porous surfaces, such as stainless steel surfaces used in the food industry (Garayoa et al., 2017; Maunula et al., 2017; Moore and Griffith, 2007), and in healthcare environments (Rawlinson et al., 2019; Claro et al., 2015). At the same time, most methods referring to porous and rough surfaces include either the collection of bulk samples of the material with methods such as scrapping and grinding or collection of water samples after immersion or rinsing. There is a need to adapt and standardise sampling methods for rough, porous and dusty materials (Verdier et al., 2014).

This study was carried out with the aim of defining a reliable sampling method for the microbiological assessment of surfaces after floods. The recovery ratios of swabbing and direct agar contact, widely used for smooth and non-porous surfaces, are compared with the indirect sampling methods of stamping and adhesive tape. Stamping was studied as a method to enumerate high numbers of bacteria by using consecutive imprints. Adhesive tape, a method previously used to sample bacteria from monument surfaces (Urzì and De Leo, 2001), meat surfaces (Fung et al., 2000) and also for collection of touch DNA from fabrics in forensic biology (Verdon et al., 2014) was adapted and tested as an alternative way to recover bacteria attached on surfaces.

3.2 MATERIALS AND METHODS

3.2.1 Indicator organisms

E. coli ATCC 25922 was chosen as the organism of study. Before each batch experiment *E. coli* was incubated in 1.3% w/v sterile Oxoid CM0001 Nutrient Broth (Oxoid Ltd., Basingstoke, UK) solution in Erlenmeyer flasks for 24 h at 37 ˚C. The concentration of

E. coli bacteria after incubation was around 10^9 CFU.mL^{-1} (stock solution). This was diluted with 0.1% peptone physiological salt solution to different working concentrations (inoculum). The concentration of the inoculum was measured by plating serial dilutions. Drops of the inoculum were transferred on to the surfaces with a micropipette and spread with a sterile glass spreader to achieve a uniform distribution. 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. All the experiments were performed in triplicate.

3.2.2 Surfaces tested

Permeable pavement blocks (10 x 20 x 8 cm3), pieces of asphalt (3 cm high) and concrete (6 cm high), and grass together with its soil (4.5 cm high) were collected from construction sites in Delft, The Netherlands. A glass panel, (0.4 cm thick), being non-porous and pH neutral, was used as control. All surface areas sampled were 100 cm^2.

3.2.3 Sampling methods

Swabbing

Sterile cotton tipped wooden stick swabs COPAN CLASSIQSwabs 150C (Copan Italia S.p.A., Brescia, Italy) were used to collect samples. Each swab was applied 12 times in one direction (each time from left to right and then from right to left) and 12 times in a 90° perpendicular direction with regard to the first swabbing direction (each time from the front to the back and vice versa). The swabs were held so that the handle created about a 30° angle with respect to the surface, they were rotated slowly each time and, as much as possible, a similar pressure was continuously applied (slightly bending the swab stick without breaking it). The swab tubes were filled with 2 mL sterile 0.1% peptone physiological salt (PPS) solution that used both as wetting solution (except when the swabs were applied dry) and as eluent. In some experiments, different concentrations of nonionic surfactant Tween 80 (Sigma-Aldrich Co., St. Louis, MO, USA) were included. After swabbing, the swabs were submerged in the eluent and vortexed for 5-10 s. The suspension was diluted, if needed, in appropriate 10-fold dilution steps in PPS solution, and the samples were plated for colony counting.

Direct agar contact

Contact plates (Simport Scientific, Beloeil, Canada) of 60 mm diameter, were filled with 18 mL of CCA to create a convex surface and let dry. Samples were taken by applying the plates gently on the sample area. The enumeration of *E. coli* was performed by counting the number of CFU after 24h of incubation at 37 ˚C (Fig. 3.1).

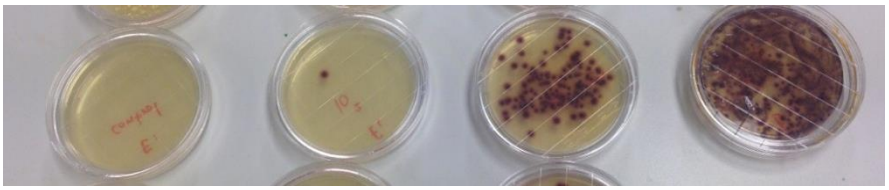


Figure 3.1 Contact plates after sampling and incubation for control (left) and different concentrations of E. coli.

Indirect Contact

Adhesive tape

A solvent-free, non-toxic paper adhesive tape (Eco Premium Masking Tape, Tesa SE, Norderstedt, Germany) was used. The width of the tape was 3.8 cm, so by selecting a length of 4.2 cm, a comparable sampling area of 16 cm^2 was chosen for enumeration, which is similar to the area of the contact plates. The tape was applied on the sampling area and then the adhesive side of the tape was kept in contact with the agar surface of CCA plates for incubation and enumeration (Fig. 3.2). Random pieces of the tape were sampled as negative controls without sterilisation, consequently no colonies were detected on the CCA plates.

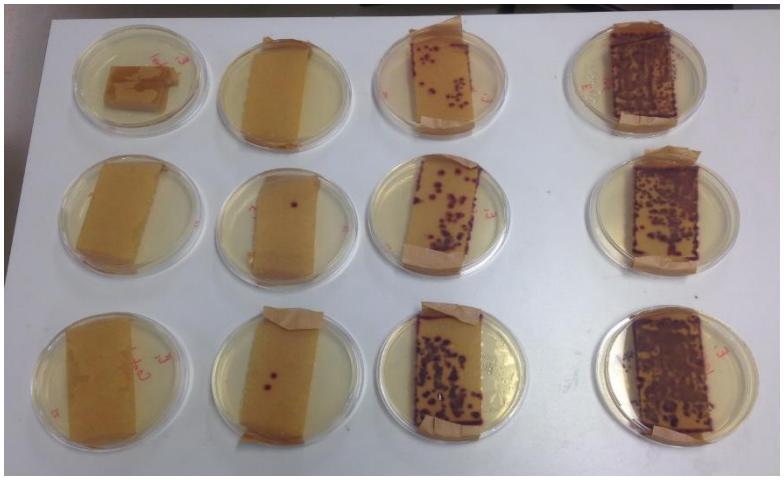


Figure 3.2 Petri dishes with adhesive tape on CCA medium after sampling and incubation. The first column is the control and the other columns contain different concentrations of E. coli. Sampling was performed in triplicate (three rows).

Stamp

Rubber caps of 7.7 cm diameter (46.6 cm^2) were used as stamps. Their flat surface was pressed on to the sample surfaces and then softly applied on the agar layer of a CCA plate

(first imprint) and subsequently immediately applied on a second plate (second imprint) (Fig. 3.3). The stamps were initially autoclaved, and then sterilised with ethanol and dried before each experiment.

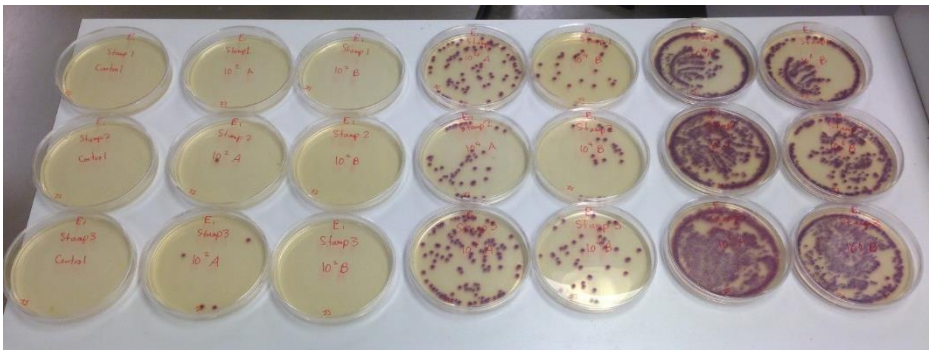


Figure 3.3 Petri dishes after stamping and incubation of E. coli samples.

3.2.4 Experimental design

Comparison of sampling methods

Glass surfaces of 10 x 10 cm, sterilised with ethanol were inoculated with 0.1 mL of three different concentrations of inoculum to achieve initial concentrations of 10^1, 10^3 and 10^5 CFU.100 cm^{-2}. The surfaces were sampled in triplicate with different sampling methods: swabbing, contact plating, adhesive tape and stamping.

Swabbing on different surfaces

Swabbing was tested on different surfaces of glass, pavement, concrete and asphalt. 1 mL of inoculum was spread on each surface of 100 cm^2 to achieve an initial concentration of around 10^5 CFU.100 cm^{-2}. The test was performed on dry surfaces (room temperature and humidity) and was repeated on water saturated surfaces, each time in triplicate. Saturation was achieved by immersing the surfaces in demineralised water and pumping out the water until it decreased to about 1 cm below the sample surface.

Wet and dry swabbing

The maximum volume of wetting solution absorbed by the swab heads (flocks) was measured by dipping dry swabs in demineralised water and weighing the difference. The importance of using the swabs dry or immersed in the PPS wetting solution was tested on glass inoculated with two different volumes of inoculum, always with a concentration of *E. coli* of around 10^5 CFU.100 cm^{-2}. Sample volumes of 0.1 mL and 1 mL were tested because they are lower and higher, respectively, than the amount of water that can be absorbed on the swabs.

Wetting solution for swabbing

The effect of Tween 80 on *E. coli* viability was tested in batch experiments in Erlenmeyer flasks, in duplicate, kept at an orbital shaker in room temperature for two days. The flasks contained 250 mL of different concentrations of Tween 80 (0, 1, 15 and 50 $g.L^{-1}$) in PPS and were inoculated with 10^7 $CFU.mL^{-1}$ of *E. coli*. The experiments were performed with flasks sterilised prior to the addition of Tween 80 and repeated with flasks sterilised after the addition of Tween 80 (by autoclaving at 121 ˚C for 15 min) to rule out any effect of the autoclaving process on the surfactant's properties.

Subsequently, the effect of Tween 80 on swabbing efficiency was tested. Glass panels were inoculated with 0.1 mL of around 10^6 $CFU.mL^{-1}$ of *E. coli*, to obtain an initial surface concentration of around 10^5 $CFU.100\ cm^{-2}$. PPS with different concentrations of Tween 80 (0, 1, 15 and 50 $g.L^{-1}$) was used as wetting solution for the swabs.

To conclude this set of experiments, the effect of Tween 80 on the of *E. coli* recovery efficiency from swabs was tested separately by directly inoculating dry swabs with 0.1 mL of around 10^5 $CFU.mL^{-1}$ with the use of a micropipette. The inoculated swabs were introduced in their tubes filled with different concentrations of Tween 80, as eluent solution. Thereafter, the suspension was plated after vortexing as described previously.

Contact plating on different surfaces

Different surfaces (glass, pavement, concrete, asphalt and grass) were sampled with direct agar contact plating. Dry surfaces were inoculated with around 10^3 $CFU.100\ cm^{-2}$. The surfaces were not sterilised, thereby negative controls were obtained prior to the inoculation to assess the potential presence of *E. coli*.

Swabbing and contact plating on different surfaces after an artificial flood

The efficiency of swabbing and contact plating on surfaces was studied under simulated flood conditions. The surfaces were not sterilised and negative controls were sampled with both methods before the flooding. The surfaces were placed in plastic tanks cleaned with ethanol and filled with artificial floodwater consisting of demineralised water inoculated with *E. coli* at a concentration of 10^4 $CFU.mL^{-1}$ up to 1 cm higher than each surface level. Samples of the floodwater were taken immediately after the flood. The flood was followed by the recession of water using a peristaltic pump at a flowrate of 1 $L.h^{-1}$, until the water level reached 1 cm below the surface's level. This stage lasted 1 h and the samples were collected after another 1 h. Samples were taken from different areas of the surfaces with contact plates and swabs. Wet swabs were used for the surfaces that dried faster (glass, pavement and concrete) and dry swabs were used for asphalt and grass that were still wet.

Data analysis

Log recovery was calculated as the ratio of the $\log_{10}$ concentration recovered to the $\log_{10}$ concentration of the inoculum, both concentrations being in CFU.100 cm^{-2}. To investigate whether the results were statistically significant, two-sample *t*-tests assuming unequal variances were performed, to compare the surface concentrations (CFU.100 cm^{-2}) of *E. coli* recovered in dry and saturated surfaces (Section 3.3.2.), or observed before and after the experiment (Section 3.3.4.), or recovered with swabbing and contact plating (Section 3.3.6.). Two-factor (sample volume and wet/dry swabs) ANOVA with replication was used in Section 3.3.3., to test if sample volumes affect the recovery ratios of wet and dry swabs. Also, a two-factor (Tween 80 concentration and time) ANOVA with replication was used in Section 3.3.4., to compare the effect of different Tween 80 concentrations on *E. coli* concentrations at different stages of the experiment. In Section 3.3.4, single factor ANOVA tests were performed, to compare the recovered *E. coli* concentrations with the use of different Tween concentrations. In all cases, the significance level, α, selected was 0.05. It was assumed that microbial concentrations were normally distributed.

