Quantitative Microbial Risk Assessment and Water Quality Modelling - On Drinking Water Sources, Recreational and Recycled Waters

Kvantitativ mikrobiell risikoanalyse og modellering av vannkvalitet – for drikkevannskilder, badevann og vann til gjenbruk

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Summary

Despite various efforts to improve water quality in all pathways, waterborne diseases are still a major public health concern in both developing and developed countries. To address the threat of waterborne diseases through different measures, understanding the level of risk is very important. In this regard, quantitative microbial risk assessment (QMRA) is an emerging modelling approach, used to estimate the risk of infection and illness from an exposure to disease causing microorganisms, and is currently promoted by the World Health Organization (WHO) to be used for setting water safety criteria and regulations. While QMRA has been useful in estimating health risk levels, and therefore highlighting risk based management options, there are various challenges to apply this method under different conditions. One of the major challenges is the shortage of quantitative data on the concentration of microbial pathogens and inconsistency in water-related pathways. Therefore, monitoring of microbial pathogens is the preferred form of information for QMRA. Currently, the potential for the integration and use of microbial water quality monitoring and modelling as an input for the QMRA framework is an important issue.

This study will enrich the QMRA approach through microbial water quality modelling. The trend, extreme microbial loads, transport, and spreading of microbial pollutants in the identified pathways of interest (drinking water source and recreational coastal water) were investigated using statistically (**Paper I**), and deterministically (**Paper II**) based water quality modelling techniques, and then coupled with the QMRA framework (**Paper III**). There is also a microbial decay rate study in seawater, which could be an input for microbial fate and transport modelling (**Paper IV**). Furthermore, this study addressed the health risk issues associated with treated greywater reuse (**Paper V**), and microbial pollutant and nutrient removal efficiency of treated greywater disposal systems (**Paper VI**). Unsaturated and saturated flow studies provided valuable insights about the removal of microbial pathogens by on-site wastewater treatment systems that could be relevant in some QMRA settings.

Paper I utilized faecal indicator organisms time series data at a drinking water treatment plant (DWTP) to evaluate the trend and to understand the probabilistic behaviour of extreme microbial loads of the drinking water source, Glomma River, Norway. Mann-Kendall's test and Sen's Slope estimator were used for trend analysis, and seasonal trends were examined through linear regression. Likewise, the probability of extreme microbial load incidents was estimated using the peak-over threshold (POT) method. The mean concentration of *Clostridium perfringens* decreased significantly during the period. Seasonal trend analysis results indicate that *Clostridium perfringens* during autumn and intestinal enterococci during spring have significantly decreasing trends. In addition, the extreme concentrations of five indicator microorganisms corresponding to selected return periods were estimated.

The second section of this study focuses on recreational coastal water quality in relation to QMRA and the details are presented in papers **II**, **III**, & **IV**. In order to understand the influence of different processes (rainfall, discharge from boats, and wind directions) on the microbial water quality of the recreational beaches, hydrodynamic modelling was carried out (**Paper II**) along the Sandvika beaches in Oslo fjord. The result of this study indicated that 1) the bathing water quality

was poor according to the EU bathing water directive up to two days after the heavy rainfall event depending on the location of the beach site, 2) the discharge from a boat up to 300-meter distance may be significant if there is a person infected with a high virulence pathogen on the boat and 3) the effect of wind in terms of spreading microbial pollutants and on the bathing water quality depends on the location of the beach from the pollution source. **Paper III** integrates hydrodynamic modelling with the QMRA framework by considering the simulation of a heavy rainfall event in the Sandvika area on 7 July 2014. The simulated *E. coli* concentration in each beach was transformed into the concentration of pathogens (Norovirus, *Campylobacter*, *Salmonella, Giardia* and *Cryptosporidium*) based on the ratio of the concentration of reference pathogens to *E. coli* in the sewer system, and then the health risk was computed using the QMRA framework. The results indicated that the risk of infection was higher for non-adults relative to adults and the highest risk was observed at the Kalvøya small and Kalvøya big beaches. Moreover, the risk of exposure to norovirus was high at all beaches; however, the proportion of infective virus should be further investigated. **Paper IV** intended to investigate mainly the decay rates of faecal indicator bacteria (FIB) and viruses in seawater and decay rate models were developed for TC, *E. coli*, IE, adenovirus, and MS2 in surface and deep seawater, which is important for fate and transport modelling and QMRA. The experiment results indicate that the decay rate coefficients were higher at 20 °C than at 4 °C, higher in surface seawater than deep seawater, and higher in the case of FIB than the virus.

In the third section of this study, the health risk of treated greywater reuse for hydroponic irrigation (**Paper V**) and the microbial pollutant and nutrient removal efficiency of treated greywater disposal systems (**Paper VI**) were investigated. In **Paper V** information about the effluent microbial water quality, the level of microbial contamination of lettuce and the bioaccumulation of heavy metals in the plant tissue were analysed. Both QMRA and chemical health risk assessment (CHRA) approaches were applied to evaluate the health risk. The probability of infection due to lettuce consumption per single exposure was estimated as 1.4×10^{-10}, 7.8×10^{-13}, and 1.3×10^{-10} in the case of *Cryptosporidium, Campylobacter*, and norovirus respectively. Moreover, CHRA results via targeted hazard quotient (THQ) indicated that the major risk contributor elements due to lettuce consumption are As, Cr and Cu. **Paper VI** investigated bacteria (TC and *E. coli),* model virus (St28B), and nutrients removal efficiency of treated greywater disposal systems, under both unsaturated and saturated flow conditions. The purpose of this study was to identify the most efficient infiltration system as a post treatment step and further to examine the removal efficiency of saturated flow conditions. Columns with 30 cm Filtralite at the top and 50 cm quarry waste "subbus" at the bottom (column-D), and Filtralite-fine sand-till soil stratified filtration systems (column-B) provided comparably better treatment performance with respect to total coliforms, *E. coli*, St28B, nutrients and organic load removal efficiency without clogging problems within the experimental period.

This study thus contributes to improving the QMRA approach and demonstrated that QMRA, in combination with microbial water quality modelling, has the potential to estimate health risks in different contexts and pathways, such as drinking water sources, recreational water and recycled water. Although different and important issues have been addressed in this thesis, many other issues still remain to be clarified in order to improve the QMRA framework.

Sammendrag

På tross av bred innsats for å forbedre vannkvaliteten langs alle smitteveier, er vannbåren sykdom fortsatt et folkehelseproblem i både utviklingsland og utviklede land. For å møte trusselen fra vannbårne sykdommer med tilpassede tiltak, er det viktig med en forståelse av risikonivået. I denne sammenhengen er kvantitativ mikrobiell risikoanalyse (QMRA) en modelleringstilnærming som får økt oppmerksomhet, og blir nå anbefalt av Verdens helseorganisasjon (WHO) for å fastsette kriterier og forskrifter for trygt vann. QMRA har vært nyttig for å estimere helserisikonivåer og derigjennom rette fokus mot risikobaserte tiltak, men det er fortsatt diverse utfordringer ved å anvende QMRA under varierte betingelser. En av de største utfordringene er mangelen på kvantitativ informasjon om konsentrasjonene av smittestoffer, og variasjonene i disse gjennom vannets transportveier. Derfor er data fra overvåkning av smittestoffer kjærkommen informasjon for QMRA. Mulighetene for å integrere og bruke data fra overvåkning og modellering av mikrobiell vannkvalitet i QMRA-rammeverket er for tiden en sentral problemstilling.

Denne avhandlingen vil styrke QMRA-tilnærmingen gjennom modellering av mikrobiell vannkvalitet. Trender, ekstreme konsentrasjoner, transport og spredning av smittestoffer i en drikkevannskilde og badevann ved kysten ble studert med statistiske (**Artikkel I**) og deterministiske (**Artikkel II**) metoder for modellering av vannkvalitet, og deretter knyttet til QMRA-rammeverket (**Artikkel III**). Det er også en studie av inaktivering av smittestoffer i sjøvann, som kan være inngangsdata for modellering av mikrobiell overlevelse og transport (**Artikkel IV**). Dessuten har avhandlingen sett på helserisiko knyttet til gjenbruk av behandlet gråvann (**Artikkel V**), samt fjerning av mikroorganismer og næringsstoffer i systemer for etterbehandling og utslipp av forbehandlet gråvann (**Artikkel VI**). Studier av umettet og mettet strømning gav verdifull innsikt i fjerningen av smittestoffer i desentraliserte systemer for avløpsbehandling, og kan i noen sammenhenger være relevant for QMRA.

Artikkel 1 benyttet tidsseriedata for fekale indikatororganismer fra et vannbehandlingsanlegg med Glomma som råvannskilde for å vurdere trender i mikrobiell vannkvalitet og for å beregne sannsynligheten for at ekstreme nivåer av mikroorganismer skal opptre i vannkilden. Trendanalyse ble gjennomført med Mann-Kendalls test og Sens estimator for helning, og sesongbaserte trender ble undersøkt med lineær regresjon. Sannsynligheten for ekstreme nivåer av mikroorganismer ble estimert med «peak-over threshold»-metoden. Den midlere konsentrasjonen av *Clostridium Perfringens* falt signifikant i løpet av perioden. Sesongbasert trendanalyse indikerer at *Clostridium Perfringens* og intestinale enterokokker har fallende trender henholdvis om høsten og våren. I tillegg ble ekstreme konsentrasjoner for fem indikatororganismer estimert for utvalgte gjentaksintervall.

Andre del av avhandlingen fokuserer på badevannskvalitet ved kysten i tilknytning til QMRA, og detaljene blir presentert i artikkel **II**, **III** & **IV**. For å forstå effekten av ulike prosesser (nedbør, utslipp fra båter og vindretninger) på den mikrobielle vannkvaliteten på badestrender, ble det gjennomført hydrodynamisk modellering (**Artikkel II**) i Oslofjorden ved strendene i Sandvika. Resultatene fra disse studiene viser at 1) badevannskvaliteten var dårlig i henhold til EUs badevannsdirektiv i opptil to dager etter kraftige nedbørshendelser, avhengig av hvor på

stranden man er, 2) utslipp fra en båt i 300 m avstand fra land kan utgjøre en helserisiko hvis en person om bord i båten er smittet med et høyinfektivt smittestoff og 3) virkningen av vind på spredningen av mikrobielle forurensninger og badevannskvalitet avhenger av plasseringen av stranden i forhold til forurensningskilden. **Artikkel III** integrerer hydrodynamisk modellering med QMRA ved hjelp av simulering av en kraftig nedbørshendelse i Sandvika-området 7. juli 2014. De simulerte *E.coli*-konsentrasjonene på hver strand ble brukt til å estimere konsentrasjonene av smittestoffer (Norovirus, *Campylobacter*, *Salmonella*, *Giardia* og *Cryptosporidium*) basert på forholdet mellom konsentrasjonen av smittestoffer og *E.coli* i avløpssystemet, og helserisikoen ble deretter beregnet ved hjelp av QMRA. Resultatene indikerte at infeksjonsrisikoen var høyere for mindreårige sammenlignet med voksne, og den høyeste beregnede risikoen gjaldt strendene på Kalvøya. Dessuten var sannsynligheten for å bli eksponert for norovirus høy på alle strendene, men andelen infektive virus bør undersøkes nærmere. **Artikkel IV** undersøker inaktiveringsrater for fekale indikatorbakterier (FIB) og virus i sjøvann, og inaktiveringsmodeller ble utviklet for totale koliforme bakterier, *E.coli*, intestinale enterekokker, adenovirus og MS2 i sjøvann fra overflaten og fra dypt vann, noe som er viktig for transportmodeller og QMRA. Resultatene fra eksperimentet indikerer at inaktiverings-koeffisientene var høyere ved 20 °C enn ved 4 °C, høyere i overflatevann enn i vann fra dypet, og høyere for FIB enn for virus.

I tredje del av avhandlingen undersøkes helserisikoen ved gjenbruk av behandlet gråvann til irrigasjon i hydroponiske systemer (**Artikkel V**) og fjerningen av næringsstoffer og mikrobielle forurensninger fra systemer for utslipp av behandlet gråvann (**Artikkel VI**). I **Artikkel V** analyseres informasjon om den mikrobielle kvaliteten på behandlet gråvann, nivået av mikrobiell forurensning i dyrket salat og bioakkumuleringen av tungmetaller i plantevev. Både QMRA og kjemisk helserisikoanalyse (CHRA) ble benyttet for å vurdere helserisikoen. Sannsynligheten for infeksjon pga. salatinntak per eksponering ble estimert til 1.4×10^{-10}, 7.8×10^{-13} og 1.3×10^{-10} for henholdsvis *Cryptosporidium, Campylobacter* og norovirus. Dessuten viser CHRA-resultatene i form av «targeted hazard quotient» at de største bidragene til helserisiko fra grunnstoffer var As, Cr og Cu. **Artikkel VI** undersøkte fjerningen av bakterier (totale koliforme bakterier og *E.coli*), modellvirus (St28B) og næringsstoffer i systemer for utslipp av behandlet gråvann, både under mettede og umettede forhold. Hensikten med studien var å identifisere det mest effektive infiltreringssystemet til bruk som etterbehandlingssteg og dessuten å undersøke fjerningseffekten ved mettede strømningsforhold. Kolonner med 30 cm Filtralite over 50 cm subbus (kolonne D), og kolonner med Filtralite over finsand over morene (kolonne B) gav forholdvis bedre fjerningseffekt for totale koliforme bakterier, *E.coli*, St28B, næringsstoffer og organisk stoff uten at det oppstod problemer med gjentetting i løpet av forsøksperioden.

Denne avhandlingen bidrar til å forbedre QMRA-metodikken og viser at QMRA i kombinasjon med modellering av mikrobiell vannkvalitet har potensiale til å estimere helserisikoen i ulike kontekster og langs ulike smitteveier, som drikkevann, badevann og gjenbruksvann. Selv om varierte og viktige problemer er behandlet i denne avhandlingen, gjenstår det å undersøke mange andre utfordringer for å forbedre QMRA-metodikken.

Dedication

This thesis is dedicated to the hundreds of thousands of innocent children perishing each year from diarrhoea due to lack of safe drinking water and adequate sanitation services.

"If there is magic on this planet, it is contained in water"
Loren Eiseley (1907-1977)

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List of Acronyms

ANOVA	Analysis of variance
BOD	Biochemical oxygen demand
CHRA	Chemical health risk assessment
COD	Chemical oxygen demand
CSO	Combined sewer overflows
DALY	Disability Adjusted Life Years
DO	Dissolved oxygen
DWTP	Drinking water treatment plant
EC	Electrical conductivity
FFU	Fluorescence forming unit
FIB	Faecal indicator bacteria
GEMSS	Generalized Environmental Modeling System for Surface waters
GI	Gastrointestinal
GPD	Generalized Pareto distribution
HPC	Heterotrophic plate counts
HRI	Health risk index
ICP-MS	Inductively coupled plasma mass spectrometry
IE	Intestinal enterococci
MPN	Most probable number
MST	Microbial source tracking
NIVA	Norwegian Institute for Water Research
NRV	Nedre Romerike vannverk
PCA	Principal Components Analysis
PCR	Polymerase chain reaction
PFU	Plaque forming unit
PLC	Programmable logic controller
POT	Peak-over Threshold
QMRA	Quantitative microbial risk assessment
qPCR	Quantitative polymerase chain reaction
RT-qPCR	Reverse-transcription quantitative polymerase chain reaction
RWQC	Recreational water quality criteria
SS	Suspended solids
SSO	Sanitary sewer overflows
TC	Total coliforms
THQ	Targeted hazard quotient
TOC	Total organic carbon
TSS	Total suspended solids

USEPA	United States Environmental Protection Agency
UV	Ultra Violet
WHO	World Health Organization
WQ	Water quality
WQM	Water quality monitoring
WWTP	Wastewater treatment plants
YLD	Years Lost due to Disability
YLL	Years of life lost

List of Publications

This thesis is based upon the following appended papers, which will be referred by their Roman numerals (I – VI) throughout the text. Published papers are reproduced with permission from the publishers.

Paper I

Eregno, F. E., Nilsen, V., Seidu, R., & Heistad, A. (2014). Evaluating the Trend and Extreme Values of Faecal Indicator Organisms in a Raw Water Source: A Potential Approach for Watershed Management and Optimizing Water Treatment Practice. *Environmental Processes, 1*(3), 287-309. https://link.springer.com/article/10.1007/s40710-014-0026-6

Paper II

Eregno, F. E., Tryland, I., Tjomsland, T., Kempa, M., & Heistad, A. (2017). Hydrodynamic modelling of recreational water quality using *Escherichia coli* as an indicator of microbial contamination. *Journal of hydrology* (Revised version submitted)

Paper III

Eregno, F. E., Tryland, I., Tjomsland, T., Myrmel, M., Robertson, L., & Heistad, A. (2016). Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Science of The Total Environment, 548–549*, 270-279. http://www.sciencedirect.com/science/article/pii/S004896971630033X

Paper IV

Eregno, F. E., Tryland, I., T., Myrmel, Wennberg, A., Oliinyk, A., Khatri, M., & Heistad, A. (2017). Decay rate of virus and faecal indicator bacteria (FIB) in seawater and the concentration of FIBs in different wastewater systems. *Microbial risk Analysis*. (Revised version submitted)

Paper V

Eregno, F., Moges, M., and Heistad, A., 2017. Treated Greywater Reuse for Hydroponic Lettuce Production in a Green Wall System: Quantitative Health Risk Assessment. *Water*, 9(7): 454. http://www.mdpi.com/2073-4441/9/7/454

Paper VI

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Other relevant publications not included in the thesis

Eregno, F.E., Grøndahl-Rosado, R.C., Nilsen, V., Seidu, R., Heistad, A. and Myrmel, M., 2014. Multiple Linear Regression Models for Estimating Microbial Load in a Drinking Water Source: Case from the Glomma River, Norway. *Vann*, 49(3): 335-350. http://vannforeningen.no/wp-content/uploads/2015/06/2014_910120.pdf

Tryland, I., **Eregno, F.E**, Braathen, H., Khalaf, G., Sjølander, I. and Fossum, M., 2015. On-Line Monitoring of *Escherichia coli* in Raw Water at Oset Drinking Water Treatment Plant, Oslo (Norway). *International Journal of Environmental Research and Public Health*, 12(2): 1788-1802. http://www.mdpi.com/1660-4601/12/2/1788

Moges, M.E., **Eregno, F.E**. and Heistad, A., 2015. Performance of biochar and filtralite as polishing step for on-site greywater treatment plant. *Management of Environmental Quality: An International Journal*, 26(4): 607-625. http://www.emeraldinsight.com/doi/abs/10.1108/MEQ-07-2014-0101

Moges, M.E., Todt, D., **Eregno, F.E**. and Heistad, A., 2017. Performance study of biofilter system for on-site greywater treatment at cottages and small households. *Ecological Engineering*, 105: 118-124. http://www.sciencedirect.com/science/article/pii/S0925857417302458

Tryland, I., Braathen, H., Wennberg, A.C., **Eregno, F.E**. and Beschorner, A.-L., 2016. Monitoring of β-d-Galactosidase Activity as a Surrogate Parameter for Rapid Detection of Sewage Contamination in Urban Recreational Water. *Water*, 8(2): 12. http://www.mdpi.com/2073-4441/8/2/65

1. Background and Conceptual Basis

Water is vital for the survival of all living things and plays an important role in the daily activities of human beings and as a whole in the development of the world economy. Human beings highly depend on the natural water systems to meet drinking, hygiene and sanitation, agriculture, bathing, and industry needs. A number of water bodies receive a varied range of pollutants from point and/or non-point sources. Currently, the natural water systems are under serious stress throughout the globe (du Plessis 2017). The discharge of untreated or partially treated wastewater and floods into natural water systems causes' water pollution and raise huge concern worldwide. Water pollution occurs when harmful substances enter into receiving water bodies, being dissolved or suspended in the water or depositing to sediments. It can be any chemical, physical or biological agents, which change the quality of water that has a harmful effect on any living thing that drinks, uses for the different purpose, or lives in it (Gray 2010). Therefore, a better understanding of the natural and anthropogenic pollution threats is critical to introduce knowledge based management options and ultimately to the survival and quality of life of the human population.

There are various sources of water pollution but the two broad categories are point sources and nonpoint sources. Point sources are discharges of contaminants confined to a small area that includes industrial and municipal wastewater outfalls, individual septic tank discharges, and hazardous waste spills. Non-point sources of pollution include contaminant sources that are distributed over large areas or are a composite of many point sources, including runoff from agricultural operations, a fallout from the atmosphere, and urban runoff (Goel 2006; Krantzberg et al. 2010). In addition, wet weather discharges that result from precipitation events, such as rainfall and snowmelt, including storm water runoff, combined sewer overflows (CSOs), and wet weather sanitary sewer overflows (SSOs). Storm water runoff collects pollutants such as oil and grease, nutrients, metals, microbial pathogens, and other toxic substances as it travels across land. CSOs and wet weather SSOs contain a mixture of raw sewage, industrial wastewater, and storm water, and can result in beach pollution, shellfish bed contamination, and aesthetic problems (Gosset et al. 2016; Shao & Chu 2013). Generally, there are several types of water pollutants that decrease water quality and the major categories are: 1) microbial pathogens (disease-causing agents), 2) organic pollutants (oxygen-demanding wastes), 3) water soluble nutrients and agriculture runoff, 4) suspended solids and sediments, 5) water-soluble inorganic pollutants (acid, salts and metals), 6) water soluble radioactive compounds and 7) thermal pollution (Clark et al. 1989).

The link between pollutant discharges into natural waters, the resulting quality of receiving water systems, the formulation of health risk assessment tools, and creation of water quality criteria to maintain the water quality of a water body, with alterations in the physical, chemical, and biological condition is at the core of this risk study and water management issues. This study provides an overview of microbial water quality monitoring, modelling and integrating such information in order to estimate the public health risk. The study designed in response to the research need to the QMRA approaches, specifically in exposure assessment and risk characterization by addressing the challenges through water quality modelling that has the

potential to estimate health risks in different contexts and pathways, such as drinking water sources, recreational water and recycled water.

1.1. Water quality monitoring and modelling

Water quality monitoring in a given water body serves as a means of gathering information about water quality characteristics of a system. If the water system does not experience major temporal or spatial variation in quality, then only a minimal monitoring system would be required in order to characterize these changes. However, if the variation or the potential for variation exists in the system's water quality, the monitoring must be designed to capture that variability (Clark 2011). Moreover, water quality modelling in a given water system is a tool that can effectively complement system wide water quality monitoring (Beck 1987). These days, the potential for the integration of water quality monitoring and modelling into a decision support system, the QMRA framework is an important issue.

1.1.1. Water quality monitoring

Water quality is a term used to express the suitability of water to sustain various water uses and any a particular water use have certain requirements for the physical, chemical or biological characteristics of water. Although many water uses have some common requirements, each water use will have its own demands and influences on water quality (Bartram et al. 1996). Water quality can be introduced by quality indicators, which can be measured in the natural water bodies and in the discharged water. These indicators includes, temperature, pH, dissolved oxygen (DO), turbidity, conductivity, total organic carbon (TOC), microorganisms, chemical oxygen demand (COD), biochemical oxygen demand (BOD), metals and non-metals, organic compounds, suspended solids, salts, nutrients (phosphates, nitrogen compounds), etc. and each indicator is measured by its specific techniques of detection (Benedini & Tsakiris 2013). The principal reason for monitoring of water quality is the need to verify whether observed water quality is suitable for intended uses, which provides empirical evidence to support the decision making process on public health and environmental safety issues. In general, water quality monitoring (WQM) provides an understanding of water quality conditions in streams, rivers, groundwater and aquatic systems; how those conditions vary in space; whether conditions are changing over time; how natural features and human activities affect those conditions; and where those effects are most pronounced. Such evaluation is necessary in order to assess the attitude of a water body for the environmental enhancement or in view of a feasible process useful to abate a dangerous contamination (Ahuja 2013; Li & Migliaccio 2010).

Information about microbial water quality is the base for QMRA framework and a key factor for decision making regarding the microbial water quality for the different purpose. The important groups of pathogenic microorganisms (pathogen of concern) in the water system in relation to QMRA are bacteria, viruses, protozoa, and helminths. Regularly, microbial water quality has been monitored using faecal indicator bacteria (FIB) that serve as surrogates for the diverse array of pathogens, co-occur in human and animal faecal material and easily measured than pathogens or host associated bacteria. The most widely used FIB are faecal coliforms, *Escherichia coli*, and *Enterococcus* and the use of FIB as a surrogate are based on the assumption that their density is correlated with microbial pathogens. However, a number of studies have found that the

assumption is not always true (McQuaig et al. 2012; Noble & Fuhrman 2001). Furthermore, FIB can originate from the non-human faecal material, in which the pathogen to FIB ratio differs from that in humans (Ervin et al. 2013). Due to an increased understanding of the diversity of waterborne pathogens, including their sources, physiology, and ecology, the use of FIB may not be as universally indicator as was once thought. In addition, the survival of pathogenic bacteria, viruses, and protozoa in different environmental conditions raised serious questions about the suitability of the FIB as an indicator of the microbiological quality of water sources. Nevertheless, QMRA approaches based on FIB will still be required due to: 1) the difficulty to monitor all known waterborne pathogens directly, 2) pathogens usually occur in very low numbers and it is difficult to account their low numbers.

Currently, there is no unified method to include the collection and analysis of a water sample for all microbial pathogens of interest. The challenges of the detection methods include the physical variances between the major pathogen groups, low concentration of pathogens in a large volume of the water sample, and the presence of inhibitors from the sample (especially if it comes from polluted water) (Straub & Chandler 2003). The most important requirements for reliable analysis are the ones which take into account specificity, sensitivity, reproducibility of results, speed, automation and low cost (KOSTIĆ et al. 2011). Therefore, powerful, sensitive and reproducible diagnostic tools are required to monitor pathogen contamination in the water of interest, not only cultivable pathogens but also detect the occurrence of viable non-culturable microorganisms as well as the presence of pathogens on biofilms (Ramírez-Castillo et al. 2015).

1.1.2. Water quality modelling

A model is a simplified representation of a complex system that stands for the basic, most important and interesting aspects of that reality. Specifically, mathematical modelling attempts to describe a dynamic physical phenomenon by mathematical relationships by imitating the real system (Dooge 1992). The development of water quality modelling tools depends on the different purposes and rely on a number of various modelling techniques. The accuracy of the models depends on the understanding of processes that occurs in the environment and rely on an appropriate choice of mathematical equations that describe the phenomena properly. In the other hand, it depends on available data sets, namely results of the measurement of water quality indicators, on which the estimation of parameters depend on (Wang et al. 2013). In order to understand the present pollutant load in the water system as well as pollutant transport in the water and filter media, mathematical models can be considered as one of the best tools and with the help of these models, the response of the water system to different scenarios can also be predicted. Mathematical water quality models have various forms and they can be empirically derived from statistical relationships, developed based on the law of conservation of mass, or the combination of the two and each model group has its own strength and limitations. Mathematical models can be classified into a variety of groups based on their characteristics, such as whether they are (1) statistical (empirical) or processes-based (mechanistic), (2) deterministic or stochastic, and (3) analytical or numerical (Clark 2011; Ji 2017).

1.1.2.1. Statistical (empirical) models and process-based (mechanistic) models

Statistical (empirical) models are usually expressed in simple mathematical relationships derived by statistically fitting equations to the observed data. Empirical models are usually easy to use

and require minimal effort and data. The weakness of empirical models is that they tend to have large standard errors of prediction, particularly when there is no sufficient data for model calibration. Empirical models are most reliable when applied within the range of observations used to construct the model, however, extrapolation from the empirical data set is uncertain (Ji 2017). Different statistical water quality studies developed models that are applied in different water systems, such as correlation, regression models, trend assessment, extreme event analysis, and multivariate statistics (Antonopoulos et al. 2001; Chen & Liu 2015; Kim et al. 2005; Pejman et al. 2009; Towler et al. 2010). On the other hand, Process-based or mechanistic water quality models formulated by a set of equations that attempt to describe all the relevant processes or mechanisms in the desired water systems. These models are capable of addressing many more details of hydrodynamic and decay dynamics in the whole processes. Extrapolation from mechanistic models usually carries higher confidence than extrapolation using empirical models because mechanistic models usually have a better representation of the physics, chemistry, and biology features of the waterbody (Ji 2017). Mechanistic water quality models have been extensively applied to diverse water systems including rivers, lakes, wetlands, and estuaries (Arhonditsis et al. 2008; Chen & Sheng 2005; Robson et al. 2008). Such modelling is a fundamental tool to support the understanding of ecosystem structures and functions, in order to support management decision through hypothesis testing and prediction

1.1.2.2. Deterministic and stochastic models

Deterministic models are intended to represent physical processes, enabling a wide range of model applications that stochastic models are unable to address. The model does not contain random (stochastic) elements and each component of the input to the model is determined exactly by mathematical equations (Loucks et al. 2005). The behaviour of every variable is spatiotemporally determined by the governing equations and the initial states of the variables. Therefore, the model seeks to simulate the physical processes through cause and effect relationships and always produces the same response to the same input (Ji 2017; Obropta & Kardos 2007), whereas, a stochastic model encompasses random (stochastic) components and the model allows for random or probabilistic elements in the relationship between two or more variables. The model uses statistical patterns of a particular phenomenon and always produces a different response to the same input (Ji 2017). In addition, stochastic models are created only from the available data set without detailed knowledge of the underlying processes and focus on reproducing certain statistical features of a waterbody (Cox et al. 2015; HUANG & Morimoto). Unlike deterministic models, stochastic models cannot be extended to analyse alternate scenarios because the stochastic model is only applicable to the specific conditions and data set upon which the model is based (Parker et al. 2009). The alternative of a hybrid approach, which applies stochastic techniques in a deterministic framework, has great potential to reduce model uncertainty and error and can potentially exploit the advantages of each method. The hybrid modelling approaches demonstrated a strong ability to improve model predictions, challenge conventional assumptions, and simplify complex models (Obropta & Kardos 2007).

1.1.2.3. Analytical and numerical models

An analytical model is the exact mathematical solution to the differential equations describing the processes in a water system. Analytical water quality modelling in a water system involves

derivation and solution of the governing partial differential equation, which describes pollutant concentration change with time and space due to convection, dispersion, decay and the loading function. The models are often limited by the assumptions used to derive their solutions. Most models for water systems are often too complicated to obtain analytical solutions, and numerical techniques are essential to finding solutions to these models (Jha & Singh 2008; Mau et al. 1996). A numerical model is a discretized version of a set of mathematical equations, such as the continuity equation and momentum equations that describe processes in a waterbody. Numerical models can be classified in terms of their representations of space and time, such categories are (1) steady state or time dependent (dynamic) and (2) Zero, one, two, or three-dimensional. The temporal characteristics include whether the model is steady state (inputs and outputs are constant over time) or time dependent (dynamic) depending on the treatment of the time derivative in the governing equations. The spatial characteristics of numerical models include the number of dimensions simulated and the spatial resolution (Ji 2017).

1.2. Quantitative microbial risk assessment

Pathogenic microorganisms are shed into the environment and the water cycle by the infected hosts (human or animal) and transported to new hosts by the water cycle and these pathogens in some case grow in aquatic ecosystems (natural or man-made) and may infect humans that come into contact or use with this water. Monitoring of microorganisms in different environments are normally carried out and tell us about the level of microbial pathogens in a given environment; however, this alone does not tell us the level of health risk. Therefore, in order to manage the risk of waterborne disease transmission, knowledge about the nature of the pathogens, their potential growth, fate and transport in the water cycle, the routes of exposure to humans and the health effects that may result from this exposure in the human population, as well as the effect of potential mitigation measures is required (EPA 2010). The method that combines all this knowledge and allows the risk manager to use the best available scientific evidence as a basis for risk management decision is quantitative microbial risk assessment **(**QMRA) framework (Figure 1) (Haas et al. 1999; Medema 2012). QMRA involves the application of risk assessment principles to estimate the consequences of planned or actual exposure to infectious microorganisms and detailed description of the QMRA methodological steps are given in different documents (Benford 2001; Haas & Eisenberg 2001; Haas et al. 1999). QMRA can be used to estimate the health risk associated with drinking water, wastewater reuse, irrigation water, recreational waters, aquifer recharge, storms water contact etc. Furthermore, it also used to assess the effect of newly engineering control measures like UV disinfection, membrane filtrations, and newly emerging pathogens like Ebola (Bibby et al. 2016; Haas et al. 1999; Schönning et al. 2007; WHO 2006; WHO 2016).

QMRA framework served as a basis for the explanation of World Health Organization (WHO) guidelines for the safety of water systems (WHO 2016). It is applied worldwide to establish guiding principle and recommendations for the quality of different water types, such as drinking water, bathing water, recycled water, surface water, and ground water to ensure public safety (WHO 2004; WHO 2006; WHO 2009). Several QMRA studies have been conducted to understand the extent of microbial risk in water related exposure pathways (Benford 2001; Eregno et al. 2016; Fewtrell & Bartram 2001; Haas et al. 1999; Haas 2000; Medema et al. 2003; Sokolova et al. 2012;

Teunis et al. 1997). The QMRA results are evaluated relative to predetermined health targets value, which is a benchmark for a given water use, set by the regulator as part of their health policy. Health based targets area milestones to guide improvement towards water safety and public health goals (WHO 2004) and regulators are responsible for the enforcement of these targets and confirm the adequacy of existing systems or the need for improvement (Kamizoulis 2008). The most common types of health based targets are health outcome targets (tolerable burdens of disease), water quality targets (guideline values for microbial hazards), performance targets (log reductions of specific pathogens), and specified technology targets (application of defined treatment processes) (O'Toole et al. 2015).

Recently, WHO adapted the QMRA framework that harmonized across the previous guidelines to facilitate the consistent application of QMRA in the practice of water supply, water reuse and recreational water (WHO 2016). The approach involves problem formulation and hazard identification, exposure assessment, health effects assessment and risk characterization (Figure 1).

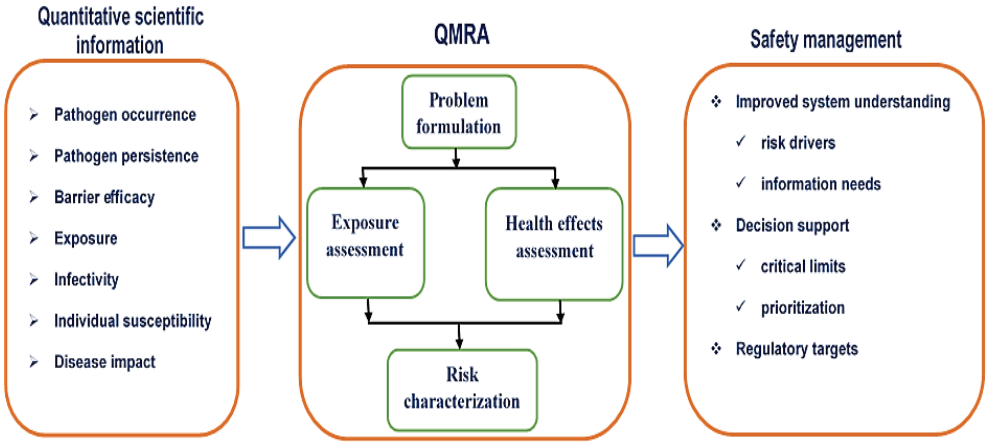

Figure 1. QMRA as a tool combining quantitative scientific information (Adapted from: WHO, 2016).

1.2.1. Problem formulation and hazard identification

Problem formulation is the initial phase of QMRA that the overall context of the risk assessment is defined in order to target the specific risk management question. The scope and the boundaries of the QMRA process are determined by answering key questions. Such as, what are the scope and purpose of the risk assessment? Which hazards, exposure pathways, hazardous events, and health outcomes should be included? What level of certainty is needed for risk management? (WHO 2016). Hazard identification involves the selection of water borne pathogen within the system boundaries that cause human illness, the type of illness caused, the identification of possible transmission routes and the significance of these routes. There is a wide range of pathogens that may be present in a given environment of interest. However, it is unrealistic to assess their presence and concentration, instead, it is common to use "index pathogens" or

"reference pathogens" that cover the behaviour of the wide range of microbial pathogens, considering the health risk of other known pathogens is adequately controlled by the system and that the system offers protection against unknown pathogens (Medema 2013). The commonly used reference pathogens are norovirus, rotavirus, adenovirus, *Cryptosporidium*, *Giardia lamblia*, *Campylobacter*, *Salmonella*, *E. coli* O57:H7 (Ferguson et al. 2008; Rosen et al. 2000; Soller et al. 2010; USEPA 2010). Besides, some research on QMRA has been carried out based on the combination of indicator organisms and reference pathogens (Eregno et al. 2016; Hamilton et al. 2006; Mara et al. 2007). However, the extrapolation procedure commonly used to transform indicator organisms to pathogens based on the predefined ratio is challenged by the weak correlation of indicator organisms and pathogens (Harwood et al. 2005; Williams & Ebel 2014).

The other important aspect in relation to microbial hazard identification is characterizing indicator organisms according to its specific origin, meaning distinguish between human and animal origin. This distinction is especially relevant because indicator organisms from the human origin are typically expected to pose a higher risk to public health than indicator organisms of animal origin. For this reason, microbial source tracking (MST) is a very noticeable tool in terms of identifying specific origin (Hagedorn et al. 2011).

1.2.2. Exposure assessment

The intention of the exposure assessment is to estimate the magnitude and frequency of exposure to each reference pathogen via the identified exposure pathways and hazardous events defined initially during problem formulation. During exposure assessment, the concentration of microbial pathogens is estimated with associated uncertainty. The estimation could contain the actual level of pathogens or the probability of occurrence derived from the data in a specified probability distribution. Therefore, exposure assessment should account for the variability of factors such as concentrations of microorganisms over time and in space, volumes ingested, etc. In addition, it can be considered as a single dose of pathogens that a consumer ingests at a certain point of time or the total amount of several exposures (Hagedorn et al. 2011). Normally, at this step, assessment perform on pathogen occurrence in source water, elimination of pathogens during treatment, the changes in microbial water quality during storage and distribution, consumption of water, and dose (exposure) estimation. Moreover, the size and nature of the population exposed the route of exposure (single or multiple), frequency, duration and the magnitude of exposure associated with the exposure routes are evaluated (Haas et al. 1999; Medema 2012; WHO 2016). The number of microbial pathogens that are swallowed (i.e. dose) during the time of exposure for a given pathway can be calculated using Equation 1:

$$D = C\ x\ 1/R\ x\ V \tag{1}$$

Where D is the dose (number of swallowed pathogens) per event, C is the concentration of pathogens in the water, R is the recovery efficiency of the enumeration method for the pathogen, and V is the volume of swallowed water per event (Hagedorn et al. 2011). Moreover, the component of exposure assessment, which is commonly performed at this step, is the assessment of microbial pollution based on a catchment survey, identifying the major sources of microbial contaminants in the catchment, and the conditions that may lead to peak events, such as heavy rainfall, season of manure application, or resuspension of sediments. In the absence of enough

information or in the case of practical difficulty, exposure assessment could potentially integrate with different modelling techniques in order to quantify microbial exposure dose (Medema 2012; WHO 2016).

1.2.3. Health effects assessment

Health effect assessment is the determination of the health outcomes associated with the level of exposure to waterborne microbial pathogens. The likelihood of an adverse health effect as a result of exposure to one or more pathogenic organisms is derived from a dose-response model (WHO 2016). Existing dose-response models have been obtained mainly from studies using healthy adult using a few strains of microbial pathogens are tested. Information about the effect of strain-to-strain variability and the influence of the immune response of the hosts is still missed. Several subgroups of the population, such as children, the elderly and a person with weakened immune systems, are more sensitive to infectious disease; currently, however, adequate data are lacking to account for this (Hagedorn et al. 2011). Homogeneously distributed microbial pathogens in water system are considered as the Poisson distributed and the probability of any microorganism to survive and start infection is the same and the dose-response relation simplifies as an exponential function (Equation 2). If, however, there is heterogeneity in the probability of individual microbial pathogen and the individual probabilities among pathogens (and hosts) are beta distributed that lead to the beta-Poisson dose–response relation (Equation 3) (Hagedorn et al. 2011). The dose-response relationships apply to a group of a certain type of pathogen, whereas the pathogen that was measured in the source water may only be a subpopulation (Hagedorn et al. 2011).

$$P_I = 1 - e^{-rD} \tag{2}$$

$$P_I = 1 - (1 + D/\beta)^{-\alpha} \tag{3}$$

Where P_I is the portion of a population experience a risk of infection, D is the dose, r is the parameter of exponential dose-response relation, and α and β are the parameters of the Beta distribution describing the variability of the pathogen survival in the host (Haas 2002).

1.2.4. Risk characterization

Risk characterization is the step that brings together all the information collected on pathogen exposure, dose-response relation, the severity etc. to generate a quantitative measure of risk. The key issues addressed in risk characterizations step are the expected health effects of the estimated dose, quantification of the risk, variability and uncertainty in the estimated risk, and sensitivity analysis. The process of risk characterization can be carried out in two ways, a point estimate (deterministic) or stochastic estimate. A point estimate of risk is when the exposure (i.e. the number of organisms ingested) combined with a point estimate of the dose-response parameters to compute the risk. This could be carried out using the best estimate, intended to obtain a measure of central tendency, or using an extreme estimate, intended to obtain a measure of consequence in some more adversely affected circumstance. Whereas, stochastic estimation of risk accounts for uncertainty, which allows the incorporation of variability that encompasses the characterization of the distribution of all data used for risk assessment, for instance, by Monte Carlo analysis. The important outcome of the risk characterization using a Monte Carlo approach

is the assessment of the relative contribution of uncertainty (the factors of imprecision and inaccuracy) and variability (intrinsic heterogeneity) to a risk estimate, that leads to differential risk among sectors of the exposed group. The risk characterisation consists of calculating the annual infection probability (P) that is linked to multiple exposures per person (Equation 4).

$$P = 1 - (1 - P_I)^n \quad (4)$$

Where P is the annual probability of infection, P_1 is the probability of infection for a single exposure of organisms and n is the frequency of exposure per year during which a person is exposed to a dose D of pathogenic agents (Asano 1998). Not all infected individuals will develop a clinical illness. The risk of illness per year for an individual is estimated using Equation 5.

$$P_{illness} = P\ x\ S\ x\ I \quad (5)$$

Where $P_{illness}$ is the annual probability of illness. P is the annual probability of infection, S *is* the proportion of the population susceptible to infection, and I is the proportion of individuals who develop symptomatic illness after infection. Infectious diseases typically have several possible health outcomes, ranging from acute self-limiting diseases to chronic disabilities or even death. These different outcomes can be combined in single composite measures such as the Disability Adjusted Life Years (DALYs), which translate the risk of illness per year for an individual to a disease burden to the community by combining information in morbidity and mortality within a time-based measure (Allotey et al. 2003) and DALYs can be calculated using Equation 6.

$$\text{DALY} = \text{YLL} + \text{YLD} \quad (6)$$

Where YLL is the years of life lost in a population due to premature mortality attributable to health condition *i*. Whereas, YLD is the healthy years of life lost in a population due to disability attributable to health condition *i*.

YLL is the summation of all fatal cases (d) due to the health outcomes (l) of a specific disease, each case multiplied by the expected individual life span (e) at the age of death (Equation 8).

$$\text{YLL} = \sum_I d_I\ x\ e_I \quad (8)$$

In order to estimate YLD for a particular cause in a particular time period, the number of incident cases in that period is multiplied by the average duration of the disease and a weight factor that reflects the severity of the disease on a scale from 0 (perfect health) to 1 (dead) (Gore et al. 2011; Murray 1994; WHO 2013) (Equation 7).

$$\text{YLD} = \sum_I n_I\ x\ t_I\ x\ W_I \quad (7)$$

The YLD is the product of the duration of the illness (t) and the disability weight (w) of a specific disease, the number of incidence cases (n) and all health outcomes (l).

1.3. QMRA and drinking water sources

Source water quality fluctuates with time and environmental conditions, such variability can have a major impact on the efficiency of drinking water treatment plants (Dechesne & Soyeux 2007). The concentrations of microbial pathogens and faecal indicators in streams and rivers are higher during the periods of rainfall-induced runoff compared to those seen simply during dry

weather conditions. Rainfall mobilises and transports non-point source of microbial pathogens via runoff and in some case, the increased flow during rainfall leads to resuspension of contaminants in the water body. Usually, heavy rainfall is the most common cause of peak pollution events, associated with high surface runoff and discharge of untreated wastewater, which may lead to high pathogen loads in the source water (Signor et al. 2007). Different epidemiologic studies have linked waterborne disease outbreaks with the periods of high precipitation (Curriero et al. 2001; Naumova et al. 2005; Rose et al. 2001). Wastewater treatment plants are also one of the sources of microbial pathogens in terms of both concentration and strain, and during the periods of high rainfall or plant failure, wastewater treatment plants (WWTP) may release significant amounts of poorly treated effluent. Moreover, sources of faecal contamination that might be a threat to drinking water sources may include agricultural practices (the use of sewage sludge as fertilizer) (Dechesne & Soyeux 2007; Signor et al. 2005; Signor et al. 2007).

Given a series of events and different activates that contribute to the release of the microbial pathogen from a single catchment, the concentration of microbial pathogens vary considerably over time. Measuring and describing this variation is an important input for QMRA model and offers a means to estimate the health risks to the consumers of treated drinking water by considering source water quality is an integral part of QMRA (Signor et al. 2007). Usually, the variation in the source water quality influences the requirements for treatment and the resulting health risk associated with the finished water. However, water supply QMRA applications have rarely explored the effects of hazardous events, such as periods of peak source water contamination or treatment failures (Signor et al. 2005). Microbial water quality monitoring of the source water is the key for QMRA application in the water supply system. However, the collection of water samples to monitor microbiological water quality, particularly to detect particular pathogens directly, is difficult, time-consuming, and expensive compared with methods to measure other water quality parameters. Since these pathogens tend to be found in very low concentrations in the source water, and there are quite a lot different pathogens, it is problematic to monitor them directly; also, pathogens are shed into the water stream inconsistently. For these reasons, direct monitoring for microbial pathogens is nearly impossible. Instead of monitoring for pathogens, "indicator" organisms, species whose presence in the water suggests that faecal contamination may have occurred are utilized. The four indicators most commonly used are total coliforms, faecal coliforms, *Escherichia coli*, and intestinal enterococci that are normally prevalent in the intestines and faeces of warm blooded animals, including humans, wildlife, farm animals and pets (de Brauwere et al. 2014; Field & Samadpour 2007).

Given the complex hydrologic dynamics of source water catchments and interactions between different activities and the natural phenomena, models may help to understand catchment dynamics and evaluate the extent of pollution, contamination scenarios, and may support best environmental practices for water safety management (Schijven et al. 2015). Moreover, a catchment model can be implemented as a tool for investigating water quality and for the communication between parties with different interests in the catchment area (Oliver et al. 2016; Schijven et al. 2015). Depend on the model structure; some models need detailed catchment survey to develop an overview of the contaminant source, fate and transport, and to understand

the contributing factors to water contamination in the catchment area. It should include details on the catchment (size, water intake, water uses, etc.) and its hydrology, hydrogeology and climate, a description of the potential sources of faecal contamination. On the other hand, some stochastics model utilizes time series water quality data generated in drinking water treatment plant (DWTP), which can be also used to characterize the source water catchment (Eregno et al. 2014).

Few pieces of research undertaken on the application of QMRA for drinking water systems by integrating water quality modelling with QMRA frameworks, which deals about the occurrence of indicator organisms and pathogens at the raw water intake at DWTP to evaluate the risk for drinking water consumption (Åström et al. 2007). Some research were conducted on the QMRA for a drinking water production chain from surface water to potable water (Schijven et al. 2011) and to quantify the impact of upstream loading events on the health risks for drinking water consumers using hydrodynamic modelling (Sokolova et al. 2015). However, there are knowledge gaps in current microbial transport and fate models in terms of capturing the spatiotemporal variation of microbial pathogen concentration, and the ability of the environment to buffer pathogen transport into waterways among others to sharpen QMRA for drinking water supply system.

1.4. QMRA and coastal recreational water

Microbial water quality is best described by both microbial water quality assessment and some modelling results. This approach provides information on the actual level of faecal pollution, on possible sources of pollution in a recreational water catchment, as well as the trend of pollution. Actually, the sources of recreational coastal water pollution include sewage, surface runoff, farming activities, industrial processes, domestic animals, and wildlife. Consequently, recreational water can be exposed to a range of disease causing microorganisms, including those naturally present in water. In order to improve the safety of bather's and introduced stricter standards for water quality, recreational water standard has been established based on epidemiology studies that have linked swimming associated illnesses with faecal indicator bacteria (FIB) densities. The theory for the establishment of recreational water quality criteria (RWQC) is those studies based on gastrointestinal (GI) illness rates in a population of bathers, who were engaged in recreational activities at a given beach, and comparison with a similar population at the same beach, who were not exposed to the water (Fujioka et al. 2015). The beach sites impacted by point sources of human faecal pollution showed increasing incidences of GI illness among bathers, who were exposed to waters with increasing concentrations of FIB. Whereas in beaches contaminated with non-point sources of FIB, no correlation exists between FIB densities and GI illness. (Calderon et al. 1991; Colford et al. 2012). Therefore, the specific source of FIB determines the health risks to bathers. In this case, it is better to use molecular methods to determine the specific source of FIB using microbial source tracking technology and combining it with QMRA (Ferguson & Signoretto 2011; Harwood et al. 2014).

The QMRA provides credible scientific analysis that can be used in conjunction with or, at times, instead of epidemiological investigations to assess the risk to human health at recreational water use. QMRA can also be applied on recreational coastal water as a complimentary for epidemiological studies in order to understand health risks of bathers, to establish recreational

water standards, and to support beach management decisions (Soller et al. 2015). QMRA studies support the findings from the epidemiology in relation to the different results between point source and non-point source of pollution in recreational water, and as viruses seem to be the pathogens of concern, human sewage inputs carry the greatest risk of infection, and infection risks seem to be greater after rainfall (Fewtrell & Kay 2015). However, the application is constrained by the lack of specific water quality data for many pathogens and the fact that the concentration of pathogen, as opposed to indicator organisms, vary according to the prevalence of specific pathogens in the contributing population and may exhibit seasonal trends (WHO 2009).

Temporal and spatial variability of the concentrations of pathogens and FIB in the recreational water considerably reflects the complexities of the dispersion processes associated with varying pollutant sources (Boehm 2007). In addition to variability in the hydrology at coastal sites, there is a particular concern at the shallow recreational water where sediment resuspension may release microbial pathogens and raise public health risk (Davies et al. 1995). The dynamics of recreational water microbiology is difficult to capture with few number of samples, similarly, not feasible to collect large sample numbers with the short time interval (Ashbolt et al. 2010). Consequently, the QMRA studies at recreational beaches are encountered by a range of uncertainties in relation to the variation of microbial contaminants in space and at the different time. However, hydrodynamics modelling appear as critical controls of dilution and export a way for estimating the dispersion of pollutants in recreational beaches (Ashbolt et al. 2010). Currently, defining the relationships between reference pathogens and indicators organisms, the quantification of pathogens using culture or PCR endpoint assays, fate and transport of FIB and pathogens in seawater are the major knowledge gaps that need to be addressed concerning QMRA application for recreational water (Harris et al. 2004).

1.5. QMRA and water recycling

Population increase, water quality deterioration, groundwater depletion, severe drought, and climate change along with an overall desire to achieve greater water sustainability have increased the demand for alternative water sources (Asano 2002). Many countries have been continuously seeking alternative water resources including the capture and use of rainwater, storm water, recycled water, and desalinated water. Compared to the other alternative water resources, recycled water could potentially provide constant water supply throughout the year, with great human benefits (Anderson et al. 2001; Huertas et al. 2008). With increasing knowledge and understanding on the advantages of recycled water, different wastewater treatment technologies have been invented in different countries with a various degree of pollutant removal efficiency. The term "water recycling" in this study involves the treatment of wastewater and redirecting the effluent into the water use scheme. As a general principle, wastewater or any marginal quality waters can be used for any purpose as long as adequate treatment is provided to meet the water quality requirements for the intended use (Figure 2). Reclaimed water can be suitable for a large variety of applications. In general, the most common reuse applications can be categorized as (1) agricultural irrigation, (2) landscape irrigation, (3) groundwater recharge, (4) industrial reuse, (5) environmental and recreational uses, (6) non-potable urban uses, and (7) indirect or direct potable reuse (Asano 2002).

As water reuse becomes an increasing interest, it is important to guarantee the safety, acceptability and reliability of recycled water for human health and the environment (Rose 2007). One important means of safeguarding the public health is to ensure that contaminants should remove up to the required levels in the treated wastewater. This means that there is a need to control the treatment processes until it constantly meets the required treatment level (Asano 2002). In addition to regulatory approaches, QMRA has been applied to provide a more rigorous assessment of health risks associated with various water reuse applications (O'Toole et al. 2007; Olivieri et al. 2014). In this regard, the advantage of QMRA is that it can estimate very low levels of risk. However, there are major sources uncertainty, which is difficult to estimate directly due to limitations of available data (Robillot et al. 2016).

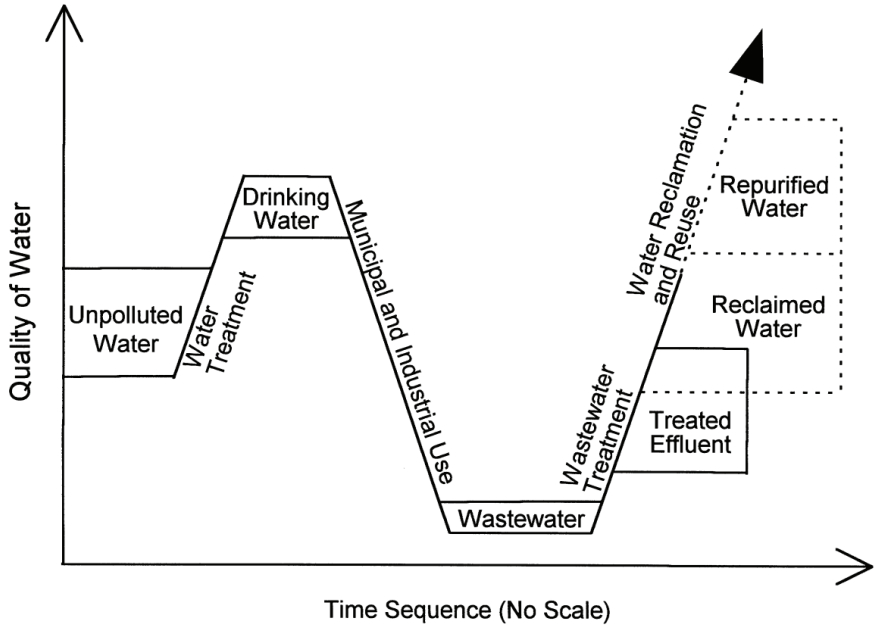

Figure 2. Water quality changes during uses of water in a time sequence. From (Asano, 2002)

Infectious diseases caused by pathogenic bacteria, viruses and parasites (e.g., protozoa and helminths) are the most common and widespread health risk associated with wastewater reuse. Among the different microbial pathogens, enteric viruses and protozoa have received the most attention because of their low dose infectivity, long-term survival in the environment, monitoring difficulties, and the limited extent of removal and inactivation that occurs in most of wastewater treatment systems (Robillot et al. 2016). A higher degree of treatment is required to produce reclaimed water that is virtually pathogen free to use for irrigation of crops that are consumed uncooked, or when reclaimed water is used for irrigation where most likely have frequent human contact (Olivieri et al. 2014). In addition, groundwater recharge with reclaimed water (water reuse

via aquifer) and direct non-potable water reuse (toilet flash) share many of the public health concerns. However, despite variations in treatment technologies, environmental buffers, aquifer have a significant impact in terms of removing most of the microbial pathogens (Rodriguez et al. 2009).

The degree of treatment and the associated water quality monitoring requirements tend to be related to the type of wastewater and the specific water reuse application (Asano 2002). The source of wastewater can be from previous uses, such as greywater, municipal wastewater, or industrial effluents. Each source of recycled water has its own characteristics and constituents, in which the concentration of chemical pollutants or the number of microbial pathogens varies significantly. Thus, recycled water from different wastewater origins poses different risk levels to human health and the environment (Chen et al. 2013; Toze 2006). Among the different wastewaters, greywater is relatively less polluted and low concentration of microbial pathogens, nitrogen, suspended solids, and turbidity compared with other sources of recycled water and it can be efficiently reused for toilet flushing, landscape and garden irrigation, recreational impoundments watering, clothes washing, as well as fire protection (Eriksson et al. 2002). Many water recycling schemes use wastewater treatment plants (WWTPs) as a common treatment process. However, WWTPs are now frequently required to produce high-quality water (alone or in conjunction with other treatment steps) that can be recycled for a variety of water recycling schemes (Robillot et al. 2016). Therefore, different treatment technologies are required to reach the required treatment level. A number of studies that address the knowledge gaps and contribute toward quantifying the health risk associated with water reuse (Ayuso-Gabella et al. 2011; Beaudequin et al. 2016; Hamilton et al. 2005; Hamilton et al. 2006; Page et al. 2010).

2. Objectives of the Study

The aim of this study is to integrate water quality modelling with quantitative microbial risk assessment (QMRA) framework. QMRA intensively uses site-specific water quality data to determine the need for different treatment barriers and to evaluate the impact to the overall health risk. Microbial water quality data is extremely useful, however, it is not always easy to collect with a required space and time dimensions, instead water quality modelling, process-based (mechanistic) models and/or statistical (empirical) models can be applied to answer questions regarding the current level of pollution as well as the future potential pollutants in the water system. Water quality modelling might be used in addition to or instead of monitoring for the reasons that modelling might be feasible in some situations where monitoring is not. The integration of microbial water quality monitoring and modelling could provide better information than one or the other alone. Currently, the progress in computational methods and technology has supported the development of advanced mathematical models for microbial water modelling in the different water systems.

The overall objective of this study is to strengthen QMRA framework through water quality modelling approach with regard to quantifying the microbial load in the water systems of interest focusing on drinking water source, recreational water, and recycled water pathways. The specific objective of this study stated with respect to each of the appended papers:

- To demonstrate the use of microbial water quality data set available at drinking water treatment plant as an input for statistically based water quality modelling to support QMRA through evaluating the effectiveness of watershed management strategies and understanding the probabilistic behaviour of extreme events. (**Paper I**)

- To evaluate the influence of processes (rainfall, discharge from boats, and wind directions) in microbial water quality at the recreational beaches and to demonstrate the importance of hydrodynamic modelling as a tool to predict the spreading of microbial contaminants and support beach management decisions. (**Paper II**)

- To develop integrated QMRA approach that combines hydrodynamic modelling of recreational water to estimate the public health risk associated with bathing after a rainfall event. (**Paper III**)

- To estimate the decay rates of viruses and FIB in seawater, to investigate the growth potential of FIB in greywater system, and to provide information about the concentration of FIB in different types of wastewater as an input for QMRA framework. (**Paper IV**)

- To assess the efficiency of an integrated greywater treatment and reuse scheme, and to evaluate the health risk associated with the production and consumption of lettuce through QMRA and a chemical health risk assessment (CHRA) approach. (**Paper V**)

- To identify efficient treated greywater disposal system in removing nutrients, virus and bacteria as a post treatment step, and to evaluate the removal efficiency of both unsaturated and saturated flow conditions in order to incorporate in the computation of safest setback distance between water sources and treated greywater disposal site. (**Paper VI**)

3. Rationale and Overview of the Thesis

Thorough and careful risk analysis is essential for making well-versed decisions in order to minimize the public health risk from different infectious waterborne diseases. However, risk analysis methods and procedures are challenged by multiple sources of uncertainty and lack of appropriate information. Water harmonized QMRA framework is a growing multidisciplinary field that combines scientific knowledge about the occurrence of microbial pathogens, their fate and transport in the water systems, the routes of exposure to human being and the different health effects that may result from this exposure, along with the effect of natural and engineered barriers and hygiene measures (WHO 2016). Since QMRA is a young risk analysis approach, the methodology used to examine the occurrence and concentration of the microbial pathogen, and their fate and transport in the water systems are potentially challenging with substantial implications on the magnitude of estimated health risk. While there are a number of QMRA studies in different environment, there is a scant research on the method of assessment about the spatiotemporal spreading of microbial pathogens under different environmental conditions, their trends and probabilistic behaviour of extreme events. This study designed in response to the research need to fuel QMRA approaches with the necessary advance specifically in exposure assessment and risk characterization by addressing such challenges.

Waterborne pathogens are transmitted to human through drinking contaminated water, ingesting contaminated water during bathing, in the preparation of food, or the consumption of food that is infected. Considering the most common pathways, the study was designed to focus on applying a QMRA framework to a drinking water source (Paper I), recreational water (Paper II, III, and IV), and recycled water (Paper V and VI). The QMRA framework encompasses four basic elements as an assessment steps mainly targeted to risk management issues, and with regard to different steps of QMRA framework, the role of individual papers are illustrated in Figure 3.

There are different opportunities for application of QMRA, and the microbial water quality data set collected from the raw water at drinking water treatment plant is one of the potential sources of information readily available to use as an input for QMRA. The application of statistical tools on such data set could potentially provide information about the pollution status of the source water and the behaviour of extreme microbial load events; at the same time, it could be possible to integrate such information with QMRA approaches. The first part of the study focuses on the exploration of valuable information on the microbial water quality data (time series) of drinking water source at DWTP through statistical analysis (**Paper I**). The statistically based water quality modelling study of the drinking water source from Glomma River provides information about the trend of changes, magnitude and frequency of extreme microbial load; it could be an input for exposure assessment step of QMRA.

The investigation of different processes (rainfall, discharge from boats, and wind directions) on the spreading of microbial contaminants in the recreational beaches using hydrodynamic modelling could also be an input for exposure assessment step in QMRA (**Paper II**). Considering the importance of hydrodynamic modelling, the study investigates the spreading of microbial pollutant using *E. coli* as an indicator in order to understand the recreational water quality under

the different condition as well as to make use of the simulated result in QMRA framework. The study that combines discharge-based hydrodynamic modelling with QMRA framework in the context of bathing water quality after rainfall event evaluated the public health risk in order to support beach management decisions (**Paper III**). This study combined data from the hydrodynamic model simulation, monitored data, and microbial pathogens from secondary data sources. The study fitted with exposure assessment, risk characterization, and risk management steps of QMRA framework. Information about the concentration, growth and decay rates of enteric microorganisms in different wastewater systems and water bodies are crucial for the fate and transport modelling of microbial pathogens and an input for exposure assessment step of QMRA. However, little information is available about the decay rates of faecal bacteria and viruses in the marine environment where many wastewater treatment plants discharge their effluent. To solve such challenges, one of the studies focused on the investigation of the decay rates of FIB and pathogenic viruses in seawater from different depths (**Paper IV**).

Water reclamation, recycling and reuse is currently a vital component of integrated water resource management, however, associated health risk has been debatable in the different part of the world. With regard to this problem, the third part of the study was designed to assess the efficiency of an integrated greywater treatment system, and evaluate the health risk associated with treated greywater reuse for irrigation (**Paper V**). The health risk assessment associated with treated greywater reuse for hydroponic lettuce production and consumption considered as exposure assessment, risk characterization, and risk management steps of QMRA framework. One of the principal objectives of wastewater treatment is to discharge the treated wastewater without causing danger; however, the degree of treatment may not be sufficient and raise concern for the contamination of source water. In order to understand the effectiveness of treated greywater disposal system in removing virus and bacteria and to evaluate removal efficiency of both unsaturated and saturated flow conditions, **Paper VI** was designed. The assessment of treated greywater disposal system in removing virus and bacteria as a post treatment step was evaluated in both unsaturated and saturated flow conditions and the result could provide information for the decision of safest setback distance between treated wastewater disposal site and drinking water sources.

Overall, the goal of this body of research was to enrich the QMRA approach through the assessment of fate and transport of microbial pathogens in the identified pathways of interest (drinking water source and recreational water) using statistically, and processes-based water quality modelling techniques coupled with the QMRA framework. Moreover, this study addressed the health risk issues associated with water reuse and treated greywater disposal systems. Both studies could give valuable insights about the effect of source separation, greywater treatment, and disposal systems.

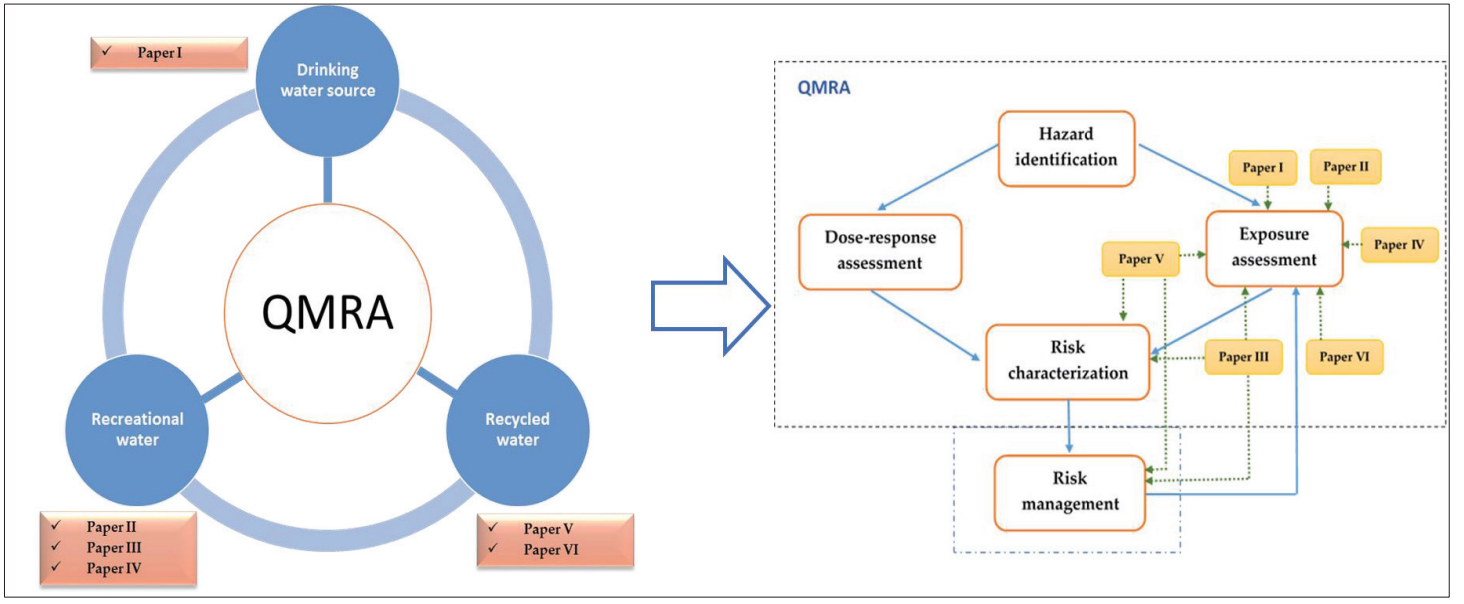

Figure 3. Conceptual framework of the study in relation to QMRA framework

4. Research Methodology

4.1. The study sites

Paper I was based on the water quality data set at Nedre Romerike Vannverk (NRV) drinking water treatment plant in Norway. NRV draws raw water from river Glomma, the largest river in Norway, with a catchment area of 41,200 km^2 (13 % of the total area of the country). The Northwest part of the river's watershed is dominated by high mountains, while the Eastern part is covered by forest (Figure 4). The central and southern parts of the watershed are covered by large agricultural areas, which constitute 5.8 % of the total catchment area. The Glomma river basin contains Lake Mjøsa, the largest lake in Norway, with a surface area of 350 km^2. The average annual flow of the river at Solbergfoss (the lower most reservoir) is 700 m^3/s. The discharge usually varies from 150 m^3/s to 3500 m^3/s during the year. Approximately 675,000 inhabitants live in the catchment area (Grizzetti B. et al. 2007).

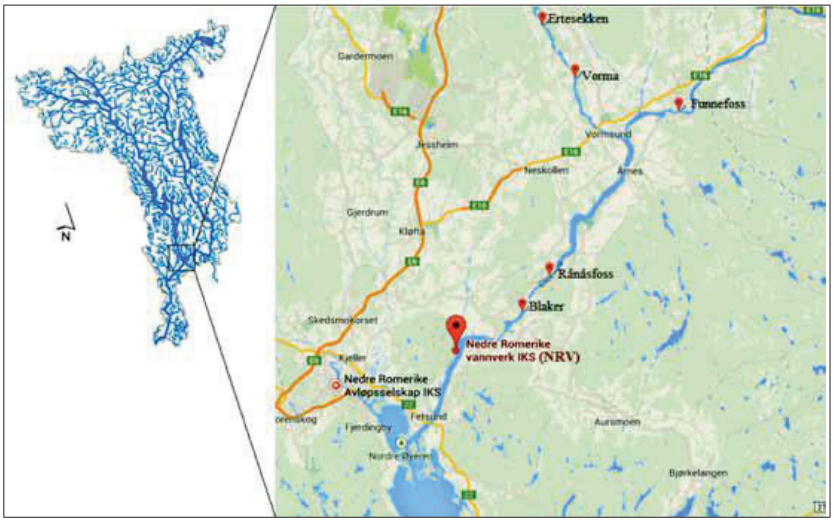

Figure 4. Glomma River basin and the NRV treatment plant .

Papers II and **III** were carried out on bathing water quality in Oslo fjord, located in Bærum municipality adjacent to Sandvika town, which is situated approximately 15 km west of Oslo, Norway (Figure 5). Six coastal beaches were selected and these are: A) Kadettangen-north, which is the extension of Kadettangen main beach close to the highway (59°53'20" N, 10°31'49" E). B) Kadettangen-main beach, which is the busiest beach as compared to the other beaches during the warm summer period (59°53'14"N, 10°31'54" E). C) Kalvøya-small beach is located close to the mouth of Sandvikselva river where it enters into the fjord, which is located at (59°53'5" N, 10°32'7" E). D) Kalvøya-big beach (59°52'58" N, 10°32'11" E) is located further south from Kalvøya small beach and is influenced by the storm flood of the river Sandvikselva. E) Kalvøya-nudist beach (59°53'22" N, 10°33'2" E), which is located on the other side of the Kalvøya island. F) Høvikodden

beach (59°53′16″ N, 10°33′10″ E) located close to where private boats are moored. Two combined municipal sewer systems (about 4000 and 1500 inhabitants connected) are located approximately 500 m from Høvikodden beach and overflow at around 6 m deep into the ocean during heavy rainfall events.

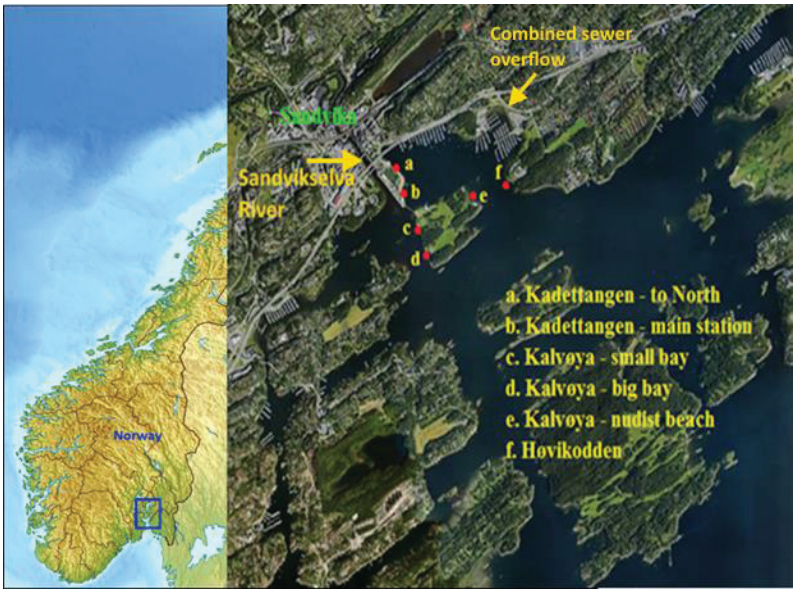

Figure 5. The study area that shows the position of the beaches in the Oslo fjord . From **Paper II**

Paper IV was microbial decay rate experiment based on seawater sample collected from Norwegian Institute for water research (NIVA) research facility at Solbergstrand in Drøbak (59°36′56.7′′N and 10°39′10.1′′E), Oslo fjord, while untreated wastewater was concurrently collected from the inlet of municipal WWTP in Drøbak (12 000 persons connected). **Papers V** and **VI** were based on an experimental setup for on greywater recycling. Source separation system was established at Norwegian University of Life Sciences (NMBU) student dormitory in Kaya, serving 48 students. The greywater system collects wastewater from washbasins, showers, kitchen sink, and laundry whereas the blackwater system, collect toilet waste separately. Both systems pumped into the REALTEK water laboratory (fløy 4) in a separate pipeline and the greywater system used for both experiments (**Paper V** & **Paper VI**).

4.2. Data collection and analysis

Paper I: To evaluate the microbial water quality trend and to understand the probabilistic behaviour of an extreme microbial load of the drinking water source, the time series data on indicator microorganisms, at drinking water plant was utilized. The data was based on weekly records of five indicator microorganisms in the NRV raw water source namely, heterotrophic plate count (HPC), *Clostridium perfringens (C. perfringens)*, intestinal enterococci (IE), *Escherichia coli (E. coli)*, and total coliforms (TC). The data for *E.coli* and coliform bacteria were from 1999 to

2013, for intestinal enterococci from 2002 to 2013, and for HPC and *C.perfringens* from 2005 to 2013. The physicochemical water quality parameters, temperature, pH, electrical conductivity, turbidity, river discharge, and colour were also utilized.

Paper II: In order to understand the influence of different processes (rainfall, discharge from boats, and wind directions) on the microbial water quality of the recreational beaches, hydrodynamic modelling, using *E. coli* as an indicator was performed. The input data for the GEMSS hydrodynamic model calibration and simulation were obtained from different sources. Meteorological and hydrological data were collected from the Norwegian meteorological institute (eKlima), the Norwegian marine data centre, Bærum municipality and direct observation. The data used for this modelling include air temperature, dew point temperature, sea temperature, wind direction, wind speed, solar radiation, cloud cover, salinity, wave height, discharge rate (flow rate) and *E. coli* concentration in the river and combined sewer overflow (CSO). The main discharge came from the Sandvikselva River and two CSO sites. The *E. coli* concentration and the corresponding water flows were monitored in the Sandviksilva River before, during and after the rainfall. Moreover, the two CSOs discharged directly into the Oslo fjord were recorded on-tile based and the time of overflow was converted into volume in order to use as input for the model, assuming that 50% of the sewage discharged in this period. In this study, the impact of boat discharge and wind direction on bathing water quality was investigated based on scenarios. The boat discharge scenario was if a single boat with 200-litre toilet tank discharges its sewage at 300 meters from the nearest beach according to the regulation of boat sewage discharge in the Oslo fjord (*E.coli* concentration 3 x 10^7 MPN/100 ml, equivalent to one day-production of *E. coli* from four persons). Moreover, the wind direction scenario was developed by adjusting the wind input data set, by tuning all winds into one direction using the average wind speed for all.

Paper III: The study considered the heavy rainfall event in the Sandvika area, specifically the rainfall event of 7 July 2014, which utilized the same hydrodynamic modelling of **Paper** II. In order to simulate for the rainfall event, *E. coli* concentration in the beach water was analysed for four consecutive days after rainfall event to evaluate the model performance. The simulated *E. coli* concentrations at each beach were transformed into the concentration of pathogens (Norovirus, *Campylobacter*, *Salmonella, Giardia* and *Cryptosporidium*) based on the concentration of reference pathogens and *E. coli* in the sewer system from the previous studies (Grøndahl-Rosado et al. 2014; Langeland 1982; Myrmel, M. et al. 2015; Robertson & Gjerde 2006). However, the concentration of *Campylobacter* was estimated based on the epidemiological data from the Norwegian Surveillance System for Communicable Diseases (MSIS).

Paper IV: For the decay rate experiment, the concentration of FIB and microbial pathogens, namely total coliforms, *E. coli*, intestinal enterococci, adenovirus (adv40), and MS2 were analysed. Seawater samples were characterized by their physicochemical parameters using standard methods. These parameters were pH, conductivity, salinity, total organic carbon, turbidity, zooplankton and algae. Zooplankton and algae were analysed by microscopy and heterotrophic plate count (HPC) was analysed by the spread plate method. Total coliforms and *E. coli* were quantified using a most probable number method (MPN) with Colilert-18 (IDEXX). IE was quantified after membrane filtration using the ISO 7899-2 method. The results were given as

colony forming units (cfu). Adenovirus (Adv40) was detected using primers and probe Brilliant III Ultra-Fast qPCR Master Mix (Agilent Technologies). In order to detect MS2, RT-qPCR was performed using the RNA UltraSense™ One-Step Quantitative RT-PCR System kit (Invitrogen, USA) and primers, probe and RT-qPCR conditions. In addition, the abundance of FIB in greywater, blackwater and combined municipal wastewater were monitored to quantify the concentration in this wastewater system.

Paper V: With the intention to investigate the health risk associated with the recycling of greywater for hydroponic lettuce production, information about effluent water quality, microbial contamination of the edible part of the plant and bioaccumulation of heavy metals in the plant tissue were analysed. Water samples were collected every two weeks from raw greywater, biofilter system effluent, filtration column effluent (green wall), and circulated irrigation water. The water samples were analysed for total phosphorus (P) and total nitrogen (N) using spectrophotometric test kits (Hach-Lange); total coliforms (TC) and *E. coli* were quantified using the most probable number method (MPN) with Colilert-18 (IDEXX). In addition, water samples were collected from the same position to analyse heavy metals by using inductively coupled plasma mass spectrometry (ICP-MS). Seven to ten replicates of the lettuce plant, from each treatment plots, were collected to evaluate the plant growth status, the surface area of a fresh leaf, fresh and dry mass of the leaf, fresh and dry mass of the plant, fresh and dry mass of the root system, and number of leaves per plant. Heavy metal bioaccumulation in the plant tissue was analysed using inductively coupled plasma mass spectrometry (ICP-MS). In addition, the plant tissue was examined for microbial assay, 25 g of composite lettuce sample from each treatment plots were collected and put into stomacher plastic bags containing 225 ml sterile buffered peptone water (0.1%), homogenized by using a stomacher for 1 minute. *E. coli* were enumerated from the homogenised supernatant using the most probable number method (MPN) with Colilert-18 (IDEXX).

Paper VI: In order to study the virus, bacteria and nutrients removal efficiency of treated greywater disposal system, both unsaturated and saturated flow media were constructed and the physicochemical properties of experimental columns and trench were characterized. The particle size distribution of the filter media on a weighted base was analysed in triplicate from each media by standard operating procedure, LS 13 320 Laser Diffraction Particle Size Analyser (Fraunhofer.rf780d optical model, Beckman Coulter, Inc. USA). Besides, the transport of conservative tracer in the unsaturated filtration media and saturated trench were examined using sodium chloride (NaCl). The breakthrough of NaCl was monitored in the form of electrical conductivity (μS/cm) using EC meter. The effluent was analysed for physicochemical parameters using standard methods: pH, EC, total phosphorus (P), total nitrogen (N) using spectrophotometric test kits (Hach-Lange, Berlin, Germany). Total suspended solids (TSS) were determined with 1.2 μm glass fiber filters (Whatman GF-C, GE Healthcare, and Little Chalfont, UK) and turbidity was measured with light scattering. The indicator microbial pollutants considered in the study were bacteria (total coliforms and *Escherichia coli)* and model virus (*Salmonella typhimurium* phage 28B (St28B)). The St28B was propagated using a host culture of *S. enterica Typhimurium* type 5. The St28B enumeration was carried out using a double-layer agar plaque assay. First, Petri dishes with 20 ml solid bottom-agar (growth medium with 1.5 % w/v

agar) were prepared. Then, 0.5 ml sample (after serial dilution in 0.9 % NaCl, if needed), 0.5 ml exponential growth-phase host culture, and 4 ml molten top-agar (growth medium with 0.65 % w/v agar), were mixed and poured over the solid agar in the petri dishes. Finally, samples were incubated at 37 °C for 18 hours and plaques were counted. Enumeration of total TC and *E. coli* was performed using Colilert-18 with Quanti-Tray/2000 (IDEXX Laboratories, USA) using the most probable number method (MPN) according to ISO 9308-2:2012.

4.3. Methodological approach

Monitoring of microbial water quality is the preferred form of information for QMRA. However, microbial water quality modelling might be feasible in some situations where monitoring is not, specifically if we need to conduct QMRA for beach sites when monitoring cannot handle the dynamic of microbial pollutants after rainfall event (Spatio-temporal spreading of microbial pollutants are very high). Moreover, modelling can be used to evaluate the trend of water quality parameters resulting from different management strategies and also can be used to predict the probabilistic behaviour of extreme microbial loads. In some cases, integrating both monitoring and modelling results of microbial water quality could provide better information than one or the other alone. Considering the above-mentioned advantages of microbial water quality modelling, and the challenges of information gaps about microbial water quality in the QMRA framework, this study was designed to apply microbial water quality modelling on different water use and to integrate that data within the QMRA framework. The overall intention was to improve the QMRA framework, focussing on three different water uses (pathways), through which humans are exposed to waterborne pathogens. The study gives special attention to QMRA framework in relation to source drinking water, recreation coastal water, and water recycling and reuse pathways.

The first study emphasis on drinking water source (**Paper I**), based on microbial water quality time series data at DWTP, statistical models were developed using Principal Components Analysis (PCA), Trend analysis and extreme event analysis. PCA is a multivariate statistical method that reduces the dimensionality of the dataset by performing a covariance analysis between factors, and this reduction is achieved by transforming the data set into a new set of variables called the principal components (Abdi & Williams 2010). It is important statistical analysis to identify the important physicochemical factors that affect the microbial quality of raw water source. Trend analysis determines whether the measured values of a water quality variable increase or decrease over time. Therefore, Mann-Kendall test, Seasonal Mann-Kendall test, Sen's Slope Estimator, and linear regression were used to analyses the trend. In addition, the most commonly used statistical approaches to estimating recurrence probabilities of extreme events, which is based on the tail distribution of the underlying variable was applied (Coles et al. 2001). Peak-over Threshold (POT) method is one of extreme value analysis technique that considers all values exceeding a certain predefined threshold, leads to the Poisson process model for threshold exceedances. Generalized Pareto distribution (GPD) used to estimate the extreme quantiles. From the GPD, the return level is derived by setting the cumulative distribution function equal to the anticipated quintile, (1–p), and then solving for the return level (McNeil 1999). Based on the principle of POT methods, the return levels of five indicator microorganisms corresponding to the selected return periods were estimated. Normally, the application of the POT method with

the intention of calculating the probability of incidence of extreme events involves the following three steps (1) select the threshold u value. (2) Fit the GP distribution function to the exceedances over threshold data, and then estimate the shape and scale parameters. (3) Compute the return level of the extreme event using estimated parameters.

The second component of this study emphasis on recreation coastal water quality in relation to QMRA (**Paper II, Paper III, and Paper IV**). In **Paper II**, the GEMSS hydrodynamic model was applied to explore the spreading of microbial pollutant, *E. coli* as an indicator, along with the Sandvika beaches. The GEMSS (Generalized Environmental Modeling System for Surface waters), which is an integrated system of 3D hydrodynamic and transport models embedded in a geographic information and environmental data system (Kolluru 1994). The model is able to simulate horizontal and vertical distributions of water velocities and temperature, salinity, water surface elevations, and water quality in rivers, lakes, reservoir, estuaries, and coastal water bodies at different spatiotemporal resolution (ENTRIX et al. 2001; Kolluru et al. 2014). The model was calibrated by making minor adjustments to the Chezy friction coefficient by comparing the observed and computed salinity level. The validated by comparing observed *E. coli* concentration against modelled values. Afterwards, the simulation was conducted for three conditions: 1) after a rainfall event, 2) boat discharge scenario, 3) wind direction scenarios. Microbial contamination of the recreational beaches was studied after rainfall events during summer 2014, and the scenarios were used to separately assess the impact of boat sewage discharge and wind directions on the recreational beaches water quality.

Paper III considered only the simulation of heavy rainfall event in the Sandvika area on 7 July 2014. The purpose of this study was to integrate hydrodynamic modelling of bathing water quality with QMRA framework. Model simulation result of *E. coli* concentration transform into microbial pathogen (Norovirus, *Campylobacter*, *Salmonella, Giardia* and *Cryptosporidium*) concentration using *E. coli* to pathogen ratio in the sewer system, and then evaluate the health risk within QMRA framework. Sensitivity analysis was performed to investigate the variation in the output that can be apportioned quantitatively to the different source of variation in the input. The variables considered in the sensitivity analysis were pathogen concentration, the human water ingestion rate in the recreational beaches during swimming, and related to the dose-response model parameters (α and β parameters in the case of beta-Poisson model and r for the exponential model).

Microbial decay rate experiment (**Paper IV**) was designed to model the decay rate of FIB and microbial pathogens, namely TC, *E. coli*, IE, adenovirus (adv40), and MS2 in surface and deep seawater (Table 1). According to the design, the concentrations were monitored initially, after 2 days and 5 days. Then, the decay rate computed from linear regression of log-transformed data, which is commonly being used to model decay coefficient. The model describes inactivation with time and the concentration time relationship, equation (9)

$$C = C_o\, e^{-kt} \tag{9}$$

Where C is the concentration of organism at time t, C_o is initial concentration of organisms at time zero, t is the independent variable time (day) and k the decay rate coefficient.

In addition to decay experiment, the abundance of FIB in greywater, blackwater and combined municipal wastewater systems were monitored. Also, the growth and reduction of FIB in greywater were investigated by incubating the water samples in the dark at 4 °C and 20 °C (three replicates of each) and TC, *E. coli*, and IE were enumerated after 0, 24, 48, and 98 hours.

Table 1. The design of decay rate experiment

Microbes	Sea water	Treatment	Replicates	Time interval for monitoring
Adenovirus Bacteriophage MS2 *E.coli* Intestinal enterococci total coliforms	Surface (1m) & deep (60m)	4°C and 20°C	3 for each	Initial (day-0), after 2 days, and after 5 days

Water recycling and reuse in relation to QMRA was investigated by considering two issues, the health risk of treated greywater reuse for irrigation (**Paper V**) and the removal efficiency of treated greywater disposal system (**Paper VI**). In **Paper V**, source separated greywater was first treated with a package greywater treatment plant (biofilter system), which encompasses a sequence of a primary settler, an unsaturated fixed-film biofilter, and a secondary clarifier. Furthermore, the effluent from the biofilter system was treated by an infiltration system. Three filtration columns (2.5 m in height and 31.5 cm in diameter) as a part of a green wall were constructed to treat the effluent from the greywater treatment plant (GWTP). The filtration columns constructed with three layers, the 1 m bottom layer is 0.8–1.6 mm diameter filtralite, the 0.3 m in the middle consists of granular activated carbon, and the 1.1 m on top of the activated carbon is 2-4 mm diameter filtralite. The top 10 cm is air space used to feed the water uniformly from the top of the column by using nozzles. The columns were run in parallel with similar intermittent loading rates of 2 min followed by a rest period of 8 h, and with a daily loading rate of 0.58 $m^3 m^{-2} d^{-1}$. The effluent from the filtration columns was collected in the bucket at the bottom of the column and used to grow lettuce hydroponically by adding human urine, which was stored for three months, as a nutrient solution. The plantation pots were mounted on the green wall's shelves and irrigated with the treated greywater from the buckets by using small submersible pumps for circulation.