3.3 RESULTS AND DISCUSSION

3.3.1 Comparison of sampling methods

In all cases the control samples on glass had no colonies, which means that sterilisation of the glass with ethanol reached *E. coli* numbers below the detection limits. The results with the use of all the different methods tested are presented in Fig. 3.4.

The contact plates' method had the highest log recovery, of 77.6% and 96.1%, at the low surface concentrations of *E. coli* of 10^1 and 10^3 CFU.100 cm^{-2}, respectively. However, the colonies were too many to be enumerated on the contact plates at the concentration of 10^5 CFU.100 cm^{-2} (Fig. 3.1). Another disadvantage was that the area that can be sampled is small (16 cm^2). Since there were limited colony counts at 10^1 CFU.100 cm^{-2}, and dilutions or concentrations (e.g. with filtration) can hardly be made with this method, it can be suggested that the range of concentrations to apply the contact plates' method appears to be limited to between 10^1-10^3 CFU.100 cm^{-2}.

Similarly, with the use of adhesive tape the highest surface concentration of *E. coli* was not possible to be enumerated (Fig. 3.2). Even at lower concentrations (log recoveries of 67.1% and 79.2% at 10^1 and 10^3 CFU.100 cm^{-2}, respectively) it was more difficult to count colonies, compared to the contact plates, because the colonies, which had to grow between the tape and the agar surface, spread and covered each other. Furthermore, the handling of the tape in a sterile way was difficult. Therefore, the use of this method appeared impractical and did not offer any significant advantages, but there is still room for optimisation. For example, the tape could be placed on the agar plate for a few seconds

and then lifted and discarded instead of remaining on the surface during incubation. Such a method was used by Fung et al. (2000) to sample meat surfaces with an easy to handle "pop-up" Scotch tape that can be operated with one hand. Although the recovery was 0.91 times higher compared to swabs, the problem of colonies growing too close to each other was also present because dilution was not possible. The same research observed that sampling of moist or uneven surfaces affected the method. It is interesting to note that adhesive sheets have also been used for direct counting of bacteria from solid surfaces with epifluorescent microscopy (Yamaguchi et al., 2003) and scanning electron microscopy (Urzì and De Leo, 2001).

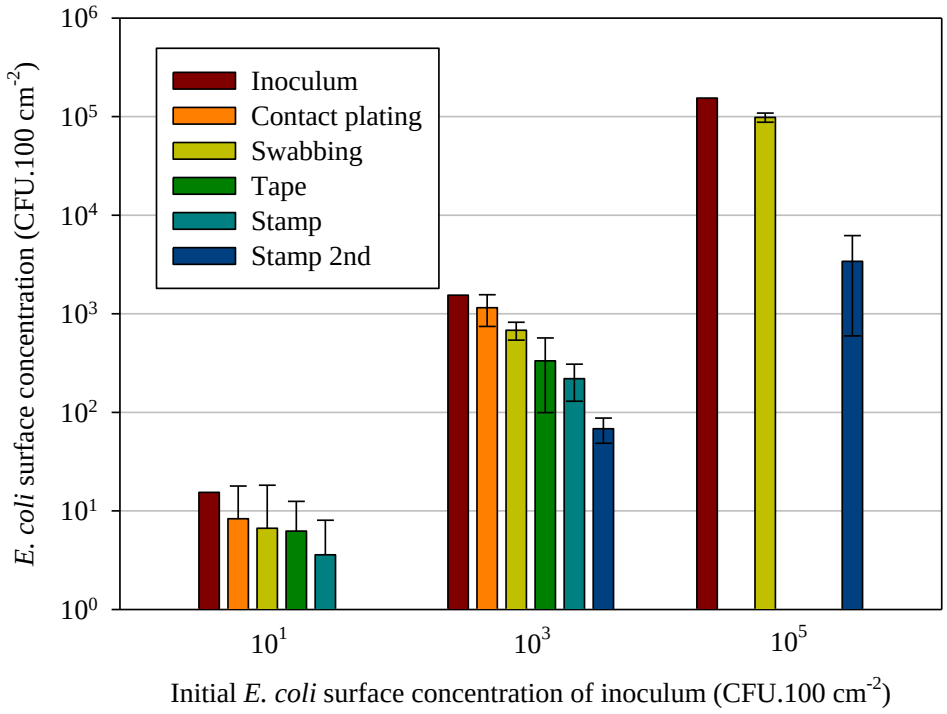


Figure 3.4 E. coli surface concentrations recovered with different methods from a glass surface compared to the surface concentration of the inoculum initially spread over of around 10^1 (left cluster), 10^3 (central cluster) and 10^5 (right cluster) CFU.100 cm^{-2}.

On the other hand, the swabbing method was reliable for the whole range of surface concentrations because the samples could be diluted after being transferred to the eluent. The log recovery percentages for this method were 69.4% for the initial surface concentration of 10^1 CFU.100 cm^{-2}, 88.9% for 10^3 CFU.100 cm^{-2} and 96.2% for 10^5 CFU.100 cm^{-2}. Since there are limited colony counts at 10^1 CFU.100 cm^{-2} (30-300 are needed on a plate) the minimum amount of *E. coli* swabbed should be 10^3 CFU.100 cm^{-2}, unless a larger area is swabbed or the membrane filtration method is used for plating low

surface concentrations. Due to the advantages of swabbing, it was studied more in depth in the following sections.

Recovery using rubber stamps was lower compared to the other methods. For an initial level of 10^1 CFU.100 cm^{-2} the first imprint had a log recovery of only 46.7% with regard to the initial bacteria and the second one was below the detection limit (suggesting that no bacteria were recovered). At the surface concentration of 10^3 CFU.100 cm^{-2}, the first imprint had a log recovery of 73.4% and the second one of 57.5%, whereas at 10^5 CFU.100 cm^{-2} the first imprint was too concentrated and the second one had a log recovery of 68.1%. The reason for the low efficiency of this indirect contact method was mainly the poor contact of the rubber on the sampled surfaces. This could be seen from the fact that the distribution of colonies on the plates was not uniform (Fig. 3.3). Previous applications of stamps faced this by using softer stamp materials, such as polyurethane foam cylinders (Tresner and Hayes, 1970) or wooden cylinders with a latex layer covered by velvet (Elek and Hilson, 1954). The advantage of the stamps was that the enumeration of higher concentrations was possible by consecutive imprints, even if the accuracy was lower. This was demonstrated in the case of the highest level of inoculum that was possible to be counted using the second imprint, while the colonies on the first one were too many to count. At 10^3 CFU.100 cm^{-2}, the second imprint had 69% less colonies than the first one. Elek and Hilson (1954) determined an average transfer factor of 1 to 40 on the primary imprint (19.9% log recovery). Furthermore, it is important to note that the stamps' surface was larger (46.5 cm^2) compared to the contact plates, but different sizes can be used. Based on these observations, the detection range of this method is 10^2-10^4 CFU.100 cm^{-2} for the first imprint and 10^3-10^5 CFU.100 cm^{-2} for the second one.

3.3.2 Swabbing on different surfaces

First of all, although the surfaces were not sterilised (except glass that was cleaned with ethanol), the concentration of *E. coli* recovered by swabbing prior to inoculation was under the detection limit. The results from the swabbing experiments are presented in Fig. 3.5.

In the tests on dry surfaces, the initial level of *E. coli* was $1.2 \cdot 10^5$ CFU.100 cm^{-2}. The log recovery on glass was 77.0%, on pavement 78.6% and on asphalt 84.8%, while no *E. coli* colonies were detected by swabbing concrete. The recovery on glass was lower compared to the previous experiment, because the volume of the inoculum was 10 higher (1 mL) and it exceeded the amount of liquid that can be absorbed by the swab. This raised the question of whether swabs are better to be used dry or to be first immersed in a wetting solution. This question is investigated in the next sections.

In the tests on saturated surfaces, the initial concentration of *E. coli* was $7.7 \cdot 10^4$ CFU.100 cm^{-2}. The log recovery on glass, pavement, concrete, asphalt and grass was 78.2%, 76.5%, 40.9%, 76.1% and 62.8%, respectively. Moore and Griffith (2007)

measured an *E. coli* sampling efficiency of 21.8% (log recovery of 66.9%) with cotton swabs on a wet stainless steel surface. Through statistical analysis (using two-sample *t*-tests assuming unequal variances), it was observed that the difference of the recovered concentrations between dry and saturated surfaces was not statistically significant for glass (p=0.13) and pavement (p=0.08), while for asphalt (p=0.01) it was significant. Saturated asphalt has lower recovery ratios than unsaturated, probably because of the excess of water accumulated and "trapped" on the voids of the rough surface; the inoculum is diluted and the volume recovered by the swab is limited. In the case of concrete, as all values on the dry surface are equal to 0, a *t*-test could not be performed, but saturation increased the recovery ratio because the dry surface is permeable and the inoculum percolated through the surface. The effect of high pH of concrete on the survival of *E. coli*, especially when concrete is new and has not undergone carbonation, is examined in Chapter 4. At the same time, the low recovery ratio on concrete could be related to the roughness of the concrete surface, twisting and compressing the cotton tip and reducing its ability to hold sample volume and entrap bacteria between the cotton fibres (Ahnrud et al., 2018).

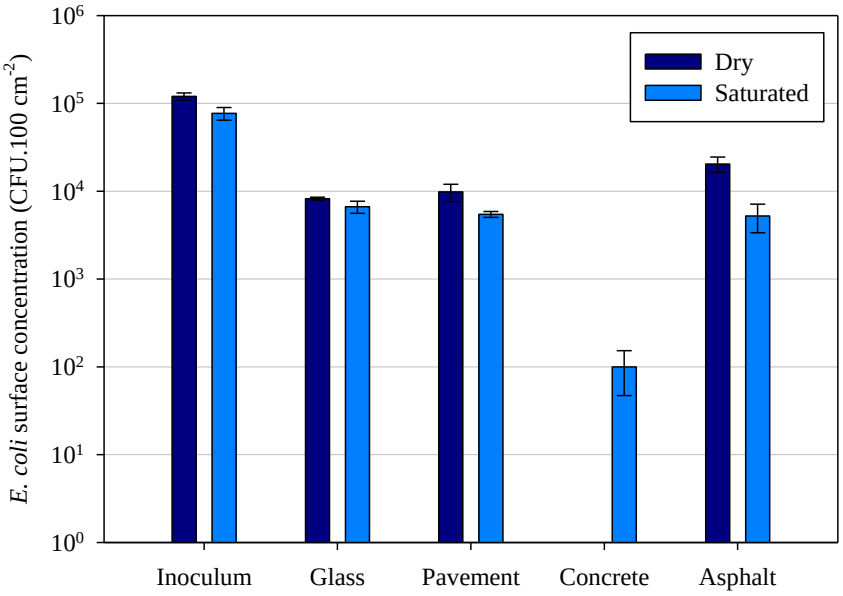


Figure 3.5 E. coli surface concentrations of the inoculum and as recovered by swabs from different surfaces, dry and saturated.

3.3.3 Wet and dry swabbing

The maximum amount of water that can be absorbed by the swab heads was estimated to be around 0.15 mL on average. It was assumed that higher sample volumes would lead to lower recovery ratios. This was confirmed with dry swabs that had a log recovery of

90.1% of *E. coli* in 0.1 mL volume samples and 81.4% in 1 mL volume samples on glass. Similarly, swabs that had been immersed in a wetting solution had a log recovery of 88.5% in 0.1 mL volume samples and 74.0% in 1 mL volume samples, both at the initial concentration of $1.3 \cdot 10^5$ CFU.100 cm^{-2}. A statistical analysis of two-factor ANOVA with replication showed that the volume of the sample was a significant factor affecting recovery (F=100.04, p=0.001), confirming the hypothesis that sample volumes higher than what the swabs can absorb lead to a lower recovery. However, the same analysis showed that immersing the swabs in a wetting solution did not have a statistically significant effect, nor the interaction between sample volumes and dryness of the swabs was significant. Therefore, it is suggested to use dry swabs on wet surfaces in order to maximise the sample volume absorbed and wet swabs to be used on dry surfaces if needed.

3.3.4 Wetting solution for swabbing

In the case of the experiments of viability of *E. coli* at different concentrations of Tween 80, a two-factor ANOVA with replication showed that there were no significant differences between the *E. coli* concentrations at different concentrations of Tween 80. The results were similar when the flasks were autoclaved prior to (F=0.57, p=0.65) and after (F=1.91, p=0.18) the addition of Tween 80. Also, two-sample t-tests assuming unequal variances showed that the surface concentration of *E. coli* was constant throughout the experiment in both cases (p=0.09 and p=0.03, respectively). Therefore, neither the different concentrations of Tween 80 tested had a statistically significant effect on *E. coli* survival, nor this was affected by autoclaving.