Three lettuce varieties, namely: (a) Lactuca sativa 'Lobjoits Green Cos'; (b) Lactuca sativa 'Red Salad Bowl' and (c) Lactuca sativa 'Australische Gele', were planted for this study, and mounted on the top, middle, and bottom shelves respectively. The first green wall column's effluent mixed with 0.3% urine for the first three weeks and then increased to 0.6% until harvesting time. The second column's effluent mixed with 0.15% of urine for the first three weeks and then increased to 0.3% until harvesting time. The third column's effluent directly irrigated from the treated greywater without urine (Figure 6). Each mix batch was circulated every 30 min using small-submerged pumps controlled by a programmable logic controller (PLC). The desired urine concentration was added into the two columns every three days. The lettuce growth analysis was performed using a plant growth index to describe the performance of the plant growth under this

experimental setup. These plant growth indexes used were (a) Specific leaf area (SLA), (b) Leaf weight ratio (LWR), (c) Leaf area ratio (LAR), and (d) Root-shoot ratio.

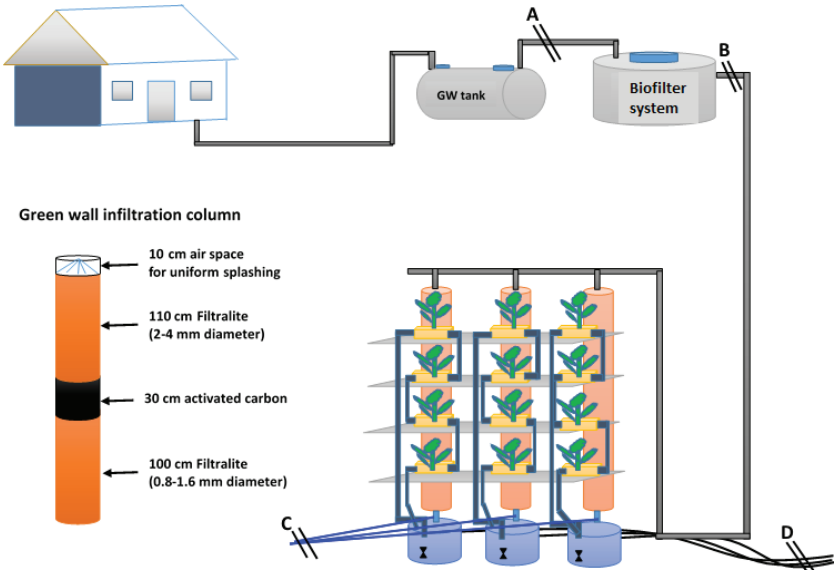

Figure 6. Greywater treatment steps, green wall and lettuce production configuration . From **Paper V**

The main exposure pathway considered in this study were operational activities in relation to lettuce production and raw lettuce consumption. Both quantitative microbial risk assessment (QMRA) and chemical health risk assessment (CHRA) approaches were applied to evaluate the health risk. The health risk was computed based on the cumulative greywater treatment efficiency, the volume of routine and accidental ingestion of treated greywater during operational activities, and the volume of lettuce consumption. The probability of infection per event was computed based on the dose-response relationship and then the annual risk of infection and illness was further calculated by estimating the frequency of exposure per year. CHRA was evaluated via targeted hazard quotient (THQ), which is a ratio between heavy metal concentration and the oral reference dose, weighted by the duration and frequency of exposure, intake rate, and body weight. $THQ < 1$ indicates that the exposed population to heavy metals through lettuce consumption is unlikely to experience visible adverse health effects. *THQ* values between 1 and 5 consider the exposed population to be at a certain level of health risk concern (Hope & Stock 1998).

Paper VI: Unsaturated infiltration systems are regularly used for the disposal of on-site treated wastewater. With the intention to investigate the treatment efficiency of soil infiltration systems as a post treatment step, four different stratified infiltration columns (Column- A, B, C, and D) with two replicate were constructed (Figure 7). Furthermore, the effluent from the A-columns (A1 & A2) pumped into the saturated trench (Figure 8), which was constructed with compacted till soil that mimics saturated ground water flow conditions. The focus of this study was to

investigate microbial pathogens and nutrient removal efficiency of different infiltration materials under unsaturated flow conditions and further evaluate the removal efficiency with saturated flow condition. The effluent from on-site greywater treatment plant (biofilter system) was pumped into infiltration columns using peristaltic pumps, which was synchronized with the biofilter system dosing-pump controlled by the level switch in the primary settling tank and the timer gives the plus interval. The actual flow rate of the peristaltic pumps were 2.5 l h^{-1} with the daily load variation from 37.5 l d^{-1} to 44.8 l d^{-1} depending on the resting time of the treatment plant, which was fluctuating from 6 to 9 hours a day, resulting in surface loading rate of 132 to 158 l m^{-2} d^{-2}. The columns were loaded using a plastic tube with inner diameter 6 mm and placed at the centre of the column 7.5 cm deep from the top layer of either filtralite in the case of column-A, B, and D or crushed stone in the case of column-C. On the other hand, the saturated flow trench was designed to receive an effluent from unsaturated column A, with the hydraulic load of 11.5 l/day (80 l/m^2 day). An average saturated cross-sectional area of the saturated trench was 0.142 m^2 and the computed water flux was 0.08 $m^3m^{-2}d^{-1}$. The water sample was withdrawn through the tube at 0.5m, 1.5m, 2.5, 3.5, 4.5, and 5m distance.

The pollutants that considered in this study was bacteria (TC and *E. coli),* model virus (St28B), and nutrients. The removal efficiency of both unsaturated disposal system and saturated flow conditions were evaluated under normal condition and with rainfall situations. The rainfall simulation in unsaturated columns experimental was to test two conditions 1) the detachment of virus particles from the infiltration media when virus shedding followed by rainfall after three days (rainfall simulation run for one hour). 2) Virus removal efficiency of infiltration media when both virus shedding and rainfall occurred simultaneously at the same time (rainfall simulation run for 17 hours).

The $\log_{10}$ reduction of TC, *E. coli*, and St28B in the infiltration media, between the concentration of column influent and effluent, was calculated as Log_{10} reduction = - Log_{10} (C/C_o), where C is column effluent concentration, C_o is column influent concentration, and the negative sign is to make the reduction positive. Moreover, a one-way analysis of variance (ANOVA) with Tukey's post hoc test was used to examine whether $\log_{10}$ reduction of TC, *E. coli*, and St28B in the effluent of representative columns are varied significantly with the infiltration system. The independent variable represented the four different type of infiltration system, A, B, C, and D columns. The dependent variable was the $\log_{10}$ reduction of TC, *E. coli*, and St28B. An alpha level of 0.05 used to determine statistical significance for all analyses. All statistical analyses were performed using Minitab 17 statistical software (State College, PA: Minitab, Inc.).

Moreover, the spatial and temporal removal rates of microbial suspension in saturated flow condition were modelled. The observed TC, *E. coli* and St28B were decreasing with respect to a certain flow length, x, and over time, t, in a saturated filter media due to the filter effect, expressed as a filter coefficient, β (m^{-1} or day^{-1}). The removal of microorganisms through porous media can be described by exponential temporal/spatial reduction equation (Equation 10) (Merkli 1975) and (Equation 11) (Iwasaki et al. 1937).

$$\mathrm{C}/Co = \lambda_o \, e^{-\beta t} \tag{10}$$

$$\mathrm{C}/Co = \lambda_o \, e^{-\beta x} \tag{11}$$

Where C is the concentration of a microbial suspension at time t and at flow length x, C_o is the initial concentration of a microbial suspension, β is filter removal coefficient, t is time and x is flow length. The model uses time-dependent removal coefficient of saturated filter media $\beta(t)$ and flow distance-dependent removal coefficient of saturated filter media or filter efficiency factor $\beta(x)$ (Matthess et al. 1988). The model fitted to the experimental data using least squares algorithms to obtain λ_o, and β.

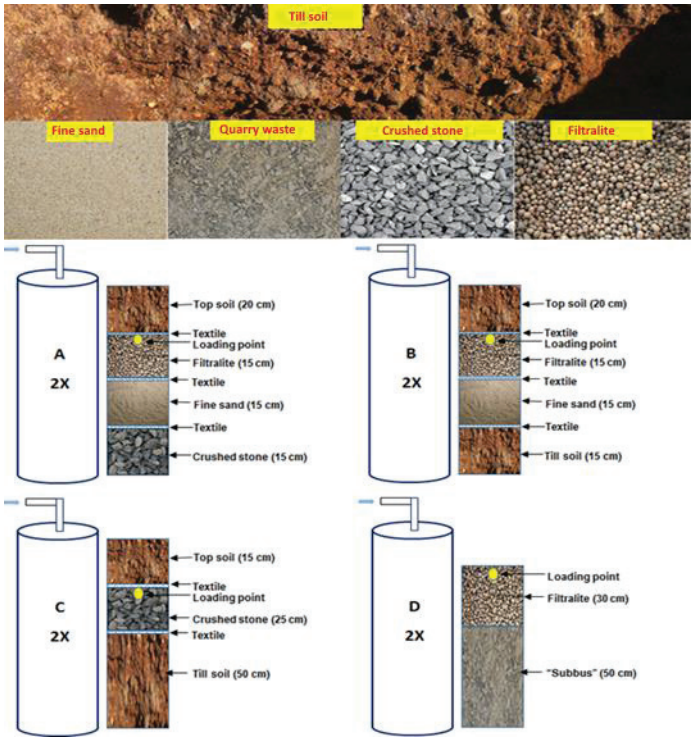

Figure 7. The infiltration media and the cros-sectional view of the columns . From **Paper VI**

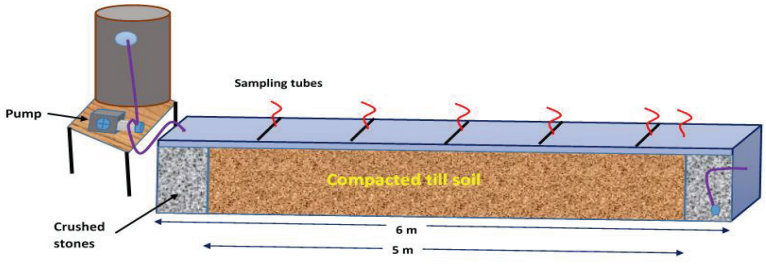

Figure 8. The sketch of saturated flow experimental trench . From **Paper VI**

5. Results and Discussion

5.1. Drinking water source

Preferably, QMRA could be based on quantitative data about the exposure of consumers to microbial pathogens. However, monitoring of pathogens in the source water is impracticable due to the difficulty in their detection and random distribution (Field & Samadpour 2007). As an alternative, faecal indicators are used by DWTPs to monitor microbial water quality. In this regards, the trend and extreme microbial load analysis using a time series data from the drinking water source through statistical tools (**Paper I**) could provide valuable information for a holistic approach to drinking water quality management that provides a comprehensive and preventive strategy from drinking water source catchment to consumer (Rizak et al. 2003).

5.1.1. The trend and extreme load of FIB in a drinking water source (Paper I)

Most of the drinking water treatment plants (DWTP) monitor microbial raw water quality (HPC, *Clostridium perfringens*, intestinal enterococci, *Escherichia coli*, and coliform bacteria) within a certain time interval (on a weekly basis) by taking a sample from the inlet in Norway. As a result, substantial time series data in a chronological order is available at DWTP and trend analysis could potentially determine whether the water quality deteriorate or improve within the time interval. Moreover, statistical extreme event analysis could provide information about the probability of extreme microbial load incidences. Therefore, it could give a good opportunity to provide quantitative information for QMRA framework, which suffers from lack of quantitative data about microbial water quality and extreme events.

The dataset was also treated using Principal Component Analysis (PCA) to extract the parameters that are most important in assessing variation in water quality. Thus, the PCA result indicated that factor 1 (F1), the coefficients of pH and EC are relatively notable, and F1 can be classified as a mineral/chemical related water quality factor. In Factor 2 (F2), the coefficients of river discharge and temperature are notable, and this factor can be a weather related factor. In Factor 3 (F3), the coefficients of turbidity and colour are significant and F3 can be the organic matter related factor. The result from the Mann-Kendall test of monthly mean microbial water quality data, the Kendall tau statistics for HPC, *C. perfringens*, IE, *E. coli*, and coliform bacteria were -0.135, −0.195, −0.015, 0.006, and -0.041 respectively. All indicators except *E. coli* have a negative trend and only *E. coli* shows a slightly positive trend. From all trend test, a statistically significant negative trend was observed in the case of *C.perfringens* ($p<0.05$). Similarly, the trend analysis result of the monthly maximum microbial load of drinking water source showed the negative trend for HPC and *C. perfringens* and a positive trend for IE, *E. coli*, and coliform bacteria. However, no trends were statistically significant. The parametric trend analysis, using linear regression, for seasonal trends of microbial water quality revealed a positive trend for all indicator microorganisms during the summer season (June-August). However, this relationship was not statistically significant ($p>0.05$). During winter, spring and autumn seasons, the microbial load showed a decreasing trend. The results also show that for *C.perfringens* during autumn (September-November) and for intestinal enterococci during spring (March-May), the raw water microbial load is significantly decreased ($p<0.05$).

Extreme microbial load events of the water source at DWTP are rare, but important for microbial risk analysis. The quantification of extreme quantiles using extreme event analysis could provide information as an input for QMRA framework. However, little work has been done for extremes in microbial water quality variables. In this study, extreme microbial load events were analyzed using the POT method to estimate return levels of five indicator microorganisms corresponding to selected return periods. From the GPD, the return level was derived by setting the cumulative distribution function equal to the anticipated quintile, and the computed return levels were illustrated in Figure 9. This study demonstrates the application of extreme value theory on a microbial water quality data set in order to provide valuable information for QMRA framework.

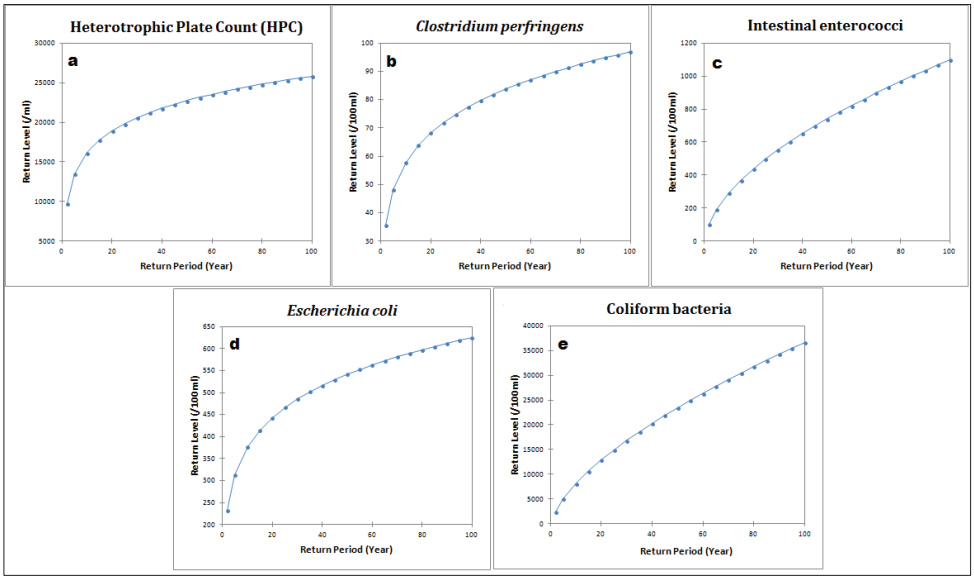

Figure 9. Return level plots for different indicator microorganisms . From **Paper I**

5.2. Recreational coastal water

Recreational coastal waters along the beaches can be polluted by runoff after rainfall event that carries a point and non-point source of microbial pollutants via surface waterways. Moreover, sewage discharge from the boats also releases microbial pollutants significantly. Varieties of illnesses have been associated with exposure to polluted recreational coastal waters and assessing the public health risk with QMRA framework is very important for risk management strategies. In this study, three connected research issues were involved, aiming for the improvement of QMRA framework on recreational coastal water. The first research themes were investigated the spatiotemporal spreading of microbial pollutants by using hydrodynamic modelling (**Paper II**). The second theme emphasized on the integration of hydrodynamic model results with QMRA framework (**Paper III**). The third research issue focused on the investigation of information,

which is crucial for fate and transport modelling of microbial pollutants and the QMRA modelling in relation to recreational water (**Paper IV**).

5.2.1. Hydrodynamic modelling of microbial recreational water quality (Paper II)

The hydrodynamic modelling was developed to understand the influence of rainfall, discharge from boats, and wind directions on the microbial pollution at the recreational beaches. The influence of rainfall was modelled based on the actual observation of rainfall episode, whereas the impact of boat discharge and wind directions were scenario based. During model calibration, a good agreement was observed between detected and computed values of salinity. In addition, the model validation was carried out based on the statistical comparison of the observed and the corresponding model simulation of *E. coli* concentration. Thus, the Nash-Sutcliffe coefficient was 0.36 and the relative volume of error was 8.4 %, which was less than 10% suggesting a reasonably close agreement between observed and simulated *E. coli* concentration. The dispersion of *E. coli* from the point sources into the beach areas were simulated for the consecutive three days after the rainfall event and the daily average *E. coli* concentration was plotted for each decay rate coefficient as shown in Figure 10. The result shows that the daily average *E. coli* concentration one day after the rainfall event was much higher than the second and the third days at all beaches and the magnitude difference was highest in the case of Kalvøya small beach followed by Kalvøya big and Kadettangen beaches. In addition, the impact of the microbial decay rate coefficient on the daily average *E. coli* concentration was substantial.

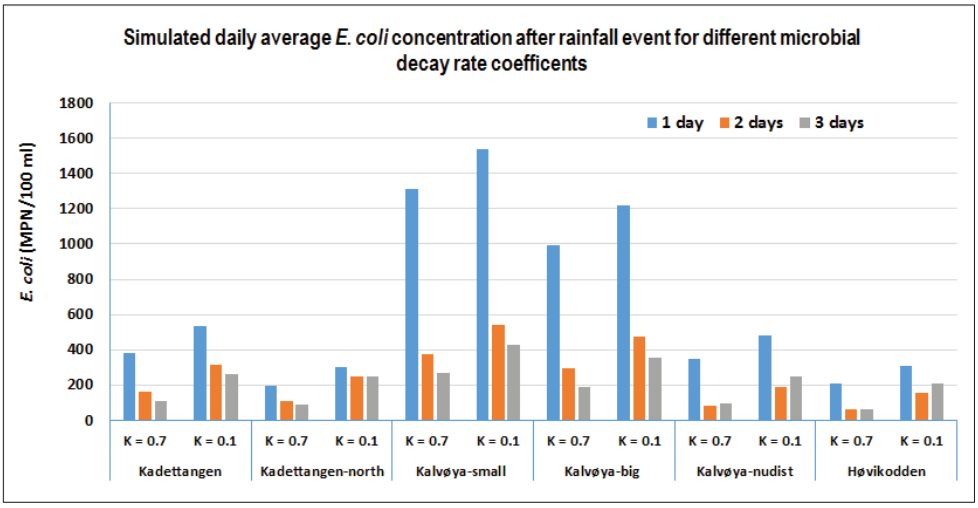

Figure 10. Simulated daily average *E.coli* concentration . From **Paper II**

The results of model simulation on the dispersion of *E. coli* at the beach sites for the top 1-meter depth shown in Figure 11. The first three hours after the heavy rainfall event, the *E. coli* dispersion was limited only to the surrounding area of Sandvikselva River and the river mouth. However,

after 24 hours of the rainfall event, the dispersion of *E. coli* increased and covered a wide area, and then the concentration was gradually reduced until the end of the third day.

In addition to the rainfall effect, the boat sewage discharge will also one of the threats of the pollution of coastal beaches. As the number of boats and beach users increases through time in Oslo fjord, the pressure on the water quality along the coastal beaches increased and it needs proper protection measures. The simulation result based on the boat discharge scenario showed that the discharge of 200-liter sewage with 3×10^7 *E. coli* per 100 ml could affect the nearby beaches by relatively low levels of *E. coli* (20-24 MPN/100 ml). Moreover, we may also predict that if there was a person infected with a high virulence pathogen on the boat, such a discharge will cause a substantial health risk on the bathers.

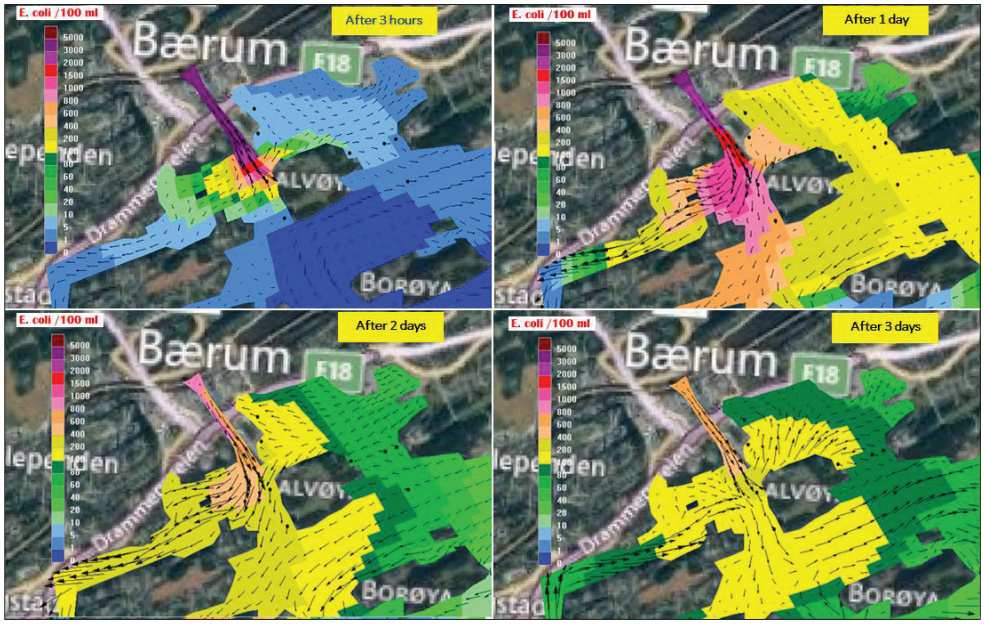

Figure 11. The top layer simulation showing the spreading of *E. coli* (K=0.7) . From **Paper II**

The key driving force for the spreading of microbial pollutant along the recreational coastal beaches is the current driven by winds or influenced by the input of fresh water from the rivers, and tidal motions. Different wind directions could have various impacts on the spreading of *E. coli* in the study area, for example, wind blowing to the west direction affected Kadettangen beach more than Høvikodden and Kalvøya nudist beach. Whereas the wind blows to the south, in the same direction as the pollution source flow, was the predominant wind direction that affected all the beaches sites. These simulations result demonstrated that the effect of wind direction highly depends on the location of the beaches relative to the main pollution source, and in this case, the river Sandvikselva was the main source of pollution.

5.2.2. Integrating hydrodynamic water quality modelling with QMRA (Paper III)

The model simulation of *E. coli* concentrations was utilized to calculate the concentration of reference pathogens based on the ratio between *E. coli* and pathogens concentration in the Norwegian sewer systems (from the previous studies). The exposure dose of pathogens was computed assuming that the ingestion rate of water is different while swimming between adult and non-adult as it mentioned in different studies. Then the health risk on bathers after rainfall for three consecutive days were computed by integrating dose-response model and exposure dose. The estimated health risk (Figure 12) in this study indicated that the probability of being infected by *Giardia, Cryptosporidium, Salmonella, and Campylobacter* after the rainfall event was below 19 per 1000 bathers (0.019) at all beaches, which is considered as an acceptable risk of gastrointestinal illness after recreational activities (WHO 2003). However, this study indicates that viral infection constitutes the main risk from exposure to bathing water after heavy rainfall. Therefore, further studies are needed regarding the determination of the infectivity of viruses to assess the risk more precisely.

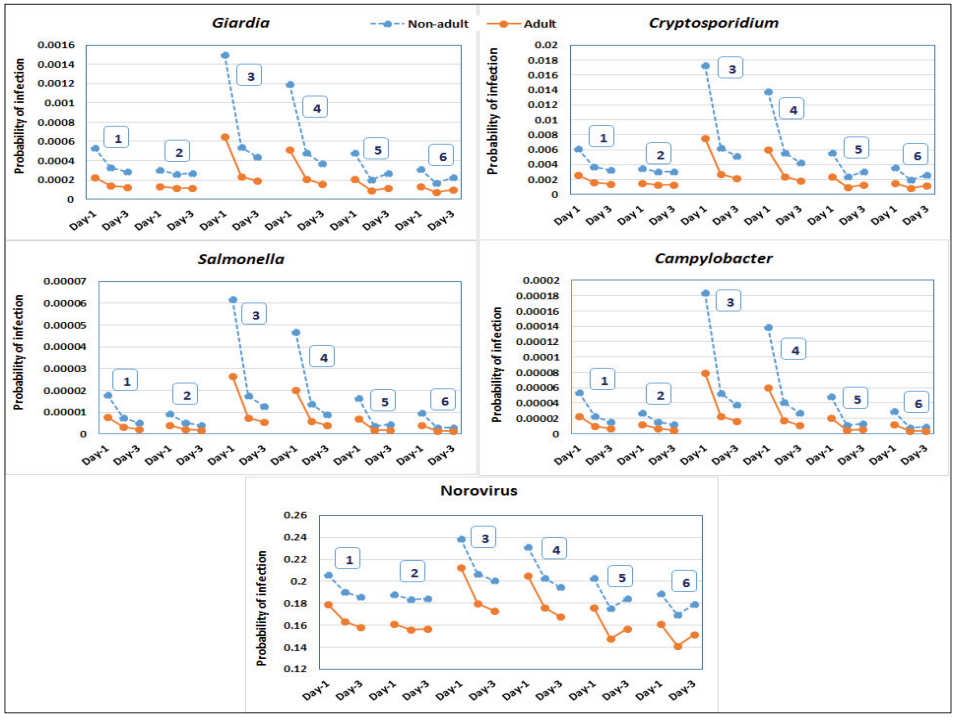

Figure 12. The average risk of infection : 1. Kadettangen, 2. Kadettangen-North, 3. Kalvøya-small, 4. Kalvøya-big, 5. Kalvøya-nudist, 6. Høvikodden. From **Paper III**

The risk of infection for a single exposure of pathogens during bathing was higher for non-adult relative to an adult because of a higher volume of water ingested. Moreover, the highest risk was

observed at the Kalvøya small and Kalvøya big beaches the first day after the rainfall event due to the location of the beaches where the incoming flood from Sandvikselva River directly affected the two beaches relatively more than the others. The probability of infection on the first day after the rainfall event was higher than on the second day. The risk of exposure to norovirus was high at all beaches since 100 % of the estimated virus concentration was considered to represent infective virus particles. However, the proportion of infective virus could be much lesser.

A discussion of uncertainties is an important part of the risk assessment that could help to evaluate the implications and limitations of the risk assessment and to estimate the degree of confidence that could be placed in the risk estimate. The QMRA modelling contains different input variables that could be a potential source of uncertainty in the output. Since we used models, model uncertainty expected as a potential source of uncertainty. In this study, the main sources of uncertainty can be classified into three categories; 1) Factors associated with the estimation of *E. coli* concentration at the beaches, which is the result of the GEMSS hydrodynamic modelling. 2) Factors associated with the volume of water intake in the recreational beaches during swimming. 3) Factors related to the dose-response model parameters (α and β parameters in the case of beta-Poisson model and r for the exponential model). Furthermore, it is important to notice that, in this hydrodynamic modelling, the input factors are associated with biological and environmental issues that are characterized by huge natural variability and, a lack of substantial knowledge. In relation to this, a sensitivity analysis was performed to investigate how variation in the output can be apportioned quantitatively to a different source of variation in the input. To visualize the change of health outcome (probability of infection) in response to different input values, computed values of norovirus at Kadettangen beach were plotted as an example by considering a change in the input values: volume of water ingested, pathogen concentration, and dose-response model parameter value (Figure 13).

5.2.3. Decay rate of virus and FIB in seawater (Paper IV)

The abundance of FIB in wastewater system, the growth potential of FIB in greywater, and the decay rate of virus and FIB in seawater were investigated in order to directly or indirectly utilize the information in the QMRA model. The results of this study indicated that blackwater contained considerably higher numbers of IE than the combined municipal wastewater and greywater, but only slightly higher numbers of *E. coli* and TC. The mean concentrations of *E. coli* and TC in the greywater was 6.8 and 6.1 $\log_{10}$ MPN/100 ml for TC and *E. coli* respectively, which is relatively high as compared with other studies (Ottoson & Stenström 2003). Moreover, all wastewater systems were analysed for the faecal sterol coprostanol and gene markers of human related *Bacteroides*, to check whether the high numbers of *E. coli* and TC in the greywater was a result of regrowth in the collection system or due to faecal contamination. The result of this study revealed that the level of coprostanol and gene markers of human related *Bacteroides* in greywater was less than or equal to 0.02% of the level in blackwater. The higher greywater to blackwater ratio of *E. coli* than of *Bacteroides* may indicate the regrowth of *E. coli* in the greywater collection system than faecal contamination. Moreover, the growth potential experimental result indicated that TC and *E. coli* had a tendency of further growth in the greywater for the first 24 h at 20 ^{o}C, but the concentration was abruptly reduced thereafter, whereas no growth was observed in the case of IE at 20 ^{o}C. None of the indicator bacteria showed any further growth at 4 ^{o}C.

Microbial decay rate experiment was performed to address the scarcity of information for decay rates of FIB and viruses at different depths of the marine environment where many wastewater treatment plants discharge their effluent. The reduction of virus and FIB was in general highest in surface seawater at 20 °C. During the five days experiment, adenovirus (PCR U/ml) was reduced by > 2 Log_{10} at 20 °C, but nearly no reduction at 4 °C. Similarly, infective adenovirus (FFU/ml) exhibited the same tendency as adenovirus (PCR U/ml) except the magnitude difference. Also, the concentration of infective MS2 (PFU/ml) virus at 4 °C in both seawaters (surface and deep) indicated an increasing pattern, however, at 20 °C a decreasing pattern observed. Moreover, the concentration of MS2 (RT-qPCR) was not reduced with a similar pattern as adenovirus (PCR U/ml) at 20 °C. In the case of FIB in surface seawater, TC, *E. coli*, and IE decreased by 1.5, 2.5, and 2.3 log_{10} at 20 °C, in the 5 days of experiment respectively and the reduction was slightly lower in deep seawater. The experiment results also shown that at 20 °C, the concentration of FIB slightly reduced in the first 2 days and then followed by 1-2 log_{10} reduction in the next 3 days. There was a large variation between the number of infectious virus and total virus particles, this indicates that analysis based on (RT)-qPCR may significantly overestimate the concentration of infectious virus.

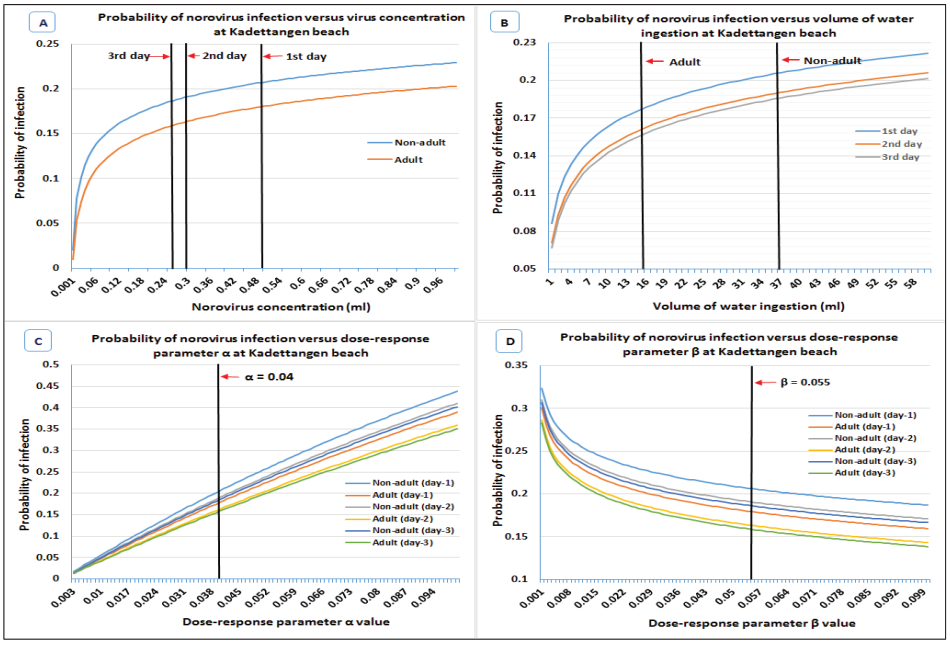

Figure 13. The sensitivity of the calculated probability of norovirus infection for single exposure at the Kadettangen beach for input variable of a) volume of water ingested b) norovirus concentration c) dose-response model parameter (α) d) dose-response model parameter (β). From **Paper III**

Table 2. Exponential decay rate coefficient (day^{-1}) for indicator bacteria and viruses in seawater (from 1 m and 60 m depths) incubated at 4 °C and 20 °C. From **Paper IV**

Microorganism	Unit	Seawater sample depth (meter)	Temperature (°C)	Method of analysis	Decay rate coefficient (*k*)
Adenovirus	PCR U/ml	1	4	qPCR	0.10
			20		1.10
		60	4		0.00
			20		0.85
Adenovirus	FFU/ml	1	4	Cultivation	0.00
			20		0.94
		60	4		0.04
			20		0.60
MS2	PCR U/ml	1	4	RT-qPCR	0.06
			20		0.30
		60	4		0.01
			20		0.14
MS2	PFU/ml	1	4	Cultivation	0.38
			20		0.79
		60	4		-0.53
			20		0.80
Intestinal enterococci	CFU/100 ml	1	4	Membrane filtration with incubation on selective media	0.05
			20		1.05
		60	4		0.03
			20		0.46
E. coli	MPN/100 ml	1	4	IDEXX Colilert 18 Quanti-Tray/2000	0.05
			20		1.13
		60	4		0.06
			20		0.73
Total coliforms	MPN/100 ml	1	4	IDEXX Colilert 18 Quanti-Tray/2000	0.12
			20		0.79
		60	4		0.15
			20		0.54

$C_t = C_o * e^{-(kt)}$ Where C_t is the concentration at any time t, C_o is the initial concentration on day 0, k is first-order decay rate coefficients, and t is the transition time

Computed exponential decay rate coefficient (day^{-1}) for FIB and viruses in deep and surface seawaters, and at 4 °C and 20 °C temperatures shown in Table 2. From this study, the decay rate coefficients were higher at 20 °C than at 4 °C. In addition, the effect of seawater difference in terms of depth was observed at 20 °C, with higher decay rate coefficients in surface seawater than in deep seawater, although due to high variations between the replicates and the differences were not always statistically significant. The results of the experiment suggest that the discharges from combined sewer overflows (CSO) and effluents from WWTP into the cold deep layers or the

surface layers during the cold winter season are expected to provide lower decay rates of FIB and viruses than in the surface layers during a warm summer. QMRA studies and microbial transport modelling in the coastal water, such as hydrodynamic modelling that can make use of these decay rates coefficients in Oslo fjords.

5.3. Water recycling and reuse

One of the major concerns associated with wastewater recycling and reuse is the quality of the wastewater in terms of microbial pathogens, which is threatening the public health when reused directly or with insufficient treatment. This potential threat can be reduced through proper treatment (Paper VI) as well as integrated with efficient utilization systems (Paper V). The promising strategy to raise the coverage of domestic wastewater reuse for irrigation, as well as other non-potable purposes, is the integration of the system with source separation principle, and the application of appropriate treatment technologies. The selection of less risky irrigation methods, such as hydroponic and drip irrigation, also reduced the risk. Moreover, the system becomes more effective and robust for domestic water use when the regular treatment system is further combined with post treatment systems (unsaturated infiltration and saturated flow steps) (Paper VI).

5.3.1. The health risk of treated greywater reuse for hydroponic lettuce production (Paper V)

In this study, *E. coli* concentration monitored in each treatment steps as well as in the produced lettuce were used to estimate the concentration of reference pathogens. The base for the estimated concentration of reference pathogen was the combination of information on *E. coli* in each treatment steps with the concentration of reference pathogens in the sewage system, which was published in different studies. Moreover, 1% of the sewage assumed to be leaked into the greywater system, therefore, the average concentration of *Cryptosporidium, Campylobacter, and* norovirus in the sewage system estimated 678.1 oocysts/100ml, 118 MPN/100ml, and $5.1x10^4$ gene copies/100ml respectively (Myrmel, M et al. 2015; Robertson et al. 2006). The concentration of reference pathogens in the irrigation water was reliant on the efficiency of greywater treatment systems, which was based on *E. coli* removal efficiency. The final estimated concentration of *Cryptosporidium, Campylobacter, and* norovirus in circulated irrigation water were 4.7E-04 oocysts/100ml, 8.2E-06 MPN/100ml, and 3.5E-08 gene copies/100ml respectively. The microbial contamination of produced lettuce was assumed as unintentional contact with irrigation water during harvesting time.

Routine and accidental ingestions are the two rout of exposure in the operation of lettuce production during irrigation and harvesting practices. Based on practical observation, routine ingestion was assumed to be 0.0001 litres per event and occurred more frequently during the irrigation practices whereas accidental ingestions were estimated about 0.001 litres per event and occurred less frequent for both operational activities. The exposure dose during operational activities was estimated from 4.7E-07 to 4.7E-08 for *Cryptosporidium,* 8.2E-09 to 8.2E-10 for *Campylobacter* and 3.5E-11 to 3.5E-12 for norovirus. The exposure dose due to lettuce consumption depends on the volume of irrigation water contaminated the lettuce and the amount of lettuce consumption. Thus, estimated exposure dose due to contaminated lettuce consumption was

2.35E-09, 1.75E-10, and 4.1E-11 in the case of *Cryptosporidium, Campylobacter* and norovirus respectively.

The computed health risk that accounts lettuce production (irrigation and harvesting) and consumption expressed in terms of probability of infection for single exposure that ranges from 2.8E-08 in the case of accidental ingestion of *Cryptosporidium* to 2.5E-12 in the case of routine ingestion of norovirus during lettuce production process. On the other hand, the probability of infection due to lettuce consumption per single exposure was estimated 1.4E-10, 7.8E-13, and 1.3E-10 in the case of *Cryptosporidium, Campylobacter*, and norovirus respectively (Table 3).

Table 3. The health risk of lettuce production and consumption . From **Paper V**

Pathogens	Rout of exposure	Activities	$P_{inf/event}$	$P_{inf/year}$	$P_{ill/expo}$	$P_{ill/year}$
Cryptosporidium	Hydroponic irrigation	Routine ingestion	2.8E-09	1.0E-06	1.1E-09	3.9E-07
		Accidental ingestion	2.8E-08	2.8E-07	1.1E-08	1.1E-07
	Lettuce harvest	Routine ingestion	2.8E-09	8.3E-08	1.1E-09	3.2E-08
		Accidental ingestion	2.8E-08	1.4E-07	1.1E-08	5.4E-08
	Lettuce consumption	Deliberate ingestion	1.4E-10	1.4E-08	5.4E-11	5.6E-09
Campylobacter	Hydroponic irrigation	Routine ingestion	1.6E-11	5.7E-09	5.2E-12	1.9E-09
		Accidental ingestion	1.6E-10	1.6E-09	5.2E-11	5.2E-10
	Lettuce harvest	Routine ingestion	1.6E-11	4.7E-10	5.2E-12	1.6E-10
		Accidental ingestion	1.6E-10	7.8E-10	5.2E-11	2.6E-10
	Lettuce consumption	Deliberate ingestion	7.8E-13	8.2E-11	2.6E-13	2.7E-11
Norovirus	Hydroponic irrigation	Routine ingestion	2.5E-12	9.3E-10	1.9E-12	6.8E-10
		Accidental ingestion	2.5E-11	2.5E-10	1.9E-11	1.9E-10
	Lettuce harvest	Routine ingestion	2.5E-12	7.6E-11	1.9E-12	5.6E-11
		Accidental ingestion	2.5E-11	1.3E-10	1.9E-11	9.3E-11
	Lettuce consumption	Deliberate ingestion	1.3E-10	1.3E-08	9.3E-11	9.7E-09

Due to the suitability of the experimental setup and the importance of the information in relation to treated wastewater reuse, the investigation further extends to chemical health risk assessment, which also helps us to compare the health risk outcome from the same spot, could help to prioritize the mitigation measures. The chemical health risk due to lettuce consumption expressed in terms of health risk index (HRI) and targeted hazard quotient (THQ), which is commonly used to evaluate non-carcinogenic health effect. The major risk contributor element due to lettuce consumption was As, Cr and Cu whereas the lowest risk contributor element in the system was Cd. The sensitivity of THQ based on intake rate of lettuce per body weight for different heavy

metals provides an opportunity to notice the effect relative to the benchmark value of THQ, which is one (Figure 14). As we can see from the graph, THQ value was above the critical value, when the value of lettuce intake rate per body weight of As, Cr, and Cu was above 1.65 g/kg, 2.05 g/kg, and 2.55 g/kg respectively. It must be noted that the THQ value computed here only signify the contribution from this specific lettuce produced in our system, whereas the contribution of heavy metals from other daily diets will potentially increase the risk. On the other hand, heavy metals bioaccumulation potential varied substantially among the different lettuce varieties. Accordingly, *Lactuca sativa 'Australische Gele'* have relatively less THQ value as compared with the other lettuce varieties and this indicates that there is an opportunity to reduce the health risk through proper selection of plant varieties.

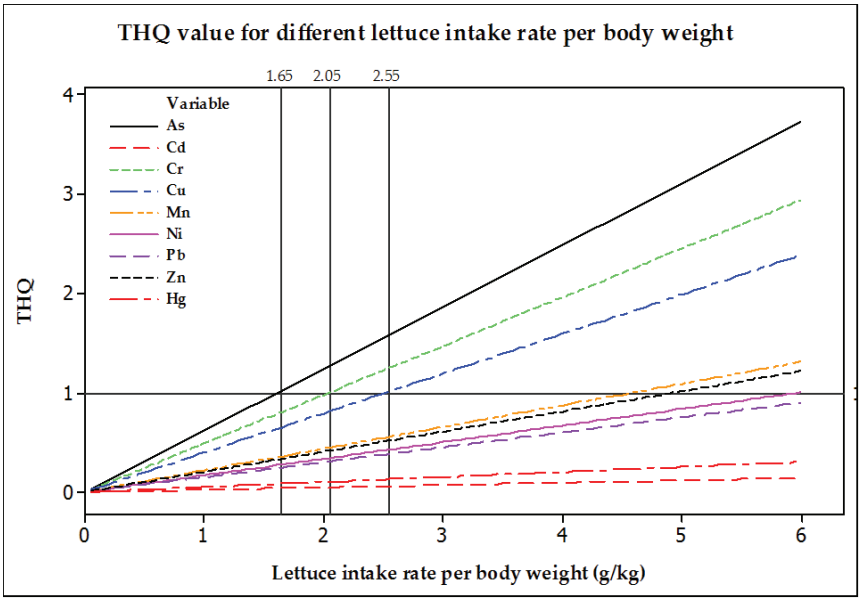

Figure 14. The sensitivity of THQ value for lettuce intake rate for a given body weight . From **Paper V**

5.3.2. Virus, FIB, and nutrients removal efficiency of treated greywater disposal system (Paper VI)

The concentration of total coliforms, *E. coli* and St28B were analysed from the effluent of unsaturated columns and compared with the concentration of the influent in order to determine the removal efficiency of the columns (Table 15). Regardless of the mechanisms, the average $\log_{10}$ MPN/100ml reduction of *E. coli* ranged from 2.6 to 3.0 in period 1 (T1) and the efficiency of all columns improved through time and reached from 2.9 to 3.4 $\log_{10}$ MPN/100ml in the fourth period (T4), however, the reduction decline to 0.4 to 3.0 at the fifth period (T5). The same happens in the case of total coliforms except for the difference in magnitude. The decline in removal efficiency of the fifth period could be explained by biofilm dispersion, which can be happened due to stress inducing conditions like alteration in nutrient availability, oxygen fluctuations, and

increase in toxic products (Kostakioti et al. 2013; Rowe et al. 2010; Sauer et al. 2004), however, to confirm the causes, further study is required.

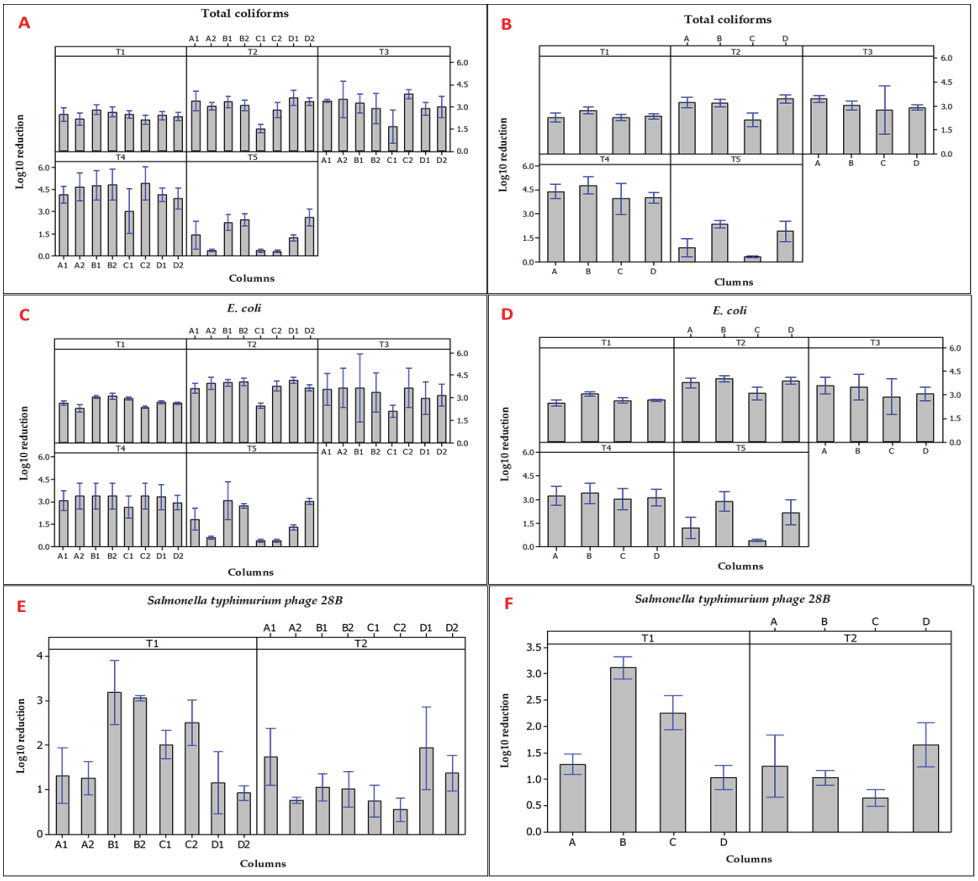

Figure 15. TC, E. coli, and St28B removal efficiency of unsaturated columns . A) TC removal efficiency of replicate columns at different period. B) TC removal efficiency of representative columns at different period. C) *E. coli* removal efficiency of replicate columns at different period. D) *E. coli* removal efficiency of representative columns at different period. E) St28B removal efficiency of replicate columns at different period, and F) St28B removal efficiency of representative columns at different period. Error bar represents 95% confidence interval of the mean. From **Paper VI**

Experiment on virus removal efficiency from unsaturated columns was carried out in two periods (T1: April 2016, and T2: February 2017) with an average inlet concentration of 1.0 x 10^5 and 4.5 x 10^5 PFU/ml respectively. The overall average $\log_{10}$ reduction of St28B were 1.8, 1.4, 1.1, and 1.9 in the representative column A, B, C, and D respectively. The initial (T1) St28B removal efficiency of columns have been relatively higher with the variation from 1.4 to 2.4 $\log_{10}$ PFU/ml in the case of column-C2 and column-A2 respectively. While during the second period (T2), the removal

efficiency of columns ranges from 0.56 to 1.94 log_{10} PFU/ml in the case of column-C2 and column-D1 respectively. The efficiency of virus removal of all columns was reduced during the second period (T2) except column-D and the reduction of removal efficiency could be explained by the saturation of adsorption surface of filter media, due to biofilm dispersion, or a combination of different factors.

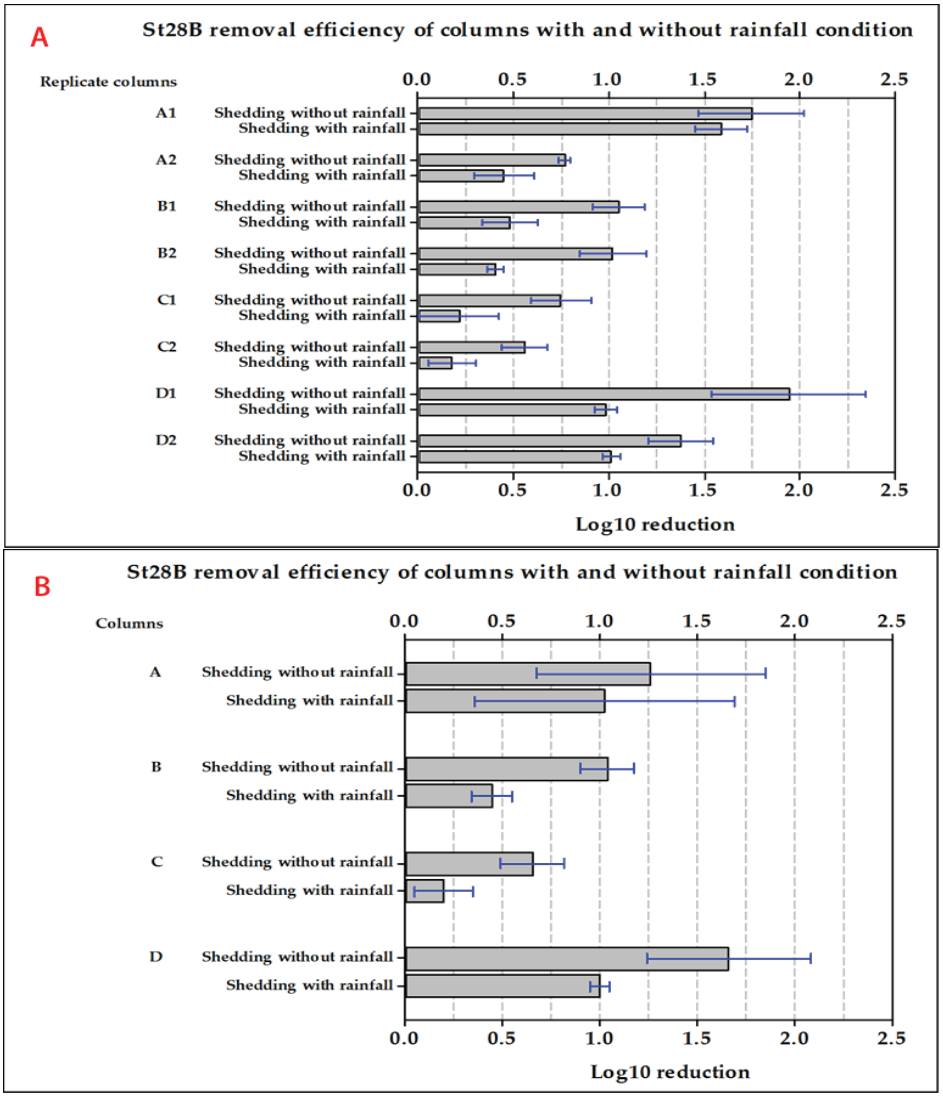

Figure 16. Variations of St28B removal efficiency when shedding simultaneously with rainfall and without rainfall in A) replicate columns, and B) representative columns. From **Paper VI**

Pollutants removal efficiency of both on-site greywater treatment plant coupled with unsaturated infiltration system for treated greywater disposal system was substantial and the magnitude of removal efficiency varied among the different infiltration systems because of the difference in filter media and its stratification. In this study, there was no single infiltration system universally efficient to remove all pollutants. Relatively, column-B (15 cm layer of each, Filtralite, fine sand, and till soil) showed the highest removal of total coliforms and *E. coli* [3-4 log reduction (99.9 - 99.99%)], whereas, the poorest removal observed in column-C (a layer of 25 cm crushed stone and 50 cm till soil) [2-3 log reduction (99 – 99.9%)]. Thus, the layering of filtralite-fins sand-till soil in the case of column-B could fever for the formation of biofilm and the order of stratified layer could resist biofilm stress conditions that favour for dispersion. The quarry west "subbus" (column-D), which was dominantly characterised by granite and amphibolite, have been relatively efficient in removing the virus. However, statistical analysis result showed that there was no significant difference ($p < 0.05$) in the removal of St28B from the columns. Besides, the St28B removal efficiency of columns was tested with rainfall condition, and Figure 16 illustrated the removal efficiency of columns when the virus was shedding with and without rainfall.

The change in water chemistry and the higher loading rate due to additional water from rainfall resulted in lower removal efficiency of St28B in all columns. The highest removal efficiency was 1.02 $\log_{10}$ PFU/ml reduction in column-A and the lowest reduction was 0.19 $\log_{10}$ PFU/ml reduction in the case of column-C, however, clogging was observed in column-A1 and that increase the residence time of water as a result the removal efficiency increases. The overall St28B removal efficiency of column A, B, C and D reduced by 19.3 %, 57.7 %, 70.8 %, and 40.4 % due to rainfall respectively as compared with the removal efficiency without rainfall.

When St28B loading into the columns ended during normal virus removal efficiency experiment, the concentration of St28B in the effluent was monitored to observe the recession phenomena by loading treated greywater as the same as the normal influent. At the 79th hour, rainwater was pumped with the same rate instead of treated greywater for one hour, the concentration of St28B in the effluent were slightly increased in the case of all columns, and it could be the sign of detachment due to the application of low ionic strength rainwater.

Total coliforms, *E. coli*, and St28B removal efficiency of saturated flow in a 5 m trench shown in Figure 17. As we can see from the plot, the drop of total coliform concentration from the initial to the end was from 5.16 $\log_{10}$ MPN/100ml to 0.33 $\log_{10}$ MPN/100ml on average. Whereas the drop in *E. coli* concentration between 0 to 3.5 m was from 4.43 $\log_{10}$ MPN/100ml to 0.05 $\log_{10}$ MPN/100ml and *E. coli* was not observed after 3.5 m.

The removal efficiency of total coliforms and *E. coli* were more rapid at the front side and slow down after 0.5 m (Figure 17 A & B), whereas the drop in the case of St28B was rather constant all over the flow distance (Figure 17 C) and 1.96 $\log_{10}$ PFU/ml reduction of St28B was observed in 5 m saturated trench.

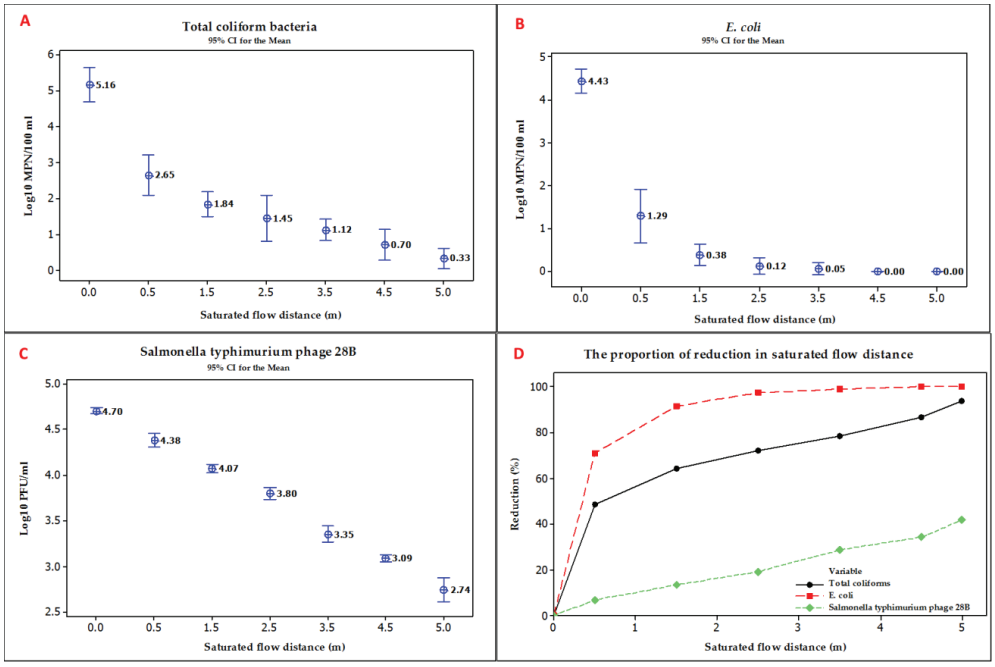

Figure 17. The removal efficiency along the saturated trench A) total coliform, B) *E. coli*, C) St28B. D) the relative removal efficiency of total coliforms, *E. coli*, and St28B (cumulative). From **Paper VI**

An attempt has been made to develop simple data driven model for simulating the subsurface transport of virus and bacteria, assuming removal of microbes through porous media can be described by exponential reduction equation, total coliforms and *E. coli* are representative bacteria and St28B is representative virus. For each non-linear exponential fittings of the data points, the corresponding exponential equation was computed and illustrated in Figure 18.

For total coliforms, *E. coli* and St28B, the computed $\beta(x)$ (the rate of removal along the flow distance) were 10.81, 10.99, and 1.11 and $\beta(t)$ (the rate of removal at a time) were 2.35, 2.39, and 0.24 respectively. The coefficient indicates the removal rate of total coliforms and *E. coli* was higher than St28B for the same flow distance and retention time. It can be used to calculate the expected removal distance or required retention time between treated greywater disposal system and water sources, considering the degree of similarity between this experimental setup and the area where we want to extrapolate in terms of hydrogeological settings and physicochemical property of filter media.

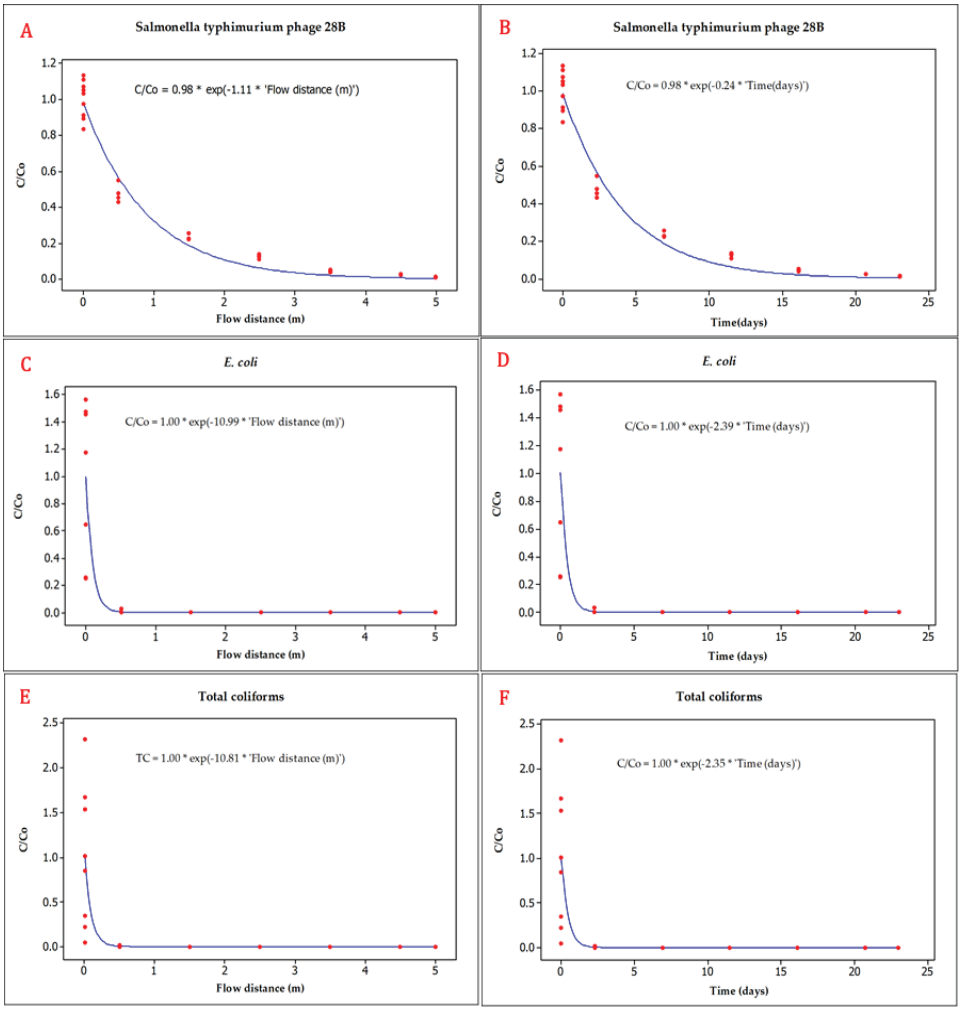

Figure 18. Data fitted 1st order exponential model expressed in terms of flow distance A) St28B, C) *E. coli*, E) total coliforms, expressed in terms of retention time B) St28B, D) *E. coli*, F) total coliform. From **Paper VI**

6. Conclusion and Future Research

This thesis presents a microbial water quality modelling and possibilities of integrating modelling results and monitoring data with QMRA framework. The overall approach was to lower the degree of assumptions on microbial concentrations through microbial water quality modelling. Moreover, the study contributes to improving QMRA approach by monitoring water quality data from water recycling processes. The following major conclusions are drawn from the research described in this thesis:

Microbial raw water quality monitoring using faecal indicator bacteria is one of the tasks of drinking water treatment plants in Norway to determine the current hygienic status of raw water. Considering the opportunity, this study demonstrates the use of time series data, through statistical trend and extreme event analysis, to evaluate the trend of microbial water quality (the effectiveness of watershed management strategies), and to understand the probabilistic behaviour of extreme microbial load events so as to make available relevant information for QMRA.

In the recreational coastal water, the fate and transport processes of microbial pathogens are highly dynamic and complex as they significantly vary in space and over time. Mainly, hydrodynamic processes and degradation mechanisms control microbial pathogens concentration in the recreational beaches. Hydrodynamic modelling of coastal water quality is a key to understand the influence of different processes (rainfall, discharge from boats, and wind directions) on the microbial concentrations at the recreational beaches. In this regard, this study demonstrates the importance of hydrodynamic modelling as a tool to identify the risk of contamination of recreational beaches in order to prioritize mitigation measures.

Significant variation of the spatiotemporal spreading of microbial contaminants at recreational beaches, specifically after rainfall events, requires frequent monitoring of microbial water quality, which is difficult in terms of operation and costly, in order to estimate the associated health risks. However, hydrodynamic modelling has the potential to overcome such problems if the modelling is reasonably calibrated, validated, and combined with supplementary information (realistic scenario). Therefore, this study demonstrates the importance of hydrodynamic modelling of microbial bathing water quality that offers a tool to understand the dispersion of microbial pathogens in the recreational beaches, and to integrate the simulation result with QMRA framework.

There is very little information on the concentration of microbes in different wastewater system, the potential for growth of microorganisms in greywater system, and the decay rates of microbial pathogens in seawater in different regions. This information is very important input for fate and transport modelling as well as QMRA framework. Thus, this study provides important information as an input for QMRA modelling in the region.

Greywater reuse can be safely carried out as a means to address current and future water scarcities; however, mainly this is contingent upon safe and reliable treatment systems and/or interventions. The configuration of greywater treatment schemes and hydroponic lettuce

production systems as a part of a green wall structure and make use of urine, as a nutrient solution was the unique feature of this study. Considering its distinctive arrangement, this study provided key information about the health risk associated with treated greywater reuse for lettuce production.

This study assessed both microbial and heavy metal health risks assessment on the same subject that enable us to observe the most critical risks to prioritize mitigation measures, and at the same time to perceive the health risk in different directions. The results of QMRA demonstrate the importance of microbial removal efficiency of integrated greywater treatment system and hydroponic irrigation scheme to minimize health risk below the health-based targets, 10^{-6} DALYs per person per year. Heavy metals risk assessment based on HRI and THQ indexes were not exceeded the permissible level (one), and as a result, the health risk concern of consuming lettuce was insignificant.

This study further reveals that heavy metal uptake rate of Lactuca sativa 'Australische Gele' was relatively less, highlighting the importance of having selected plant varieties with minimum heavy metals uptake in order to reduce health risk. Therefore, the selection of potential varieties should be considered in future studies.

By considering all the benefits that may arise from this scheme, this study points out some vital health risk minimizing strategies that may potentially further reduce health risks and these include 1) Improving microbial and heavy metal removal efficiency of greywater treatment systems through appropriate research approaches. 2) Growing plant varieties that have the potential of reduced heavy metal bioaccumulation and 3) Taking regulatory measures on consumable goods that can potentially release heavy metals at the household level.

The construction of on-site treated greywater disposal system from selected filter media with different stratification operate as a post treatment step and greatly reduced microbes, nutrients, and organic load. The removal of total coliforms and *E. coli* in all infiltration system were relatively higher as compared to St28B. Among the range of infiltration systems tested, column with 30 cm Filtralite at the top and 50 cm quarry waste "subbus" at the bottom (Column-D), and Filtralite-fine sand-till soil stratified filtration system (Column-B) provided comparably better treatment performance with respect to total coliforms, *E. coli*, St28B, nutrients and organic load removal efficiency without clogging problem during the experimental period.

The removal efficiency of unsaturated infiltration system during rainfall was investigated through experiment. The result demonstrated that St28B removal efficiency of the infiltration systems was significantly reduced when virus released simultaneously with the rainfall event. The magnitude of efficiency reduction ranges from 19 % to 70 %, depending on the infiltration system.

Saturated flow experiment using compacted till soil deposit (5 m) with a water retention time of 23 days can significantly eliminate indicator bacteria (total coliforms and *E. coli*) and the removal efficiency was relatively high in the front 0.5 meter and then it reduced along the trench. While the removal rate of St28B was decreased constantly along the flow length, only 1.96-$\log_{10}$ reduction of virus (St28B) after 23 days retention time was observed. This result indicates that

appropriate setback distance will be essential to rely upon the saturated flow as efficient treatment barriers for viruses.

QMRA has the potential to estimate the health risk in different context and pathway, such as drinking water source, recreational water, recycled water, etc. Although different and important issues have been resolved in this thesis, many others issues still remain to be clarified in order to improve QMRA framework. Therefore, based on the limitations of this study, the following issues need further attention:

- The statistical water quality analysis based on the time series microbial water quality data should be an integral part of QMRA at the DWTP and further investigation is required on the technique to integrate this information.
- Hydrodynamic microbial water quality modelling is an important tool to estimate the spreading of microbial pathogens in the recreational coastal water. The hydrodynamic and microbial degradation processes are the two main factors for the concentration of microbial pathogens at specific position and time. Investigation on the impact of the spatial and temporal resolution of hydrodynamic modelling will further strengthen the approach.
- The variation of microbe removal efficiency of different unsaturated infiltration systems and saturated flow conditions was relatively higher depending on the filter material, the quality of influent, and water flow condition. However, these need more attention regarding when and how the removal efficiency would fluctuate and how they will affect the risk of source water contamination.
- Based on the removal efficiency of unsaturated infiltration columns and saturated flow conditions, future study should investigate the safest setback distance of the disposal sites from drinking water source and integrate this concept with QMRA framework.

References

Abdi, H. & Williams, L. J. (2010). Principal component analysis. *Wiley interdisciplinary reviews: computational statistics*, 2 (4): 433-459.

Ahuja, S. (2013). *Monitoring water quality: Pollution assessment, analysis, and remediation*: Newnes.

Allotey, P., Reidpath, D., Kouamé, A. & Cummins, R. (2003). The DALY, context and the determinants of the severity of disease: an exploratory comparison of paraplegia in Australia and Cameroon. *Social science & medicine*, 57 (5): 949-958.

Anderson, J., Adin, A., Crook, J., Davis, C., Hultquist, R., Jimenez-Cisneros, B., Kennedy, W., Sheikh, B. & Van der Merwe, B. (2001). Climbing the ladder: a step by step approach to international guidelines for water recycling. *Water Science and Technology*, 43 (10): 1-8.

Antonopoulos, V. Z., Papamichail, D. M. & Mitsiou, K. A. (2001). Statistical and trend analysis of water quality and quantity data for the Strymon River in Greece. *Hydrology and Earth System Sciences Discussions*, 5 (4): 679-692.

Arhonditsis, G. B., Papantou, D., Zhang, W., Perhar, G., Massos, E. & Shi, M. (2008). Bayesian calibration of mechanistic aquatic biogeochemical models and benefits for environmental management. *Journal of Marine Systems*, 73 (1): 8-30.

Asano, T. (1998). *Wastewater Reclamation and Reuse: Water Quality Management Library*, vol. 10: CRC Press.

Asano, T. (2002). Water from (waste) water–the dependable water resource (The 2001 Stockholm Water Prize Laureate Lecture). *Water science and technology*, 45 (8): 23-33.

Ashbolt, N. J., Schoen, M. E., Soller, J. A. & Roser, D. J. (2010). Predicting pathogen risks to aid beach management: the real value of quantitative microbial risk assessment (QMRA). *Water research*, 44 (16): 4692-4703.

Åström, J., Petterson, S., Bergstedt, O., Pettersson, T. J. & Stenström, T. A. (2007). Evaluation of the microbial risk reduction due to selective closure of the raw water intake before drinking water treatment. *Journal of Water and Health*, 5 (S1): 81-97.

Ayuso-Gabella, N., Page, D., Masciopinto, C., Aharoni, A., Salgot, M. & Wintgens, T. (2011). Quantifying the effect of Managed Aquifer Recharge on the microbiological human health risks of irrigating crops with recycled water. *Agricultural water management*, 99 (1): 93-102.

Bartram, J., Ballance, R. & Organization, W. H. (1996). Water quality monitoring: a practical guide to the design and implementation of freshwater quality studies and monitoring programs.

Beaudequin, D., Harden, F., Roiko, A. & Mengersen, K. (2016). Utility of Bayesian networks in QMRA-based evaluation of risk reduction options for recycled water. *Science of the Total Environment*, 541: 1393-1409.

Beck, M. B. (1987). Water quality modeling: a review of the analysis of uncertainty. *Water Resources Research*, 23 (8): 1393-1442.

Benedini, M. & Tsakiris, G. (2013). *Water quality modelling for rivers and streams*: Springer Science & Business Media.

Benford, D. (2001). *Principles of Risk Assessment of Food and Drinking Water Related to Human Health*: ILSI Europe.

Bibby, K., Casson, L. & Haas, C. N. (2016). *Risks from Ebola discharge from hospitals to sewer workers*.

Boehm, A. B. (2007). Enterococci Concentrations in Diverse Coastal Environments Exhibit Extreme Variability. *Environmental Science & Technology*, 41 (24): 8227-8232.

Calderon, R. L., Mood, E. W. & Dufour, A. P. (1991). Health effects of swimmers and nonpoint sources of contaminated water. *International journal of environmental health research*, 1 (1): 21-31.

Chen, W.-B. & Liu, W.-C. (2015). Water quality modeling in reservoirs using multivariate linear regression and two neural network models. *Advances in Artificial Neural Systems*, 2015: 6.

Chen, X. & Sheng, Y. P. (2005). Three-dimensional modeling of sediment and phosphorus dynamics in Lake Okeechobee, Florida: Spring 1989 simulation. *Journal of environmental engineering*, 131 (3): 359-374.

Chen, Z., Ngo, H. H. & Guo, W. (2013). Risk control in recycled water schemes. *Critical reviews in environmental science and technology*, 43 (22): 2439-2510.

Clark, R. B., Frid, C. & Attrill, M. (1989). *Marine pollution*, vol. 4: Clarendon Press Oxford.

Clark, R. M. (2011). *Modeling water quality in distribution systems*: American Water Works Association.

Coles, S., Bawa, J., Trenner, L. & Dorazio, P. (2001). *An introduction to statistical modeling of extreme values*, vol. 208: Springer.

Colford, J. M., Schiff, K. C., Griffith, J. F., Yau, V., Arnold, B. F., Wright, C. C., Gruber, J. S., Wade, T. J., Burns, S. & Hayes, J. (2012). Using rapid indicators for Enterococcus to assess the risk of illness after exposure to urban runoff contaminated marine water. *Water research*, 46 (7): 2176-2186.

Cox, T. J., Turner, D. F., Pelletier, G. J. & Navato, A. (2015). Stochastic Water Quality Modeling of an Impaired River Impacted by Climate Change. *Journal of Environmental Engineering*, 141 (11): 04015035.

Curriero, F. C., Patz, J. A., Rose, J. B. & Lele, S. (2001). The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948–1994. *American journal of public health*, 91 (8): 1194-1199.

Davies, C. M., Long, J. A., Donald, M. & Ashbolt, N. J. (1995). Survival of fecal microorganisms in marine and freshwater sediments. *Applied and Environmental Microbiology*, 61 (5): 1888-1896.

de Brauwere, A., Ouattara, N. K. & Servais, P. (2014). Modeling fecal indicator bacteria concentrations in natural surface waters: a review. *Critical reviews in environmental science and technology*, 44 (21): 2380-2453.

du Plessis, A. (2017). Global Water Quality Challenges. In *Freshwater Challenges of South Africa and its Upper Vaal River: Current State and Outlook*, pp. 13-44. Cham: Springer International Publishing.

Dechesne, M. & Soyeux, E. (2007). Assessment of source water pathogen contamination. *Journal of Water and Health*, 5 (S1): 39-50.

Dooge, J. (1992). Hydrologic models and climate change. *Journal of Geophysical Research: Atmospheres*, 97 (D3): 2677-2686.

ENTRIX, I., J. E. Edinger Associates, I., & & Gahagan and Bryant Associates, I. (2001). Hydrodynamic and Water Quality Modeling and Feasibility Analysis of Indian River, Rehoboth Bay and Little Assawoman Bay. *Delaware's Inland Bays Flushing Study Project* Delaware Department of Natural Resources and Environmental Control

EPA. (2010). Quantitative Microbial Risk Assessment to Estimate Illness in Freshwater Impacted by Agricultural Animal Sources of Fecal Contamination, EPA 822-R-10-005.

Eregno, F. E., Nilsen, V., Seidu, R. & Heistad, A. (2014). Evaluating the Trend and Extreme Values of Faecal Indicator Organisms in a Raw Water Source: A Potential Approach for Watershed Management and Optimizing Water Treatment Practice. *Environmental Processes*, 1 (3): 287-309.

Eregno, F. E., Tryland, I., Tjomsland, T., Myrmel, M., Robertson, L. & Heistad, A. (2016). Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Science of the Total Environment*, 548–549: 270-279.

Eriksson, E., Auffarth, K., Henze, M. & Ledin, A. (2002). Characteristics of grey wastewater. *Urban water*, 4 (1): 85-104.

Ervin, J. S., Russell, T. L., Layton, B. A., Yamahara, K. M., Wang, D., Sassoubre, L. M., Cao, Y., Kelty, C. A., Sivaganesan, M., Boehm, A. B., et al. (2013). Characterization of fecal concentrations in human and other animal sources by physical, culture-based, and quantitative real-time PCR methods. *Water Research*, 47 (18): 6873-6882.

Ferguson, C. M., Charles, K. & Deere, D. A. (2008). Quantification of microbial sources in drinking-water catchments. *Critical Reviews in Environmental Science and Technology*, 39 (1): 1-40.

Ferguson, D. & Signoretto, C. (2011). Environmental persistence and naturalization of fecal indicator organisms. In *Microbial Source Tracking: Methods, Applications, and Case Studies*, pp. 379-397: Springer.

Fewtrell, L. & Bartram, J. (2001). Water quality: guidelines, standards and health: assessment of risk and risk management for water-related infectious diseases.

Fewtrell, L. & Kay, D. (2015). Recreational water and infection: a review of recent findings. *Current environmental health reports*, 2 (1): 85-94.

Field, K. G. & Samadpour, M. (2007). Fecal source tracking, the indicator paradigm, and managing water quality. *Water research*, 41 (16): 3517-3538.

Fujioka, R. S., Solo-Gabriele, H. M., Byappanahalli, M. N. & Kirs, M. (2015). US recreational water quality criteria: a vision for the future. *International journal of environmental research and public health*, 12 (7): 7752-7776.

Goel, P. (2006). *Water pollution: causes, effects and control*: New Age International.

Gore, F. M., Bloem, P. J. N., Patton, G. C., Ferguson, J., Joseph, V., Coffey, C., Sawyer, S. M. & Mathers, C. D. (2011). Global burden of disease in young people aged 10–24 years: a systematic analysis. *The Lancet*, 377 (9783): 2093-2102.

Gosset, A., Ferro, Y. & Durrieu, C. (2016). Methods for evaluating the pollution impact of urban wet weather discharges on biocenosis: a review. *Water research*, 89: 330-354.

Gray, N. F. (2010). *Water technology: an introduction for environmental scientists and engineers*: IWA Publishing.

Grizzetti B., Bouraoui F., Bianchi M., Barkved L., Berge D., Campbell D., Dan Kim N., Gooch G., Lana-Renoult N., Nesheim I., et al. (2007). Managing data in Integrated Water Resources Management projects: the STRIVER case European Commission Joint Research Center. Institute for Environment and Sustainability. Rural, Water and Ecosystem Resources Unit (JRC-EC)

Grøndahl-Rosado, R. C., Tryland, I., Myrmel, M., Aanes, K. J. & Robertson, L. J. (2014). Detection of Microbial Pathogens and Indicators in Sewage Effluent and River Water During the Temporary Interruption of a Wastewater Treatment Plant. *Water Quality, Exposure and Health*, 6 (3): 155-159.

Haas, C. & Eisenberg, J. (2001). Risk assessment. In Lorna Fewtrell & Bartram, J. (eds) *Water Quality: Guidelines, Standards and Health: Assessment of risk and risk management for water-related infectious disease* London: IWA Publishing.

Haas, C. N., Rose, J. B. & Gerba, C. P. (1999). *Quantitative microbial risk assessment*. New York: John Wiley & Sons.

Haas, C. N. (2000). Epidemiology, microbiology, and risk assessment of waterborne pathogens including Cryptosporidium. *Journal of food protection*, 63 (6): 827-831.

Haas, C. N. (2002). Conditional dose-response relationships for microorganisms: Development and application. *Risk Analysis*, 22 (3): 455-463.

Hagedorn, C., Blanch, A. R. & Harwood, V. J. (2011). *Microbial source tracking: methods, applications, and case studies*: Springer Science & Business Media.

Hamilton, A., Stagnitti, F., Boland, A.-M. & Premier, R. (2005). Quantitative microbial risk assessment modelling for the use of reclaimed water in irrigated horticulture. *WIT Transactions on Biomedicine and Health*, 9.

Hamilton, A. J., Stagnitti, F., Premier, R., Boland, A.-M. & Hale, G. (2006). Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. *Applied and environmental microbiology*, 72 (5): 3284-3290.

Harris, E., Falconer, R. & Lin, B. (2004). Modelling hydroenvironmental and health risk assessment parameters along the South Wales Coast. *Journal of Environmental Management*, 73 (1): 61-70.

Harwood, V. J., Levine, A. D., Scott, T. M., Chivukula, V., Lukasik, J., Farrah, S. R. & Rose, J. B. (2005). Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Applied and Environmental Microbiology*, 71 (6): 3163-3170.

Harwood, V. J., Staley, C., Badgley, B. D., Borges, K. & Korajkic, A. (2014). Microbial source tracking markers for detection of fecal contamination in environmental waters: relationships between pathogens and human health outcomes. *FEMS microbiology reviews*, 38 (1): 1-40.

Hope, B. & Stock, M. (1998). Guidance for use of probabilistic analysis in human health risk assessments. *Oregon DUSEPArtment of Environmental Quality, Portland, OR*.

HUANG, Z. & Morimoto, H. Water pollution models based on stochastic differential equations.

Huertas, E., Salgot, M., Hollender, J., Weber, S., Dott, W., Khan, S., Schäfer, A., Messalem, R., Bis, B., Aharoni, A., et al. (2008). Key objectives for water reuse concepts. *Desalination*, 218 (1): 120-131.

Iwasaki, T., Slade, J. J. & Stanley, W. E. (1937). Some Notes on Sand Filtration [with Discussion]. *Journal (American Water Works Association)*, 29 (10): 1591-1602.

Jha, R. & Singh, V. P. (2008). Analytical water quality model for biochemical oxygen demand simulation in River Gomti of Ganga Basin, India. *KSCE Journal of Civil Engineering*, 12 (2): 141-147.

Ji, Z.-G. (2017). *Hydrodynamics and water quality: modeling rivers, lakes, and estuaries*: John Wiley & Sons.

Kamizoulis, G. (2008). Setting health based targets for water reuse (in agriculture). *Desalination*, 218 (1-3): 154-163.

Kim, J. H., Kim, R. H., Lee, J., Cheong, T. J., Yum, B. W. & Chang, H. W. (2005). Multivariate statistical analysis to identify the major factors governing groundwater quality in the coastal area of Kimje, South Korea. *Hydrological Processes*, 19 (6): 1261-1276.

Kolluru, V. (1994). *GEMSS: Generalized Environmental Modeling System for Surfacewaters*. Available at: http://gemss.com/index.html (accessed: May, 23).

Kolluru, V. S., Prakash, S. & Hodgins, D. (2014). *Evaluation of Urbanization and Impacts on Water Quality in Nottawasaga Bay Using an Integrated 3-D Modeling Framework*. 978-88-9035-744-2, San Diego, California, USA.

Kostakioti, M., Hadjifrangiskou, M. & Hultgren, S. J. (2013). Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. *Cold Spring Harbor perspectives in medicine*, 3 (4): a010306.

KOSTIĆ, T., Stessl, B., Wagner, M. & Sessitsch, A. (2011). Microarray analysis reveals the actual specificity of enrichment media used for food safety assessment. *Journal of food protection*, 74 (6): 1030-1034.

Krantzberg, G., Tanik, A., do Carmo, J. S. A., Indarto, A., Ekdal, A., Gurel, M., pehlivanoglu Mantas, E., Wang, Z., Wang, G. & Zhao, C. (2010). *Advances in water quality control*: Scientific Research Publishing, Inc. USA.

Langeland, G. (1982). Salmonella spp. in the working environment of sewage treatment plants in Oslo, Norway. *Applied and environmental microbiology*, 43 (5): 1111-1115.

Li, Y. & Migliaccio, K. (2010). *Water quality concepts, sampling, and analyses*: CRC Press.

Loucks, D. P., Van Beek, E., Stedinger, J. R., Dijkman, J. P. & Villars, M. T. (2005). *Water resources systems planning and management: an introduction to methods, models and applications*: Paris: Unesco.

Mara, D., Sleigh, P., Blumenthal, U. & Carr, R. (2007). Health risks in wastewater irrigation: comparing estimates from quantitative microbial risk analyses and epidemiological studies. *Journal of water and health*, 5 (1): 39-50.

Matthess, G., Pekdeger, A. & Schroeter, J. (1988). Persistence and transport of bacteria and viruses in groundwater—a conceptual evaluation. *Journal of Contaminant Hydrology*, 2 (2): 171-188.

Mau, R. E., Boulos, P. F. & Bowcock, R. W. (1996). Modelling distribution storage water quality: An analytical approach. *Applied Mathematical Modelling*, 20 (4): 329-338.

McNeil, A. J. (1999). Extreme value theory for risk managers. *Departement Mathematik ETH Zentrum*.

McQuaig, S., Griffith, J. & Harwood, V. J. (2012). Association of fecal indicator bacteria with human viruses and microbial source tracking markers at coastal beaches impacted by nonpoint source pollution. *Applied and environmental microbiology*, 78 (18): 6423-6432.

Medema, G., Hoogenboezem, W., Van der Veer, A., Ketelaars, H., Hijnen, W. A. & Nobel, P. (2003). Quantitative risk assessment of Cryptosporidium in surface water treatment. *Water Science and Technology*, 47 (3): 241-247.

Medema, G. (2012). Microbial Risk Assessment of Pathogens pathogen in Water pathogen in water. In *Encyclopedia of Sustainability Science and Technology*, pp. 6605-6633: Springer.

Medema, G. (2013). Microbial Risk Assessment of Pathogens in Water. In Laws, E. A. (ed.) *Environmental Toxicology: Selected Entries from the Encyclopedia of Sustainability Science and Technology*, pp. 361-401. New York, NY: Springer New York.

Merkli, B. (1975). *Untersuchungen über Mechanismen und Kinetik der Elimination von bakterien und Viren im Grundwasser*.

Murray, C. J. (1994). Quantifying the burden of disease: the technical basis for disability-adjusted life years. *Bulletin of the World Health Organization*, 72 (3): 429-445.

Myrmel, M., Lange, H. & Rimstad, E. (2015). A 1-Year Quantitative Survey of Noro-, Adeno-, Human Boca-, and Hepatitis E Viruses in Raw and Secondarily Treated Sewage from Two Plants in Norway. *Food and Environmental Virology*, 7 (3): 213-223.

Naumova, E. N., Christodouleas, J., Hunter, P. R. & Syed, Q. (2005). Effect of precipitation on seasonal variability in cryptosporidiosis recorded by the North West England surveillance system in 1990–1999. *Journal of Water and Health*, 3 (2): 185-196.

Noble, R. T. & Fuhrman, J. A. (2001). Enteroviruses detected by reverse transcriptase polymerase chain reaction from the coastal waters of Santa Monica Bay, California: low correlation to bacterial indicator levels. *Hydrobiologia*, 460 (1): 175-184.

O'Toole, J., Leder, K. & Sinclair, M. (2007). Recycled water and human health effects. *Australian family physician*, 36 (12): 998.

O'Toole, J., Sinclair, M., Gibney, K. & Leder, K. (2015). Adoption of a microbial health-based target for Australian drinking water regulation. *Journal of water and health*, 13 (3): 662-670.

Obropta, C. C. & Kardos, J. S. (2007). Review of urban stormwater quality models: deterministic, stochastic, and hybrid approaches. *JAWRA Journal of the American Water Resources Association*, 43 (6): 1508-1523.

Oliver, D. M., Porter, K. D., Pachepsky, Y. A., Muirhead, R. W., Reaney, S. M., Coffey, R., Kay, D., Milledge, D. G., Hong, E. & Anthony, S. G. (2016). Predicting microbial water quality with models: over-arching questions for managing risk in agricultural catchments. *Science of the Total Environment*, 544: 39-47.