With an initial concentration of $2.9 \cdot 10^5$ CFU.100 cm^{-2}, the log recovery of *E. coli* without addition of Tween 80 was 85.6% and it was found to be linearly decreasing as the Tween 80 concentration increased (log recovery=$0.85-0.002 \cdot C_T$, R^2=0.99, where C_T is the concentration of Tween 80 in $g.L^{-1}$), reaching the lowest recovery of 75.2% at 50 $g.L^{-1}$ of Tween 80, the highest concentration tested. This decrease was found to be statistically significant by a single factor ANOVA (F=4.65, p=0.04). This finding is interesting given the fact that Tween has been proposed by many as one of the best wetting solutions for swabbing and wiping surfaces. For example, You et al. (2019) observed that cotton swabs had an overall higher recovery efficiency (up to three times) of dried *E. coli* and *Staphylococcus aureus* samples from a desk surface with the use of 1% Tween 20 and 1% glycerol on phosphate-buffered saline (PBS) than with plain PBS or with a commercial solution. Valentine et al. (2008) studied different wetting solutions for wiping and swabbing *B. subtilis* bacterial endospores from stainless steel and vinyl surfaces. They concluded that phosphate-buffered saline with 0.3% Tween was a better collection solution than sterile water or phosphate-buffered saline without Tween for all the tested swab and wipe types used, except for the Dacron / polyester swab, which had the same results with the use of water. Therefore they used the Tween solution for swabbing sterile

porous (polyester upholstery fabric and a commercial carpet) and nonporous (plastic laminate countertop, finished oak wood flooring and an activated computer monitor screen) surfaces, with the highest recovery being around 8% (CFU.mL^{-1}) on laminate.

With an initial amount of $2.3 \cdot 10^4$ CFU inoculated per swab, a single factor ANOVA, showed (F=4.39, p=0.09) that Tween 80 did not have a statistically significant effect on the recovered concentrations from swabs when used as eluent. The average log recovery ratio was 90.6%. This suggests that the negative effect of Tween on the *E. coli* recovery was not related to the detachment of bacteria from the swabs, but rather to the swabbing process. It is possible that the drying of the inoculum may have caused sub-lethal damage to the cellular membranes, which increases the sensitivity of cells to substances and conditions (Moore and Griffith, 2007) such as agitation during swabbing.

3.3.5 Contact plating on different surfaces

The surface concentration of the inoculum was $2.3 \cdot 10^3$ CFU.100 cm^{-2}. The log recovery ratios were 61.3% on glass, 73.2% on pavement, 63.5% on asphalt and 73.1% on glass. Recovery on concrete was below the detection limit. It was noted that the agar surface could not reach some of the voids, especially on the asphalt surface, that swabs could reach.

3.3.6 Swabbing and contact plating on different surfaces after an artificial flood

The concentration of *E. coli* in flooding water was between 1.6 and $2.3 \cdot 10^4$ CFU.mL^{-1}. The negative control was below detection limit for the swabbing method, while a few colonies were recovered by contact plating. The log recoveries on glass, pavement, concrete, asphalt and grass were 43.3%, 58.9%, 21.0%, 59.0% and 44.8% respectively for the swabbing method and 41.0%, 55.6%, 37.3%, 57.6% and 37.0% respectively for contact plating. It must be noted here, that as the *E. coli* were in suspension in the flooding water the recovery ratios in this experiment were calculated by assuming that the bacteria settled vertically in a uniform way on top of the sampled surfaces when the water receded. Therefore, these recovery ratios cannot be compared to the previous experiments in absolute terms because the hydraulic characteristics of the different surfaces could also play a role on the amount of bacteria that were actually present on the sampled surfaces. For example, the relatively low recoveries on glass could be attributed to the fact that the amount of water that remained on the glass was low, although it was horizontal. What can be concluded from this experiment is that similar trends were observed by both methods. In fact, two-sample t-tests assuming unequal variances showed that the means were equal for both methods on each of the surfaces (p=0.76 for glass, p=0.32 for pavement, p=0.18 for concrete, p=0.13 for asphalt and p=0.09 for grass). The highest recoveries were achieved on pavement and asphalt. Concrete had the lowest recovery,

probably because it dried fast. The recovery on grass was not high because only the top layer was being sampled. In previous research on different surfaces in hospital rooms, such as floor, bed linen, door handles, and toilet seats, Lemmen et al. (2001) detected *E. coli* contamination with a frequency of 30.0% with the use of swabs, while with contact plates only 5.0% contamination was detected. Similar results were found for other gram-negative rods, while for gram-positive cocci, contact plates showed a higher recovery compared to swabbing. Therefore, a combination of methods is suggested for the assessment of overall contamination.

3.3.7 Strengths and limitations

This work provides data on the recovery ratios of different methods on materials like concrete, asphalt and grass, for which the scientific literature is very limited.

A limitation of this research was the comparison of only four methods. For example, alternative types of swabs, some of which are presented in section 1.5., could possibly be sturdier and provide better results that cotton tipped swabs, especially on rough surfaces.

3.4 CONCLUSIONS

Based on the results of the tests, the following conclusions can be made:

- On a flat, smooth and non-porous surface like glass, swabbing was the best of the tested methods for a wide range of concentrations. Contact plates had the highest recovery, but a low range of inoculum levels. Tape and stamps had technical challenges and no advantages over contact plating or swabbing.
- Moisture on glass and pavement did not affect swabbing recovery ratios, but the accumulation and "trapping" of water in the voids of asphalt surface resulted in dilution of the samples, while the fact that concrete surfaces did not trap water led to no recovery of *E. coli*.
- The maximum amount of water absorbed by the swabs was 0.15 mL. Swabbing any amount of sample higher than that reduced the recovery ratio. Dry swabs are proposed for wet surfaces and wet swabs for dry surfaces.
- The addition of Tween 80 in wetting solution and eluent did not result in higher recovery. It did not have any statistically significant effects on the survival of *E. coli* and on its recovery from swabs, while it decreased the overall swabbing efficiency.
- Swabbing and contact plating were confirmed as reliable methods to sample and enumerate the presence of *E. coli* after flooding on different urban surfaces.

4

INACTIVATION OF *E. COLI* AS FAECAL INDICATOR ORGANISM ON DIFFERENT SURFACES AFTER URBAN FLOODS

A better understanding of the effects of different urban and recreational surfaces on the inactivation of water-borne pathogens that can cause infections after urban floods if released from surcharged combined sewers and other sources of faecal contamination is needed. The inactivation of faecal indicator *E. coli* was studied under controlled exposure to simulated sunlight on a range of different surfaces found in urban environments: gravel, sand, asphalt, pavement blocks, concrete, playground rubber tiles and grass, using glass as control. The surfaces were inoculated with artificial flooding water containing 10^5 CFU.mL^{-1} of *E. coli* and sampled periodically using the sterile cotton swab technique, after lowering the water level. The results show that dark inactivation was not statistically significant for any surface, suggesting that chemical composition and pH (varying between 6.5±0.8 and 9.2±0.4) did not affect the inactivation rates. The highest light-induced inactivation rates for *E. coli* after the floodwater recession, observed on rubber (>3.46 h^{-1}) and asphalt (2.7 h^{-1}), were attributed to temperature stress and loss of surface moisture.

This chapter is based on: Scoullos, I.M., Lopez Vazquez, C.M., van de Vossenberg, J. and Brdjanovic, D.: Die-off of E. coli as fecal indicator organism on different surfaces after urban floods. J. Environ. Manage., 250, 109516, doi:10.1016/j.jenvman.2019.109516, 2019. (IF=4.9)

4.1 INTRODUCTION

In the previous chapter, it was demonstrated that the die-off of *E. coli* is governed by sunlight; die-off is higher under higher solar irradiance, longer duration of daylight and low TSS concentrations. The effect of different surfaces on the solar inactivation of faecal indicators has not been studied in depth in the literature. The need for further research on the influence of real-world conditions on the inactivation processes has been identified (Nelson et al., 2018).

The objective of this chapter is to better understand and quantify the effects of different surface types found in urban and sub-urban open areas, including storm water detention and retention basins, on the inactivation of indicator organisms under different light conditions, immediately after floods. The research hypothesis is that light irradiation affects bacterial growth, because of damage of light to the cells, but also because light affects the temperature and moisture of different surfaces. The novelty of this research is the focus on the importance of the surface type on the aforementioned processes in order to predict the concentration of pathogens and ultimately to facilitate the decision-making process to assess public health risk and safety conditions.

4.2 MATERIALS AND METHODS

4.2.1 Experimental setup

Samples of gravel of two particle sizes (sieved between 2.5-4.0 mm as very fine gravel and 1.4-2.24 mm as very coarse sand, respectively), pavement blocks (10 x 20 x 8 cm3), pieces of asphalt (3 cm high) and concrete (6 cm high), and grass together with its soil (4.5 cm high) were collected from construction sites in Delft, The Netherlands. Additionally, new playground rubber tiles (2.5 cm high) were used, consisting of styrene-butadiene rubber granulate, heat-bonded with moisture resistant polyurethane binder (Granuflex, Amsterdam, The Netherlands). A glass panel (0.4 cm thick), being non-porous and pH neutral, was used as control. All the above mentioned surfaces were fitted in plastic containers of 27×37 cm2 and 12 cm height. These containers were placed in larger containers with a temperature control system set at 20 ˚C. The inner containers were covered with a Perspex lid to eliminate evaporation. The experimental setup is presented in Fig. 4.1 and in Fig. 4.2.

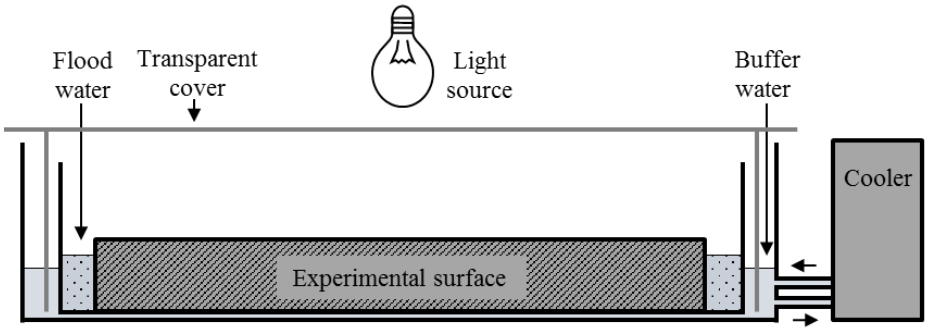


Figure 4.1. Schematic representation of a reactor exposed to artificial light. Two such reactors were used in parallel.

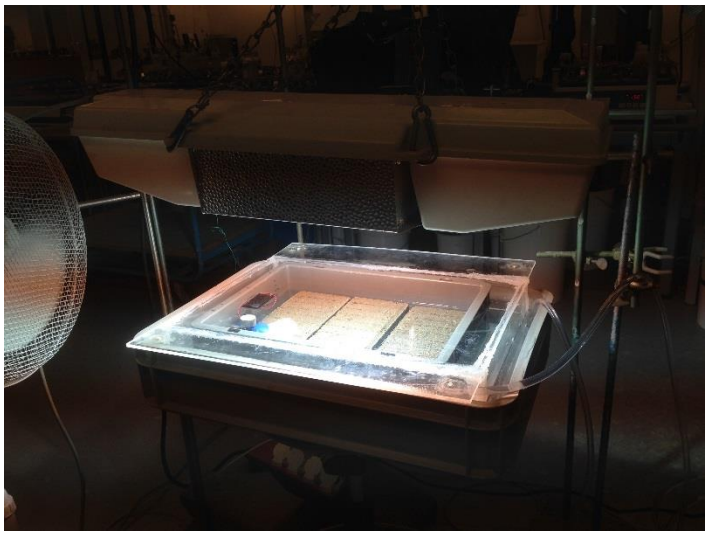


Figure 4.2 Experimental setup used for the study of the inactivation of E. coli under artificial light and dark conditions. Two such reactors were used in parallel.

4.2.2 Indicator organism

E. coli ATCC 25922 was chosen as a proxy of faecal microorganisms. Before each batch experiment *E. coli* was incubated in 1.3% w/v sterile Oxoid CM0001 Nutrient Broth (Oxoid Ltd., Basingstoke, UK) solution in Erlenmeyer flasks for 24 h at 37 ˚C. After incubation, the concentration of the inoculum was estimated at $3 \cdot 10^9$ CFU.mL^{-1}. The initial concentration of *E. coli* for the experiment was around 10^5 CFU.mL^{-1}, based on the typical concentrations of *E. coli* and enterococci observed in raw sewage: between 10^4 CFU.mL^{-1}, the maximum event mean concentration of the first flush of urban storm water runoff after dilution with storm water (Hathaway and Hunt, 2010), and 10^6 CFU.mL^{-1} (Mark et al., 2015).

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. 10-fold dilution steps were performed using 0.1% peptone physiological salt solution. The plates were spread in triplicate.

4.2.3 Swabbing

Sterile cotton tipped wooden stick swabs COPAN CLASSIQSwabs 150C (Copan Italia S.p.A., Brescia, Italy) were used to collect samples from designated 10×1 cm^2 areas on all surfaces, in triplicate. Each swab was applied 20 times, while being rotated. For sampling dry surfaces, the swabs were moistened with sterile 0.1% peptone physiological salt solution prior to swabbing. For wet surfaces, swabbing was performed with dry swabs. In both cases, after swabbing, the swabs were submerged in 2 mL of 0.1% peptone physiological salt eluent and vortexed for 5-10 s. The suspension was diluted and/or plated for colony counting. In the case of grass, swabbing was performed, to the extent possible, only on the grass leaves, not touching the soil.

4.2.4 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. Apart from a few peaks in the area of visible light (with the highest one at 540 nm) the spectrum of these metal halide lamps is continuous (Calin and Parasca, 2008). The light intensity was fixed at 690 $W.m^{-2}$. The photon flux, in $\mu mol.m^{-2}.s^{-1}$, 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; this limitation was taken into account when calculating the lamp irradiance spectrum. The light intensity was calculated as discussed in Section 2.2.4, taking into account the spectral power distribution of the lamp.

4.2.5 Experimental design

Batch experiments in flasks

The effect of different surface materials on *E. coli* inactivation was tested in Erlenmeyer flasks in dark condition. The flasks contained 10 g of the different surface materials and 250 mL of demineralised water and were inoculated with *E. coli* at a concentration of 10^5 $CFU.mL^{-1}$. In a second set of flasks 25 mM HEPES Buffer Solution (Life Technologies Europe B.V., Bleiswijk, The Netherlands) were added as buffering agent to achieve an initial pH of around 7.0 in order to eliminate the effect of pH of the different materials. The *E. coli* concentration and pH were measured daily.

Flood cycle on different surfaces

Each container was filled on a pulse with the flooding water up to a height of 2 cm above the surface. Water was pumped out with the use of a peristaltic pump at a rate of 1 $L.h^{-1}$ until the water surface reached 1 cm below the surface, for approximately 2 h, depending on the surface subject to study. At this point (time 0) the lamp was turned on for 6 h followed by a dark period of 18 h.

4.2.6 Sampling and physicochemical parameters of study

pH was measured in the water phase with a handheld pH-meter (WTW pH 323, WTW GmbH, Weilheim, Germany) and on the experimental surfaces with pH paper (Fisherbrand pH-Fix Test Strips, Fisher Scientific, Landsmeer, The Netherlands). Temperature was measured every hour (fluctuating between 20 and 28 ˚C, data not shown) with a glass thermometer.

The total organic carbon (TOC) concentration of samples was determined using a carbon analyser TOC-L_{CPN} (Shimadzu, Tokyo, Japan). Porosity (%) and gravimetric water content (%) of the surfaces were derived by measuring the mass and volume of the surfaces in dry condition (heated at 105 ˚C for 24 h) and after saturation in water. For measuring the dry volume, the surfaces were covered watertight with paraffin film and immersed in water; the mass and volume of the paraffin used were taken into account.

4.2.7 Data analysis

In all the experiments where the inactivation of *E. coli* was addressed, the inactivation rates were calculated using the first order exponential inactivation Chick-Watson equation (Eq. 1.1). The inactivation was considered to start at time 0 (time of inoculation) with no lag period. The inactivation rate was assumed to be constant in each of the batch experiments because of their short duration, adopting the traditional approach (Wu et al., 2016). In the experiments with light and dark phases, the inactivation rates were calculated separately for each phase.

To investigate whether the difference of concentrations at the beginning and the end of each experimental phase was statistically significant, two-sample *t*-tests assuming unequal variances were performed, with a significance level, α, of 0.05. The null hypothesis was that the concentrations of *E. coli* before and after each phase (light or dark) came from distributions with equal population means. It was assumed that microbial concentrations are normally distributed.

To describe the effect of pH on the maximum specific growth rate $k_{max}(pH)$ (h^{-1}) the Cardinal pH Model equation (CPM, Eq. 1.3, Rosso et al., 1995) was used. The parameters were calculated for the best fit to the experimental data with a minimum sum of squared errors.

4.3 RESULTS AND DISCUSSION

4.3.1 Batch experiments in flasks

The initial pH of water in flasks without buffer, measured 24 h after the immersion of the surface materials in water, ranged from pH 5.4±0.4 for the control to 10.9±0.5 for pavement. In HEPES buffer all the initial pH values were between 7.0±0.1 and 8.2±0.4.

The data from the experiment in the pH unbuffered flasks fitted best to Equation 2 for parameters with the values of pH_{min}=4.6, pH_{max}=6.8, pH_{opt}=5.8 and k_{opt}=0.01 h^{-1}; the square of the Pearson product moment correlation coefficient was equal to r^2=0.96. It is known that *E. coli* species are sensitive to changes in internal pH in an alkaline environment and can maintain pH homeostasis in the external pH range of 4.5 to 7.9 (Booth, 1985). The pH of the surrounding environment affects the net charge of the organism's interface with the environment. This may lead to denaturing proteins and nucleic acids or affect the organisms' net charge and transport. Most organisms exhibit the greatest stability at near-neutral pH (Sinclair et al., 2012). The data from the experiment with HEPES did not fit well to the curve using the same parameters (r^2=0.09). This shows that pH is not the only parameter affecting the concentration of *E. coli* in the flasks and underlines the need to better understand the effect of the materials on the cells. The regression curve is presented together with the two experiments' data in Fig. 4.3.

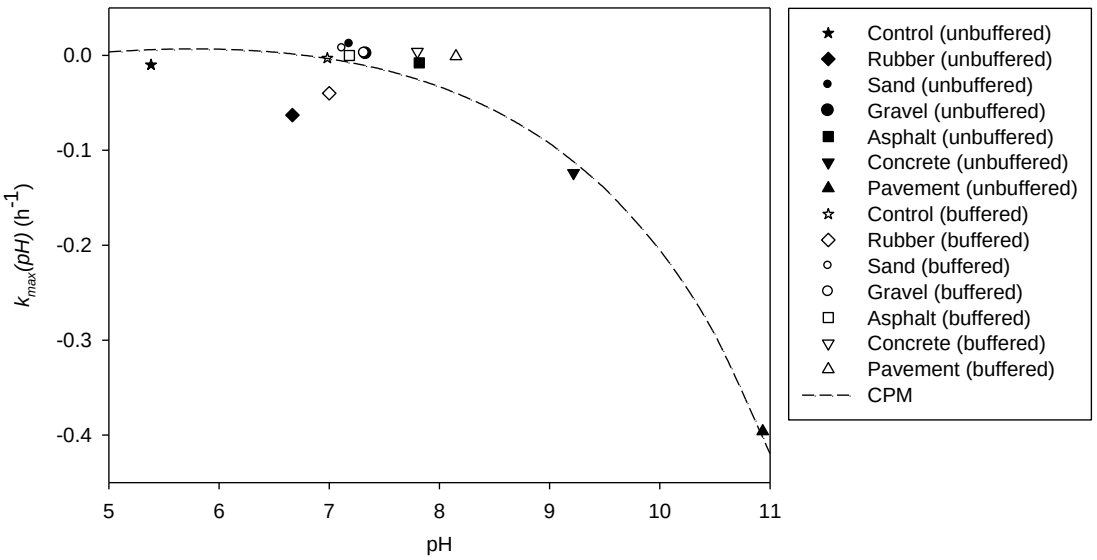


Figure 4.3 The effect of pH of water with different surface materials in batch experiments in flasks with and without the addition of HEPES, on $k_{max}(pH)$, of E. coli, compared to the CPM curve that best fits the data (r^2=0.96). The experimental data for gravel with and without HEPES coincide. The experiments took place in room temperature of 20 ˚C.

The only material that did not fit well to the CPM curve, either with or without buffer, was rubber, which had a higher inactivation rate with (0.04 h^{-1}) and without (0.06 h^{-1}) the use of HEPES. The level of TOC for rubber, 7.46 $mg.L^{-1}$, standing out from the rest, which were all between 0.92 and 1.83 $mg.L^{-1}$, accounts in large part for organic compounds that are inhibitory to *E. coli*, as toxicity of styrene-butadiene rubber leachate has been observed on different organisms (Krüger et al., 2013).

4.3.2 Flood cycle on different surfaces

The results from sampling in the water phase around the surfaces (Fig. 4.4) and from swabbing the surfaces (Fig. 4.5) are presented in Table 4.1.

The value of pH measured around the surfaces (Table 4.1) was around 7.0 for most materials, except pH 7.9±0.1 for pavement and pH 9.2±0.4 in the case of concrete. It has to be noted that in the flask experiment (Fig. 4.3) the pH of pavement was higher, 10.9±0.5, because the pavement blocks were freshly ground and the interior of the blocks had not undergone carbonation, the process of hydroxide anions of calcium hydroxide being replaced over time by carbonate anions resulting in the formation of calcium carbonate (Pade and Guimaraes, 2007). In the case of concrete, as opposed to pavement blocks, no difference between the pH of water around the surface and the pH of water in the flasks with ground sample was observed.

In the case of transparent glass used as a control surface, there were no viable cells recovered with swabbing after three hours of exposure to light and the surface was fully dry. The experiment was performed separately in dark and there was no statistical significant difference between the initial and the final concentrations, therefore the inactivation was solely caused by light. Also, in the floodwater below and around the glass the bacteria died more rapidly (k_D>2.27 h^{-1}) during the light phase compared to all other materials, because the glass is transparent and thin and the whole reactor was shallow and directly exposed to light.

On rubber, there were no viable cells recovered by swabbing after three hours of exposure to light, which could be partly attributed to evaporation as the surface appeared dry. However, in the surrounding floodwater a relatively high inactivation rate of *E. coli* was observed (k_D=0.96 h^{-1}), suggesting an effect of the rubber's constituents on the removal of the bacteria, as also observed in the experiment in flasks. Canepari et al. (2018) confirmed the presence of significant bio-accessible amounts of toxic metals in styrene-butadiene rubber. Furthermore, rubber was the only one among the tested surfaces that had a statistically significant dark inactivation rate in swabbed samples, and the highest

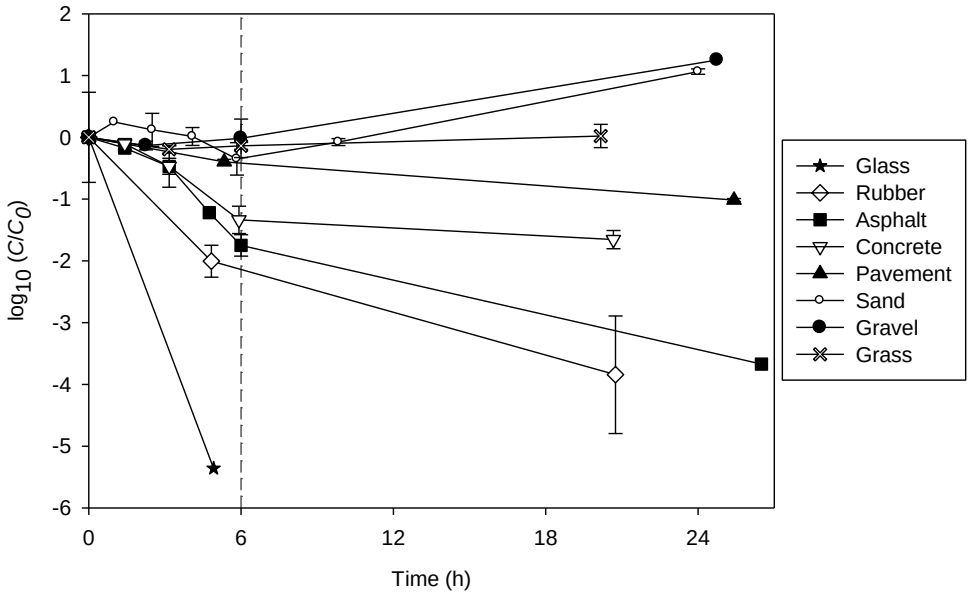


Figure 4.4 Inactivation of E. coli in water samples around different flooded surfaces. The vertical line at 6 h signifies the transition from the light to the dark phase. The detection limit corresponds to $log_{10}(C/C_0) = -4$.

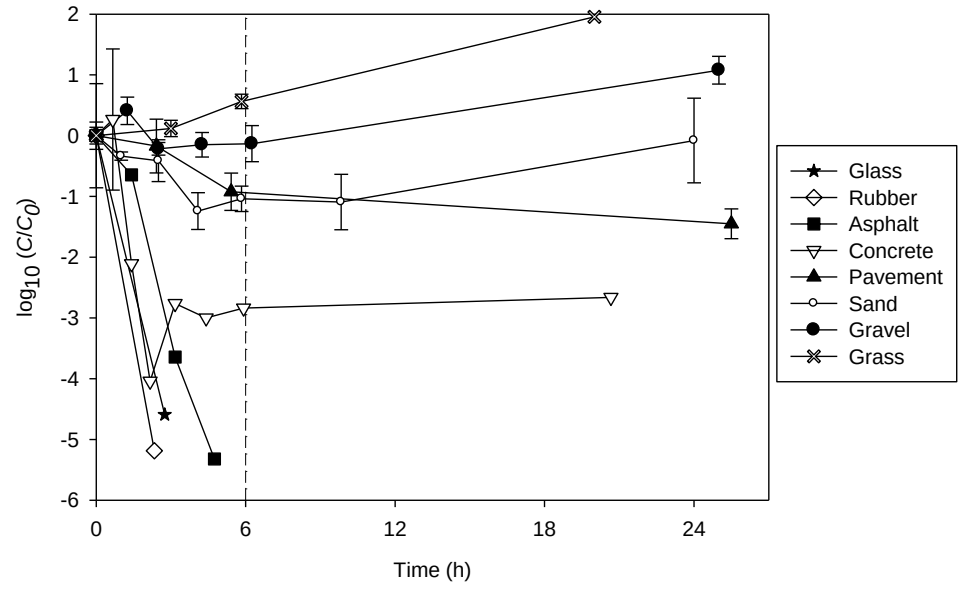


Figure 4.5 Inactivation of E. coli on different surfaces, recovered by swabbing. The vertical line at 6 h signifies the transition from the light to the dark phase. The detection limit corresponds to $log_{10}(C/C_0) = -4$.