Olivieri, A. W., Seto, E., Cooper, R. C., Cahn, M. D., Colford, J., Crook, J., Debroux, J.-F., Mandrell, R., Suslow, T. & Tchobanoglous, G. (2014). Risk-based review of California's water-recycling criteria for agricultural irrigation. *Journal of Environmental Engineering*, 140 (6): 04014015.

Ottoson, J. & Stenström, T. A. (2003). Faecal contamination of greywater and associated microbial risks. *Water Research*, 37 (3): 645-655.

Page, D., Dillon, P., Toze, S., Bixio, D., Genthe, B., Cisneros, B. E. J. & Wintgens, T. (2010). Valuing the subsurface pathogen treatment barrier in water recycling via aquifers for drinking supplies. *water research*, 44 (6): 1841-1852.

Parker, G., Droste, R. & Rennie, C. (2009). An objective test of stochastic behavior in riverine water quality models. *Water Science and Technology*, 59 (1): 159-165.

Pejman, A., Bidhendi, G. N., Karbassi, A., Mehrdadi, N. & Bidhendi, M. E. (2009). Evaluation of spatial and seasonal variations in surface water quality using multivariate statistical techniques. *International Journal of Environmental Science & Technology*, 6 (3): 467-476.

Ramírez-Castillo, F. Y., Loera-Muro, A., Jacques, M., Garneau, P., Avelar-González, F. J., Harel, J. & Guerrero-Barrera, A. L. (2015). Waterborne pathogens: detection methods and challenges. *Pathogens*, 4 (2): 307-334.

Rizak, S., Cunliffe, D., Sinclair, M., Vulcano, R., Howard, J., Hrudey, S. & Callan, P. (2003). Drinking water quality management: a holistic approach. *Water Science and Technology*, 47 (9): 31-36.

Robertson, L. J. & Gjerde, B. K. (2006). Fate of Cryptosporidium Oocysts and Giardia Cysts in the Norwegian Aquatic Environment over Winter. *Microbial Ecology*, 52 (4): 597-602.

Robertson, L. J., Hermansen, L. & Gjerde, B. K. (2006). Occurrence of Cryptosporidium oocysts and Giardia cysts in sewage in Norway. *Applied and Environmental Microbiology*, 72 (8): 5297-5303.

Robillot, C., Le-Clech, P., Pype, M.-L., Sidhu, J., Khan, S. & Monis, P. (2016). National Validation Framework for Water Recycling: Overview of Priority Research, 192220272X: Australian Water Recycling Centre of Excellence.

Robson, B. J., Hamilton, D. P., Webster, I. T. & Chan, T. (2008). Ten steps applied to development and evaluation of process-based biogeochemical models of estuaries. *Environmental Modelling & Software*, 23 (4): 369-384.

Rodriguez, C., Van Buynder, P., Lugg, R., Blair, P., Devine, B., Cook, A. & Weinstein, P. (2009). Indirect potable reuse: a sustainable water supply alternative. *International journal of environmental research and public health*, 6 (3): 1174-1203.

Rose, J. (2007). Water reclamation, reuse and public health. *Water science and technology*, 55 (1-2): 275-282.

Rose, J. B., Epstein, P. R., Lipp, E. K., Sherman, B. H., Bernard, S. M. & Patz, J. A. (2001). Climate variability and change in the United States: potential impacts on water- and foodborne diseases caused by microbiologic agents. *Environmental Health Perspectives*, 109 (Suppl 2): 211-221.

Rosen, B. H., Croft, R., Atwill, E., Wade, S. & Stehman, S. (2000). *Waterborne pathogens in agricultural watersheds*: US Department of Agriculture, Natural Resources Conservation Service, Watershed Science Institute.

Rowe, M. C., Withers, H. L. & Swift, S. (2010). Uropathogenic Escherichia coli forms biofilm aggregates under iron restriction that disperse upon the supply of iron. *FEMS microbiology letters*, 307 (1): 102-109.

Sauer, K., Cullen, M. C., Rickard, A. H., Zeef, L. A. H., Davies, D. G. & Gilbert, P. (2004). Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. *Journal of Bacteriology*, 186 (21): 7312-7326.

Schijven, J., Derx, J., de Roda Husman, A. M., Blaschke, A. P. & Farnleitner, A. H. (2015). QMRAcatch: Microbial quality simulation of water resources including infection risk assessment. *Journal of environmental quality*, 44 (5): 1491-1502.

Schijven, J. F., Teunis, P. F. M., Rutjes, S. A., Bouwknegt, M. & de Roda Husman, A. M. (2011). QMRAspot: A tool for Quantitative Microbial Risk Assessment from surface water to potable water. *Water Research*, 45 (17): 5564-5576.

Schönning, C., Westrell, T., Stenström, T. A., Arnbjerg-Nielsen, K., Hasling, A. B., Høibye, L. & Carlsen, A. (2007). Microbial risk assessment of local handling and use of human faeces. *Journal of water and health*, 5 (1): 117-128.

Shao, H. & Chu, L. (2013). Water-quality Engineering in Natural Systems: Fate and Transport Processes in the Water Environment. *CLEAN–Soil, Air, Water*, 41 (8): 829-830.

Signor, R., Roser, D., Ashbolt, N. & Ball, J. (2005). Quantifying the impact of runoff events on microbiological contaminant concentrations entering surface drinking source waters. *Journal of water and health*, 3 (4): 453-468.

Signor, R., Ashbolt, N. & Roser, D. (2007). Microbial risk implications of rainfall-induced runoff events entering a reservoir used as a drinking-water source. *Journal of Water Supply: Research and Technology-AQUA*, 56 (8): 515-531.

Sokolova, E., Åström, J., Pettersson, T. J., Bergstedt, O. & Hermansson, M. (2012). Estimation of pathogen concentrations in a drinking water source using hydrodynamic modelling and microbial source tracking. *Journal of water and health*, 10 (3): 358-370.

Sokolova, E., Petterson, S. R., Dienus, O., Nyström, F., Lindgren, P.-E. & Pettersson, T. J. R. (2015). Microbial risk assessment of drinking water based on hydrodynamic modelling of pathogen concentrations in source water. *Science of The Total Environment*, 526: 177-186.

Soller, J., Bartrand, T., Ravenscroft, J., Molina, M., Whelan, G., Schoen, M. & Ashbolt, N. (2015). Estimated human health risks from recreational exposures to stormwater runoff containing animal faecal material. *Environmental modelling & software*, 72: 21-32.

Soller, J. A., Schoen, M. E., Bartrand, T., Ravenscroft, J. E. & Ashbolt, N. J. (2010). Estimated human health risks from exposure to recreational waters impacted by human and non-human sources of faecal contamination. *Water Research*, 44 (16): 4674-4691.

Straub, T. M. & Chandler, D. P. (2003). Towards a unified system for detecting waterborne pathogens. *Journal of Microbiological Methods*, 53 (2): 185-197.

Teunis, P. F. M., Medema, G. J., Kruidenier, L. & Havelaar, A. H. (1997). Assessment of the risk of infection by Cryptosporidium or Giardia in drinking water from a surface water source. *Water Research*, 31 (6): 1333-1346.

Towler, E., Rajagopalan, B., Gilleland, E., Summers, R. S., Yates, D. & Katz, R. W. (2010). Modeling hydrologic and water quality extremes in a changing climate: A statistical approach based on extreme value theory. *Water Resources Research*, 46 (11).

Toze, S. (2006). Water reuse and health risks—real vs. perceived. *Desalination*, 187 (1): 41-51.

USEPA. (2010). Quantitative Microbial Risk Assessment to Estimate Illness in Freshwater Impacted by Agricultural Animal Sources of Fecal Contamination.

Wang, Q., Li, S., Jia, P., Qi, C. & Ding, F. (2013). A review of surface water quality models. *The Scientific World Journal*, 2013.

WHO. (2003). Guidelines for safe recreational water environments: Volume 1 Coastal and Fresh Water.

WHO. (2004). *Guidelines for drinking-water quality*, vol. 1: World Health Organization.

WHO. (2006). *Guidelines for the safe use of wastewater, excreta and greywater in agriculture and aquaculture*: World Health Organization.

WHO. (2009). Addendum to the WHO guidelines for safe recreational water environments. Volume 1, Coastal and fresh waters: list of agreed updates.

WHO. (2013). WHO methods and data sources for global burden of disease estimates 2000-2011.

WHO. (2016). Quantitative microbial risk assessment: application for water safety management. *Quantitative microbial risk assessment: application for water safety management.*

Williams, M. S. & Ebel, E. D. (2014). Estimating the correlation between concentrations of two species of bacteria with censored microbial testing data. *International journal of food microbiology*, 175: 1-5.

Appended Papers

Paper I

Eregno, F. E., Nilsen, V., Seidu, R., & Heistad, A. (2014). Evaluating the Trend and Extreme Values of Faecal Indicator Organisms in a Raw Water Source: A Potential Approach for Watershed Management and Optimizing Water Treatment Practice. *Environmental Processes, 1*(3), 287-309.

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ORIGINAL ARTICLE

Evaluating the Trend and Extreme Values of Faecal Indicator Organisms in a Raw Water Source: A Potential Approach for Watershed Management and Optimizing Water Treatment Practice

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Abstract This study demonstrates the use of microbial load time series, through trend and extreme event analysis, to evaluate the effectiveness of watershed management strategies and to understand the probabilistic behaviour of extreme events. Heterotrophic plate count (HPC), *Clostridium perfringens*, intestinal enterococci, *Escherichia coli*, and coliform bacteria, were monitored from 1999 to 2012 at Nedre Romerike Vannverk (NRV) drinking water treatment plant, which takes its source water from Glomma River, Norway. Mann-Kendall test, Seasonal Mann-Kendall test, and Sen's Slope Estimator were used for trend analysis over years and also seasonal trends were examined through linear regression. Mann-Kendall test results show a decreasing trend for all indicator microorganisms except *Escherichia coli*. Seasonal trend analysis results also indicate that *Clostridium perfringens* during autumn and intestinal enterococci during spring have a significantly decreasing trend. An increasing trend was observed for all pathogens during the summer season. Trend analysis results offer insights and crucial perspective for policy makers and planners to evaluate the existing watershed management strategies. Moreover, extreme microbial load events in the raw water was analysed using the POT method to estimate return levels of extreme indicator microbial load corresponding to selected return periods. It is of importance to calculate the return period of extreme microbial load events for the purpose of designing optimal pathogen barriers and performing risk analysis.

Keywords Trend analysis · Extreme event analysis · Indicator microbial load · Microbial water quality · Raw water quality

1 Introduction

Deterioration of the quality of water in a river system can significantly affect human health, the ecosystem and recreational activities. Increasingly complex management decisions require

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different water-quality data analyses that provide information for multiple purposes, including trend analyses to distinguish enhancement or worsening in water quality with time. Water quality trend analysis is necessary to determine if water quality has consistently improved, deteriorated, or remained constant over long time scales. Trend analysis also allows the detection of any significant change in water quality, which helps to enable the identification of potential causal mechanisms of such changes. Such assessments can highlight the effectiveness of environmental improvement strategies, reveal issues requiring attention, or uncover areas that need further research and evaluation (Reay 2009).

Increasing availability of reliable and long-term microbial water quality data in a drinking water treatment plant is potentially important for the statistical analysis of trends, extreme events, and degree of association with different physicochemical parameters. Trend can be tested for existence, extent and significance. The purpose of this paper is to apply statistical methods to assess changes in microbial water quality and to identify the magnitude of changes that would allow water management authorities to take adequate measures.

Statistical techniques can be used to analyse trends in biological and chemical water quality dataset. Such statistical methods can be usually categorized into parametric and non-parametric methods. Parametric methods are commonly underpinned by assumptions on data distribution and independence of the data. Non-parametric statistical methods are based on the ranks of the observations more willingly than the values themselves (Helsel and Hirsch 2002).

The physicochemical and environmental variables of water quality trend have been detected using Mann-Kendall test, seasonal Mann-kandell test, and regression analysis, in various studies, providing insight into ecological and environmental changes, as well as a basis for instituting a water resources safety strategy. Trend detection methods have not yet been applied on microbial water quality analysis, although there have been applications, for example, to promote pollutant monitoring and preservation of water resources at catchment scale (Luo et al. 2011), evaluate water quality of lakes (Yenilmez et al. 2011), get information on regulatory issues (Li et al. 2010), and identify the dominant pollutant factors in water bodies (Yin and Hu 2009), among others. However, this study was conducted to detect the trends of microbial source water quality, which is quite different from other studies.

The other essential statistical analysis with regard to water quality is dealing with extreme events. The purpose of extreme value analysis is to quantify the stochastic behaviour of a process at strangely high or low levels. The stochastic behaviour of extreme events can be analysed by their probability distribution function. Estimating the probability of extreme events has become an important statistical discipline in applied science and is frequently used in environmental process modelling (Deoliveira 1988).

Many fields of modern science have to deal with events which are rare but have significant consequences. Extreme value theory is considered to provide the basis for the statistical modelling of such extremes. The potential of extreme value theory applied to meteorology, hydrology, insurance or finance, and water and air pollution has been recognized (Simiu and Heckert 1996; Freudenthal 1976; Konecny and Nachtnebel 1985; Jiang and Zhuang 2011; Huang and Batterman 2003; Kuchenhoff and Thamerus 1996; Ercelebi and Toros 2009; Ross 1987; Klugman 1997).

The proficient management of severe risks regarding the microbial concentration in the raw water requires an understanding of the probabilistic behaviour of extreme events. Frequency analysis of extreme load events can be used to obtain such understanding, and provide sufficient quantile estimates. The most widely used statistical methods of extreme value

analysis are based on either block maxima or peak-over-threshold representation of extremes (Katz et al. 2002; Naess 1998). The Block Maxima (BM) statistical method considers maxima values in random intervals and the distribution of these maximum values converges to the Generalized Extreme Value (GEV) distribution. On the other hand, the Peak-Over-Threshold (POT) statistical method is based on the theorem that the distribution of random variables that exceed sufficiently high threshold value converges to the Generalized Pareto (GP) distribution (Ross 1987; Falk and Guillou 2008; Mazas and Hamm 2010a; Hosking and Wallis 1987). In recent studies, such methods are used to handle the probabilistic behaviour of extreme values of microbial water quality (Persson and La 2012; Hadas et al. 2004).

A Block maximum sample is constructed by extracting a series of maximum values of microbial load in each year, i.e., only one event per year is retained. On the other hand, the POT approach retains all peak values that exceed the threshold value. Thus, the POT approach is not restricted to only one event per year. The most important advantage of POT analysis is that it allows for a more coherent selection of extreme events to consider as a peak event, and provides the possibility to control the number of severe microbial load occurrences to be included in the analysis by appropriate selection of the threshold (Lang et al. 1999; Simiu and Heckert 1996). Due to the limited number of observation years and the above mentioned advantages, we selected here to apply the POT analysis.

In this study, four different kinds of statistical trend analysis were used to identify significant changes in the microbial load time series of the drinking water source. These are the following: (1) Mann-Kendall Test: a non-parametric trend analysis that compares the change in the dataset ranks over time; (2) Sen's slope estimate: a non-parametric estimate of the change in the variable over time by calculating the slope; (3) Seasonal Kendall Test: a non-parametric method that first groups the data by month of the year, and then the Mann-Kendall test is performed on each group of monthly data; and (4) Linear Regression: a parametric trend analysis that computes the slope of the observations over time using ordinary least squares regression. The paper provides information about the trend of changes, magnitude and frequency of extreme microbial load in the Glomma River at Nedre Romerike Vannverk (NRV) drinking water treatment plant in Norway. Knowledge of such information is essential to evaluate the catchment management strategies to abate microbial pollution and also to develop safety plans in drinking water treatment plants.

2 Study Area and Detaset

2.1 Study Area

This study was based on raw water quality data from NRV drinking water treatment plant located at 59°57′18.9 "latitude and 11°1′29" longitude in Norway. NRV draws raw water from river Glomma, the largest river in Norway, with a catchment area of 41,200 km^2 (13 % of the total area of the country). The northwestern part of the river's watershed is dominated by high mountains, while the eastern part is covered by forest. The central and southern parts of the watershed are covered by large agricultural areas, which constitute 5.8 % of the total catchment area. The Glomma river basin contains Lake Mjøsa, the largest lake in Norway, with a surface area of 350 km^2. The average annual flow of the river at Solbergfoss (the lower-most reservoir) is 700 m^3/s. The discharge usually varies from 150 m^3/s to 3500 m^3/s during the year. Approximately 675,000 inhabitants live in the catchment area (Grizzetti et al. 2007).

2.2 Data

Data used for the analysis were extracted from secondary raw water quality data on indicator organisms from 1999 to 2013. This study used weekly records of five indicator microorganisms in the NRV raw water source namely, Heterotrophic plate count (HPC), *Clostridium perfringens* (*C.perfringens*), intestinal enterococci, *Escherichia coli* (*E.coli*), and coliform bacteria. The physico-chemical parameters were river discharge, temperature, pH, electrical conductivity, turbidity, and colour. For *E.coli* and coliform bacteria, weekly records were available from 1999 to 2013, for intestinal enterococci from 2002 to 2013, and for HPC and *C.perfringens* from 2005 to 2013. However, some records were missing and, during analysis, the missing values were treated as such (not filled with mean or neighbourhood values). In this study, Principal Components Analysis (PCA), Trend analysis, POT analysis and their graphical presentations were performed in Addinsoft's XLSTAT 2012 and R statistical software (Heffernan 2012; XLSTAT 2012).

3 Statistical Analysis

The main statistical analyses undertaken in this study were Principal Components Analysis (PCA), Trend analysis and extreme event analysis (POT analysis).

3.1 Descriptive and Principal Components Analysis

Descriptive statistics is useful for exploring and examining the basic features of the data prior to applying statistical tests. The descriptive statistics of the physicochemical, as well as the microbial load detaset, was carried out to explore the feature of the detaset.

Principal Components Analysis (PCA) is a multivariate statistical method that reduces the dimensionality of the detaset by performing a covariance analysis between factors, and this reduction is achieved by transforming the detaset into a new set of variables called the principal components (Hait and Schektman 1980). In this study, PCA was performed to identify the most important physicochemical factors that affect the microbial quality of raw water source. For the purpose of this study, the physicochemical parameters and indicator microbial data were subjected to the analysis. PCA analysis was performed using XLSTAT 2012 software.

3.2 Trend Analysis

3.2.1 Mann-Kendall Test

The Mann-Kendall trend test is a non-parametric statistical analysis used for identifying trends in time series data. The statistical analysis contrasts the relative magnitudes of data points rather than the data values themselves. The result of the test is not decided by the magnitude of the data points but by the ranking of individual data points (Gilbert 1987). The fundamental principle of Mann–Kendall test is to investigate the signs of all pairwise differences of the data values, ranked in chronological order. The benefit of this statistical analysis is that the data do not need to coincide with any particular distribution. Both the Kendall tau coefficient (τ) and Mann-Kendall coefficient (S) are used to identify rank correlation. Kendall τ is defined as the actual rating score of correlation divided by the maximum probable score. To find the rating

score for a time series, the detaset is sorted in ascending order according to time (Hirsch et al. 1982; Antonopoulos et al. 2001). The test statistics S is defined as:

$$S = \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} \operatorname{sign}(x_j - x_i) \tag{1}$$

where S is the rating score (Mann-Kendall sum); x is the observation value; n is the number of observation values in the series; i and j are counters; *sign* is a function having values of -1, 0, or +1 if (x_i-x_j) is negative, zero, or positive, respectively. The Kendall τ is calculated as:

$$\tau = \frac{S}{S_{max}} \tag{2}$$

where the maximum value of S is calculated as:

$$S_{max} = 0.5n(n-1) \tag{3}$$

A negative value of S or τ is a sign of a decreasing trend, and a positive value indicates an increasing trend. However, it is essential to compute the probability connected with S or τ and the sample size, n, to calculate the statistical significance of the trend. In the case where there is no trend in either ranking, the distribution of S can be well approximated by a normal distribution with mean zero and variance calculated as:

$$Var(S) = \frac{n(n-1)(2n+5) - \sum_{i=1}^{m} t_i(i)(i-1)(2i+5)}{18} \tag{4}$$

provided that $n \geq 10$ and t_i is the number of ties up to sample i. Then, the normal distribution parameter (the Mann-Kendall statistic, *Z*) is calculated as:

$$Z = \begin{cases} \frac{1}{\sqrt{Var(S)}}(S-1) & \text{if} \quad S > 0 \\ 0 \quad \text{if} \quad S = 0 \\ \frac{1}{\sqrt{Var(S)}}(S+1) & \text{if} \quad S < 0 \end{cases} \tag{5}$$

The Mann-Kendall statistic, Z, at this point follows a normal distribution. A negative (positive) value of Z signifies a downward (upward) trend. Significance levels α are applied for testing either an increasing or decreasing monotone trend. If Z values appear greater than $Z_{\alpha/2}$, where α shows the significance level, then the trend is considered as significant.

3.2.2 Seasonal Mann-Kendall Test

The seasonal Mann-Kendall Test is a modified version of Mann-Kendall test that accounts for seasonality by computing the Mann-Kendall test on each of m seasons independently, in order to identify seasonal trend. Monthly scores are then added to get the test statistic. By adding the variances of the Kendall score statistic for each month, the variance of the test statistic is obtained. The normal approximation can be used to evaluate significance level. It carries all the robust statistical properties offered by the Mann-Kendall test. The Seasonal Mann-Kendall test is established on the basis that the trend is cyclically varying in relation to the seasons of the year. It is used to analyse time series data for the possible existence of an upward or downward

trend, at a particular significance level, while accounting for the effect of seasonality (Hirsch et al. 1982; Yu et al. 1993). Kendall's S statistics S_i for each season are added to form the overall statistic S:

$$S = \sum_{i=1}^{m} S_i \tag{6}$$

3.2.3 Sen's Slope Estimator

Sen's slope statistical trend analysis is a non-parametric procedure. Its statistic is computing the median slope of each point-pair slope in the detaset. To obtain an estimate of the slope Q, the slopes of all pairs in the detaset are calculated for each time period by the equation (Sen 1968):

$$Q = \frac{x_j - x_k}{j - k} \tag{7}$$

where Q is the slope between data points k and j, x_k is the data measurement at time k, x_j is data measurement at time j, j is the time after time k. If there are n values x_j in the time series data, we get as many as $N=n(n-1)/2$ slope estimates Q. Its sign reflects the trend direction, while its value reflects how steep the trend is. Sen's slope estimator is then calculated by choosing the middle-ranked slope as follows;

$$Q = \begin{cases} Q_{\frac{N+1}{2}} & \text{if} \quad N \quad \text{is} \quad \text{odd} \\ \frac{1}{2}\left(Q_{\frac{N}{2}} + Q_{\frac{N+2}{2}}\right) & \text{if} \quad N \quad \text{is} \quad \text{even} \end{cases} \tag{8}$$

where N is the number of calculated slopes.

3.2.4 Linear Regression Test

Linear regression is the most popular parametric statistical method to identify a monotonic trend in a time series detaset. Let y_i denote the response variable observed at time, t_i. A conventional linear regression model for trend analysis is given by:

$$y_i = b_0 + b_1 t_i + e_i \tag{9}$$

The parameter b_1 in a linear regression model expresses the rate of change of y_i in time. The slope coefficient (b_1) is statistically tested under the null hypothesis that it is equal to zero. The t-statistic on b_1 is tested to decide if it is significantly different from 0. If the slope is non-zero (upward or downward slope), the null hypothesis of zero slopes over time is rejected, and one can conclude that there exist a linear trend in y over time. Besides of providing a measure of significance based on the hypothesis test on the slope, it also gives the magnitude of the rate of change (Abaurrea et al. 2011).

3.2.5 Test Statistics

All the statistical tests were based on testing the null hypothesis. The null hypothesis (H_o) is that no change has occurred in microbial concentration of a variable over time, or that no trend is present. The alternative hypothesis (H_1) is that a change has occurred over time, or that an increasing or decreasing trend in the concentration of a variable is evident. The statistical tests

determine the probability (*p*-value) of the statistics and the slope of the trend line. The smaller the *p*-value is, the greater the weight of evidence against H_o. A significance level α=0.05 corresponds to 95% confidence level.

3.3 Extreme Event Analysis

3.3.1 Peak-Over-Threshold Method and Generalized Pareto Distribution

Extreme Value Theory is used to model the tail return levels and then incorporate this into risk assessment. Two main approaches are proposed in the literature: Block maximum and Peak-Over-Threshold method (Kasperski and Hoxey 2008; Katz et al. 2002; Deoliveira 1988). The Block Maximum picks the highest value per year and does not include all extreme values because any second highest would be dropped out of the sample. The POT method considers all values exceeding a certain predefined threshold. In this study, we apply the POT method based on the GP distribution to describe tail behaviour that enables us to estimate the extreme quantiles.

If an unidentified distribution function F of a random variable X over a high threshold value u is considered, the cumulative distribution function $F_u(y)$ of exceedances of X above a high threshold value is defined as (Embrechts et al. 1997):

$$F_u(y) = \Pr(X - u \le y | X > u) = \frac{F(x) - F(u)}{1 - F(u)}, \quad 0 \le y \le x_F - u \tag{10}$$

where X is a random variable, u is a given threshold, y=x - u are the excesses, and x_F is the right end point of F. As the threshold value u gets large, the Extreme Value Theory provides us with the underlying distribution of $F_u(y)$, i.e., the distribution of the excess $F_u(y)$ approximated by F(y,k,α) (Iii 1975):

$$F_u(y) \approx F(y, k, \alpha), \quad u \to \infty \tag{11}$$

where:

$$F(y, k, \alpha) = \begin{cases} 1 - \left(1 - k\frac{y}{\alpha}\right)^{1/k}, & k \neq 0 \\ 1 - \exp\left(-\frac{y}{\alpha}\right), & k = 0 \end{cases} \tag{12}$$

where α and *k* are scale and shape parameters, x>0 for k≤0, and x ϵ [0,α/k] for k>0. This expression is the same as the GP distribution (Castillo and Hadi 1997).

3.3.2 Threshold Selection

The values of extreme quantile estimation greatly depend on the choice of the threshold. The selection of the threshold value is a trade-off between variance and bias. A high threshold value would produce a reduced amount of excesses used to formulate the model, leading to large variance. A low threshold value would lead to a violation of the asymptotic foundation of the model, leading to bias (Klugman 1997; Ross 1987). The right position is the lowest possible threshold for which the excesses follow the GP distribution. Two methods are available for this: A simple graphical method that plots the estimated shape parameter *k* as function of threshold. When the threshold is high enough, the shape parameter should be approximately independent of threshold. The graphs are plotted focusing on the extreme

values where the threshold value is expected. The second method is the plot of mean excesses as a function of threshold. The mean excess function describes the expected overshoot of a threshold given that exceedances occur. The interpretation of the plot is explained by various authors (Klugman 1997; Mazas and Hamm 2010b; Wong and Li 2010). In these studies, it is stated that if the observed plot follows a rationally straight line with positive gradient over a distinct value of u, then this is a sign that the excesses over this threshold follow a GP distribution with positive shape parameter. This property was used as a criterion for the selection of threshold in this study (Leadbetter and Rootzen 1988).

3.3.3 Return levels (quantile)

The return level X_T is defined as the probability p that X_T is exceeded in any given year or the level that is likely to be exceeded on the average once every $1/p$ year. In extreme value statistics terminology, X_T is the return level related to the return period T=1/p (Katz et al. 2002). From the GP distribution, the return level is derived by setting the cumulative distribution function equal to the anticipated quintile, (1–p), and then solving for the return level (El-Aroui and Diebolt 2002). Suppose that the GP distribution is an appropriate model for y, the N period return level is denoted as follows:

$$X_T = \frac{\alpha}{k}\left\{1 - \left\{\frac{-\log\left(1-\frac{1}{T}\right)}{\lambda}\right\}^k\right\} + u \tag{13}$$

where X_T is the return level, T is the return period; λ is the number of exceedances per year.

3.3.4 Model Validation

Graphical model validation is a vital step in the model building process, since it allows the investigator to have confidence in the inference obtained. We use two methods of validation for assessing the quality of a fitted GP distribution model; these are probability plots and quantile plots. A GP distribution model fits the data well, if a probability plot and a quantile plot of the model and data points could be approximated by a straight line (Katz et al. 2002).

4 Result and Discussion

4.1 Descriptive Statistics of Water Quality Parameters

The summary of descriptive statistics of the water quality variables, based on the weekly data from 2008 to 2012, is presented in Table 1. Among the physicochemical variables, the highest observation ranges were observed in river discharge (2314.8 m^3/s), while the lowest were observed in pH (1.7). Among indicator microbial load detaset, HPC recorded the highest median value of 365/mL while *C.perfringens* load in the source water exhibited the least median value of 5/100 mL. The standard deviation around the means is substantially high in HPC, coliform bacteria, *E.coli* and intestinal enterococci with values 1920.4, 291.7, 47.5, and 23.5, respectively. This could be a result of complex land use in the catchment area and seasonal variation of different anthropogenic and physicochemical factors surrounding the study area.

The numerical values of the correlation coefficient, r, for physicochemical water quality parameters and indicator microbial load records during the period of five years (from 2008 to 2012) are tabulated in Table 2. A highly positive correlation is observed between pH and

Table 1 Descriptive statistics of physicochemical and microbial raw water quality variables (records from 2008 to 2012)

Statistic	River discharge (m^3/s)	Temperature (°C)	pH	Turbidity (NTU)	Electrical Conductivity (mS/m)	Colour (mg pt/L)	HPC (/mL)	*C.perfringens* (/100 mL)	Intestinal enterococci (/100 mL)	*E.coli* (/100 mL)	Coliform bacteria (/100 mL)
Minimum	136.5	1.0	5.7	0.2	1.3	3.0	1.0	1.0	1.0	1.0	1.0
Maximum	2451.2	21.5	7.4	124.0	7.6	87.0	14000	59.0	140.0	240	2400
Range	2314.8	20.5	1.7	123.8	6.3	84.0	13999	58.0	139.0	239	2399
1st Quartile	424.8	2.2	6.5	0.7	3.0	22.0	157.5	1.0	1.0	1.0	15.0
Median	576.8	7.5	7.1	1.6	4.1	29.0	365.0	5.0	6.5	25.0	165.0
3rd Quartile	879.0	13.3	7.2	3.2	4.5	36.0	850.0	8.0	25.0	70.0	260.0
Mean	700.5	8.2	6.9	4.4	3.8	30.7	971.1	6.3	16.4	41.6	211.8
Variance	154696.7	34.1	0.1	141.8	0.9	156.7	3688094	57.7	553.1	2253	85080
St. deviation	393.3	5.8	0.4	11.9	1.0	12.5	1920.4	7.6	23.5	47.5	291.7
Skewness	1.5	0.3	−1.0	7.3	−0.2	1.3	4.4	3.6	2.8	1.5	4.0
Kurtosis	2.5	−1.3	−0.1	62.7	0.4	3.1	22.8	18.8	9.9	2.3	21.9

Table 2 Correlation coefficients among physicochemical and microbial raw water quality variables (records from 2008 to 2012)

Variables	River discharge	Temperature	pH	Turbidity	EC	Colour	HPC	*C.perfringens*	Intestinal enterococci	*E.coli*	Coliform bacteria
River discharge	1.00										
Temperature	0.62	1.00									
pH	0.19	0.27	1.00								
Turbidity	0.04	0.09	−0.27	1.00							
EC	−0.14	−0.07	0.62	0.32	1.00						
Colour	0.08	0.00	0.04	−0.12	−0.17	1.00					
HPC	−0.12	−0.12	0.58	−0.04	0.48	0.31	1.00				
C.perfringens	−0.05	−0.10	0.68	−0.09	0.67	0.23	0.72	1.00			
Int. enterococci	−0.14	−0.18	0.51	−0.10	0.55	0.16	0.68	0.67	1.00		
E.coli	−0.09	−0.11	0.75	−0.14	0.73	0.18	0.75	0.85	0.83	1.00	
Coliform bacteria	0.12	0.15	0.79	−0.13	0.63	0.16	0.78	0.77	0.74	0.89	1.00

indicator microorganism load (from r=0.51 to r=0.79), and EC and indicator microorganism load (from r=0.48 to r=0.73), while a very weak negative correlation was observed between turbidity and indicator microorganism load (from r=-0.04 to r=-0.14).

4.2 Principal Component Analysis

Principal Component Analysis (PCA) was carried out to identify the most important physicochemical factors that affect the microbial quality of source water. When five source water microbial load records and six physicochemical variables were reduced to four principal components with eigenvalue greater than one, the identified four components accounted for around 86 % of the variance in the detaset. Table 3 presents rotation factor correlation coefficients of original water quality parameters. Based on the first of the four principal components, Factor 1 (F1), the coefficients of pH and EC are relatively notable, and F1 can be classified as a mineral related water quality factor. In Factor 2 (F2), the coefficients of river discharge and temperature are remarkable, and this factor can be classified as weather related factor. In Factor 3 (F3), the coefficients of turbidity and colour are significant and F3 can be classified as organic matter related factor. Finally, Factor 4 (F4) is almost the same as factor 3. Figure 1 shows the PCA biplot obtained from the first two principal components, accounting for 64.3 % of the variance in the detaset. It can be seen that pH, EC, turbidity and colour are more correlated with microbial water quality parameters, and river discharge and temperature are weakly correlated with microbial water quality parameters, but highly correlated to each other.

4.3 Trend analysis

In order to visualize the annual fluctuation of each indicator microbial load records, annual mean values±standard deviations are plotted in Fig. 2. As one can see from Fig. 2, the annual fluctuation in 2002 and 2003 for intestinal enterococci, *E.coli*, and coliform bacteria, in 2009

Table 3 Rotation factor correlation coefficients for physicochemical and microbial raw water quality parameters (records from 2008 to 2012)

Variables	F1	F2	F3	F4
River discharge	0.007	**0.881**	0.013	0.043
Temperature	0.012	**0.895**	0.153	−0.047
pH	**0.815**	0.280	0.002	−0.353
Turbidity	−0.092	0.006	**0.778**	0.609
EC	**0.750**	−0.174	0.546	−0.036
Colour	0.197	0.111	**−0.651**	0.656
HPC	**0.835**	0.144	−0.059	0.290
C.perfringens	**0.894**	−0.093	−0.047	0.054
Intestinal enterococci	**0.826**	−0.226	−0.074	0.054
E.coli	**0.965**	−0.130	−0.039	−0.037
Coliform bacteria	**0.933**	0.149	−0.024	−0.061
Eigenvalue	5.256	1.817	1.364	1.026
Variability (%)	47.778	16.522	12.398	9.328
Cumulative %	47.778	64.300	76.698	86.026

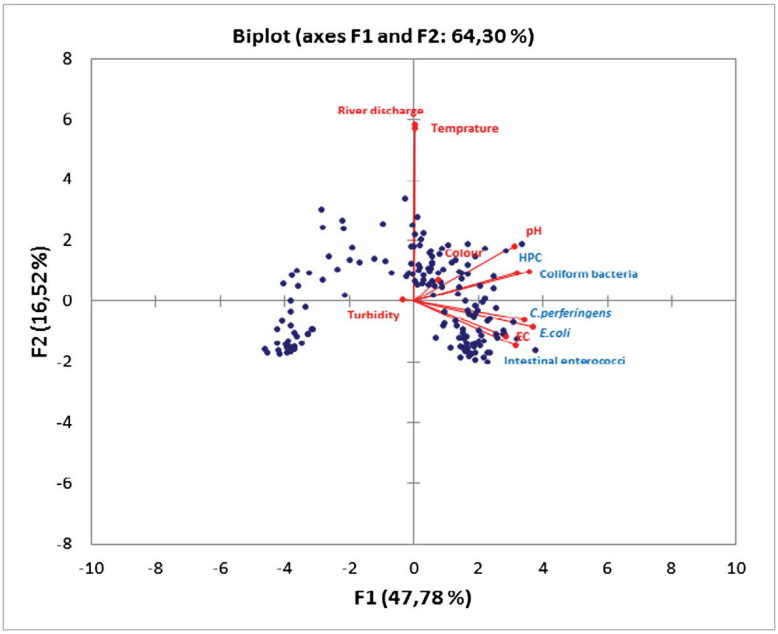

Fig. 1 PCA biplot of physicochemical and microbial water quality variables

and 2011 for *C.perfringens*, and in 2009 for HPC was relatively high. As we can see from the monthly average microbial load in the raw water (Fig. 3), the trend in microbial load was characterized by strong seasonal fluctuations with low load during the summer season (June, July, and August) for all indicator microorganisms; this indicates that microbial concentration per unit volume of water is reduced due to the summer flood dilution effect.

4.3.1 Mean monthly microbial load in the raw water

To present the monthly mean value fluctuation of each indicator microbial load records, by taking year 2012 as a representative year, monthly mean values±standard deviations are plotted in Fig. 4. It is shown that the monthly mean value fluctuation for the representative year, 2012 was high, and it is relatively very high during March and July for all indicator microbial load records. Both high monthly and annual mean value fluctuation indicates that non-parametric trend tests are likely to be more powerful than parametric techniques in this study.

Statistical results from trend analysis are given in Table 4. Based on the Mann–Kendall test, the Null Hypothesis of no trend was rejected for only *C.perfringens*. In other words, statistically significant trend was observed for *C.perfringens* ($p<0.006$). The Mann-Kendall test Statistic (S), revealed conformity in the magnitude of the statistic, which indicates that there was a decreasing trend for all indicator microbial organisms except *E.coli*. The decreasing trend of Sen's Slope Estimator, except for *E.coli*, also supports the Mann-Kendall test result. The Seasonal Mann-Kendall test is applied in order to test whether the trend is cyclical, varying with the seasons in the year or not. In all microbial load cases, there was not any statistically significant trend. However, the magnitude varied, showing a decreasing trend in the case of HPC, *C.perfringens*, close to zero in the case of intestinal enterococci and coliform bacteria, and increasing in the case of *E.coli*.

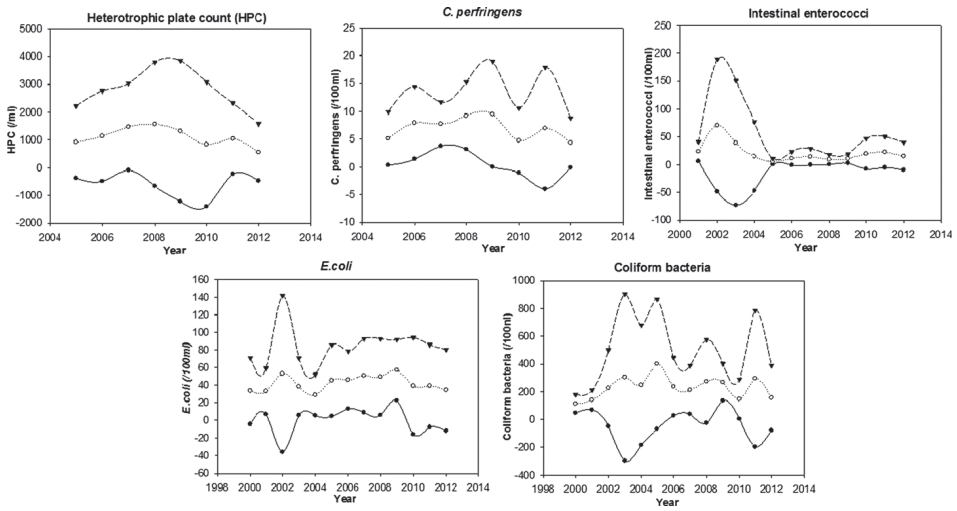

Fig. 2 The fluctuation of annual mean values of source water microbial load (annual mean value±STDEV)

The downward trend of the indicator microbial organisms in the trend analysis indicates an improvement in microbial water quality. This observed trend may be attributed to improvements in wastewater treatment and watershed management within the Glomma basin. Even though the upward trend in *E.coli* load in the raw water is not statistically significant (p>0.913), there is a need for strategies to be implemented to reduce the trend.

4.3.2 Monthly maximum microbial load in the raw water

Trend analysis of monthly maximum microbial load was carried out in order to investigate only trend in extreme events. Figure 5 presents the monthly maximum microbial load for each indicator microorganism. The Mann-Kendall trend test results for monthly maximum microbial load indicates an evidence of a rising trend for intestinal enterococci, *E.coli*, and coliform bacteria,

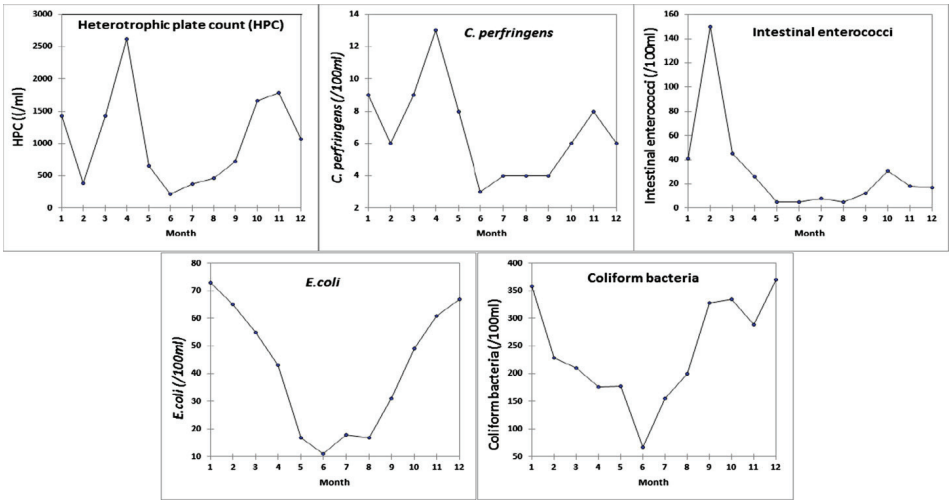

Fig. 3 Monthly average indicator microbial load in the raw water

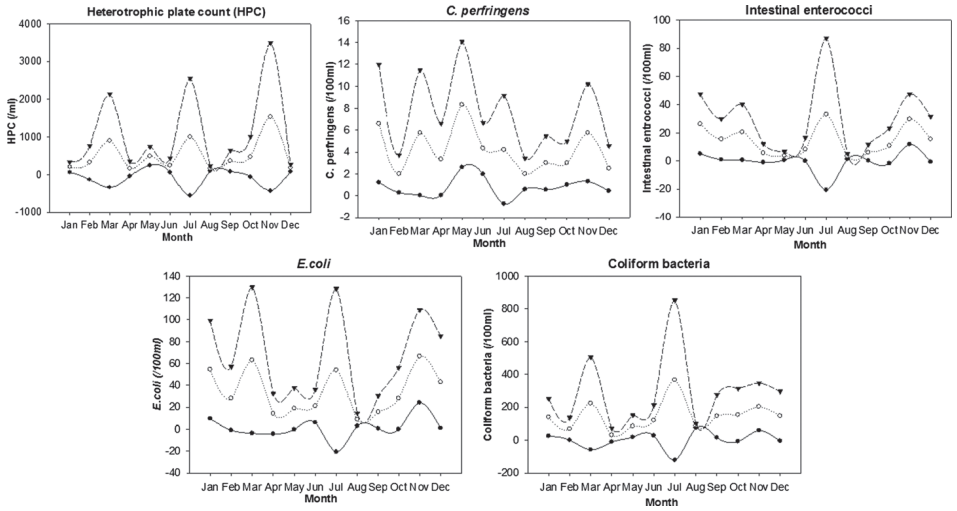

Fig. 4 The fluctuation of mean monthly values of source water microbial load (monthly mean value±STDEV) for year 2012

and a negative trend for HPC and *C.perfringens*. However, there was no statistical significance ($p>0.415$) for any of the microbial organisms (Table 5).