*Table 4.1 Experimental parameters on surfaces under artificial sunlight of 690 W.m^{-2} (two-sample t-tests assuming unequal variances, α=0.05, comparing log_{10} values of microbial concentrations at the beginning and end of each phase). Values noted with an * were measured in separate experiments. The number of samples (n) is indicated.*

Surface	Porosity	pH	Surface k_D (h^{-1})		Floodwater k_D (h^{-1})	
			Light	Dark	Light	Dark
Glass	0%	7.1±0.1	>2.44 (n=2)	0.17* (p=0.23, n=2)	>2.27 (n=2)	-0.01* (p=0.32, n=2)
Rubber	15%	6.8±0.5	>3.46 (n=2)	0.19* (p=0.04, n=2)	0.96 (p=0.01, n=2)	0.27 (p=0.01, n=2) (0.01*) (p=0.60, n=2)
Asphalt	4%	6.8±0.3	2.70 (n=4)	-0.02* (p=0.30, n=3)	0.68 (p=0.00, n=5)	0.22 (p=0.00, n=2) (0.03*) (p=0.09, n=3)
Concrete	14%	9.2±0.4	0.90 (p=0.04, n=7)	-0.03 (p=0.62, n=2)	0.54 (p=0.01, n=4)	0.05 (p=0.15, n=2)
Pavement	1%	7.9±0.1	0.40 (p=0.03, n=3)	0.06 (p=0.10, n=2)	0.17 (p=0.00, n=2)	0.07 (p=0.00, n=2)
Sand	9%	6.8±0.3	0.46 (p=0.00, n=5)	-0.13 (p=0.23, n=3)	0.16 (p=0.96, n=5)	-0.18 (p=0.01, n=3)
Gravel	15%	6.9±0.5	0.12 (p=0.36, n=5)	-0.15 (p=0.01, n=2)	-0.002 (p=0.70, n=3)	-0.16 (p=0.00, n=2)
Grass	-	6.5±0.8	-0.22 (p=0.22, n=3)	-0.23 (n=2)	0.05 (p=0.04, n=3)	-0.03 (p=0.37, n=2)

dark inactivation rate (k_D=0.27 h^{-1}) in the water samples, although a repetition of the same experiment in dark (without prior exposure to light) showed no significant inactivation. This observation, together with the difference of inactivation rates between dark and light conditions in the water phase, suggest that exposure of rubber tiles to light and consequently to a high temperature, induced by its dark colour, increased the stronger inactivation of *E. coli*.

The effect of asphalt was similar to the effect of rubber, but on a lower magnitude. Under exposure to light, *E. coli* had an inactivation rate of 2.70 h^{-1} (R^2=0.92) on the surface and 0.68 h^{-1} (R^2=0.94) in the surrounding water. In the dark phase, there was no significant inactivation on the surface, but an inactivation rate of 0.22 h^{-1} was observed in the water. In a separate experiment without prior exposure to light, no statistical significant difference was found between the initial and the final concentrations in dark conditions in the surrounding water. Therefore, it is suggested that the chemical composition of asphalt plays a minor role. The effect of its dark colour, which increased the surface temperature up to a maximum of 60.7±1.3 ˚C after 285 min of exposure to light was stronger (despite that the surrounding floodwater temperature did not exceed 23±1.4 ˚C). The temperature dependence of *E. coli* has been studied thoroughly and is commonly expressed as an exponential increase of the inactivation rate with increasing temperature, assuming applicability of the first order exponential inactivation model (Blaustein et al., 2013). This assumption is also supported by Mendez et al. (2011), who suggest that low concentrations of FIB in water collected from a metal roof might be related to the low emissivity of the metal, leading to higher surface temperatures on the roof, which increase the inactivation rate of these organisms.

In the case of concrete, although the pH was 9.2±0.4, much higher compared to the other surfaces, we did not observe dark inactivation on the surface or in the surrounding water. Light inactivation is, however, significant on the surface (maximum temperature 44.6±0.9 ˚C at the end of the light phase) and around it (30.5±0.7 ˚C), as can be seen in Table 4.1 and in Fig. 4.4 and 4.5. Knowledge on the impact of cement chemistry and surface characteristics such as roughness and texture (controlled by chemical composition of mortar admixtures as well as the type of curing applied) on the adhesion and growth of microorganisms is very limited (Grengg et al., 2018).

While in the dark there was an insignificant or low inactivation, the light inactivation rate on and around the pavement blocks was less than half of the rate for concrete, as shown in Table 4.1. It was concluded that this was related to the observation that the surface of concrete dried fast under exposure to light, while the surface of pavement remained wet. The difference in drying rates can be attributed to faster draining of concrete after the recession of floodwater due to the difference in porosity of the two surfaces. The coefficient of permeability increases with an increase in porosity and the exact relationship is under debate, presented either as a power function (Xu et al., 2018; Kayhanian et al., 2012), a linear relationship (Zhong and Wille, 2015; Bhutta et al., 2012) or an exponential relationship (Sata et al., 2013).

On sand and gravel, there was no significant inactivation, except on the surface of sand under exposure to light (maximum surface temperature 46.6±1.1 ˚C at the end of the light phase, compared to 28.5±0.7 ˚C measured in the water). Some growth of *E. coli* in dark condition was observed (Fig. 4.4 and Fig. 4.5), which was however not always statistically significant according to the *t*-test (see Table 4.1). Both surface samples,

especially sand, have a high standard error. The cause of the variation was the practical limitations of the swabbing method. Even though swabbing was performed gently in order to sample only the top layer that was relatively dry, it was unavoidable to also sample lower grains that were both protected from the light and wet. The presence of organic compounds was not higher than in the other surfaces (TOC of 1.25 $mg.L^{-1}$ for sand and 1.38 $mg.L^{-1}$ for gravel). The results agree with previous research showing that *E. coli* can survive for many weeks in sand, especially in cooler fine-grain sand (Staley et al., 2016), if the conditions and/or their physiological capabilities allow them to replicate (Whitman et al., 2014). Also, Beversdorf et al. (2007) observed that high doses of UV irradiation had no effect on *E. coli* in 30 cm deep sand plots, as opposed to a 94-99% reduction in cell numbers in control liquid cultures.

Although the concentration of *E. coli* had an apparent growth in dark conditions on the surface of grass leaves, which all kept some droplets of water until the end of the experiment, the increase of concentrations was not statistically significant in the light phase. The measurement of TOC on the grass surface was not possible as it was affected by the soil below. Page et al. (2015) calculated a mean inactivation rate of 0.169 d^{-1} (or 0.007 h^{-1}) by combining the data for *E. coli* and *E. faecalis* in summer and winter on grass surfaces of a sporting oval in Perth, Australia, irrigated with secondary treated effluent seeded with microorganisms. The solar radiation was 1440-1580 $W.m^{-2}$ during winter and 1440-1640 $W.m^{-2}$ during summer, much higher compared to 690 $W.m^{-2}$ used in our research. The high variability (standard deviation of 0.126 d^{-1}) was attributed to the environmental conditions of temperature and shading, including the shading created by the green grass leaves, which can absorb more than 90% of radiation (Sidhu et al. 2008). These observations are in agreement with the lower inactivation rates observed in this study, indicating that the shading effects caused by the grass leaves decreased considerably the inactivation effect of solar radiation due to light absorption.

The experimental results suggested a link between the surface moisture and the concentration of bacteria. Sudden changes in moisture are stressful to microbes because they must expend energy to regulate osmotic pressure to their microenvironment (Csonka, 1989). Although gravimetric water content in dark and light conditions was investigated as a measure of surface moisture, the mass of water in the surfaces above the water level (1 cm) did not exceed 4% of the overall mass in any sample, therefore this method was not reliable. The possibility to measure the electric resistance of the surfaces to quantify the surface moisture level was also explored, but when the surfaces dried out, the resistance was too high to measure, except in the case of concrete, which is also conductive when dry. Therefore, it was not possible to determine a quantitative relation between moisture and inactivation rate. Taking these limitations into account, future research can be conducted by keeping the moisture of the surfaces constant throughout the experiment. For example, with the use of a bioclimatic chamber, Ordaz et al. (2019) observed a faster inactivation of *E. coli* inoculated on cellulose filters and on flat cork

coupons for 25 days at 25 ˚C and 65% relative humidity (5.8 log CFU and 1.8 log CFU, respectively) than at 10 ˚C and 95% relative humidity (2.9 log CFU and 1.2 log CFU, respectively).

Furthermore, the effect of porosity was investigated and the data, presented in Table 4.1, suggest that, apart from the differences between concrete and pavement blocks due to the indirect effect of porosity on surface moisture, as discussed above, there was no direct relation between porosity and *E. coli* concentration. Therefore, the concentration of the indicator organism depends on the moisture, temperature and pH of different surfaces.

4.3.3 Strengths and limitations

This work provides data and qualitative observations related to the inactivation of *E. coli* on different surfaces, for which the scientific literature is very limited.

Apart from the limitations related to the lack of a reliable means of measuring surface moisture that were discussed above, the temperature control system did not maintain a constant temperature throughout the experiments that took place under exposure to light. As a result, temperature rose up to 60.7±1.3 ˚C on asphalt, and the maximum temperatures differed depending on the surfaces, making comparison difficult. Also, it was difficult to achieve a uniform temperature distribution due to the presence of the surrounding cooling water and to the small distance of the light source. A proposed solution to that would be the use of bioclimatic chambers with controlled temperature and relative humidity conditions. This would also allow the removal of the Perspex lid that was necessary to eliminate evaporation. Although light irradiance was measured below the lid in order to compensate for the irradiance that was reflected or attenuated, it is probable that the absorbance spectrum of Perspex had an effect on the incident radiation. This could have lead to a reduced penetration of UV wavelengths, which in turn could lead to an underestimation of the inactivation rates under the studied light intensity.

Other limitations were the lack of replicate experiments, the short duration of the experiments for 24 h and the small sample size.

4.4 CONCLUSIONS

Based on the examination of *E. coli* inactivation on surfaces after floodwater recession, the following conclusions can be made:

- Photo-inactivation was minor compared to the temperature stress and evaporation reducing surface moisture.

- The impact of high pH was significant only in the case of ground pavement blocks (pH 10.9±0.5), corresponding to fresh concrete, while the outer surface had lower pH (7.9±0.1) due to carbonation.
- Porosity was not directly related to *E. coli* inactivation, apart from affecting soil moisture.

5

INACTIVATION OF SURROGATE ORGANISMS ON DIFFERENT URBAN SURFACES AFTER URBAN FLOODS

This chapter contributes to a better understanding of the effects of urban and recreational surfaces on the inactivation of surrogates for enteric pathogens under controlled exposure to simulated 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 faecal indicator bacteria *E. coli*, *B. subtilis* spores chosen as surrogates for *C. parvum* oocysts and *Giardia* cysts, and bacteriophages MS2 as surrogates 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 inactivation 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 the inactivation rate after flooding. It also suggests that given the sunlight conditions, concentration of indicator organisms and type of surface, the fate of water-borne pathogens after a flood could be estimated.

This chapter is based on: Scoullos, I.M., Adhikari, S., Lopez Vazquez, C.M., van de Vossenberg, J. and Brdjanovic, D.: Inactivation of indicator organisms on different surfaces after urban floods. Sci. Total Environ., 704, 135456, doi:10.1016/j.scitotenv.2019.135456, 2020. (IF=5.6)

5.1 INTRODUCTION

Urban and recreational surfaces have a possibility to act as a reservoir of faecal contamination after urban floods. The objective of this chapter was to better understand and quantify the effects of different surface types on the inactivation of surrogate organisms after urban floods. The inactivation of *E. coli*, *B. subtilis* spores, and bacteriophage MS2 was studied under controlled exposure to simulated sunlight on artificially flooded concrete, asphalt, pavement blocks and glass as control. Similar conditions can be met in all kinds 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 research took into account the pH of the water that is exposed to these surfaces and its role on the survival of the organisms.

5.2 MATERIALS AND METHODS

5.2.1 Experimental setup

The experimental setup consisted of two open batch reactors in which different surfaces were tested (Fig. 5.1 and Fig. 5.2). Pieces of asphalt and concrete and pavement blocks were collected from 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 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 in Chapter 4 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.

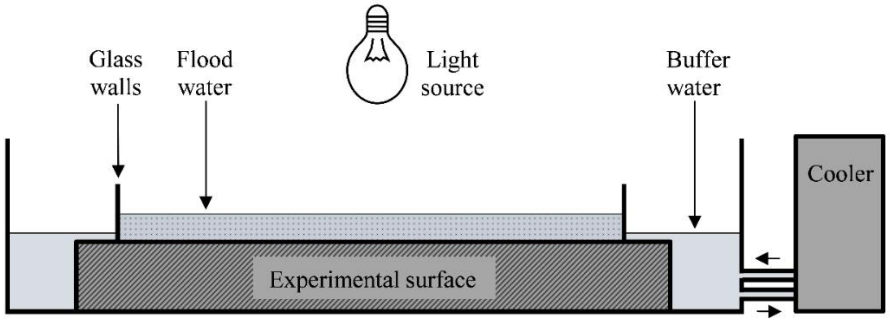


Figure 5.1. Schematic representation of a reactor exposed to artificial light. Two such reactors were used in parallel. In the experiments under real sunlight the cooler was not used.

Figure 5.2. Experimental setup of the reactors used for the study of the inactivation of surrogate organisms under artificial light and dark conditions.

5.2.2 Surrogate organisms

The organisms studied were *E. coli* (ATCC 25922), *B. 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 a surrogate for viral contamination.

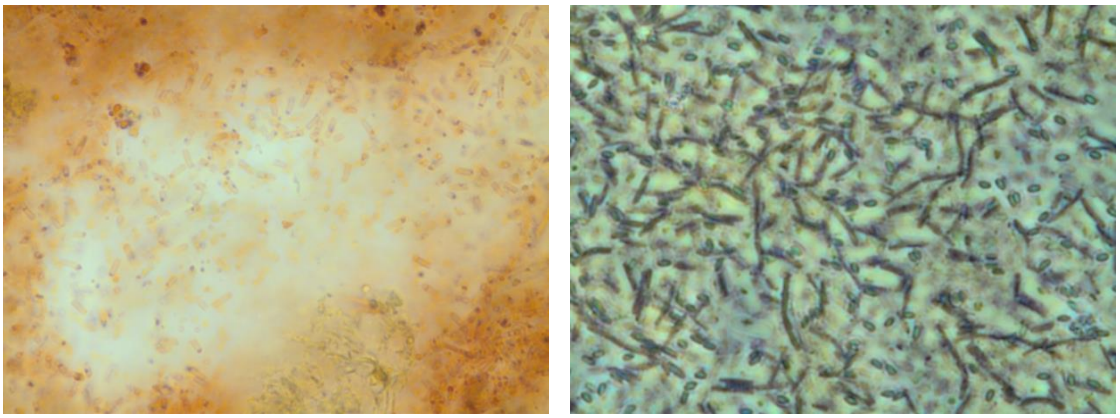
E. coli

Before each batch experiment *E. coli* was incubated in 1.3% w/v sterile Oxoid CM0001 Nutrient Broth (Oxoid Ltd., Basingstoke, UK) solution in Erlenmeyer flasks for 24 h at

37 ˚C. After incubation, the concentration of the inoculum was around $3 \cdot 10^9$ CFU.mL^{-1}. The initial concentration of *E. coli* for the experiment was around 10^5 CFU mL^{-1}, based on the typical concentrations of *E. coli* and enterococci observed in raw sewage: between 10^4 CFU.mL^{-1}, the maximum event mean concentration of the first flush of urban storm water runoff after dilution with storm water (Hathaway and Hunt, 2010), and 10^6 CFU.mL^{-1} (Mark et al., 2015). The enumeration of *E. coli* in the samples was performed by counting the number of colony forming units (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. The plates were spread in triplicate.

B. subtilis

The *B. subtilis* spores were enumerated with the spread plate method. The spores' propagation was adapted from US EPA (2006), UV Disinfection Guidance Manual For the Final Long Term 2 Enhanced Surface Water Treatment Rule (LT2ESWTR). The resulting culture was stained and examined under the microscope (Fig. 5.3) to confirm the presence of free spores without the vegetative cells (Chang et al., 1985). The *B. subtilis* spore suspension was assayed in triplicates by spreading it over plate count (PC) agar plates. Appropriate dilutions of the samples were made with 0.001 M phosphate buffer solution (PBS). They were plated and spread uniformly on PC plates and incubated for 24 hours at 37 ˚C.



a b

Figure 5.3. B. subtilis spores under 100x magnification. Before pasteurization (a), the spores can be seen inside vegetative cells. After pasteurization and sonication (b), spores (green in colour) are released from inactivated cells (brown in colour).

MS2 bacteriophages

The enumeration of MS2 phage was done by double layer agar method following ISO 10705-1:1995. *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 dilute sample. The solution was mixed and poured uniformly over the surface of Tryptone Yeast Glucose Agar (TYGA) plates and incubated for 18h ± 2h at 37 ˚C. Sterile filter pipette tips were used in all steps to avoid contamination.

5.2.3 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 in Chapters 2 and 4 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; this limitation was taken into account when calculating the lamp irradiance spectrum. Light intensity was calculated considering the spectral power distribution of the lamp, as done in Chapter 2 using the same lamp. 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.

5.2.4 Experimental design

Inactivation of E. coli, B. subtilis spores and MS2 bacteriophages on urban 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 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.

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".

5.2.5 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 in the first part, while in the second part samples were taken every 2h. Samples were taken once in two hours in both parts under dark conditions. 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 water on 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.

5.2.6 Data analysis

The inactivation of organisms was calculated based on the "Chick-Watson" first-order exponential inactivation equation (Eq. 1.1). The sample for initial concentration of the organisms was measured immediately after the sample was poured into the water pool on the surfaces. The log survival rate of organisms $\ln(C_t/C_0)$ 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 experiments was analysed using *t*-test for paired two samples for means with a significance level of α=0.05, comparing the $\log_{10}$ values of microbial concentrations at the beginning and the end of each experimental phase. In the case of *B. subtilis* spores, the test was carried out for the overall experiment, between the beginning of the light phase (0 h) and the end of the dark phase (24 h). To compare inactivation rates between *E. coli* and MS2 under exposure to light, two-factor ANOVA tests with replication were used (α=0.05). In these tests, the $\log_{10}$ values of the concentrations of *E. coli* at the beginning and the end of the experimental phase were compared to the respective values of MS2. It was assumed that microbial concentrations were normally distributed.

5.3 RESULTS

5.3.1 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. 5.4.a. The graph shows that there was no significant 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 5.1.

The difference between the concentrations before and the ones after 24 h in dark conditions at 33 ˚C on glass was not statistically significant for any of the three organisms (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.

5.3.2 Inactivation of *B. subtilis* 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. 5.4.b). 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.

Table 5.1 Summary of all inactivation rates under artificial light, sunlight and dark conditions for all organisms (t-test for paired two samples for means, α=0.05, comparing log_{10} values of microbial concentrations at the beginning and end of each phase, or of the overall experiment). The number of samples (n) is indicated.

Surfaces	Average pH	*E. coli*			*B. subtilis* spores	MS2 bacteriophages		
		Light		Dark	Overall	Light		Dark
		Inactivation rate (h^{-1})	R^2	Inactivation rate (h^{-1})	Inactivation rate (h^{-1})	Inactivation rate (h^{-1})	R^2	Inactivation rate (h^{-1})
Artificial light (320 $W.m^{-2}$ for 6 h)								
Glass	6.9	0.78 (p=0.01, n=7)	0.90	0.02 (p=0.59, n=3)	0.02 (p=0.47, n=9)	0.64 (p=0.08, n=4)	0.99	0.04 (p=0.12, n=3)
Pavement	8.1	0.36 (p=0.04, n=7)	0.78	0.07 (p=0.27, n=5)	0.03 (p=0.03, n=11)	0.18 (p=0.77, n=4)	0.91	0.05 (p=0.51, n=3)
Concrete	9.6	1.85 (p=0.04, n=8)	0.96	0.02 (p=0.19, n=4)	-0.01 (p=0.17, n=12)	0.39 (p=0.06, n=4)	0.99	0.05 (p=0.07, n=4)
Asphalt	7.6	1.01 (p=0.047, n=7)	0.93	0.01 (p=0.65, n=4)	-0.05 (p=0.19, n=11)	1.29 (p=0.02, 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 (p=0.01, n=5)	0.84	0.08 (p=0.97, n=2)	-0.01 (p=0.93, n=6)	0.11 (p=0.15, n=4)	0.91	0.02 (p=0.50, n=2)

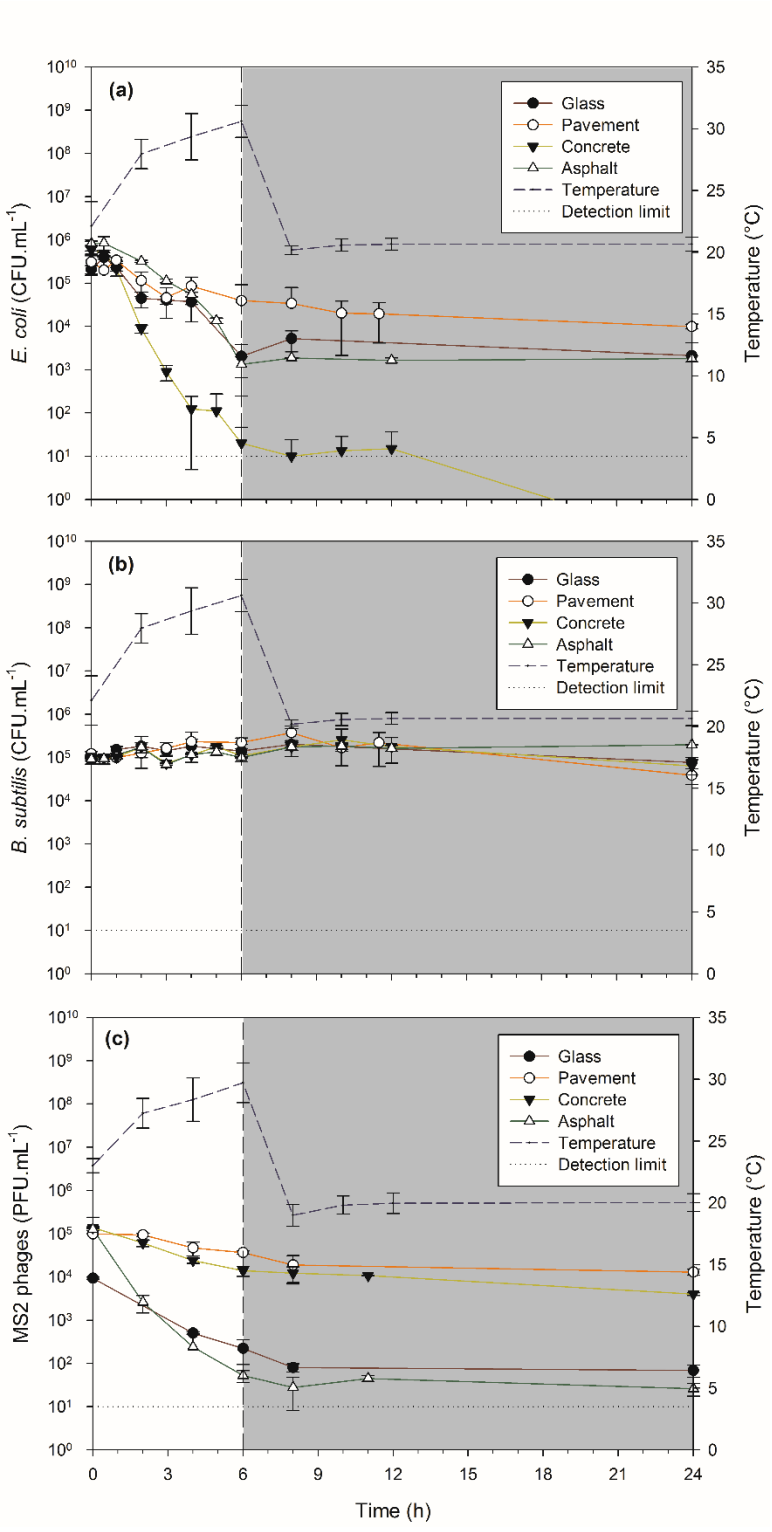


Figure 5.4 Inactivation of E. coli (a), B. subtilis (b) and MS2 bacteriophages (c) on different surfaces. The white area represents the light phase and the grey area represents the dark phase.

5.3.3 Inactivation of MS2 bacteriophages under artificial light and dark conditions

The inactivation of MS2 bacteriophages on all the surfaces is depicted in Fig. 5.4.c. The *t*-tests performed for MS2 bacteriophages in dark and in light conditions, for all surfaces except asphalt, indicated that the difference between the initial and the final MS2 concentrations at each phase was not statistically significant. In the case of asphalt, the inactivation in dark 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. The summary of all the inactivation rates can be seen in Table 5.1.

The trends followed by the inactivation rate of all three 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.

5.3.4 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. 5.5. 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.

The results show that the difference between the initial and the final concentrations was not statistically significant for any of the three organisms under dark or light conditions, except *E. coli* having an inactivation rate of 1.03 h^{-1} under exposure to light. 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 5.1 summarises all the inactivation rates of the three organisms for the experiment conducted under natural sunlight and dark conditions.