The Sen's slope test for HPC showed a decreasing slope, while for *E.coli* and coliform bacteria an increasing slope. However, there was no change in the slope for *C.perfringens* and intestinal enterococci. The result of the Mann-Kendall test and Sen's slope trend analysis have revealed that the extreme values of *E.coli* and coliform bacteria have an increasing trend even though there was no statistical significance.

Table 4 Mann-Kendall, Sen's slope and Seasonal Mann-Kendall trend test for monthly mean value of microbial load in raw water

Mann-Kendall test	HPC	*Clostridium perfringens*	Intestinal enterococci	*Escherichia coli*	Coliform bacteria
Kendall tau	−0.135	−0.195	−0.015	0.006	−0.041
S	−577	−830	−129	69	−463
Var (S)	90785	90704	264238	386275	386302
p-value (two tailed)	0.056	0.006	0.803	0.913	0.457
alpha	0.05	0.05	0.05	0.05	0.05
Conclusion	Decreasing	Sign. decreasing	Decreasing	Increasing	Decreasing
Sen's slope	−3.48	−0.041	−0.004	0.006	−0.147
Seasonal Mann-Kendall					
Kendall tau	−0.143	−0.175	0.011	0.034	0.005
S'	−36	−44	6	27	4
p-value (two tailed)	0.129	0.062	0.943	0.607	0.953
alpha	0.05	0.05	0.05	0.05	0.05
Conclusion	Decreasing	Decreasing	Increasing	Increasing	Increasing

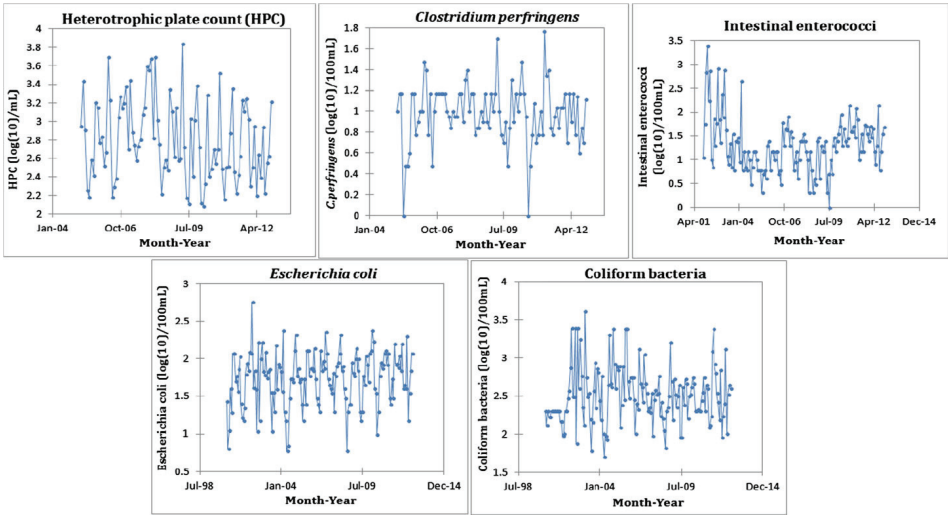

Fig. 5 Time series plot of monthly maximum indicator microbial load in the raw water

A Seasonal Kendall test was carried out in this study to test whether the extreme microbial load trends are affected by seasonal changes. The results of the Seasonal Kendall test on the microbial organisms showed no statistically significant trend. There was evidence of a rising trend for intestinal enterococci, *E.coli* and coliform bacteria, and a decreasing trend for HPC. There was no change in trend of *C.perfringens*.

Table 5 Mann-Kendall, Sen's slope, and Seasonal Mann-Kendall trend test for monthly maximum value of microbial load in raw water

Mann-Kendall test	HPC	*Clostridium perfringens*	Intestinal enterococci	*Escherichia coli*	Coliform bacteria
Kendall tau	−0.051	−0.06	0.038	0.097	0.042
S	−218	−244	329	1088	469
Var (S)	90740	88887	263011	385904	385485
p-value (two tailed)	0.471	0.415	0.522	0.80	0.451
alpha	0.05	0.05	0.05	0.05	0.05
Conclusion	Decreasing	Decreasing	Increasing	Increasing	Increasing
Sen's slope	−2.679	0	0	0.136	0.202
Seasonal Mann Kendall					
Kendall tau	−0.068	0.004	0.050	0.128	0.054
S'	−17	0.00	32	99	42
p-value (two tailed)	0.487	1.00	0.482	0.051	0.415
alpha	0.05	0.05	0.05	0.05	0.05
Conclusion	Decreasing	No trend	Increasing	Increasing	Increasing

4.3.3 Seasonal trend analysis of microbial load in the raw water

According to the Norwegian Meteorological Institute, the four seasons are defined as four equal-length periods of 3 months in the region and this study adopts the same period: winter (December-February), spring (March-May), summer (June-August), and autumn (September-November).

Seasonal trends in data were examined through linear regression analysis. The trend was determined by calculating a linear regression line using the least squares fit method. The slope of the linear regression line in this study is used to evaluate a trend direction of the mean microbial load for each season and the statistical summary of the linear regression analysis is presented in Table 6. The regression result revealed a positive trend for all indicator microorganisms during the summer season (June-August). However, this relationship was not statistically significant ($p>0.215$). During winter, spring and autumn seasons, the microbial load showed a decreasing trend. The results also show that for *C.perfringens* during autumn (September-November) and for intestinal enterococci during spring (March-May), the raw water microbial load is significantly decreased ($p<0.016$). The possible reasons for the increasing trend for microbial concentration during the summer season could be the intensification of anthropogenic activities and also the alteration of precipitation patterns during the summer time. The speculated anthropogenic summer activities may include different outdoor recreational and on farm activities. Therefore, attention should be given to reverse the situation.

Table 6 Linear regression statistical trend tests for seasonal microbial load in raw water

Microbial pathogen	Season	Slope (b_1)	Standard error	t	Pr>|t|	α	Trend
HPC	winter	−237.146	208.2	−1.139	0.306	0.05	Decreasing
	Spring	−255.415	103.0	−2.480	0.056		Decreasing
	Summer	22.111	31.4	0.704	0.513		Increasing
	Autumn	−115.413	82.5	−1.399	0.221		Decreasing
Clostridium perfringens	winter	−0.782	0.8	−1.004	0.362	0.05	Decreasing
	Spring	−1.054	0.6	−1.785	0.134		Decreasing
	Summer	−0.313	0.3	−1.020	0.355		Decreasing
	Autumn	−1.022	0.2	−5.291	0.003		Significantly Decreasing
Intestinal enterococci	winter	−1.464	1.5	−0.979	0.353	0.05	Decreasing
	Spring	−6.212	2.1	−2.960	0.016		Significantly Decreasing
	Summer	0.469	0.4	1.063	0.316		Increasing
	Autumn	−2.163	2.1	−1.021	0.334		Decreasing
Escherichia coli	winter	−1.420	2.3	−0.627	0.544	0.05	Decreasing
	Spring	1.055	0.8	1.339	0.207		Increasing
	Summer	0.669	0.5	1.315	0.215		Increasing
	Autumn	−1.255	0.9	−1.394	0.191		Decreasing
Coliform bacteria	winter	−20.144	12.1	−1.661	0.125	0.05	Decreasing
	Spring	−3.731	6.9	−0.536	0.603		Decreasing
	Summer	3.043	5.1	0.594	0.564		Increasing
	Autumn	5.088	12.9	0.394	0.701		Increasing

4.4 Extreme Events Analysis

The application of extreme value theory based on Generalized Extreme Value (GEV) distribution for water quality management through risk analysis, design and operation of west water treatment plant and sewer system was discussed (Thas et al. 1997). Particle counts in raw and treated water were subjected to numerical analysis of GEV followed by a recurrence curve to suggest a threshold value that is crucial for water quality monitoring (Persson and La 2012). In this study, extreme value theory was applied through POT method on the microbial source water quality detaset in order to analyse the risk and optimize water treatment practices.

The application of the POT method with the intention of calculating the probability of incidence of extreme events involves the following three steps: (1) select the threshold u value; (2) fit the GP distribution function to the exceedances over threshold data, and then estimate the shape and scale parameters; and (3) compute the return level of the extreme event using estimated parameters.

4.4.1 Threshold (u) selection

Figure 6 shows the sample mean excess plots corresponding to the selected microbial organisms in the raw water. Since, reasonable threshold values are obtained from linearity between mean excess values and excess over thresholds, based on a closer inspection of the plots, we suggest the values: u=1800/mL for HPC, u=9/100 mL for *C.perfringens*, u=29/100 mL for intestinal enterococci, u=90/100 mL for *E.coli*, and u=330/100 mL for coliform bacteria. These values are located at different sections of the mean excess plots that are roughly linear, leaving respectively 38, 62, 42, 47, and 70 observations of the tail section for further analysis (Table 7). The other criterion to select the threshold is a threshold stability plot which presents the shape parameter against threshold values and the exceedances with 95 % confidence interval (Fig. 7). As we can see from Fig. 7, a reasonable stable position close to the highest threshold is the position of our threshold values; hence, our threshold values look reasonable.

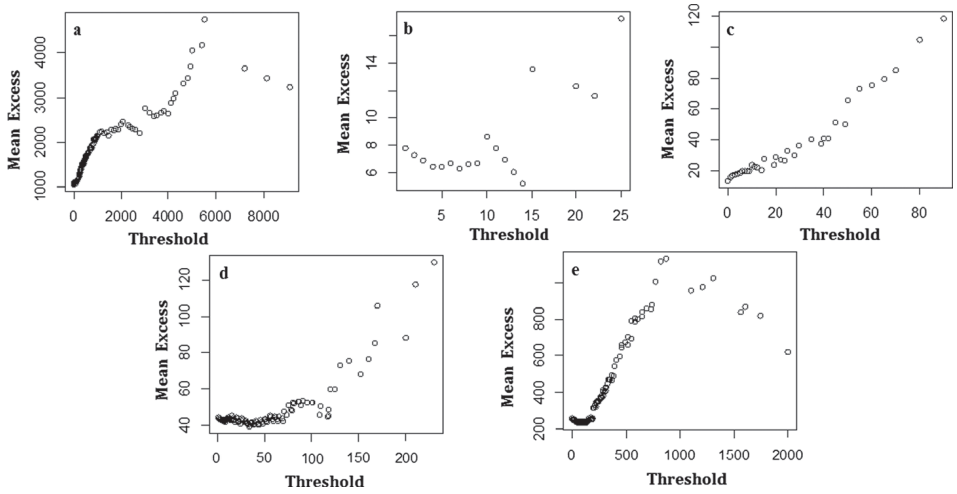

Fig. 6 Mean residual life plot for: **a** HPC; **b** *Clostridium perfringens*; **c** Intestinal Enterococci; **d** *Escherichia coli*; **e** Coliform bacteria

Table 7 Estimated parameters of GP distribution fit to microbial load detaset to compute extreme load frequency

Parameters	HPC	*Clostridium perfringens*	Intestinal enterococci	*Escherichia coli*	Coliform bacteria
Total N	298	302	456	547	547
Threshold value (u)	1800	9	29	90	330
N above threshold	38	62	42	47	78
Shape parameter (k)	0.09	0.16	0.57	0.15	0.64
Scale parameter (α)	2408.3	5.9	13.8	45.3	218.7
No. of exceedances per year (λ)	5.4	8.9	3.5	3.9	6.5

4.4.2 Estimation of Parameters and Model Validation

After determining the threshold values using the exceedances detaset, the GP distribution parameters were estimated using R statistical software. There are different numerical techniques available for the estimation of the parameters of the extreme value distributions. From the different methods, the Maximum Likelihood (ML) technique tends to be the selected one since it is relatively general and more flexible than other methods (Hosking and Wallis 1987), and was applied in this study.

Table 7 shows the scale (α) and shape (k) parameters with their corresponding threshold value for each indicator microbial organisms in the raw water. The shape parameters have negative value and are close to zero for all microbes, while the scale parameters have a huge variation among the different microbes. The number of exceedances per year (λ), which is used to compute the return level, is calculated by dividing the number of observations over the threshold value by the number of years the observations are carried out.

Figure 8 shows the probability plots (a), and Quantile plots (b), for HPC (1), *C.perfringens* (2), intestinal enterococci (3), *E.coli* (4), and coliform bacteria (5). The plots are used to test

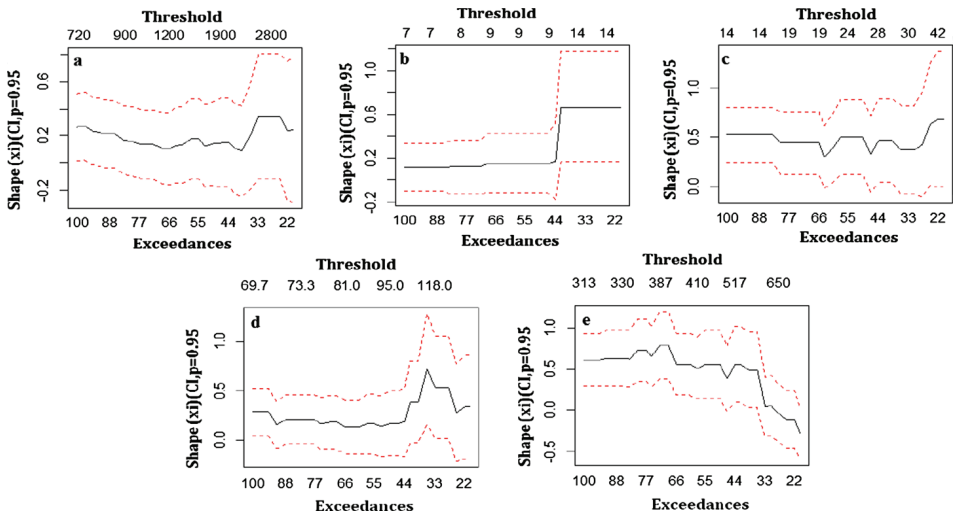

Fig. 7 Threshold stability plots for: **a** HPC; **b** *Clostridium perfringens*; **c** Intestinal Enterococci; **d** *Escherichia coli*; **e** Coliform bacteria

whether the exceedances detaset follows the Generalized Pareto Distribution or not. The probability and quantile plots of GP distribution model and microbial load data are close to linear, with relatively slight debate in the case of *C.perfringens* and intestinal enterococci. We can see that all diagnostic plots show valid results for all microbial load data. Therefore, the model can be used to predict extremes through return levels for different return periods.

4.4.3 Return level of the extreme event

Based on the parameter values which are estimated by the maximum likelihood method, and assuming that extreme microbial load data are from stationary processes, return levels are provided in Fig. 9. The return levels gradually increase for higher return periods. From the above results one would expect, for example, HPC loads in the raw water to exceed about 16151/mL on the average once every 10 years, and about 22739/mL on the average once every 50 years, or *E.coli* in the raw water to exceed about 376/100 mL on the average once every 10 years, and about 542/100 mL on the average once every 50 years. The applicability of this result for drinking water treatment plans is that the design of the microbial pathogen barriers should consider these extreme events in order to minimize microbial risk.

4.5 Practical Implications and Limitations of the Study

Monitoring of source water microbial load is a common practice in water treatment plants, and long records of microbial concentration data are available. Such datasets may be used in statistical analysis for various purposes. The findings of this study reveal a number of practical applications of value for watershed management, water treatment practices, and risk analysis in relation to microbial source water quality.

Source water microbial load trend analysis may reveal existing trends in the time series that could have a direct association with deterioration or improvement in watershed management practices. Results from such analyses can be valuable as a first evaluation of watershed

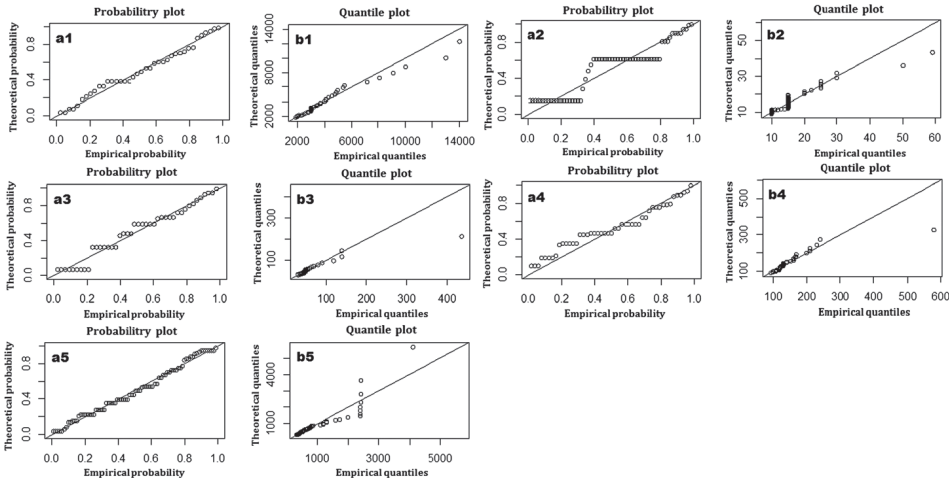

Fig. 8 Non-exceedance probability plots for GP distribution fit to detasets of: (a1) HPC; (a2) *Clostridium perfringens*; (a3) Intestinal Enterococci; (a4) *Escherichia coli*; (a5) Coliform bacteria; and non-exceedance quantile plots for GP distribution fit to detasets of: (b1) HPC; (b2) *Clostridium perfringens*; (b3) Intestinal Enterococci; (b4) *Escherichia coli*; (b5) Coliform bacteria

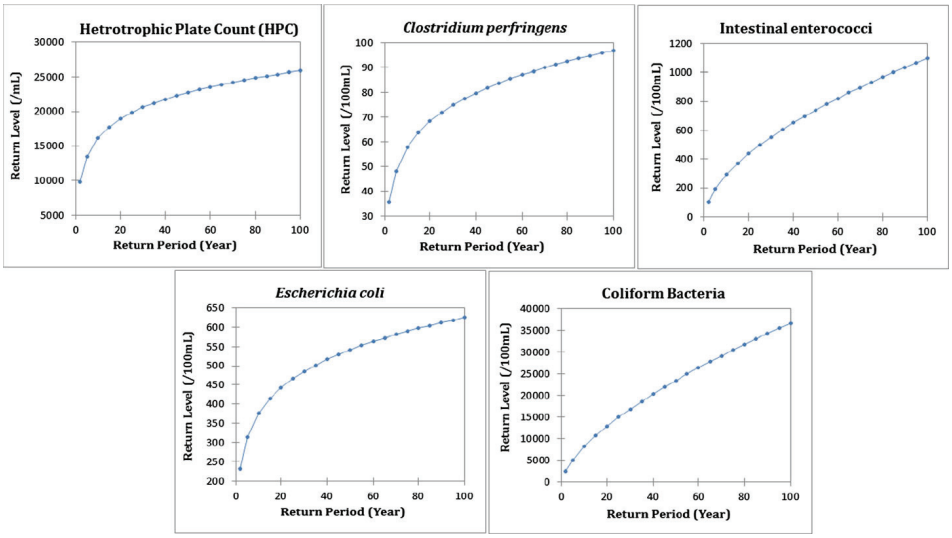

Fig. 9 Return level plots for different indicator microorganisms

management and for recommending further examinations of the causes of any observed trends, as well as monitoring the results of management interventions.

Extreme source water microbial load events at drinking water treatment plants are rare, but important for microbial risk analysis. The quantification of extreme quantiles using extreme value theory assists in determining suitable treatment steps for pathogen removal based on risk assessments. These techniques are regularly used in the areas of storm, flood and high wind events. However, little work has been done for extremes in microbial water quality variables. This study demonstrates the application of extreme value theory on a microbial water quality detaset. Quantitative microbial risk models rely on knowing the probabilities of extreme microbial load events and also include a probabilistic representation of the performance of pathogen treatment barriers. Therefore, integrating extreme event analysis in risk models, may give opportunities for optimization of drinking water treatment with respect to microbial risk.

Even though this study generated important findings in the field of statistical analysis of microbial water quality, a number of caveats need to be noted and we have to acknowledge the limitations. The main limitations are expressed as follows:

- The monitored microbial detaset at the drinking water treatment plant was provided as a working document, and could not be checked for errors. Therefore, any data quality issues such as missing, erroneous, extreme and duplicate values will affect the results.
- There are also limitations associated with changes in laboratory analytical techniques. When the analytical techniques change due to, e.g., technological advancement, this could also be a source of variation in the results.
- Since the temporal resolution of data points are weekly time steps, there will be some limitation associated with capturing variation of microbial load in the river system.

5 Summary and Conclusions

Analytical tools should be developed to properly evaluate catchment management strategies, design appropriate microbial barrier mechanisms in drinking water treatment plants, and monitor water quality improvements or deterioration at different catchment scale. Thus, integrated studies of water quality and statistics are crucial to create analytical tools to support the decision making processes. In this study, statistical principles were applied for trend and extreme value analysis of microbial loads in a drinking water source, in order to demonstrate how the microbial load time series in drinking water treatment plants could be used as a potential source of information for catchment management and pathogen barrier design in the treatment plant.

Monitoring of microbial loads in the raw water is the main task of drinking water treatment plants to determine the current hygienic status of raw water. At the same time there is an opportunity to use microbial load time series for trend analysis in order to know whether watershed management strategies have beneficial effect or not. The analytical results of monthly mean microbial load trend analysis indicate that there is a decreasing trend for all indicator microorganisms except *E.coli*. Seasonal trend analysis results confirmed a decreasing trend for all indicator microorganisms except *E.coli* during the spring season and an increasing trend for all pathogens during the summer. Therefore, this study could offer insights and crucial perspective as a case study for policy makers and planners in helping them to evaluate the existing catchment management strategies. Besides, it could assist decision making processes in order to identify priorities of catchment management focal points.

Extreme microbial load events in the raw water were analyzed using the Peak-Over-Threshold (POT) method to estimate return levels of five indicator microorganisms corresponding to selected return periods. The Generalized Pareto distribution was implemented to extrapolate raw water microbial load to levels more extreme than those observed. The analytical results indicate that the generalized pareto distribution function seems to provide an adequate fit to the microbial load extreme detaset above a certain threshold value. Diagnostic plots (p-p plots and q-q plots) indicate that the Generalized Pareto distribution is an appropriate model and that the underlying assumptions are reasonable for the data analyzed. In other words, it is possible to apply extreme value theory to obtain reliable prediction of extreme microbial load for drinking water treatment plant based on their own record, so as to explore the risk of extreme microbial load and to propose equivalent mitigation measures.

Complete removal of microbial pathogen from the raw water is one of the main tasks of drinking water treatment plants. In achieving the goal, it needs to build proper pathogen barriers that will provide safe drinking water quality even during extremely high microbial load events of raw water. However, the pathogen load of the raw water varies from day to day in an apparently random way. Thus, the analysis of extreme events could be applied as a tool to help drinking water treatment engineers to deal with the likely occurrence of extreme loads. The significance of calculating the return period of extreme microbial load events is to determine the probability that an extreme pathogen load event will occur, for the purpose of optimal pathogen barrier design and microbial risk analysis.

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References

Abaurrea J, Asin J, Cebrian AC, Garcia-Vera MA (2011) Trend analysis of water quality series based on regression models with correlated errors. J Hydrol 400(3-4):341–352. doi:10.1016/j.jhydrol.2011.01.049

Antonopoulos VZ, Papamichail DM, Mitsiou KA (2001) Statistical and trend analysis of water quality and quantity data for the Strymon River in Greece. Hydrol Earth Syst Sci 5(4):679–691

Castillo E, Hadi AS (1997) Fitting the generalized Pareto distribution to data. J Am Stat Assoc 92(440):1609–1620

Deoliveira JT (1988) Stochastic-processes of extremes - definitions and statistics. Adv Appl Probab 20(1):7

El-Aroui M-A, Diebolt J (2002) On the use of the peaks over thresholds method for estimating out-of-sample quantiles. Comput Stat Data An 39(4):453–475. doi:10.1016/S0167-9473(01)00087-1

Embrechts P, Klüppelberg C, Mikosch T (1997) Modelling extremal events: for insurance and finance. Springer, Berlin, pp 283–358

Ercelebi SG, Toros H (2009) Extreme value analysis of Istanbul air pollution data. Clean-Soil Air Water 37(2):122–131

Falk M, Guillou A (2008) Peaks-over-threshold stability of multivariate generalized Pareto distributions. J Multivar Anal 99(4):715–734

Freudenthal AM (1976) Extreme value risk analysis in structural design of reactor components. Nucl Eng Des 37(1):179–181

Gilbert RO (1987) Statistical Methods for Environmental Pollution Monitoring. Wiley, New York, pp 204–252

Grizzetti B. BF, Bianchi M., Barkved L., Berge D., Campbell D., Dan Kim N., Gooch G., Lana Renoult N., Nesheim I., Machado M., Manasi S., Rieu-Clarke A., Stålnacke, P. and Tjomsland T. (2007) Managing data in Integrated Water Resources Management projects: the STRIVER case. European Commission Joint Research Center. Institute for Environment and Sustainability. Rural, Water and Ecosystem Resources Unit (JRC-EC). doi:http://kvina.niva.no/striver/Portals/0/documents/STRIVER_D21.pdf

Hadas O, Corradini MG, Peleg M (2004) Statistical analysis of the fluctuating counts of fecal bacteria in the water of Lake Kinneret. Water Res 38(1):79–88. doi:10.1016/j.watres.2003.09.004

Hait JR, Schektman Y (1980) Characterization of principal directions and components in principal components analysis (PCA) - similar components analysis. Biometrics 36(2):368–368

Heffernan JE (2012) An Introduction to Statistical Modeling of Extreme Values. Package 'ismev'. http://cran.r-project.org/web/packages/ismev/ismev.pdf.

Helsel DR, Hirsch RM (2002) Statistical Methods in Water Resources. In: Techniques of Water Resources Investigations, vol Book 4. vol chapter A3. U.S. Geological Survey, p 522

Hirsch RM, Slack JR, Smith RA (1982) Techniques of trend analysis for monthly water-quality data. Water Resour Res 18(1):107–121. doi:10.1029/Wr018i001p00107

Hosking JRM, Wallis JR (1987) Parameter and quantile estimation for the generalized Pareto distribution. Technometrics 29(3):339–349. doi:10.2307/1269343

Huang YL, Batterman S (2003) An extreme value analysis of pollutant concentrations in surface soils due to atmospheric deposition. Hum Ecol Risk Assess 9(7):1729–1746

Iii JP (1975) Statistical inference using extreme order statistics. Ann Stat 3(1):119–131. doi:10.2307/2958083

Jiang YY, Zhuang QL (2011) Extreme value analysis of wildfires in Canadian boreal forest ecosystems. Can J Forest Res 41(9):1836–1851

Kasperski M, Hoxey R (2008) Extreme-value analysis for observed peak pressures on the Silsoe cube. J Wind Eng Ind Aerod 96(6–7):994–1002

Katz RW, Parlange MB, Naveau P (2002) Statistics of extremes in hydrology. Adv Water Resour 25(8–12):1287–1304

Klugman S (1997) Practical analysis of extreme values. Insur Math Econ 21(1):91–92. doi:10.1016/S0167-6687(97)00022-X

Konecny F, Nachtnebel HP (1985) Extreme value processes and the evaluation of risk in flood analysis. Appl Math Model 9(1):11–15

Kuchenhoff H, Thamerus M (1996) Extreme value analysis of Munich air pollution data. Environ Ecol Stat 3(2):127–141

Lang M, Ouarda TBMJ, Bobee B (1999) Towards operational guidelines for over-threshold modeling. J Hydrol 225(3–4):103–117

Leadbetter MR, Rootzen H (1988) Extremal theory for stochastic processes. Ann Probab 16(2):431–478

Li YH, Gao H, Li LY, Wang X (2010) Analysis of the surface water quality status and developing trend of the Yellow River Basin in the early 21st century. Proceedings of the 4th International Yellow River Forum on Ecological Civilization and River Ethics, Vol Ii:113-122

Luo PP, He B, Takara K, Razafindrabe BHN, Nover D, Yamashiki Y (2011) Spatiotemporal trend analysis of recent river water quality conditions in Japan. J Environ Monit 13(10):2819–2829. doi:10.1039/C1em10339c

Mazas F, Hamm L (2010a) The peaks-over-threshold statistical theory for the estimation of extreme sea-states. Part 1: a review of available methods. Houille Blanche 4:96–102

Mazas F, Hamm L (2010b) The peaks-over-threshold statistical theory for the estimation of extreme sea-states. Part 2: case studies. Houille Blanche 4:103–110

Naess A (1998) Statistical extrapolation of extreme value data based on the peaks over threshold method. J Offshore Mech Arct 120(2):91–96

Persson J, La KM (2012) On selection of threshold values in alarm-systems for drinking water monitoring. VATTEN – J Water Manag Res 1(68):43–51

Reay WG (2009) Water quality within the York river estuary. J Coastal Res: Spec Issue 57:23–39

Ross WH (1987) A peaks-over-threshold analysis of extreme wind speeds. Can J Stat 15(4):328–335

Sen PK (1968) Estimates of the regression coefficient based on Kendall's Tau. J Am Stat Assoc 63(324):1379–1389. doi:10.1080/01621459.1968.10480934

Simiu E, Heckert NA (1996) Extreme wind distribution tails: A "peaks over threshold" approach. J Struct Eng ASCE 122(5):539–547

Thas O, Vanrolleghem P, Kops B, VanVooren L, Ottoy JP (1997) Extreme value statistics: potential benefits in water quality management. Water Sci Technol 36(5):133–140. doi:10.1016/S0273-1223(97)00498-8

Wong TST, Li WK (2010) A threshold approach for peaks-over-threshold modeling using maximum product of spacings. Stat Sini 20(3):1257–1272

XLSTAT (2012) Running a partial least squares (PLS) discriminant analysis with XLSTAT-PLS. http://www.xlstat.com/en/learning-center/tutorials/running-a-partial-least-square-pls-discriminant-analysis-with-xlstat-pls.htm. Accessed May, 27 2014

Yenilmez F, Keskin F, Aksoy A (2011) Water quality trend analysis in Eymir Lake, Ankara. Phys Chem Earth 36(5-6):135–140. doi:10.1016/j.pce.2010.05.005

Yin X, Hu GH (2009) Trend analysis of surface water quality in Hunan Province. 2009 Proceedings of International Conference on Environmental Science and Information Application Technology, Vol 3,:154–157. doi: Doi 10.1109/Esiat.2009.158

Yu Y-S, Zou S, Whittemore D (1993) Non-parametric trend analysis of water quality data of rivers in Kansas. J Hydrol 150(1):61–80. doi:http://dx.doi.org/10.1016/0022-1694(93)90156-4

Paper II

Eregno, F. E., Tryland, I., Tjomsland, T., Kempa, M., & Heistad, A. (2017). Hydrodynamic modelling of recreational water quality using Escherichia coli as an indicator of microbial contamination. *Journal of hydrology* (Revised version submitted)

Hydrodynamic modelling of recreational water quality using *Escherichia coli* as an indicator of microbial contamination

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Abstract

Microbial contamination of recreational beaches is often at its worst after heavy rainfall events due to storm floods that carry faecal matter and other pollutants from the watershed. Similarly, overflows of untreated sewage from combined sewerage systems may discharge directly into coastal water or via rivers and streams. In order to understand the effect of rainfall events, wind-directions and tides on the recreational water quality, Generalized Environmental Modeling System for Surface waters (GEMSS); an integrated 3D hydrodynamic model was applied to assess the spreading of *Escherichia coli (E. coli*) at the Sandvika beaches, located in the Oslo fjord. The model was also used to theoretically investigate the effect of discharges from septic tanks from boats on the water quality at local beaches. The model makes use of microbial decay rate as the main input representing the survival of microbial pathogens in the ocean, which vary widely depending on the type of pathogen and environmental stress. The predicted beach water quality was validated against observed data after a heavy rainfall event using Nash-Sutcliffe coefficient (E) and the overall result indicated that the model performed quite well and the simulation was in - good agreement with the observed *E. coli* concentrations for all beaches. The result of this study indicated that: 1) the bathing water quality was poor according to the EU bathing water directive up to two days after the heavy rainfall event depending on the location of the beach site. 2) The discharge from a boat at a 300-meter distance to the beaches only slightly increased the *E. coli* levels at the beaches but such discharges may be significant if there was a person infected with highly virulence pathogen on the boat. 3) The spreading of microbial pathogens from its source to the different beaches depended on the wind speed and the wind direction.

Key words: *Escherichia coli*; faecal contamination; water quality modelling; GEMSS model; recreational water.

1. Introduction

Surface runoff after heavy rainfall potentially transports a large number of faecal matter and microbial pathogens to the coastal ocean and poses public health concerns for beach-users (Ahn et al., 2005; Noble et al., 2003). The sources of microbiological pollution of coastal waters vary from place to place (Colford Jr et al., 2007). The major pathways of faecal contamination of coastal environments are sewage discharges including partially treated sewage, combined sewer overflows, storm water discharges, sewage network failures, polluted river discharges, possible runoff from agricultural activities and specific discharges that could come from ships, wild birds, bathers, and sediments (Clark et al., 1989; Vikas and Dwarakish, 2015). Several studies have shown that coastal bathing waters located near river estuaries are often highly contaminated after rainfall events, due to high discharges from the river (Billen and Garnier, 1997; Ludwig et al., 2009; Tilburg et al., 2015). An increasing concern about the bathing water quality at coastal beaches has inspired to the application of hydrodynamic models as a tool to understand the processes that affect the spreading of microbial contaminants in the coastal water, and to predict the effect of changing conditions (Davies et al., 2009; Rodriguez et al., 2004). Significant variation of the spatial and temporal spreading of microbial contaminants at recreational beaches requires frequent in-situ monitoring, which is difficult in terms of operation and costly (Enns et al., 2012; Kinzelman et al., 2006). Hydrodynamic modelling has the potential to overcome such problems if the modelling have reasonable spatial and temporal resolution and combined with supplementary information (Bruni et al., 2015; Holt et al., 2005).

A hydrodynamic model is a comprehensive approach to mimic water dynamic processes generated by a number of different drivers. The base of the model concept is the numerical solution of the governing equations of conservation of momentum and mass, which is the set of equations that describe the motion of fluids (Liu et al., 2007). Coastal hydrodynamic modelling are applied at several localities to understand different ecological problems by using a range of model configurations and forcing (Ferrarin and Umgiesser, 2005; Fossati and Piedra-Cueva, 2013; Gao et al., 2015; Henderson et al., 2001; Zacharias and Gianni, 2008). The models utilize a wide range of meteorological data, river inflow and tidal signals. Hydrodynamic modelling, including *E. coli* or specific microbial pathogens, as a constituent and microbial decay rate as a model parameter, is a useful tool to describe the temporal and spatial variability of microbial concentrations, even below the detection limits of analytical methods. Furthermore, hydrodynamic modelling is used to explore the effect of different scenarios and situations in order to support management decisions. Numbers of the published work about hydrodynamic water quality modelling have focused on nutrients in relation to eutrophication and sediment transportation (Kock Rasmussen et al., 2009; Park et al., 2005; Tkalich et al., 2002; Tufford and McKellar, 1999). Some studies include microbial water quality modelling using faecal coliform and *E. coli* as constituents (Bougeard et al., 2010; Sokolova et al., 2014).

The effects of heavy rainfalls on the microbial water quality at coastal beaches are of particular concern in the Oslo fjord where the storm water from urban areas and farmland catchments directly

discharge into the fjord. Moreover, overflow from the combined sewer system and pumping stations during and after rainfall event discharge a significant amount of untreated sewage water into the fjord. This study was designed to characterize the temporal and spatial variations of faecal indicator bacteria (*E. coli*) after rainfall events at the recreational beaches adjacent to the Sandvika urban settlement receiving discharges from river Sandvikselva and overflows from the local combined sewer system. The GEMSS hydrodynamic model was set up and applied at the Sandvika recreational coastline in order to visualize the impacts of different scenarios and discharges after rainfall events. The overall objective of this study was to understanding the influence of different processes (rainfall, discharge from boats, and wind directions) on the microbial concentrations at the recreational beaches, and to demonstrate the importance of hydrodynamic modelling as a tool to identify the risk of contamination of recreational beaches in order to prioritize mitigation measures.

2. Study area

The study area is located in Bærum municipality, south of Sandvika, Norway. Several bathing areas, river and streams inflow, and urban settlement along the coastline characterize the study area. During the summer time, a significant number of bathers who carry out different recreational activities like boating, swimming and various sports frequently visits the beaches (Figure 1). For this study, six beach-sites were included. Kadettangen main beach and Kadettangen to north, which is the largest and most crowded beach during summer, are connected to Kalvøya Island with a bridge. The island has bays with sand beaches with swimming facilities. These are Kalvøya small, Kalvøya big, and Kalvøya nudist beach. Høvikodden beach is a sandy beach on the mainland east of Kalvøya nudist beach. The main source of pollution in the area is river Sandvikselva, in particular - after rainfall events when combined sewer overflows (CSOs) discharge into the river. In addition, two CSOs discharge directly to the fjord. The catchment area of river Sandvikselva include boreal forest (83 %), which is the upper parts of the catchment, and the lower parts are dominated with farmland (6.5 %) and urban settlement (10.5 %) (Nizzetto et al., 2016).

3. GEMSS hydrodynamic model

The complexity of physical processes governing the transport and fate of an introduced constituent, such as bacteria, in the ocean circulation system suggests the use of advanced hydrodynamic models (Ji, 2017). For this study, the GEMSS model was used to explore the hydrodynamic and related microbial water quality along the Sandvika coastlines. GEMSS is an integrated system of 3D hydrodynamic and transport models embedded in a geographic information and environmental data system (GIS), grid generator and editor, control file generator, 2-D and 3-D post processing viewers, and meteorological and flow data processor to support 3-D modelling. The model is able to simulate horizontal and vertical distributions of water velocities and temperature, salinity, water surface elevations, and water quality in rivers, lakes, reservoir, estuaries, and coastal water bodies at different spatiotemporal resolution (ENTRIX et al., 2001; Kolluru et al., 2014). The model has

been applied in different regions for various water quality problems (Dargahi and Setegn, 2011; Goetchius and Salmun, 2002; Na and Park, 2006; Wu et al., 2001).

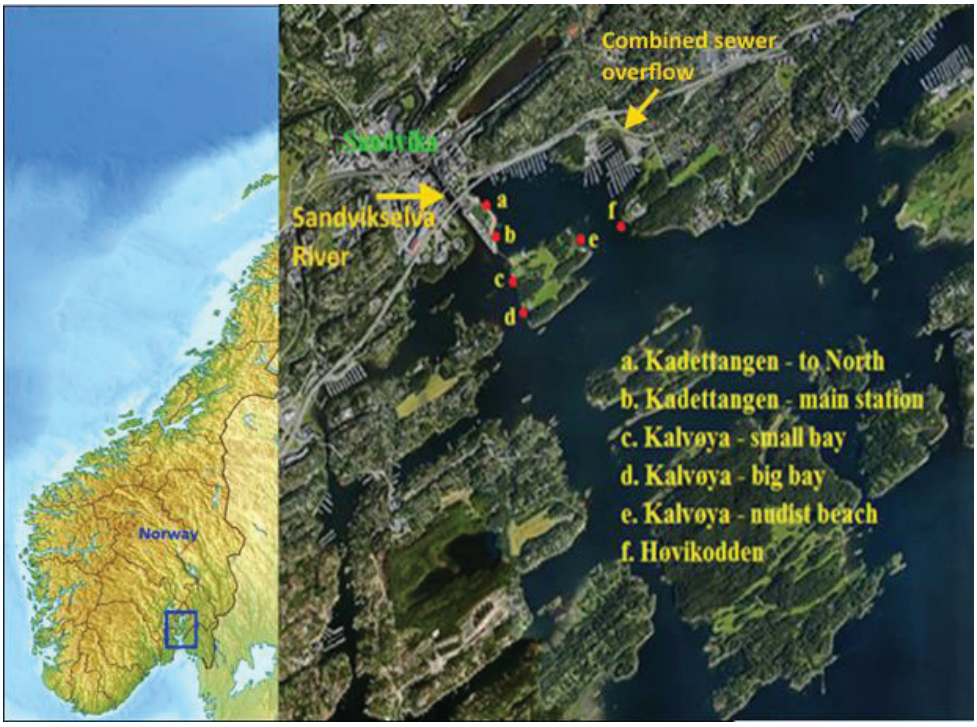

Figure 1. The map of the study area and the position of the beaches (Source: http://www.maps-of-europe.net/maps-of-norway/ and google map)

The GEMSS model uses GLLVHT (Generalized, Longitudinal-Lateral-Vertical Hydrodynamic and Transport) that computes time-varying velocities, water surface elevations, and water quality constituent concentrations in different water bodies (ERM, 2006). The hydrodynamic and transport relationships used in GLLVHT was developed from the horizontal momentum balance, continuity, constituent transport and the equation of state (Edinger and Buchak, 1980). The details of the model can be found in the technical documentation of GEMSS (ERM, 2006) and also a detailed description of the model and its application can be found in the peer reviewed publications (Dargahi and Cvetkovic, 2011; ENTRIX et al., 2001; Kolluru et al., 2014).

4. Boundary conditions and model application

The horizontal and vertical model domains were defined from the shorelines and the bathymetry data. The Inner Oslofjord (Figure 2) was divided into calculation cells. Horizontally the cells were of variable size. The vertical layers were 1 m thick down to 20 m below surface and thereafter 10

m thick. The horizontal resolution was more detailed in the Sandvika area that was of special interest in this research. In this area, the average horizontal grid size was around 100 m x 100 m. For each cell, the results were calculated forward in time with steps of some minutes.

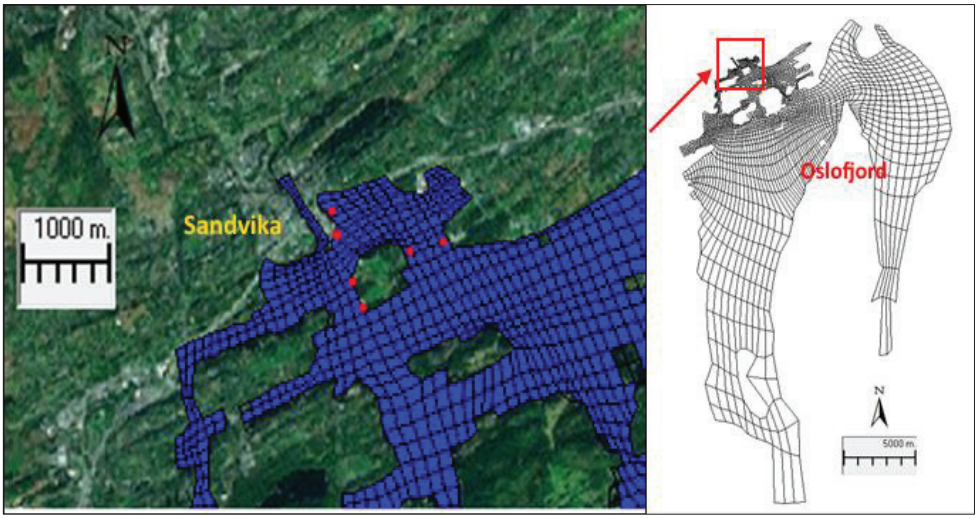

Figure 2. Oslofjord shoreline, the model grid of the study area and the position of the six beach sites (red dots).