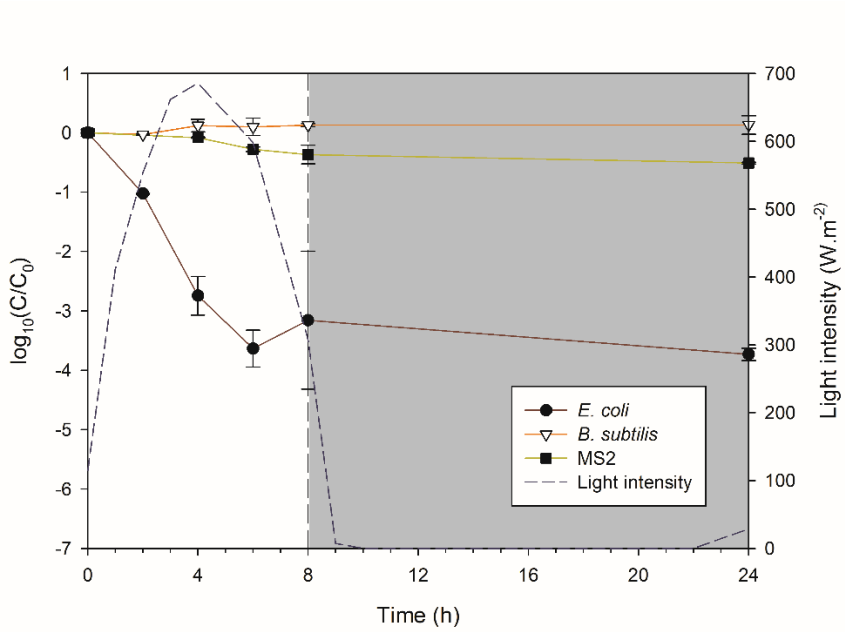


Figure 5.5 Inactivation of E. coli, B. subtilis and MS2 on pavement under natural sunlight (white area) and dark (grey area).

5.4 DISCUSSION

5.4.1 *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 (Chapter 4) 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 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* was not inactivated 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* inactivation is also supported by the fact that the inactivation rate on pavement is relatively similar to the rates measured in Chapter 2 in clear artificial flood water under exposure to the same lamp and light intensity for a duration of 6 hours per day (0.21 h^{-1}) or 12 hours per day (0.37 h^{-1}).

5.4.2 *B. subtilis* spores

B. subtilis spores had a constant concentration on all surfaces (except a small inactivation 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 was low or non-detectable 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 et al., 2016).

5.4.3 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, the ANOVA tests with replication showed that on concrete and glass under light conditions MS2 was more resistant than *E. coli* (F=38.4, p=0.004 for concrete and F=110.2, p=0.001 for glass), and there was no statistical significance when comparing the decrease of concentration of the two organisms on pavement (F=0.68, p=0.46). However, 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). 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 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, was unlikely 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 concentration 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).

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

The inactivation 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; US EPA, 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.

5.4.5 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 indicators for environmentally stable pathogens capable of surviving as spores or oocysts (Dias et al., 2018; Headd et al., 2016). For this reason, it is also necessary to develop low cost, fast and reliable detection techniques for these indicators.

Further research can be undertaken with different kinds of organisms, in particular helminth eggs because they are very resistant (Nelson, 2003), cysts and oocysts, or with a combination of 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.

5.4.6 Strengths and limitations

Although this study provides a better understanding of the inactivation of surrogates for bacterial and viral contamination and parasite oocysts on different surfaces after urban floods, for which the scientific literature is very limited, the persistence of the surrogate organisms has not been examined side-by-side with the persistence of the respective pathogens. Therefore, future experiments must study how well these organisms represent the persistence of pathogens under identical experimental conditions.

Similarly to the previous chapter, the temperature control system did not maintain a constant temperature throughout the experiments that took place under exposure to light. On the positive side, as expected, the maximum temperatures were lower in this setup thanks to the direct contact of the surface to the buffer water and the constant presence of water in the sampling wells on top of the surfaces. The presence of this water provided also a solution to the problem of evaporation that was observed in the previous chapter, which allowed the removal of the Perspex cover in this setup. Of course, it was still difficult to achieve a uniform temperature distribution due to the presence of the surrounding cooling water and to the small distance of the light source and an ideal solution would be the use of bioclimatic chambers with controlled temperature and relative humidity conditions.

Also, the lack of replicate experiments and the short duration of the experiments for 24 h were limitations that need to be mentioned.

5.5 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 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.

6

OUTLOOK

Knowledge and understanding of the parameters and processes that affect the inactivation of water-borne pathogens after floods, can lead to better estimation of public health risks.

6.1 REFLECTIONS

In the context of adaptation to climate change and the related to it extreme events it is necessary to better understand the phenomena linked to contamination of waters with water-borne pathogenic microorganisms after urban floods. Three main research questions were posed at the beginning of this thesis and were addressed in the previous chapters.

6.1.1 Sampling methods

The question of selecting a reliable and simple method for collecting samples from different urban surfaces after the recession of flood water was studied in Chapter 3. Although several methods are described for smooth surfaces like stainless steel benches in the food industry and in hospitals, literature about rough and porous surfaces was limited. The experiments showed that swabbing and contact plating are reliable methods to sample and enumerate the presence of *E. coli* on different urban surfaces after flooding. Swabbing was the best of the tested methods for a wide range of concentrations. Contact plates had the highest recovery, but only for a limited range of *E. coli* concentrations. Tape and stamps had technical challenges and no advantages over contact plating or swabbing. These conclusions lead to the use of the swabbing method in Chapter 4. Moreover, these methods are simple, of low cost and already commercially available, and can therefore be easily used *in situ* for the collection of samples in tests after floods. Although swabbing and contact plating are methods widely in use already, the novelty of this study was to assess them on different surfaces of the urban environment for which literature was extremely limited.

This study can be expanded to include more surface types. A table of recovery ratios for swabbing on different surfaces can allow direct conversion of plate counts into actual surface concentrations of pathogens. Also, as attachment, detachment and inactivation depend on the organisms, the study of further indicator organisms, such as protozoan cysts or oocysts, bacterial spores, bacteriophages or other viruses and helminth eggs will be useful. Different methods, such as sponges, wipes, vacuum methods and different kinds of swab tips can also be tested. Standardisation of sampling methods in the future, considering the relevant species, the aim of the study, the limits of detection and other parameters will be helpful in assessing microbial proliferation on different surfaces (Verdier et al., 2014) and in addressing public health risks after floods.

Of course, these results are not relevant only in the case of urban floods, but also in any other case where the detection of pathogens is required on urban surfaces such as areas around pit latrines or septic tanks, especially after emptying operations, with an impact on the society and the environment.

6.1.2 Inactivation of indicator organisms in water

The second research question was related to the effect of solar radiation and total suspended solids on the inactivation of indicator organisms that originate from the surcharging of combined sewer systems and was studied in Chapter 2. Under laboratory controlled conditions it became evident that inactivation of faecal indicators *E. coli* in waters was linearly proportional to light intensity and duration of exposure to light. As light attenuation coefficient was proportional to the concentration of suspended solids, the inactivation rate decreased exponentially with an increase in TSS concentration. It is noteworthy that even if bacteria were not found to be attached on particles, they were protected from photo-inactivation thanks to the presence of particles for a period of a few days. The outcome of these findings is that by measuring the initial concentration of *E. coli* and the pH of flooding water, and sunlight conditions, it is possible to predict the fate of faecal coliforms after a flooding event. Of course, water quality was also confirmed as an important parameter that needs to be taken into account, underlining the increased complexity of natural systems. The experimental results provided a good understanding of the inactivation of *E. coli* in the water phase that was a necessary step before studying the effect of different surface types.

6.1.3 Inactivation of surrogate organisms on surfaces

The third research question was about the effects of solar radiation on the inactivation of different organisms on urban surfaces. As no inactivation of *E. coli*, *B. subtilis*, and MS2 bacteriophages was observed under dark conditions, it was concluded that the sole effect of pH and other physical or chemical properties of the urban surfaces (pavement, concrete and asphalt) was not significant (Chapter 5). Under dark conditions only the composition of playground rubber tiles had a minor negative effect on *E. coli*. Also, the impact of high pH was significant only in the case of ground pavement blocks (pH 10.9±0.5) corresponding to fresh concrete, while in older concrete the outer surface had lower pH (Chapter 4). This underlines the high risk of water-borne diseases in places that are not exposed directly to sunlight, such as in flooded buildings, between tall buildings, in the shadow and in cloudy weather. Extreme local values of pH can increase inactivation, but these are not so common and the use of materials with a permanent high pH or other antimicrobial properties is something that needs to be further investigated.

Examination of the inactivation of *E. coli* after the recession of flood water in Chapter 4 indicated that photo-inactivation was minor compared to temperature stress and evaporation that reduced the surface moisture of the tested surfaces. For this reason, in Chapter 5 the surfaces were kept flooded with 1 cm of flood water, under controlled temperature. *E. coli* had the highest inactivation under light exposure followed by MS2 bacteriophages, while *B. subtilis* spores were stable, practically not inactivated on any of the tested surfaces, with the exception of asphalt. In fact on asphalt the inactivation of *E.*

coli and MS2 under light conditions was similar, due to much faster inactivation of MS2. This can be attributed to the combined impact of light and the leachate of asphalt, which is much more evident on MS2.

The high resistance of *B. subtilis* spores to the conditions applied leads to the conclusion that *E. coli* is not the most suitable indicator for all human 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, oocysts or helminth eggs, in order to assess the public health risk and safety conditions after urban floods.

This thesis examined the contribution of urban surfaces to pathogen inactivation under different conditions of exposure to light, for which literature is extremely limited. The sets of inactivation rates provided in the thesis can be used for the assessment and reduction of health risks through better planning. Of course this is an extremely complex issue with a variable set of situations, depending on the location, the climate characteristics, the institutional frameworks present, etc. Although many aspects still remain to be studied, this thesis provided a new approach by correlating the issue of public health risks due to water-borne diseases with the presence of different urban surfaces.

6.1.4 Strengths and limitations of this thesis

The novelty of this thesis was the study of the inactivation of surrogate organisms for bacterial and viral contamination and parasite oocysts after urban floods, for which the scientific literature is very limited. A better understanding of the complex processes taking place was achieved, but some research gaps still remain. The following section attempts to describe only a few of the potential practical applications and further research directions that can follow up this thesis.

Of course, it is also important to indicate the limitations of this work. In terms of the setups used, the main limitation was the difficulty to maintain constant temperature under exposure to light. Although in Chapter 5 this was improved compared to Chapter 4, an ideal solution would be the use of bioclimatic chambers with controlled temperature and relative humidity conditions. Although some tests at a constant temperature of 33ºC for 24 h in dark conditions in the incubator showed that all three organisms maintained constant concentrations, the effect of temperature needs to be taken into account and studied separately. Another limitation was the absence of replicate experiments in most cases, the small duration of experiments (in Chapters 4 and 5), and the small sample sizes used.

6.2 RECOMMENDATIONS FOR PRACTICAL APPLICATIONS AND SUGGESTIONS FOR FURTHER RESEARCH

Further research is necessary on the chemical effects of different materials on faecal pathogens. The effect of asphalt constituents on the inactivation of pathogens, for instance, needs to be studied further, as asphalt is a very common surface in urban areas and it is known that leaching is a common process related to it.

The effect on pathogen removal of carbonation and other processes related to the weathering of materials and in particular of concrete should be further investigated. Water in the pores of fresh concrete is saturated with calcium hydroxide, and contains sodium hydroxide and potassium hydroxide, having a typical pH of around 13-14. On the contrary, a fully carbonated part has usually a pH as low as 8.4. An easy method to assess the pH of the concrete other than pH paper is by spraying a phenolphthalein indicator solution which turns purple at pH higher than 9. The denser is the colour, the higher is the pH. Using this method, automatic detection of carbonated regions is possible with the use of image-processing algorithms (Choi et al., 2017). A non-toxic curcumin-based solution that changes from red to yellow in carbonated zones has also been proposed (Chinchón-Payá et al., 2016). These tests can be applied as part of on-site collection of data, especially in urban areas where concrete of different ages is an abundant surface. There are still many research gaps concerning the combined effect of the above and other factors, such as pH, and sunlight irradiation.

Future research is also necessary on the processes taking place within the sewer system, including decentralised, on-site sanitation systems such as pit latrines or septic tanks, as well as on the spread of indicator organisms from the sewerage network to urban surfaces and to the water sources and supply infrastructures under various conditions in both developed and developing countries. All that can provide valuable information on the initial presence of different concentrations of pathogens, nutrients available, pH and other water quality characteristics immediately after extreme rainfall events. In combination with the results of the present work, this can lead to a more holistic approach in addressing the critical issue of contamination of the environment with pathogens during and after urban/peri-urban floods.

Furthermore, many elements of the present thesis could be combined with research on irrigation of agricultural or recreational/forested lands and gardens by using non-conventional water resources such as storm waters stored in various types of reservoirs or treated wastewaters. In conclusion, the present work offers many opportunities for further research projects of high application potential.

6.2.1 Contribution to public health measures, policy frameworks and the SDGs

This thesis has showed that the use of FIBs alone for the assessment of public health risks is not sufficient. For instance, the viruses MS2 and spores of *B. subtilis* were not always inactivated, while *E. coli* concentration was within the required limits. Therefore, it is necessary to take additional measures, by introducing more resilient indicator organisms for the safety standards. For this reason, more categories of organisms need to be studied, such as helminth eggs and parasite cysts/oocysts that were not included in the present research. The detection of any organisms used as indicators, however, needs to be low cost, reliable and fast. The development of commercial kits for their detection in the field, similar to the kits used for *E. coli* detection, is an alternative that needs to be considered.

Moreover, additional practices can be suggested in order to allow public access to certain areas. Such examples may be chemical or physicochemical disinfection of areas, including through natural inactivation mechanisms allowing for certain number of days after the surfaces dry out, or even more advanced methods such as bacteriophage therapy, which has the advantage of reducing only specific targeted host bacteria (Ye et al. 2019). For this, the design/development and use of models that could predict possible risks would be extremely useful. Such an approach is explained in the next section. The above measures have to be complimented by policy frameworks, education and training in order to achieve behavioural change.

The results of the present thesis and the models to be developed can be very useful if properly taken into account for drafting new or amending existing policies connected to floods, covering a wide spectrum of plans related directly or indirectly to protection and amelioration of public health. Such plans and policy frameworks are the following: planning for adaptation to climate change; urban planning; physical planning; integrated urban water management; integrated water resources management; integrated coastal zone management; ecosystem based approach; environmental planning; sustainable development planning including contribution to achieve the SDGs; “nexus planning” for security of the water-energy-food-ecosystems nexus; and combinations of the above as suggested in the integrative methodological framework .

More specifically related to the SDGs, this work can be actually applied to various of the target communities of SDG 13 on climate action, as well as on a number of targets of SDG 3 on good health and well-being, and more specifically on SDG 3.3 on water-borne diseases, on SDG 6 on clean water and sanitation, on SDG 4 on education, and more specifically on target 4.7 on education for sustainable development and many more.

The results allow for a series of proposals for further work and useful applications addressing different target groups in both developing and developed countries. The following three can be regarded as the most important target groups. First, decision

makers in cooperation with those directly responsible for addressing risks from floods and extreme weather events. They could use the results in predicting the needed time for safe use of water in infrastructures and the environment. Second, local authorities, designers of new cities or new neighbourhoods who may incorporate, if not already in practice, or prioritise the use of appropriate drainage schemes and construction materials that allow for a rapid drying of surfaces and inactivation of microbes. Third, formal, non-formal and informal educators, such as schools, local authorities and NGOs, who could inform the public on how to enhance safety and avoid disasters related to the impact of floods. This may be part of an education for sustainable development (ESD) intervention. The role of ESD is fundamental in reducing health risks related to floods. Basic elements should be integrated in the relevant education for ESD curricula. A variety of projects could be proposed for formulating appropriate formal and non-formal education messages or courses, identifying simultaneously the suitable subjects in the curricula to make the interventions. Training for trainers on the interventions may be also considered in cooperation with academic or in-service training institutions.

However the difficulty of obtaining data in the field is an important limiting factor. In view of the above the lack of data, particularly for specific districts, towns, or even neighbourhoods could be complemented by "citizen science" on the basis of some protocols and guidelines to be issued in synergy among local authorities, academic institutions and other stakeholders such local NGOs. These data could include qualitative and quantitative elements e.g. on surfaces, plant cover, paving materials, open spaces, vulnerable surface and/or shallow underground detention and retention systems, wetlands etc.

6.2.2 Urban planning

Contemporary flood management strategies try to go beyond floodwater control by integrating multi-faceted methods in multi- and intra-disciplinary approaches (O'Neill, 2018). In this framework it is important to use sustainable blue-green storm water and nature-based solutions (Bush and Doyon, 2019) and to design appropriate urban surfaces using sustainable pavement materials with low environmental impact and with overall societal benefit (Plati, 2019), such as permeable concrete (Chandrappa and Biligiri, 2016). Also, it is necessary to consider the selection process as a high impact city-level driver (Berndtsson et al., 2019) when planning measures to address urban flood risk. Based on the present work, the chosen materials in flood prone areas or areas with higher public attendance or interaction with vulnerable groups such as children and old people, or places such as schools and hospitals, should not only be based on their hydraulic characteristics concerning the management of flood water quantity, but also based on their role in removing pathogens. This could be, for instance, the use of darker materials that induce higher surface temperatures, materials with higher pH and materials that dry faster, keeping in mind of course all other safety and environmental regulations connected

with materials used. This perspective should also be incorporated in sustainable approaches to integrated urban flood management, as one of the criteria for the selection of appropriate sustainable drainage systems, based on local factors (Lashford et al., 2019).

6.2.3 Flood-related public health risk mapping

One of the goals of this work was to provide a better understanding of the concentration of water-borne pathogens after floods, eventually allowing the development of mathematical expressions that can be incorporated into existing flooding models. An effective overall model or combination of models would describe and ultimately predict the fate of the indicator organisms and their potential dispersion in urban environments leading to public health risks taking into account the inactivation of pathogens on specific tested surfaces (Yakirevich et al., 2013; Jonsson and Agerberg, 2014). The design of a model and a resulting warning system should take into account not only the local climate, surface types, plant cover, hydraulic characteristics and latitude of a region but also periodically altering factors like cloud cover.

An integrated approach would consist of the following components:

- **Surface flood model**. There is a wide variety of one- (1D), two- (2D) and three-dimensional (3D) hydraulic flood models for river channels and floodplains. It is worthy to note that most of these models take into account not only the topography, but also surface roughness, because both topography and roughness coefficients affect the flow area and velocity (Liu et al., 2019). The new generation of urban flood simulations aim to not only accurately replicate the water flows, but also to include associated events, such as flows on street networks, water entering buildings, transport of sediment and pollutants in urban environments, for which dedicated experimental data are virtually unavailable (Mignot et al., 2019). Some of these models take into account the vertical flow interactions between the sewer system and the urban floodplain (Seyoum et al., 2012).
- **Pathogen transport model**. Transport of sediment and contaminants in porous media and in rivers has been studied (Walters et al., 2014), but applications on non-permeable urban flood plains are very limited.
- **Inactivation model**. Several existing models can be adapted to take into account the different inactivation rates of pathogens on different surfaces. For example, it is important to know with relative accuracy the presence of concrete surfaces, both relatively recent (high pH) and older ones. Surface maps may be taken from land use maps and other GIS applications. In this approach it is also important to include soil moisture as an important parameter reducing inactivation. Advancements in infrared thermography, allowing high resolution spatio-temporal mapping of surface temperature and moisture by remote sensing

(Schwarz et al., 2018) give in the long term further potential to a real-time public health warning and safety system.

- **Quantitative Microbial Risk Assessment (QMRA)**. The health risks of extreme flood events can be assessed by QMRA, a tool to estimate the risk of disease. It relies on several assumptions, especially regarding water quality and hydrological conditions due to insufficient quantitative data. The results of the present work may reduce the uncertainty of some of the model's predictions. However, often, the lack of epidemiological data also prevents the validation of QMRA (Curriero et al., 2001; ten Veldhuis et al., 2010; Andersen et al., 2013).

The outcomes of the above models, combining the public health components (contamination, spread and inactivation of water-borne and vector-borne pathogens) and monitoring modules, after being calibrated and validated *in situ*, could be extremely useful in risk mapping and classification of surfaces and areas into zones of different risk levels in an integrated model. This system could easily be applied in cities and peri-urban or rural areas for the prediction of the minimum time needed for safe inactivation of pathogens. This can be a very useful component of adaptation to climate change practices, from forecasting of risks connected to extreme events under different climate change scenaria, to early warning for implementation of upfront decisions to minimize the potential public health issues, or providing guidance for the management of the situation during or after floods and properly educating and informing key stakeholders and the public at large on health risks associated with flooding events and ways to reduce them.

In conclusion, the predicted by all climatic models increase of the frequency and intensity of floods connected to climate change, on the one hand, and the rapid increase of population, particularly in developing countries and in poor urban and peri-urban areas, on the other, are expected to lead to higher exposure to water-borne pathogens and to enhanced public health risks. The aforementioned conditions and factors make the issue of assessing the fate of pathogens after floods increasingly important, requiring an in-depth understanding. The obtained through the present thesis knowledge of the relevant processes constitutes a concrete contribution towards this direction. Based on this knowledge, no regret measures (physical/technical, institutional and educational/cultural) could be proposed. These may include, *inter alia*, both nature-based solutions and appropriate infrastructures for retention/detention of flood water, improved capacity of drainage systems, guidelines, regulations and incentives for use of suitable surfaces and employment of efficient procedures allowing more rapid inactivation of pathogens, followed and combined with controlled access to potentially contaminated areas. At the same time, capacity building of different stakeholders involved through training and education together with advocacy are necessary in order to achieve behavioural changes and meaningful public participation in proactive and contingency management schemes. Due to the complexity of the phenomena and management activities involved it is necessary to adopt an integrated, multi- and trans-disciplinary approach. A model-based

methodology capitalising on all the aspects investigated through the present thesis can offer a useful next step.

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LIST OF ACRONYMS

AWI	Air Water Interface
CCA	Chromocult Coliform Agar
CFU	Colony Forming Units
CSO	Combined Sewer Overflow
DO	Dissolved Oxygen
FIB	Faecal Indicator Bacterium
MDGs	Millennium Development Goals
PBS	Phosphate-saline Buffer Solution
PC	Plate Count
PFU	Plaque Forming Units
QMRA	Quantitative Microbial Risk Assessment
ROS	Reactive Oxygen Species
SDGs	Sustainable Development Goals
TPB	Triple Phase Boundary
TYGA	Tryptone Yeast Glucose Agar
TYGB	Tryptone Yeast Glucose Broth
UV	Ultra Violet
2D	Two Dimensional

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ABOUT THE AUTHOR

Iosif Marios Scoullos, born in Marousi, Athens, Greece, is a Chemical Engineer, graduate of the National Technical University of Athens, Greece. With a strong interest for the protection of the environment and its resources, his thesis in Biotechnology and the Environment was related to the production of second generation biofuels, entitled "Contribution of the inhibitors which are produced during the hydrothermal pre-treatment of biomass on the growth and ethanol production by the fungus *Fusarium oxysporum*".

Having understood that in order to achieve great changes it is not only technological means which are necessary, but also appropriate management tools and interdisciplinary cooperation, he studied MPhil in Technology Policy at the University of Cambridge, UK, focusing on environment and sustainable development issues.

Since the problem of water is one of the most difficult, though needed, issues to be addressed in the beginning of the 21st century he decided to focus on this with a PhD in Environmetal Biotechnology and Sanitary Engineering at the Delft University of Technology and the IHE Delft Institute for Water Education. He received an Academy of Athens Scholarship from the Vasiliki Bekiari-Vekri Bequest and obtained an Alexander S. Onassis Public Benefit Foundation Research Grant.

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Scoullos, I.M., Lopez Vazquez, C.M., van de Vossenberg, J., Hammond, M. and Brdjanovic, D.: Effect of artificial solar radiation on the die-off of pathogen indicator organisms in urban floods. Int. J. Environ. Res., 13, 107-116, doi:10.1007/s41742-018-0160-5, 2019.

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The SENSE Research School declares that **Iosif Marios Scoullos** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 36.2 EC, including the following activities:

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- Environmental research in context (2014)
- SENSE Summer academy (2014)
- Research in context activity: 'Representative of IHE PhD Fellows' Association Board (PAB) in IHE PhD Programme Committee (June 2015-March 2016)'

Other PhD and Advanced MSc Courses

- PhD start-up course, TU Delft (2014)
- Hydrology and hydraulics, IHE Delft (2013)
- Urban drainage and sewerage, IHE Delft (2014)
- Conventional wastewater treatment, IHE Delft (2014)
- Modelling of wastewater treatment processes and plants, IHE Delft (2014)

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- *Effect of simulated solar radiation on the die-off of indicator organisms.* Soil contamination symposium: scope, advances and challenges, 1 September 2017, Wageningen, The Netherlands

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In the last ten years (2009-2019), flooding caused the death of over 48,000 people, and affected over 697 million people globally. This is expected to increase as a result of climate change, population growth and urbanisation. Floods can cause infections due to the release of water-borne pathogens from surcharged combined sewers and other sources of faecal contamination on urban surfaces such as concrete, asphalt, gravel, pavement, playground rubber tiles and grass. Using laboratory experiments with faecal indicator bacteria *Escherichia coli*, and with *Bacillus subtilis* spores, and MS2 bacteriophages under controlled exposure to simulated sunlight, this research contributes towards a better understanding of the environmental parameters that affect the concentration of pathogens in contaminated shallow water bodies and on different urban surfaces. Also, several sampling methods are assessed for the recovery of bacteria from flood-prone urban surfaces. This study suggests that given the sunlight conditions after an urban flood, the concentration of indicator organisms and of total suspended solids and the surface type it is possible to estimate the fate of selected pathogens. The observations and results presented in this study contribute to the development of policy-making tools for rapid implementation of appropriate measures to mitigate public health risks after flooding.

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