The input data for the hydrodynamic model calibration and simulation were obtained from the Norwegian meteorological institute (eKlima), the Norwegian marine data centre, Bærum municipality and direct observation. The data set included flow data (river discharge and combined sewer overflow (CSO)), forced meteorological data (air temperature, dew point temperature, seawater temperature, cloud cover, pressure, wind speed, and wind direction), and water quality parameters (temperature, salinity, and *E. coli*) (Table 1). Some small streams flow into the Sandvika beach but only Sandviksilva River that has significant discharge and two small CSOs discharging directly to the fjord was included in the model as point source inflow. *E. coli* concentrations and corresponding water flow monitored in the river and CSOs were used as an input to the model. For the two small CSOs, the registered time of overflow was used as input to the model, assuming that 50% of the sewage was discharged in this period.

The water level difference, mainly due to tides, at the southern open boundary was about 1 m. Oslo and Bærum municipality were regularly measured water flow and faecal indicator bacteria in the rivers around Oslofjord. For this study, detailed measurements of *E. coli* were done in the Sandvika area during and after the precipitation, observed on 7 July 2014 (Table 2), and both discharges and *E. coli* concentrations in the river and CSOs used as an input for this model, as described in Eregno *et al*. (2016).

5. Model simulation

First, the model was validated by comparing observed *E. coli* concentration against modelled values. Afterwards, the simulation was conducted for three conditions: 1) after a rainfall event, 2) boat discharge scenario, 3) wind direction scenarios. Microbial contamination of the recreational beaches was studied during and after rainfall events during summer 2014. We used two scenarios to separately assess the impact of boat sewage discharge and wind directions on the recreational beaches water quality. The boat discharge scenario was if a single boat with 200-litre toilet tank discharges its sewage at 300 meters from the nearest beach (assuming *E.coli* concentration is 3 x 10^7 MPN/100 ml, equivalent to one day-production of *E. coli* from four persons) (Al Baz et al., 2008). The wind direction scenario was developed by adjusting the wind input data set, which was carried out by tuning all winds into one direction using the average wind speed for all.

Table 1. The ranges of input data variables used for GEMSS modelling

Description	Unit	Min	Max	Remark
Meteorological data				
Air temperature	^oC	6.5	31.9	
Dew point temperature	^oC	-0.7	19.5	
Ocean water temperature	^oC	16.6	25.2	
Cloud cover	tenths	0.0	10.0	
Atmospheric pressure	mm of Hg	970	1016	
Wind speed	m/s	0.2	7.8	
Wind direction	degrees	0	359	
Water surface elevation	m	-0.83	0.86	
Water quality				
temperature	^oC	6.6	22.5	
salinity	ppt	20.1	27.6	
E. coli	MPN/100ml	5 x 10^2	3 x 10^4	River
	MPN/100ml	7.5x10^4	7.5x10^4	CSO
	MPN/100ml	1.5 X 10^9	1.5 X 10^9	Swan faeces[a]
	MPN/100ml	3 X 10^7	3 X 10^7	Boat sewage
Flow data				
River discharge	m^3/s	0.94	26.5	
Combined sewer overflow (CSO)	m^3/s	0.017	0.045	
Swan faeces	m^3/s	6.9 X 10^{-8}	6.9 X 10^{-8}	
Boat discharge	m^3/s	3.3 X 10^{-4}	3.3 X 10^{-4}	

[a] Swan faeces Assumption: 20 swans x 300 g faeces each per day, 1.5 x 10^7 *E.coli* /g at Kadettangen beaches

6. Water quality modelling results

6.1. Model validation

Model calibration in this hydrodynamic modelling is the processes of finding a set of optimal variables to yield the best agreement between the predicted and observed variable of interest. The model was calibrated by making minor adjustments to the boundary conditions and by adjusting the Chezy friction coefficient. Comparisons of the observed and computed salinity level were made to evaluate the performance of the calibration processes. A good agreement observed between observed and computed values of salinity during the period of calibration. This testifies that the model calculates the overall water movements in the fjord in a realistic way (Tjomsland et al., 2014).

To ensure that the simulated spatiotemporal spreading of *E. coli* at the beach sites was realistic, the model results were validated by comparing the simulated *E. coli* concentrations with monitored data for four consecutive days after the rainfall event at all beach sites. Model validation was based on the statistical comparison of daily observations of *E. coli* and the corresponding model simulation results summarized for all beaches and shown in Table 3. The value of Nash-Sutcliffe coefficient, which ranges from -∞ to one, was 0.36 and the relative volume of error was 8.4 %, which was less than 10% suggesting a reasonably close agreement between observed and simulated *E. coli* concentration. The error for minimum value was much higher compared with the maximum value.

6.2. Simulation of the spreading of *E. coli* after the rainfall event

The two main sources of faecal contamination of the Sandvika beaches that were considered in this work were the river Sandvikselva and a CSOs discharging close to Høvikodden beach. In addition, swan droppings at each beach site were included, while other potential minor sources were ignored. The simulations showed that the impact of swan faeces was negligible due to the dilution effect. To be able to model the local impact of swan faces a much denser grid than 100x100 meter is required. Analysis of water samples showed a significant impact of swan faeces on the local water quality.

The survival of *E. coli* after it is discharged into coastal water depends on many abiotic and biotic factors like sunlight, temperature, salinity, competitive bacteria, viruses, predators etc. (Rozen and Belkin, 2001; Stewart et al., 2008). To illustrate how the different decay rates may affect the simulated concentration at the beaches, *E. coli* was given two different decay coefficients ($k = 0.7$ and $k = 0.1$, representing half-life time of 1 day and 1 week) representing the sensitive and resistant organisms. The spreading of *E. coli* from the sources into the beach areas were simulated for the consecutive three days after the rainfall event and the daily average *E. coli* concentration was plotted for each decay rate coefficient as shown in Figure 3. According to the simulation results, the daily average *E. coli* concentration one day after the rainfall event was much higher than the second and the third days at all beaches and the magnitude difference was highest in the case of Kalvøya small beach followed by Kalvøya big and Kadettangen beaches.

Table 2. The characteristics of the rainfall episode used for simulations

Met. Stations	Distance from Sandvika (km)	Direction from Sandvika	Rainfall episode 1 (July 7, 2014)						
			Time rain started	Time rain stopped	Amount of rainfall (mm)	Duration of rainfall (minute)	Average rainfall intensity (mm/hr.)	Days and amount of precipitation before the rainfall event	
								Days	Rainfall (mm)
Asker	8.26	SW	12:00	14:00	21.8	111	11.78	6	7.8
Blindern	11.66	NE	10:00	14:00	18.1	142	7.65	2	9.3
Bygdøy	8.89	NE	11:00	15:00	17.9	-	-	10	17.6

Table 3. Statistical parameters used for comparison of measured and modelled *E. coli concentrations*

E. coli	Log_{10} (MPN/100ml)				Root mean square error (RMSE)	Root volume of error (RV_E) %	Nash-Sutcliffe coefficient
	Min	Max	Mean	StDv			
Simulated	0.74	3.33	2.16	0.57	0.79	8.4	0.36
Observed	1.48	3.84	2.35	0.63			

In addition, the impact of the microbial decay rate coefficient on the daily average *E. coli* concentration was substantial. If we take Kadettangen and Kalvøya small beaches as an example, the change in half-life time from 1 day to 1 week resulted in an increase in daily average *E. coli* concentration by 38.6 %, 95.0 %, and 146.7 % at Kadettangen beach and 17.3 %, 44.9 %, and 61.1 % at Kalvøya small beach for the first, the second, and the third days respectively.

The simulation results of the spreading of *E. coli* at the beach sites in graphics form for the top 1-meter depth are shown in Figure 4. As we can see from the figure, the first three hours after the heavy rainfall event the *E. coli* dispersion was limited only to the surrounding area of river Sandvikselva and the river mouth. However, 24 hours after the rainfall event that caused increased discharges of *E. coli* via the river, the affected coastal area was increased and then the concentration was gradually reduced until the end of the third day.

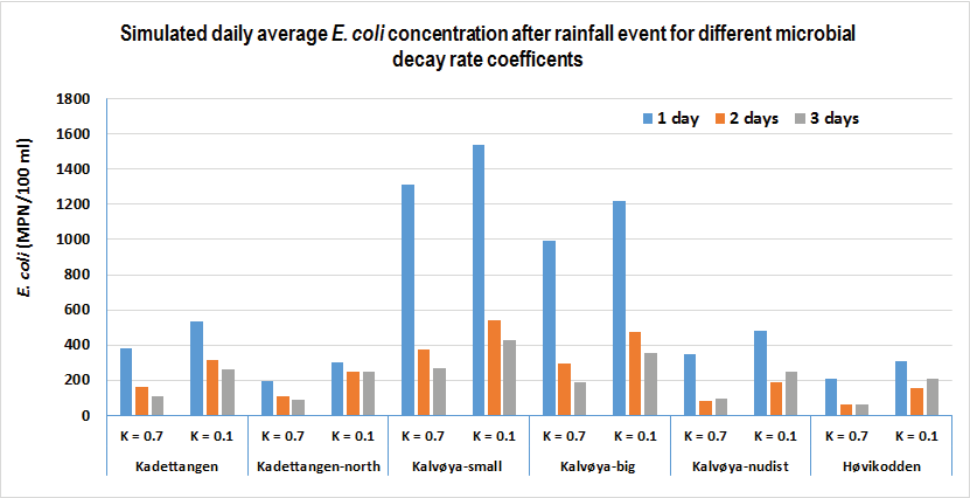

Figure 3. Simulated daily average *E.coli* concentration after the rainfall event for three-consecutive days using decay rate coefficient of K=0.7 and K=0.1, representing half-life time of 1 day and 1 week respectively.

6.3. The vertical distribution of *E. coli* concentration at the beaches

The stratification of the *E. coli* concentration is complicated by its temporal and spatial variations within and among the beaches water column. In order to simplify the presentation of the simulation result, the vertical distribution of the *E. coli* concentration at a specific time (15:00 the day after the rainfall event) at two beaches is shown as an example in Figure 5. As shown in the plot the situation was quite different at the two beaches. *E. coli* stratification had a two-layer structure in the case of Høvikodden, the surface layer (0-3.5 m depth) and the bottom layer (3.5-7.5 m depth). The bottom layer *E.coli* concentration was increased from 64 MPN/100 ml at 3.5 m to 950 MPN/100 ml at 7.5 m depth while the surface layer varied from 64 MPN/100 ml at 3.5 m to 150 MPN/100 ml at the surface. The highest *E. coli* concentration at the bottom layer was caused by

the CSOs discharging to the deep water around the Høvikodden. In the case of Kadettangen beach, the depth is relatively shallow and the *E. coli* concentration decreased with depth, from 495 MPN/100 ml at the surface to 425 MPN/100 ml at 1.5 m depth.

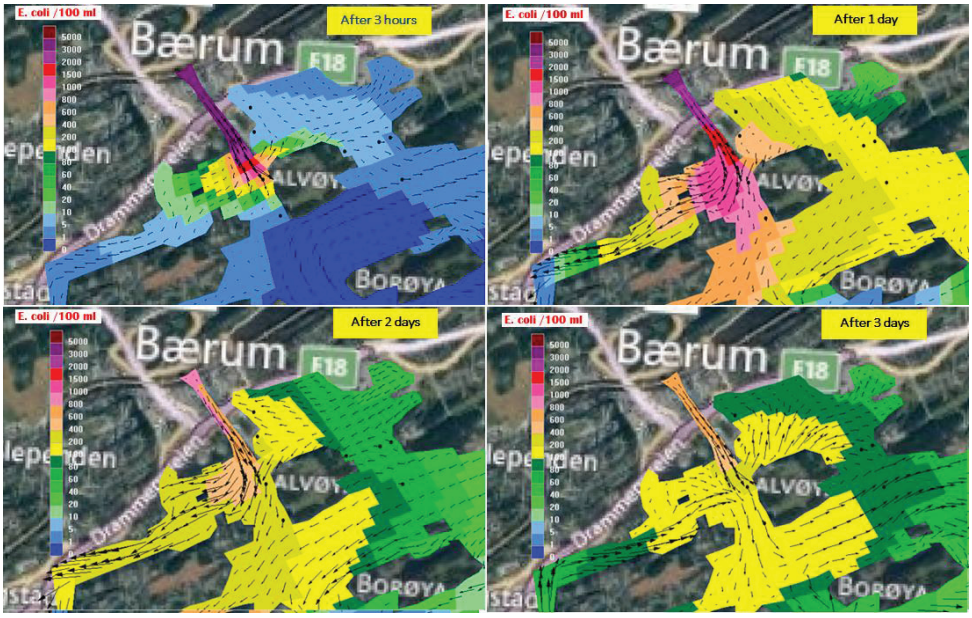

Figure 4. The top layer simulation graphics showing the spreading of *E. coli* at different time intervals after the rainfall event (K=0.7)

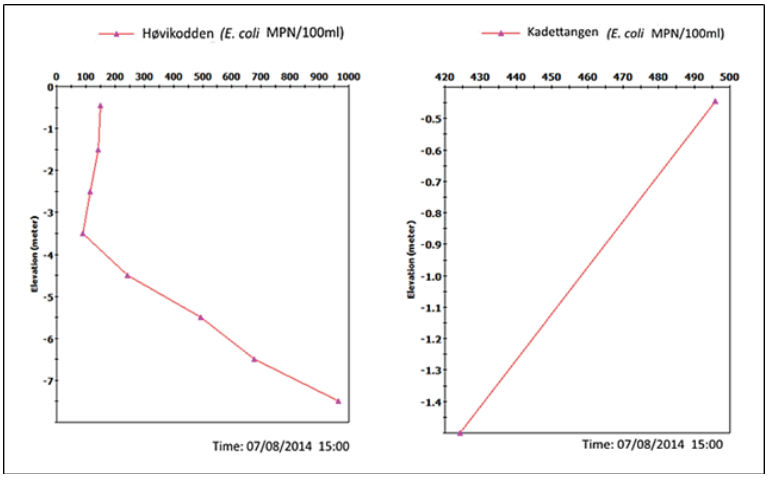

Figure 5. Plots of simulated vertical stratification of *E. coli* concentration in the water column

6.4. Scenario with discharge from a septic tank from a boat

In the Inner Oslo fjord, there are several thousand private boats/yachts. As the number of boats and beach users increases through time, the pressure on the water quality along the coastal beaches increase and need proper protection measures.

The spreading of *E. coli* from the discharge of boat sewage was simulated in order to investigate its impact on the recreational water quality of the beaches under different situations. The distance between the discharging point and the beaches, 300 meters, as the minimum required distance from the mainland, according to the regulation of boat sewage discharge in the Oslo fjord. The simulation result showed that the discharge of 200-liter sewage with 3 x 10^7 *E. coli* per 100 ml will most probably affect the nearby beaches by relatively low levels of *E. coli* (20-24 MPN/100 ml) (Figure 6). Moreover, we may also predict that if there was a person infected with a high virulence pathogen on the boat, such a discharge will cause a substantial health risk for the beach swimmers (Eregno et al., 2016). In this study, the realistic simulation of *E. coli* concentration from the boat discharge scenario could severely limit by the constraints on the coarse spatial resolution, therefore, relatively smaller resolution, the effect of different volume of discharge from different distance and directions could be investigated in the future study.

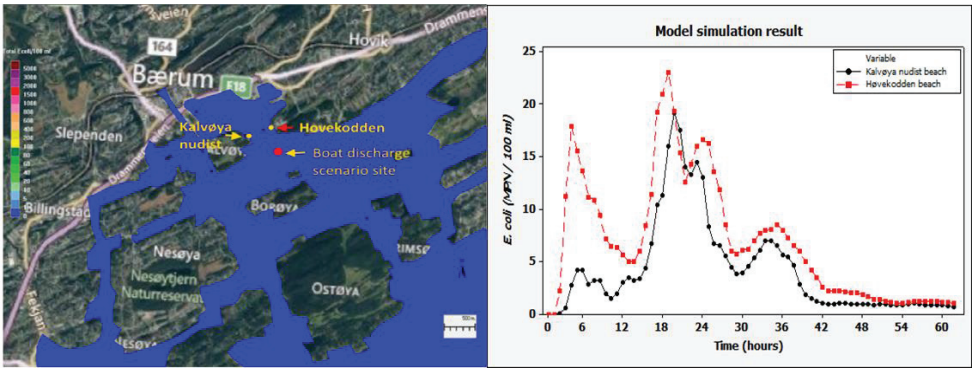

Figure 6. Boat discharge site, beaches and simulated *E.coli* concentration at the beaches (K=0.7)

6.5. Scenarios with different wind directions

The key factors for the spreading of pollutants in the coastal environments are wind and current and the influence of the wind on the spreading of microbial pollutants because of ocean circulation was an important aspect of this hydrodynamics study. In this regard, the spreading of *E. coli* from its source was investigated using different wind directions scenarios. In these simulations, the four scenarios were N-, S-, E-, W-winds, representing wind blowing to north, south, east and west respectively. Simulation using the actual observation was denoted by natural simulation. In Figure 7 a relative magnitude of simulated *E. coli* concentration associated with the scenarios with different wind directions, are presented. Different wind directions were shown to have various

impacts on the spreading of *E. coli* in the study area and as we can see from the Figure 7, wind blowing to west affected Kadettangen beach more than Høvikodden and Kalvøya nudist beach. Whereas the wind that was blowing to the south, in the same direction as the pollution source flow, was the predominant wind direction that affected all the beaches sites. These simulations demonstrated that the effect of wind direction depended on the location of the beaches relative to the main pollution source, and in this case, the river Sandvikselva was the main source of pollution.

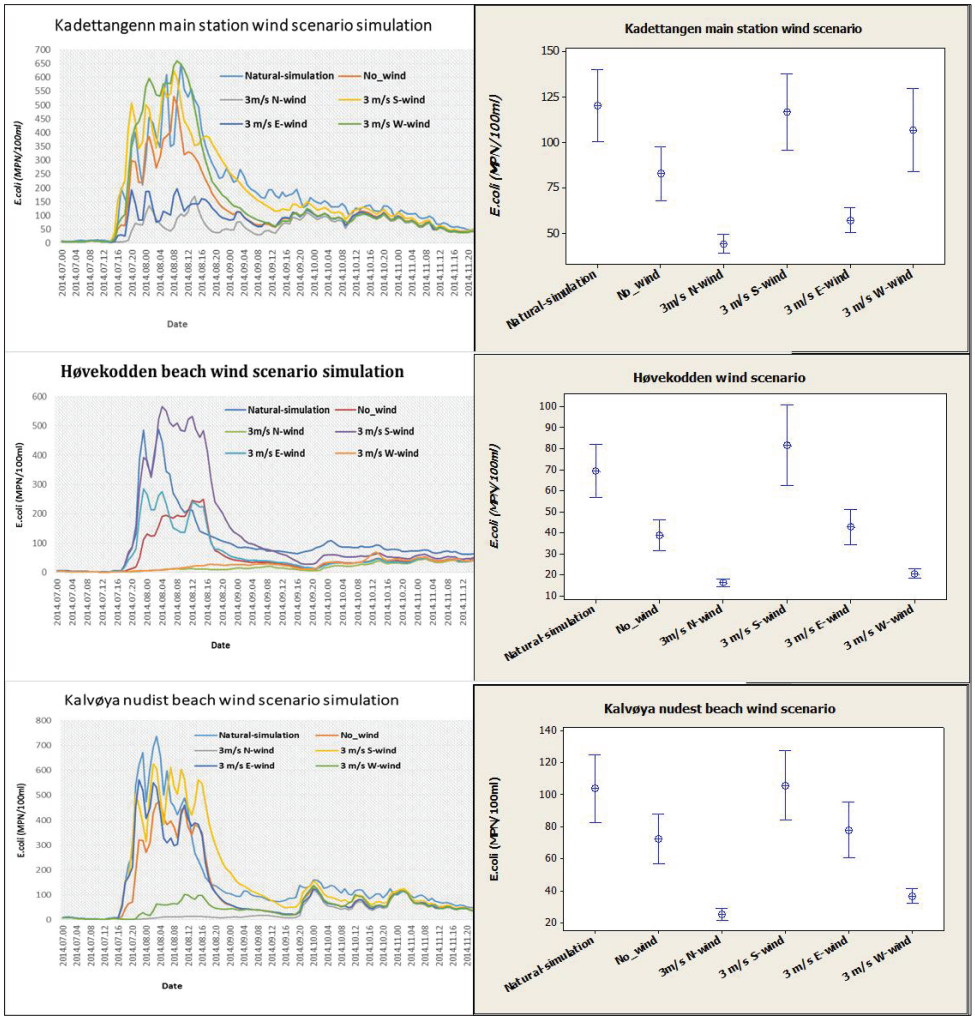

Figure 7. Predicted *E. coli* concentration at Kadettangen, Høvikodden, and Kalvøya nudist beaches, based on scenarios with different wind directions, the left plot shows the simulated time series and the right plot shows statistical summary (mean and standard deviation)

7. Conclusion

For beaches exposed to short-term pollution during/after heavy rainfall events, it is useful to know the main factors affecting the water quality, to make decisions about whether to warn against swimming or not and eventually for how many days. To study this the three-dimensional GEMSS hydrodynamic model was set up for the Inner Oslofjord and successfully applied to simulate the spatial and temporal spreading of *E. coli* after a heavy rainfall event in the Sandvika area. The model was used to demonstrate the impact of different wind directions on the spreading of *E. coli*, as well as for investigating the effect of a discharge from a septic tank on a boat.

The results of the simulations lead to the following conclusions; 1) the risk of microbial contamination was high after one day of heavy rainfall as compared with the second and third days and the level of risk was highest at Kalvøya small beach due to the position of the beach that It is located close to the river inlet and the incoming river discharge pass through the beach. 2) the risk of microbial contamination of the local beaches from a boat, emptying the septic tank at a 300-meter distance from the beaches, could substantial if a person infected with highly virulence pathogen was on the boat, although the minor increase in *E. coli* was modelled (from only one boat). However, the simulation could severely limit by the constraints on the coarse spatial resolution, therefore, relatively smaller resolution, the effect of different volume of discharge from a different distance and directions recommended in the future study. 3) The spreading of microbial contaminants from its source highly depended on wind speed and direction and the extent of pollution depended on the location of the beach relative to the source. 4) Hydrodynamic modelling offers a useful tool for understanding the spatial-temporal spreading of microbial pathogens at recreational beaches in order to prioritize mitigation measures and as a whole for beach management.

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References

Ahn, J.H., Grant, S.B., Surbeck, C.Q., DiGiacomo, P.M., Nezlin, N.P. and Jiang, S., 2005. Coastal water quality impact of stormwater runoff from an urban watershed in southern California. Environmental science & technology, 39(16): 5940-5953.

Al Baz, I., Otterpohl, R. and Wendland, C., 2008. Efficient Management of Wastewater. Springer.

Billen, G. and Garnier, J., 1997. The Phison River plume: coastal eutrophication in response to changes in land use and water management in the watershed. Aquatic microbial ecology, 13(1): 3-17.

Bougeard, M., Le Saux, J.-C., Perenne, N., Baffaut, C., Robin, M. and Pommepuy, M., 2010. Modeling of Escherichia Coli fluxes on a catchment and the impact on coastal water and shellfish quality. Journal of the American water resources association(JAWRA-09-0162-P): JAWRA-09-0162-P.

Bruni, G., Reinoso, R., van de Giesen, N., Clemens, F. and ten Veldhuis, J., 2015. On the sensitivity of urban hydrodynamic modelling to rainfall spatial and temporal resolution. Hydrology and Earth System Sciences, 19(2): 691.

Clark, R.B., Frid, C. and Attrill, M., 1989. Marine pollution, 4. Clarendon Press Oxford.

Colford Jr, J.M., Wade, T.J., Schiff, K.C., Wright, C.C., Griffith, J.F., Sandhu, S.K., Burns, S., Sobsey, M., Lovelace, G. and Weisberg, S.B., 2007. Water quality indicators and the risk of illness at beaches with nonpoint sources of fecal contamination. Epidemiology: 27-35.

Dargahi, B. and Cvetkovic, V., 2011. Hydrodynamic and Transport Properties of Saltsjö Bay in the Inner Stockholm Archipelago. Journal of Coastal Research: 572-584.

Dargahi, B. and Setegn, S.G., 2011. Combined 3D hydrodynamic and watershed modelling of Lake Tana, Ethiopia. Journal of Hydrology, 398(1-2): 44-64.

Davies, S., Mirfenderesk, H., Tomlinson, R. and Szylkarski, S., 2009. Hydrodynamic, Water Quality and Sediment Transport Modeling of Estuarine and Coastal Waters on the Gold Coast Australia. Journal of Coastal Research: 937-941.

Edinger, J.E. and Buchak, E.M., 1980. Numerical hydrodynamics of estuaries, Estuarine and Wetland Processes. Springer, pp. 115-146.

Enns, A.A., Vogel, L.J., Abdelzaher, A.M., Solo-Gabriele, H.M., Plano, L.R., Gidley, M.L., Phillips, M.C., Klaus, J.S., Piggot, A.M. and Feng, Z., 2012. Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions. Water research, 46(7): 2237-2246.

ENTRIX, I., J. E. Edinger Associates, I., & and Gahagan and Bryant Associates, I., 2001. Hydrodynamic and Water Quality Modeling and Feasibility Analysis of Indian River, Rehoboth Bay and Little Assawoman Bay, Delaware Department of Natural Resources and Environmental Control

ERM, 2006. GEMSS-HDM Hydrodynamic and Transport Module. In: ERM.inc (Editor), Technical Documentation, Pennsylvania.

Ferrarin, C. and Umgiesser, G., 2005. Hydrodynamic modeling of a coastal lagoon: the Cabras lagoon in Sardinia, Italy. Ecological Modelling, 188(2): 340-357.

Fossati, M. and Piedra-Cueva, I., 2013. A 3D hydrodynamic numerical model of the Río de la Plata and Montevideo's coastal zone. Applied Mathematical Modelling, 37(3): 1310-1332.

Gao, G., Falconer, R.A. and Lin, B., 2015. Modelling the fate and transport of faecal bacteria in estuarine and coastal waters. Marine Pollution Bulletin, 100(1): 162-168.

Goetchius, K.M. and Salmun, H., 2002. Modeling the Fate and Transport of Atrazine in the Upper Chesapeake Bay, 15 th ASCE Engineering Mechanics Conference.

Henderson, A., Gamito, S., Karakassis, I., Pederson, P. and Smaal, A., 2001. Use of hydrodynamic and benthic models for managing environmental impacts of marine aquaculture. Journal of Applied Ichthyology, 17(4): 163-172.

Holt, J.T., Allen, J.I., Proctor, R. and Gilbert, F., 2005. Error quantification of a high-resolution coupled hydrodynamic–ecosystem coastal–ocean model: part 1 model overview and assessment of the hydrodynamics. Journal of Marine Systems, 57(1): 167-188.

Ji, Z.-G., 2017. Hydrodynamics and water quality: modeling rivers, lakes, and estuaries. John Wiley & Sons.

Kinzelman, J.L., Dufour, A.P., Wymer, L.J., Rees, G., Pond, K.R. and Bagley, R.C., 2006. Comparison of multiple point and composite sampling for monitoring bathing water quality. Lake and Reservoir Management, 22(2): 95-102.

Kock Rasmussen, E., Svenstrup Petersen, O., Thompson, J.R., Flower, R.J. and Ahmed, M.H., 2009. Hydrodynamic-ecological model analyses of the water quality of Lake Manzala (Nile Delta, Northern Egypt). Hydrobiologia, 622(1): 195.

Kolluru, V.S., Prakash, S. and Hodgins, D., 2014. Evaluation of Urbanization and Impacts on Water Quality in Nottawasaga Bay Using an Integrated 3-D Modeling Framework, 978-88-9035-744-2, San Diego, California, USA.

Liu, W.C., Kuo, J.T., Young, C.C. and Wu, M.C., 2007. Evaluation of marine outfall with three-dimensional hydrodynamic and water quality modeling. Environmental Modeling & Assessment, 12(3): 201-211.

Ludwig, W., Dumont, E., Meybeck, M. and Heussner, S., 2009. River discharges of water and nutrients to the Mediterranean and Black Sea: major drivers for ecosystem changes during past and future decades? Progress in Oceanography, 80(3): 199-217.

Na, E.H. and Park, S.S., 2006. A hydrodynamic and water quality modeling study of spatial and temporal patterns of phytoplankton growth in a stratified lake with buoyant incoming flow. Ecological Modelling, 199(3): 298-314.

Nizzetto, L., Butterfield, D., Futter, M., Lin, Y., Allan, I. and Larssen, T., 2016. Assessment of contaminant fate in catchments using a novel integrated hydrobiogeochemical-multimedia fate model. Science of the Total Environment, 544: 553-563.

Noble, R.T., Weisberg, S.B., Leecaster, M.K., McGee, C.D., Dorsey, J.H., Vainik, P. and Orozco-Borbon, V., 2003. Storm effects on regional beach water quality along the southern California shoreline. Journal of Water and Health, 1(1): 23-31.

Park, K., Jung, H.-S., Kim, H.-S. and Ahn, S.-M., 2005. Three-dimensional hydrodynamic-eutrophication model (HEM-3D): application to Kwang-Yang Bay, Korea. Marine Environmental Research, 60(2): 171-193.

Rodriguez, H.N., Cope, B. and Peene, S.J., 2004. Hydrodynamic and water quality modeling of ward cove, Alaska. Estuarine and Coastal Modeling, Proceedings: 628-645.

Rozen, Y. and Belkin, S., 2001. Survival of enteric bacteria in seawater. FEMS microbiology reviews, 25(5): 513-529.

Sokolova, E., Pettersson, T.J. and Bergstedt, O., 2014. Hydrodynamic modelling and forecasting of microbial water quality in a drinking water source. Journal of Water Supply: Research and Technology-Aqua, 63(3): 189-199.

Stewart, J.R., Gast, R.J., Fujioka, R.S., Solo-Gabriele, H.M., Meschke, J.S., Amaral-Zettler, L.A., del Castillo, E., Polz, M.F., Collier, T.K. and Strom, M.S., 2008. The coastal environment and human health: microbial indicators, pathogens, sentinels and reservoirs. Environmental Health, 7(2): S3.

Tilburg, C.E., Jordan, L.M., Carlson, A.E., Zeeman, S.I. and Yund, P.O., 2015. The effects of precipitation, river discharge, land use and coastal circulation on water quality in coastal Maine. Royal Society open science, 2(7): 140429.

Tjomsland, T., Tryland, I. and Kempa, M., 2014. Modellert spredning av E. coli i Indre Oslofjord. Innledende arbeid for å studere effekt av vind og strømningsforhold. 6703-2014, NIVA, Oslo.

Tkalich, P., Pang, W. and Sundarambal, P., 2002. Hydrodynamics and eutrophication modelling for Singapore Straits, Proceedings of 7th Workshop on Ocean Models for the APEC Region (WOM-7), Singapore, pp. 5-1.

Tufford, D.L. and McKellar, H.N., 1999. Spatial and temporal hydrodynamic and water quality modeling analysis of a large reservoir on the South Carolina (USA) coastal plain. Ecological Modelling, 114(2–3): 137-173.

Vikas, M. and Dwarakish, G., 2015. Coastal pollution: a review. Aquatic Procedia, 4: 381-388.

Wu, J., Buchak, E., Edinger, J. and Kolluru, V., 2001. Simulation of cooling-water discharges from power plants. Journal of environmental management, 61(1): 77-92.

Zacharias, I. and Gianni, A., 2008. Hydrodynamic and dispersion modeling as a tool for restoration of coastal ecosystems. Application to a re-flooded lagoon. Environmental Modelling & Software, 23(6): 751-767.

Paper III

Eregno, F. E., Tryland, I., Tjomsland, T., Myrmel, M., Robertson, L., & Heistad, A. (2016). Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Science of The Total Environment, 548–549*, 270-279.

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Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events

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HIGHLIGHTS

- River flooding and overflow from combined sewer system after rainfall event contaminate the recreational beaches
- Hydrodynamic modelling was used to identify the spatial-temporal spreading of microbial pathogens
- The public health risk was computed as a probability of infection using dose-response relationships of QMRA framework

GRAPHICAL ABSTRACT

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ABSTRACT

This study investigated the public health risk from exposure to infectious microorganisms at Sandvika recreational beaches, Norway and dose–response relationships by combining hydrodynamic modelling with Quantitative Microbial Risk Assessment (QMRA). Meteorological and hydrological data were collected to produce a calibrated hydrodynamic model using *Escherichia coli* as an indicator of faecal contamination. Based on average concentrations of reference pathogens (norovirus, *Campylobacter*, *Salmonella*, *Giardia* and *Cryptosporidium*) relative to *E. coli* in Norwegian sewage from previous studies, the hydrodynamic model was used for simulating the concentrations of pathogens at the local beaches during and after a heavy rainfall event, using three different decay rates. The simulated concentrations were used as input for QMRA and the public health risk was estimated as probability of infection from a single exposure of bathers during the three consecutive days after the rainfall event. The level of risk on the first day after the rainfall event was acceptable for the bacterial and parasitic reference pathogens, but high for the viral reference pathogen at all beaches, and severe at Kalvøya-small and Kalvøya-big beaches, supporting the advice of avoiding swimming in the day(s) after heavy rainfall. The study demonstrates the potential of combining discharge-based hydrodynamic modelling with QMRA in the context of bathing water as a tool to evaluate public health risk and support beach management decisions.

1. Introduction

Poor microbial water quality at recreational beaches poses a health risk to bathers. A variety of illnesses have been associated with contaminated recreational water, including gastrointestinal-, eyes-, ears-, skin-

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and respiratory diseases (Haile et al., 1999). The major sources of microbial contamination of coastal waters are sewage discharges, including sewage outfall, combined sewer overflows (CSOs), storm water discharges, sewage network failures, polluted river discharges, runoff from agricultural activities and urban areas, and specific local discharges that could come from ships, wild birds, bathers, and sediments (Pompepuy et al., 2009).

Discharges from CSOs and increased runoff during heavy rainfall may transport a large number of microbial pathogens to the coastal areas, and their influence on beach water quality may be a serious problem in some areas. Local public health authorities may issue warnings to avoid contact with recreational water at affected coastal beaches up to three days following a storm event (Ackerman and Weisberg, 2003; Noble et al., 2003). Increasing concern about bathing water quality, and the requirement in the EU bathing water directive (EU, 2006) of including surveillance, early warning systems, and monitoring if beaches are subject to short-term pollution, has prompted the use of hydrodynamic models as tools for better understanding the transport and fate of microbial pathogens in the coastal waters, and for predicting the effect of changing weather- and discharge conditions on the water quality at the beaches. Hydrodynamic modelling is a comprehensive approach representing coastal water dynamics, and the models are based on equations that describe the motion of fluids. These numerical computational models can be used to simulate water surface elevation, current velocity, temperature, and salinity, as well as transport and fate of microbial pathogens included in a coupled transport model (Kollurua et al., 2014). This approach has been applied to simulate the fate and transport of faecal indicator bacteria (*Escherichia coli*) (Kacikoc and Beyhan, 2014; Sokolova et al., 2014; Tufford and McKellar, 1999), and to predict the concentration distributions of faecal indicator organisms in coastal waters (Huang et al., 2015).

Effective management of the public health risk at beaches exposed to short-term pollution, requires, ideally, real-time information about the occurrence of specific pathogens. Since it is impossible to monitor the level of microbial pathogens on a continuous basis, discharge-based hydrodynamic modelling provides a potential alternative and can provide a quantitative and consistent approach to estimate microbial pathogen concentration under a wide range of conditions. Quantitative Microbial Risk Assessment (QMRA) has been used in several studies to estimate the health impacts related to recreational water use. By applying QMRA, the risk of illness/infection from a particular pathogen can be calculated through four assessment steps: hazard identification, exposure assessment, dose–response effects, and final risk characterization (Duffy et al., 2006; Jaykus, 1996). Such risk assessments are useful for informing regulatory bodies about the management of beaches, and whether swimming during a certain period after the rainfall event is safe for bathers or involves an unacceptable risk of infection. However, in order to assess exposure it is necessary to have information about the concentrations of pathogens in the bathing water, and these data are often lacking.

The purpose of the study described here was to demonstrate the approach of combining the hydrodynamic modelling technique with quantitative microbial risk assessment, based on pathogen concentrations in the sewer system typical for Norwegian conditions. To the best of the authors' knowledge, few studies have been conducted on the hydrodynamic modelling of microbial water quality in combination with QMRA in order to calculate public health risk (Andersen et al., 2013; Sokolova et al., 2015). As data sources and hydrodynamic modelling routines improve over time, this approach as a tool for estimating health risk following specific events could become more accurate and valuable.

2. Methods

2.1. Study area

The study sites were located in Bærum municipality, adjacent to Sandvika town, which is situated approximately 15 km west of Oslo, Norway. Six coastal beaches were used as sampling sites for *E. coli* (Fig.1). These were: a) Kadettangen—north, which is the extension of Kadettangen main beach close to the highway (59°53′20″ N, 10°31′49″ E); b) Kadettangen—main beach, which is the busiest beach as compared to the other beaches during the warm summer period (59°53′14″ N, 10°31′54″ E). c) Kalvøya—small beach is located close to the mouth of Sandvikselva river where it enters into the fjord, which is located at (59°53′5″ N, 10°32′7″ E). d) Kalvøya—big beach (59°52′58″ N, 10°32′

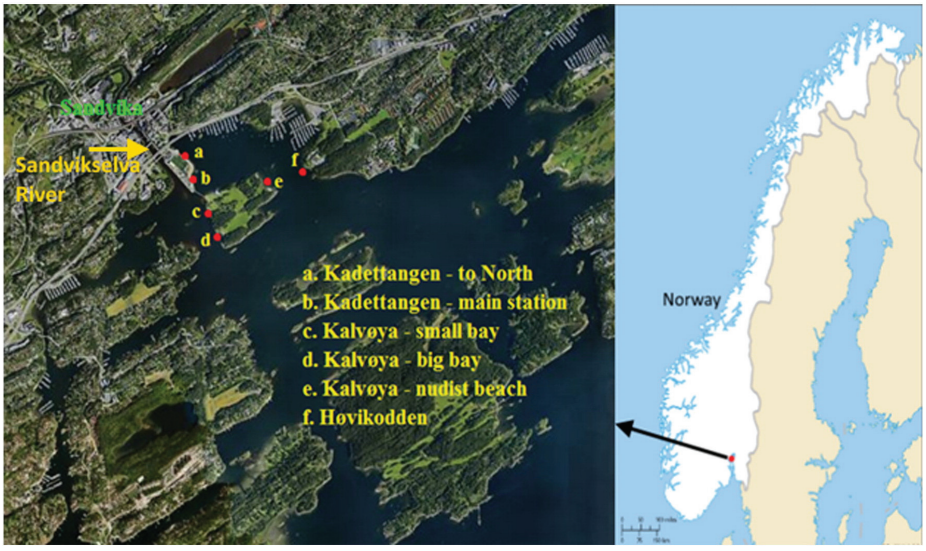

Fig. 1. Location of Sandvika beaches and sampling sites.

11″ E) is located further south from Kalvøya small beach and is influenced by the storm flood of the river Sandvikselva. e) Kalvøya—nudist beach (59°53′22″ N, 10°33′2″ E) is located on the other side of the Kalvøya island. f) Høvikodden beach is located at (59°53′16″ N, 10°33′10″ E) close to where private boats are moored. Two combined municipal sewer systems (about 4000 and 1500 inhabitants connected) are located approximately 500 m from Høvikodden beach and overflow at around 6 m deep into the ocean during heavy rain.

The beaches are supplied with an inland freshwater feed from river Sandvikselva. The river originates at the intersection of two other rivers, Lomma and Isielva, with a total catchment area of 225 km^2. The land use of the catchment area is forest land, wetlands, residential, and farmland (cropland and animal farm). There are some on-site wastewater treatment plants in the catchment area. Associated with varied land uses of the catchment area, the potential sources of faecal contamination of the river include wildlife, farm operations, rural and urban runoff, and agricultural drainage (Bærum, 2012). During heavy rain the main sources of faecal contamination are assumed to be several CSOs that have the river as recipient and increased leakage from the sewer system from the urban areas near the outlet of the river. Significant amounts of faecal contamination are transported from the river to the fjord: typical values at the outlet of the river during dry summer-days are 10^2–10^3 *E. coli* per 100 ml (water-flow about 1 m^3/s) and during heavy summer-rain about 10^4 *E. coli* per 100 ml (water-flow about 3 m^3/s).

2.2. Rainfall event

The heavy rainfall event that is considered in this study, was the highest rainfall during the summer of 2014 in the Sandvika area. On 7 July 2014, from 17 mm to 22 mm precipitation fell on the western side of the Oslo fjord area within less than three hours (Table 1). The rainfall intensity was recorded at Asker and Blindern meteorology stations as 11.8 and 7.7 mm/h respectively. The intensive rainfall overloaded the sewer system and led to combined sewer overflow (CSO) into the river Sandvikselva and directly into the fjord. At the two CSOs with outlet directly to the fjord the time of overflow was measured by Bærum municipality, and the duration at each of the CSO was two hours during this rainfall episode.

2.3. Collection of water samples

Water samples were collected in 0.5 l sterile bottles from the river and the six beaches (Fig. 1) immediately after the rainfall event and then every 24 h for three consecutive days. The samples were taken by holding the bottles about 30 cm beneath the surface in water that was 50–100 cm deep. The samples were transported to the laboratory within 2 h, stored at 4 °C and analysed within 24 h.

2.4. Enumeration of E. coli in rivers and beaches

E. coli was quantified using a most probable number method (MPN) with colilert-18 medium (IDEXX) and Quantitray 2000 (IDEXX) according to ISO 9308-2:2012.

2.5. Hydrodynamic modelling

The model used to simulate the transport and decay of microbial pathogens after the rainfall event in this study was the GEMSS hydrodynamic and transport model (GEMSS-HDM). GEMSS is an integrated system of 3D hydrodynamics and transport models embedded in a geographic information and environmental data system (GIS), grid generator and editor, control file generator, 2-D and 3-D post processing viewers, and meteorological and flow data processor to support 3-D modelling (Entrix et al., 2001; Kollurua et al., 2014). The model uses GLLVHT (Generalized, Longitudinal-Lateral-Vertical Hydrodynamic and Transport) that computes time-varying velocities, water surface elevations, and water quality constituent concentrations in different water bodies. The hydrodynamic and transport relationships used in GLLVHT developed from the horizontal momentum balance, continuity, constituent transport and the equation of state. Edinger and Buchak were first presenting the theoretical basis of the model (Edinger and Buchak, 1980). The governing equations are described in the technical documentation of GEMSS (Edinger and Buchak, 1980; ERM, 2006).

The model was set up by generating the grids that define the spatial extent and bottom topography of the model domain. The boundary conditions of the hydrodynamic model were obtained from the Norwegian meteorological institute (eKlima), the Norwegian marine data centre, Bærum municipality and direct observation. The data used for this modelling include air temperature, dew point temperature, sea temperature, wind direction, wind speed, solar radiation, cloud cover, salinity, wave height, discharge rate (flow rate) and *E. coli* concentration from the river and combined sewer system. The main discharge came via river Sandvikselva. *E. coli* concentrations and corresponding waterflows monitored in the river before, during and after the rainfall for consecutive four days were used as input to the model (e.g. the *E. coli* concentrations monitored in the river were 36,000, 5900, 1700 and 5500 MPN/100 ml, after the heavy rainfall and 3 consecutive days respectively). For the two small CSOs discharging directly to the fjord, the registered time of overflow was used as input to the model, assuming that 50% of the sewage was discharged in this period. For modelling of discharges of reference pathogens, it was assumed that most of the *E. coli* detected in the river Sandvikselva and in the CSOs during heavy rain came from sewage. Based on the *E. coli* in the river and on the average concentrations of reference pathogens relative to *E. coli* in typical Norwegian sewage, taken from previous studies (Table 2), the concentrations of reference pathogens in the faecal discharges were calculated. The model simulation was run for three days after the heavy rainfall event by using first order decay rate of microbial pathogens as a parameter $K = 0.7$ (1 day half-life), $K = 0.1$ (1 week half-life), and $K = 0.023$ (1 month half-life) assuming that various microbial pathogens have different resistances to environmental stress (Ngazoa et al., 2008; Pachepsky et al., 2014; Peng et al., 2008; Robertson et al., 1992; Robertson and Gjerde, 2006). The model simulations of *E. coli* concentration for the upper (1 to 2 m) profile, that is commonly utilized for recreation, was considered as representative grid points for each beach site.

Table 1
The characteristics of rainfall episode used for this study.

Met. stations	Distance from Sandvika (km)	Direction from Sandvika	Rainfall episode (July 7, 2014)					Days and amount of two rainfall events (>5 mm) before the rainfall episode			
			Time rain started	Time rain stopped	Amount of rainfall (mm)	Duration of rainfall (min)	Average rainfall intensity (mm/h)	Days	Rainfall (mm)	Days	Rainfall (mm)
Asker	8.26	SW	12 AM	2 PM	21.8	111	11.78	6	7.8	18	7.6
Blindern	11.66	NE	10 AM	2 PM	18.1	142	7.65	2	9.3	10	9.3
Bygdøy	8.89	NE	11 AM	3 PM	17.9	–	–	10	17.6	11	17.6

Table 2
Dose–response parameters for each pathogen used for risk assessment.

Pathogens	Models	Parameters	References
Campylobacter	Beta-Poisson	$\alpha = 0.15, \beta = 7.9$	Teunis et al. (1999)
Salmonella	Beta-Poisson	$\alpha = 0.33, \beta = 139.9$	Rose and Gerba (1991)
Cryptosporidium	Exponential	$r = 0.2$	WHO (2011)
Giardia	Exponential	$r = 0.0199$	Haas et al. (1999)
Norovirus	Beta-Poisson	$\alpha = 0.04, \beta = 0.055$	Teunis et al. (2008)

2.6. Quantitative Microbial Risk Assessment (QMRA)

Quantitative Microbial Risk Assessment (QMRA) is a framework with the possibility of taking into account all available information from epidemiological studies, dose–response models, microbial pathogen monitoring data, discharge-based hydrodynamic modelling, and exposure assessment to forecast the probability of infection due to exposure to waterborne pathogens in recreational beaches. Based on the methodological framework, the following steps are described sequentially: 1) identifying pathogens of concern (hazard identification); 2) exposure assessment; 3) dose–response relationships; 4) characterizing the infection risk.

2.6.1. Identifying pathogens of concern (hazard identification)

Five reference pathogens were included in this QMRA study (Appendix A): *Campylobacter* and *Salmonella*, which are the leading infectious bacterial pathogens in the Bærum municipality according to the Norwegian Surveillance System for Communicable Diseases (MSIS, 2015) Moreover, *Campylobacter* is the most common cause of bacterial gastroenteritis in Norway and several waterborne outbreaks have been reported (Kvitsand and Fiksdal, 2010). *Cryptosporidium* spp. and *Giardia duodenalis*, which are important protozoa in the region because of their abundance in the wastewater. Although both pathogens are abundant in the region, *Giardia* occurs more frequently and at higher concentrations in Norwegian sewage than *Cryptosporidium* (Robertson et al., 2006). Norovirus was selected as reference virus because high concentrations were found in sewage in the region (Myrmel et al., 2015).

2.6.2. Exposure assessment

The amount of pathogens ingested during a single exposure depends on the volume of water swallowed during the recreational activity and the concentration of pathogens in the water. In this study the

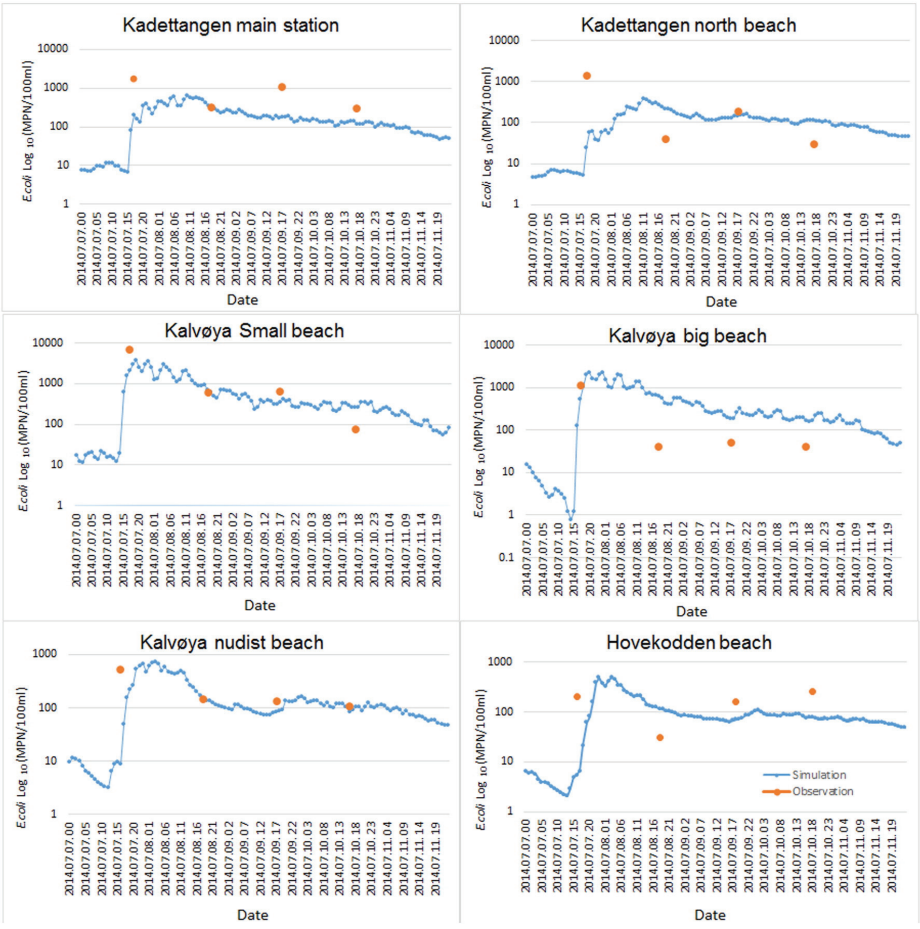

Fig. 2. Comparison of observed and simulated *E. coli* concentration in each beach site using decay rate K = 0.7.

Table 3
Average *E. coli* concentration (MPN/100 ml) estimated with different decay rates.

1st order decay rate	After rainfall event	Kadettangen	Kadettangen—north	Kalvøya—small	Kalvøya—big	Kalvøya—nudist	Høvikodden
K = 0.7	1 day	383	194	1311	990	345	205
	2 days	161	110	372	294	79	60
	3 days	107	87	267	190	96	65
K = 0.1	1 day	531	300	1538	1219	482	306
	2 days	314	249	539	473	191	158
	3 days	264	245	430	356	249	209
K = 0.0233	1 day	552	317	1565	1247	501	321
	2 days	339	273	565	501	212	176
	3 days	295	277	461	388	280	239

concentrations of pathogens in the water were calculated based on the model simulations. Water ingestion rates found in the literature varied depending on recreational versus competitive swimmers, adult versus children, occupational versus sport divers, limited contact versus full-contact recreational activities (Dorevitch et al., 2011; Rijal et al., 2011). For this study water ingestion rates of 16 ml for adults and 37 ml for children per daily recreational swimming were used (Dufour et al., 2006).

2.6.3. Dose response model

The dose response analysis is the method of deriving the mathematical relationship describing the risk of infection (although not necessarily illness) after exposure to the pathogen of interest. The response of a human population after exposure of waterborne pathogens is variable, reflecting the fact that the incidence of disease depends on the virulence of the pathogen, health and immune status of the host, and the water intake (Buchanan et al., 2000). In this study, the reference dose response models used were either the exponential or beta-Poisson models: the exponential dose response curve, in which the probability of infection for a group of people given an average dose d is computed by $p = 1 - e^{-rd}$, and the average dose for infection $ID_{50} = -\ln(0.5) / r$. The beta-Poisson curve calculate the probability of infection using the formula, $p = 1 - (1 + d / \beta)^{-\alpha}$, and $ID_{50} = \beta(2^{1/\alpha} - 1)$, where r, α, and β are dose response parameters (Haas et al., 1999), which are described in Table 2.

2.6.4. Risk characterization

Risk characterization is the final stage and the core result of this study that estimates the adverse public health risk as a consequence of exposure to the microbial pathogens in the recreational beaches after the rainfall event. The risk was calculated as the probability of infection per single exposure during the three consecutive days after the rainfall event. The risk estimations were divided into age categories (adult versus non-adult), based on differences in the amount of water ingested.

2.7. Sensitivity analysis for estimated risk

Mathematical methods in combination with graphical methods were used to investigate how variations in the input parameters affected the output. The effect on QMRA outputs by individually varying only one of the model inputs across its range of plausible values, while keeping all other inputs at their nominal values, was calculated. The difference in the model output due to the change in the input variable is referred as the sensitivity of the model to that particular input variable. Graphical representation of sensitivity analysis was used to give a visual indication of how an output is affected by variation in inputs (Geldermann and Rentz, 2001; Saltelli et al., 2000; Viscusi, 1991).

Sensitivity coefficients for each QMRA output (probability of infection) were derived by dividing the change in the probability of infection by the change in each input variable for a single exposure as

$$S_i = \frac{P_i - P_j}{x_i - x_j}$$

where, S_i is the sensitivity coefficient, x is input variable value, and P is probability of infection for input variable value x.

3. Results and discussion

3.1. Hydrodynamic model calibration and validation

In this study, model parameter selection was attuned within a range of feasible values that were determined from field measurements as a background to obtain the best agreement between predicted and observed values of *E. coli* concentrations. The calibration was performed by running the model from June 1st to July 30th, 2014. The goodness of fit was evaluated based on the combination of statistical coefficient with a visualization of results plotted for the individual observation data points against GEMSS model generated values of *E. coli* concentration as the results shown in Fig. 2. One of the statistical coefficients used to evaluate the goodness of fit of the model was Nash–Sutcliffe coefficient (E) that ranged from −∞ to 1 and the overall computed value (0.36) indicated that the model can simulate the microbial water quality with reasonable accuracy. This can also be seen from Fig. 2 where the predicted and observed *E. coli* concentrations are plotted. Some observations had a higher concentration of *E. coli* than the model prediction and this may be explained by the presence of swan faeces or storm water discharges that were not included as input to the model, but that may influence the water quality locally. In general, the best fit was observed at Kalvøya nudist beach, which is located furthest away from such potential sources of contamination (Fig. 2).

Table 4
Literature values of the average concentration of pathogens in the sewage system in the Oslo area and the ratio pathogen:*E. coli* used as a input to the model.

Microbial pathogens and indicator per 100 ml	Concentration in the WWTP influent and sewage system		Ratio pathogen:*E. coli*
	Average concentration	References	
E. coli	5.9×10^6	Grøndahl-Rosado et al. (2014)	1:1
Giardia	759.5	Robertson et al. (2006)	1.3×10^{-4}
Cryptosporidium	678.1	Robertson et al. (2006)	1.2×10^{-4}
Norovirus	5.1×10^5	Myrmel et al. (2015)	8.64×10^{-2}
Salmonella	3.16×10^2	Langeland (1982)	5.4×10^{-5}
Campylobacter	–	Estimate of this study	2.0×10^{-5}

Table 5
Calculated pathogen concentration at the beaches using K = 0.7 for *Salmonella* and *Campylobacter* and K = 0.0233 for other pathogens.

Microbial pathogen	Number of days after rainfall event	Unit	Kadettangen	Kadettangen—north	Kalvøya—small	Kalvøya—big	Kalvøya—nudist	Høvikodden
Giardia	1	(cysts/100 ml)	7.18E−02	4.12E−02	2.03E−01	1.62E−01	6.51E−02	4.17E−02
	2	(cysts/100 ml)	4.41E−02	3.55E−02	7.35E−02	6.51E−02	2.76E−02	2.29E−02
	3	(cysts/100 ml)	3.84E−02	3.60E−02	5.99E−02	5.04E−02	3.64E−02	3.11E−02
Cryptosporidium	1	(oocysts/100 ml)	6.62E−02	3.80E−02	1.88E−01	1.50E−01	6.01E−02	3.85E−02
	2	(oocysts/100 ml)	4.07E−02	3.28E−02	6.78E−02	6.01E−02	2.54E−02	2.11E−02
	3	(oocysts/100 ml)	3.54E−02	3.32E−02	5.53E−02	4.66E−02	3.36E−02	2.87E−02
Norovirus	1	(gene copies/100 ml)	4.75E+01	2.73E+01	1.35E+02	1.07E+02	4.31E+01	2.76E+01
	2	(gene copies/100 ml)	2.92E+01	2.35E+01	4.86E+01	4.31E+01	1.82E+01	1.51E+01
	3	(gene copies/100 ml)	2.54E+01	2.38E+01	3.96E+01	3.34E+01	2.41E+01	2.06E+01
Salmonella	1	MPN/100 ml	2.07E−02	1.05E−02	7.08E−02	5.35E−02	1.86E−02	1.11E−02
	2	MPN/100 ml	8.69E−03	5.94E−03	2.01E−02	1.59E−02	4.27E−03	3.24E−03
	3	MPN/100 ml	5.78E−03	4.70E−03	1.44E−02	1.03E−02	5.18E−03	3.51E−03
Campylobacter	1	MPN/100 ml	7.66E−03	3.88E−03	2.62E−02	1.98E−02	6.90E−03	4.10E−03
	2	MPN/100 ml	3.22E−03	2.20E−03	7.44E−03	5.88E−03	1.58E−03	1.20E−03
	3	MPN/100 ml	2.14E−03	1.74E−03	5.34E−03	3.80E−03	1.92E−03	1.30E−03

3.2. Effect of including different decay rates in the model simulations

The effect of using different decay rates of *E. coli* (as a model of pathogens with different decay rates) was significant, i.e. 1.2 to 3.7 times higher numbers when a half-life of 1 month was used instead of 1 day (Table 3), but since the results were evaluated for only 3 days after the rainfall, dilution had a higher effect on bacterial reduction than decay rate. The pathogen number was calculated from the simulated *E. coli* concentration using the decay rate most relevant for the actual pathogen (i. e. K = 0.7 for *Campylobacter* and *Salmonella* and K =

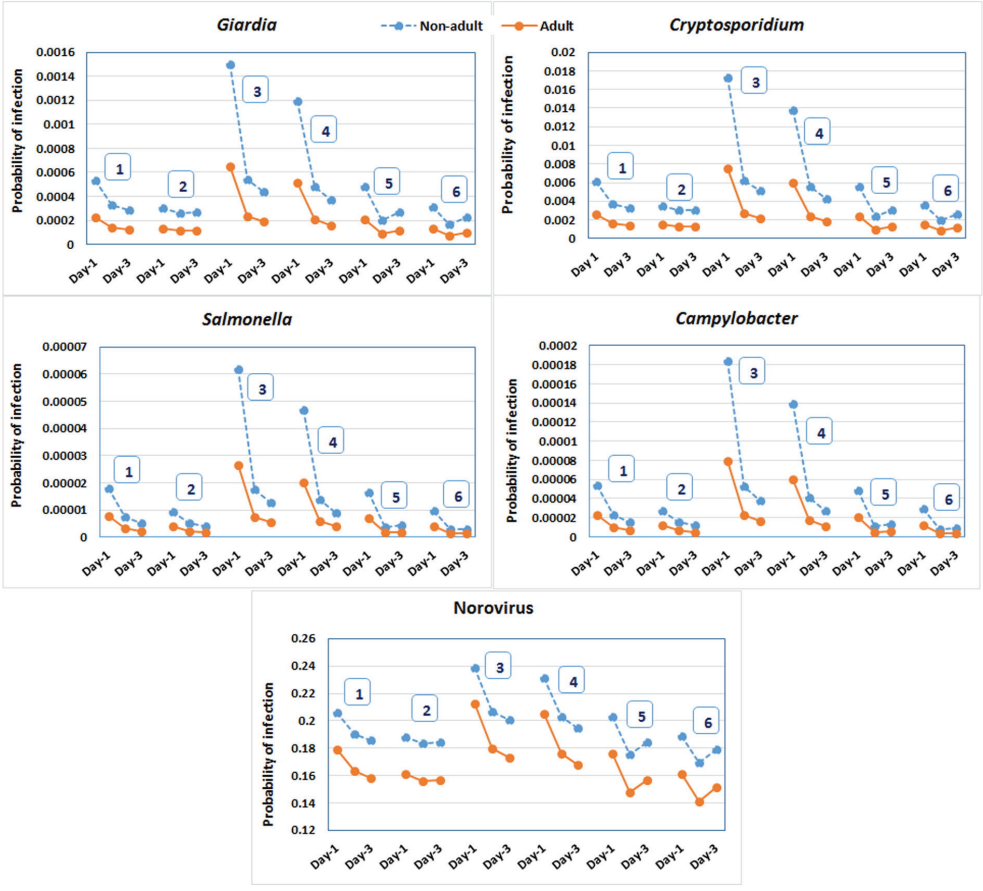

Fig. 3. The average risk of infection at: 1. Kadettangen, 2. Kadettangen-north, 3. Kalvøya-small, 4. Kalvøya-big, 5. Kalvøya-nudist, and 6. Høvikodden.

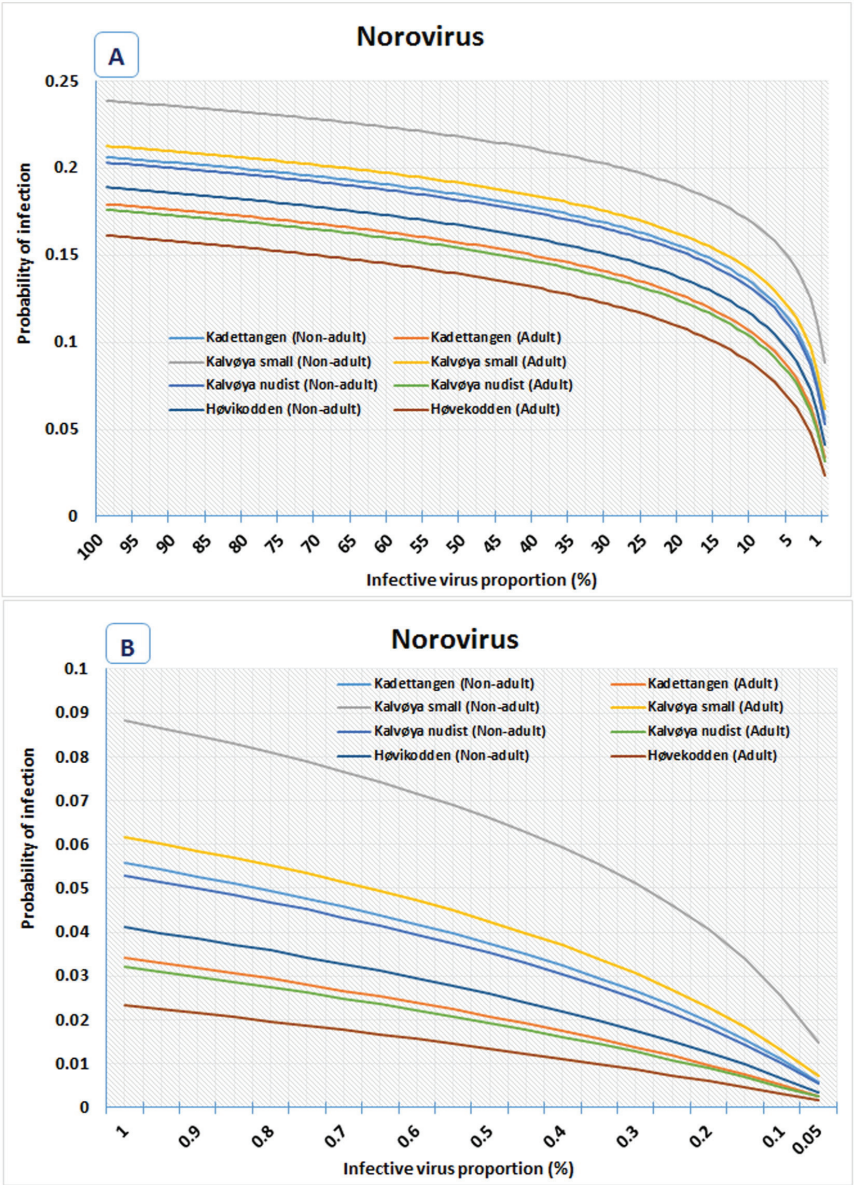

Fig. 4. The probability of being infected with norovirus (on day 1) versus the proportion of infective virus: A. (100% to 1%) and B. (1% to 0.05%).

0.023 for *Giardia*, *Cryptosporidium* and norovirus) multiplied by the pathogen:*E. coli* ratio typical for Norwegian sewage as shown in Table 4. This approach is based on the assumption, which obviously is not always true, that the pathogen:*E. coli* ratio was constant in all the faecal discharges used as input to the model during the rainfall event.

According to a study on 40 WWTP influent in Norway (Robertson et al., 2006), the concentration of *Giardia* cysts ranged from 4000 to 28,000 with the average value of 7595 cysts/l and *Cryptosporidium* oocysts ranged from 4000 to 24,000 with the average value of 6781 oocysts/l (Robertson et al., 2006). Myrmel et al. (2015) assessed the norovirus concentration in the influent of two WWTP. Norovirus concentrations ranged from 2.6×10^5 to 2.1×10^7 genome copies/l, with the average value of 5.1×10^6 genome copies/l (Myrmel et al., 2015). The average concentration of *E. coli* in the sewage system was 5.9×10^6 MPN/100 ml based on unpublished results and results from Grøndahl-Rosado et al. (2014). The average concentration of *Salmonella* in three sewer systems in Oslo was 316 MPN/100 ml (Langeland, 1982).

Published information about the concentration of *Campylobacter* in the Norwegian sewage system was not identified and therefore

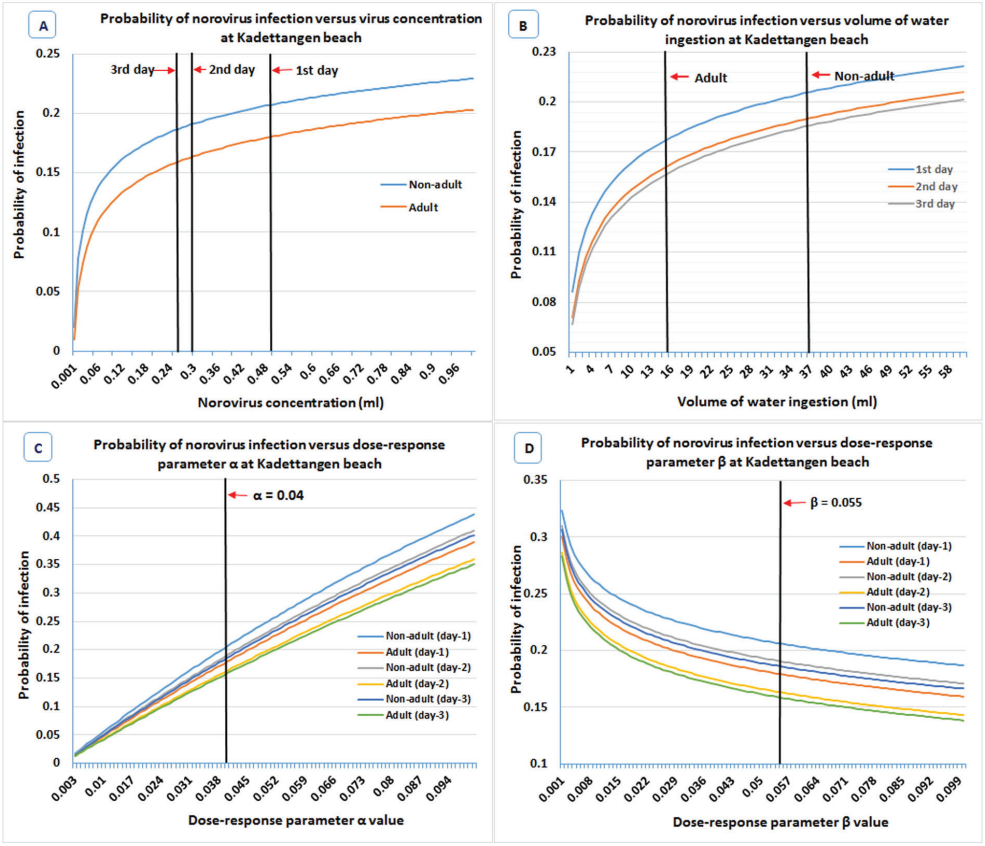

Fig. 5. The sensitivity of the calculated probability of norovirus infection for single exposure at the Kadettangen beach for input variable of A) volume of water ingested, B) norovirus concentration, C) dose–response model parameter (α), and D) dose–response model parameter (β).

epidemiological information was used to estimate the concentration in sewage. According to the epidemiological survey in MSIS (MSIS, 2015), two people of 100,000 were infected in the municipality during the rainfall event. If we assume a similar average production of *Campylobacter* and *E. coli* among the infected people (WHO, 2011), then the average ratio *Campylobacter*: *E. coli* were 2:100,000. Based on these assumptions, the calculated average pathogen concentrations at the beaches, for the three days after the heavy rainfall, were as shown in Table 5.

3.3. Health risk characterization

The final stage of bathing water risk assessment in this study was the risk characterization, which is the integration of dose–response model and exposure in order to calculate the risk. The overall risk of infection for a single exposure of pathogens during bathing was higher for non-adult relative to adult because of higher volume of water ingested. Moreover, the highest risk was observed at the Kalvøya small beach and Kalvøya big beaches the first day after the rainfall event due to the location of the beaches where the incoming flood from river Sandvikselva directly affected the two beaches relatively more than the other beaches.

The QMRA in the present study (Fig 3) indicated that the probability of being infected by *Giardia*, *Cryptosporidium*, *Salmonella, and Campylobacter* after the rainfall event was below 19 per 1000 bathers (0.019) at all beaches, which is considered as an acceptable risk of gastrointestinal illness after recreational activities (WHO, 2003). The probability of infection on the first day after the rainfall event was higher than on the second day. However, the decrease in risk from the second to the third day was relatively less. The risk of exposure to norovirus was high at all beaches since 100% of the estimated virus concentration was considered to represent infective particles.

3.4. Uncertainty and sensitivity analysis

A discussion of uncertainties is an important part of the risk characterization to evaluate the implications and limitations of the risk assessment and to estimate the degree of confidence that can be placed in the risk estimate. This study involved a combination of two modelling approaches, GEMSS hydrodynamic modelling and dose–response model of QMRA, which use different variables as an input. It is obvious that the combination of discharge-based hydrodynamic modelling with QMRA is challenged by the uncertainty caused by measurement error, knowledge gaps, and/or natural variability. Since we make use of models, model uncertainty in all steps is expected as a potential source of uncertainty.

Sensitivity analysis was performed to investigate how variation in the output can be apportioned quantitatively to different source of variation in the input. QMRA modelling involves different input variables that can be a potential source of uncertainty in the output and the analysis was based on some of these input variables, as examples to

illustrate the principle of sensitivity analysis. In general, the classification of uncertainty in this study consists of three main types: 1) Factors associated with the estimation of pathogen concentration at the beaches, which is the result of the GEMSS hydrodynamic modelling and all the uncertainties therein, in particular in the concentration of pathogens and *E. coli* in the sewer system (and river) used as discharge input to the GEMSS model and the maintenance of infectivity of virus particles after they are discharged into seawater. 2) Factors associated with the human water intake in the recreational beaches during swimming. 3) Factors related to the dose–response model parameters (α and β parameters in the case of beta-Poisson model and r for exponential model). Furthermore, it is important to notice that, in this hydrodynamic modelling, the input factors are associated with biological and environmental issues that are characterized by huge natural variability and, in general, a lack of knowledge. For example, wind direction will strongly influence the surface currents and the transport of pathogens in coastal water (Bouchalová et al., 2013; Chan et al., 2013) and sunlight, as well as biological- and physicochemical water quality, has a strong influence on the decay rate of the pathogens (Hipsey et al., 2008).

The infectivity of virus particles was not included in the calculations above since the virus quantification was based on molecular detection (RT-qPCR). Therefore, in follow-up calculations an assumption that from 100% to 0.05% of the estimated number of virus particles was infective was used as input to QMRA and this resulted in a possible range of infection risk from 23.8% to 1.5% at Kalvøya small (non-adult) and from 16.1% to 0.16% at Høvekodden (adult) as shown in Fig 4. In these calculations the virus concentrations in the river and CSOs used as input to the model was based on the average virus:*E. coli* ratios found in Norwegian sewage (i. e. about 1 log lower number of viruses than *E. coli*). The concentrations of viruses in sewage may, in worst-case, be close to the *E. coli* concentration (Myrmel et al., 2015), but the fraction of infective norovirus is unknown. This illustrates the difficulties in estimating the risk associated with norovirus in faecally contaminated water.

Further sensitivity analysis involved the utilization of a set of parameter values upside and downside from the reference value of inputs, in order to visualize the corresponding change in the output. To visualize the change of health outcome (probability of infection) in response to different input values, computed values of norovirus at Kadettangen beach were plotted as an example by considering change in the input values: volume of water ingested, pathogen concentration, and dose–response model parameter value (Fig 5).

As can be seen from Fig. 5B, when the estimated volume of water ingested is increased from 16 ml to 37 ml, the risk of norovirus infection for a single exposure rises from 17.9% to 20.6%, from 16.3% to 19.1%, and from 15.9% to 18.6% for the first, second, and third days respectively. The other source of uncertainty that was included in this sensitivity analysis was norovirus concentration (Fig. 5A). The estimated concentration of norovirus after the first day of heavy rainfall was 0.47 GC/ml at the Kadettangen beach. If the estimated concentration was increased or decreased by one log (0.047 to 4.7 GC/ml), the probability of infection (assuming that all virus particles were infective) ranged from 13.1% to 27.6% for non-adult and from 10.2% to 25.1% for adult. A one log increase may represent a worst case based on maximum numbers found in sewage. A one log reduction may represent lower concentrations of norovirus in the sewage, but since the fraction of infective viruses in the sewage and on the beaches is unknown, one log reduction may also overestimate the actual risk. The choice of appropriate dose–response model is also a critical step and one of the sources of uncertainty. The sensitivity of norovirus infection for the dose–response model parameter values was evaluated using α parameter value (Fig. 5C) or β parameter value (Fig. 5D). It can be seen from the graphs that the risk of norovirus infection was more sensitive to a slight change in the α parameter value than the β parameter value by observing the slope of the lines. If the α value of the beta-Poisson model ranged from 0.02 to 0.06, the sensitivity coefficient was 4.6 for non-adults and 4.1 for adults. While if the β parameter value ranged from 0.0275 to 0.0825, the sensitivity coefficient was −0.63 for non-adults and −0.65 for adults.

Three different values of first order decay coefficient (K) were used to characterized environmental inactivation of microbial pathogens ($K = 0.7$, $K = 0.1$, $K = 0.0233$). The sensitivity of the computed risk was evaluated as an example at Kadettangen beach for *Cryptosporidium* and norovirus and the result is plotted in Appendix B. The reduction of K value from 0.7 to 0.1 resulted in an increase in the probability of *Cryptosporidium* and norovirus infection, whereas further reduction of K value to 0.0233 resulted in a lower increase, which is natural since the investigations were performed for only three days after the rainfall event. In general, there is a knowledge gap regarding which decay rates that should be used for infective viruses when they are discharged into coastal water, and this is a topic for further research.

One of the largest sources of uncertainty is the pathogen concentration in the sewer system and Appendix C shows the variation of computed probability of infection for *Cryptosporidium* based on the minimum, average, and maximum concentrations in sewage (Robertson et al., 2006). If the maximum concentration was used, the calculated probability of infection was unacceptable for non-adults at Kalvøya small beach the day after the rainfall event, but acceptable after two days and acceptable at the other beaches also one day after the rainfall event.

4. Conclusion

The risk assessment method presented here is an integration of QMRA with discharge-based hydrodynamic modelling of microbial bathing water quality as an attempt to improve the understanding of spatial–temporal spreading of microbial pathogens after rainfall events at recreational beaches. Continuous monitoring of pathogens at each beach after flooding and discharges from CSOs, as an input for QMRA is costly and, in general, impractical. Therefore, hydrodynamic model simulation can be a potential substitution for direct monitoring of microbial pathogen concentration. The integration of hydrodynamic modelling with QMRA and evaluation of the associated uncertainties could contribute to the improvement of the QMRA technique for recreational waters.

The results of this study indicate an acceptable risk for the bacterial and parasitic reference pathogens if people go swimming at the Sandvika recreational beaches one day after the modelled rainfall event. The study confirms that viral infection constitutes the predominant risk from exposure to bathing water after heavy rainfall and sewage overflows at beaches in urban areas. However, further studies are needed regarding the determination of the infectivity of viruses to assess the risk more precisely. The highest infection risk at the two beaches Kalvøya small and Kalvøya big, due to the position of the beach, suggests specific attention for mitigation measures and further in-depth risk assessment at local level. The study supported the general advice of warning against swimming at the Sandvika beaches the day (s) after heavy rainfall.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2016.01.034.

References

Ackerman, D., Weisberg, S., 2003. Relationship between rainfall and beach bacterial concentrations on Santa Monica bay beaches. J Water Health 1, 85–89.

Andersen, S.T., Erichsen, A.C., Mark, O., Albrechtsen, H.J., 2013. Effects of a 20 year rain event: a quantitative microbial risk assessment of a case of contaminated bathing water in Copenhagen, Denmark. J. Water Health 11, 636–646.

Bærum, 2012. Hovedplan Vannforsyning og Avløp 2012–2021 (Bærum Municipality, Oslo) p. 52.

Bouchalová, M., Wennberg, A., Tryland, I., 2013. Impact of rainfall on bathing water quality — a case study of Fiskevollbukta, Inner Oslofjord, Norway. VANN 4, 491–498.

Buchanan, R.L., Smith, J.L., Long, W., 2000. Microbial risk assessment: dose–response relations and risk characterization. Int. J. Food Microbiol. 58, 159–172.

Chan, S.N., Thoe, W., Lee, J.H.W., 2013. Real-time forecasting of Hong Kong beach water quality by 3D deterministic model. Water Res. 47, 1631–1647.

Dorevitch, S., Panthi, S., Huang, Y., Li, H., Michalek, A.M., Pratap, P., et al., 2011. Water ingestion during water recreation. Water Res. 45, 2020–2028.

Duffy, G., Cummins, E., Nally, P., O'Brien, S., Butler, F., 2006. A review of quantitative microbial risk assessment in the management of *Escherichia coli* O157: H7 on beef. Meat Sci. 74, 76–88.

Dufour, A.P., Evans, O., Behymer, T.D., Cantu, R., 2006. Water ingestion during swimming activities in a pool: a pilot study. J. Water Health 4, 425–430.

Edinger, J.E., Buchak, E.M., 1980. Numerical hydrodynamics of estuaries. Estuarine and Wetland Processes. Springer, pp. 115–146.

Entrix, I., J. E. Edinger Associates I, Gahagan and Bryant Associates I, 2001. Hydrodynamic and Water Quality Modeling and Feasibility Analysis of Indian River, Rehoboth Bay and Little Assawoman Bay. Delaware's Inland Bays Flushing Study Project Delaware Department of Natural Resources and Environmental Control.

ERM, 2006. In: ERM.inc (Ed.), GEMSS-HDM Hydrodynamic and Transport Module (Technical Documentation, Pennsylvania).

EU, 2006. Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union L64.

Geldermann, J., Rentz, O., 2001. Integrated technique assessment with imprecise information as a support for the identification of best available techniques (BAT). OR-Spektrum 23, 137–157.

Grøndahl-Rosado, R.C., Tryland, I., Myrmel, M., Aanes, K.J., Robertson, L.J., 2014. Detection of microbial pathogens and indicators in sewage effluent and river water during the temporary interruption of a wastewater treatment plant. Water Quality, Exposure and Health 6, 155–159.

Haas, C.N., Rose, J.B., Gerba, C.P., 1999. Quantitative Microbial Risk Assessment. John Wiley & Sons.

Haile, R.W., Witte, J.S., Gold, M., Cressey, R., McGee, C., Millikan, R.C., et al., 1999. The health effects of swimming in ocean water contaminated by storm drain runoff. Epidemiology 10, 355–363.

Hipsey, M.R., Antenucci, J.P., Brookes, J.D., 2008. A generic, process-based model of microbial pollution in aquatic systems. Water Resour. Res. 44, 1–26.

Huang, G., Falconer, R.A., Lin, B., 2015. Integrated river and coastal flow, sediment and escherichia coli modelling for bathing water quality. Water 7, 4752–4777.

Jaykus, L.A., 1996. The application of quantitative risk assessment to microbial food safety risks. Crit. Rev. Microbiol. 22, 279–293.

Kacikoc, M., Beyhan, M., 2014. Hydrodynamic and water quality modeling of Lake Egirdir. Clean-Soil Air Water 42, 1573–1582.

Kollurua, V.S., Prakasha, S., Hodginsb, D., 2014. Evaluation of urbanization and impacts on water quality in Nottawasaga Bay using an integrated 3-D modeling framework. Proceedings of the 7th International Congress on Environmental Modelling and Softwar, San Diego, California, USA.

Kvitsand, H.M.L., Fiksdal, L., 2010. Waterborne disease in Norway: emphasizing outbreaks in groundwater systems. Water Sci. Technol. 61, 563–571.

Langeland, G., 1982. *Salmonella* spp. in the working environment of sewage treatment plants in Oslo, Norway. Appl. Environ. Microbiol. 43, 1111–1115.

MSIS. Norwegian Surveillance System for Communicable Diseases (MSIS). 2015, Norway.

Myrmel, M., Lange, H., Rimstad, E., 2015. A 1-year quantitative survey of noro-, adeno-, human boca-, and hepatitis E viruses in raw and secondarily treated sewage from two plants in Norway. Food and Environmental Virology 7, 213–223.

Ngazoa, E., Fliss, I., Jean, J., 2008. Quantitative study of persistence of human norovirus genome in water using TaqMan real-time RT-PCR. J. Appl. Microbiol. 104, 707–715.

Noble, R., Weisberg, S., Leecaster, M., McGee, C., Dorsey, J., Vainik, P., et al., 2003. Storm effects on regional beach water quality along the southern California shoreline. J. Water Health 1, 23–31.

Pachepsky, Y., Blaustein, R., Whelan, G., Shelton, D., 2014. Comparing temperature effects on *Escherichia coli*, *Salmonella*, and *Enterococcus* survival in surface waters. Lett. Appl. Microbiol. 59, 278–283.

Peng, X., Murphy, T., Holden, N.M., 2008. Evaluation of the effect of temperature on the die-off rate for *Cryptosporidium parvum* oocysts in water, soils, and feces. Appl. Environ. Microbiol. 74, 7101–7107.

Pompepuy, M., Guyader, S.L., Catherine, M., Saux, J.C.L., Joanny, M., Camus, P., 2009. Impacts of microbiological contamination on the marine environment of the North-East Atlantic. Monitoring and Assessment Series. OSPAR COMMISSION.

Rijal, G., Tolson, J., Petropoulou, C., Granato, T., Glymph, A., Gerba, C., et al., 2011. Microbial risk assessment for recreational use of the Chicago Area Waterway System. J. Water Health 9, 169–186.

Robertson, L.J., Gjerde, B.K., 2006. Fate of *Cryptosporidium* oocysts and *Giardia* cysts in the Norwegian aquatic environment over winter. Microb. Ecol. 52, 597–602.

Robertson, L.J., Campbell, A., Smith, H., 1992. Survival of *Cryptosporidium parvum* oocysts under various environmental pressures. Appl. Environ. Microbiol. 58, 3494–3500.

Robertson, L.J., Hermansen, L., Gjerde, B.K., 2006. Occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in sewage in Norway. Appl. Environ. Microbiol. 72, 5297–5303.

Rose, J.B., Gerba, C.P., 1991. Use of risk assessment for development of microbial standards. Water Sci. Technol. 24, 29–34.

Saltelli, A., Tarantola, S., Campolongo, F., 2000. Sensitivity analysis as an ingredient of modeling. Stat. Sci. 15, 377–395.

Sokolova, E., Pettersson, T.J.R., Bergstedt, O., 2014. Hydrodynamic modelling and forecasting of microbial water quality in a drinking water source. J. Water Supply Res Technol. 63, 189–199.

Sokolova, E., Petterson, S.R., Dienus, O., Nyström, F., Lindgren, P.-E., Pettersson, T.J., 2015. Microbial risk assessment of drinking water based on hydrodynamic modelling of pathogen concentrations in source water. Sci. Total Environ. 526, 177–186.

Teunis, P.F., Nagelkerke, N.J., Haas, C.N., 1999. Dose response models for infectious gastroenteritis. Risk Anal. 19, 1251–1260.

Teunis, P.F., Moe, C.L., Liu, P., Miller, S.E., Lindesmith, L., Baric, R.S., et al., 2008. Norwalk virus: how infectious is it? J. Med. Virol. 80, 1468–1476.

Tufford, D.L., McKellar, H.N., 1999. Spatial and temporal hydrodynamic and water quality modeling analysis of a large reservoir on the South Carolina (USA) coastal plain. Ecol. Model. 114, 137–173.

Viscusi, W.K., 1991. Uncertainty — a guide to dealing with uncertainty in quantitative risk and policy analysis. In: Morgan, M.G., Henrion, M., Small, M. (Eds.), Journal of Economic Literature 29, pp. 1172–1174.

WHO, 2003. Guidelines for Safe Recreational Water Environments: Coastal and Fresh Waters vol. 1. World Health Organization.

WHO, 2011. Guidelines for Drinking-water Quality. WHO Chronicle, p. 38.

Appendix A.

Reference waterborne pathogens: concentrations in sewage, occurrence in human infections, and their potential sources in Norway

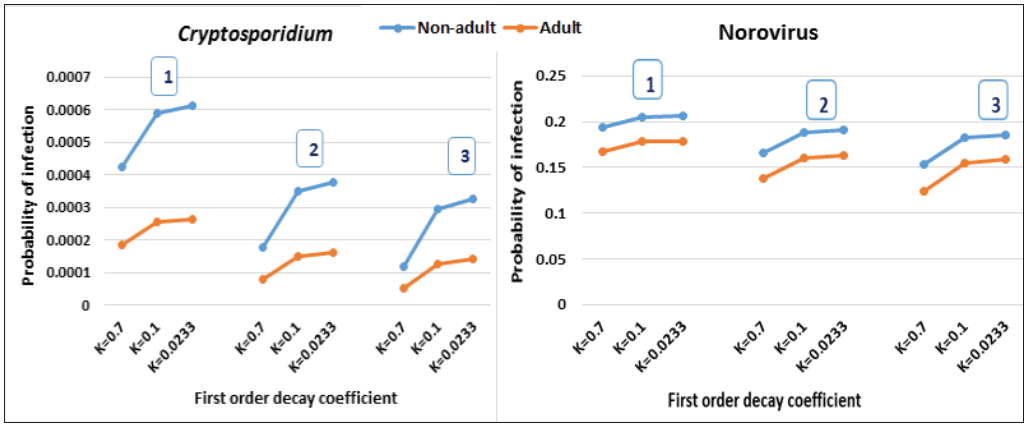
	Concentration in Untreated sewage		Cases/year reported [a] (2005-2014)		Animal source [e]
Pathogens	Range	Mean	Bærum municipality	Norway	
Bacteria					
Campylobacter (CFU/100ML)	NA	NA	58 - 92	2588 - 3389	Zoonosis
Salmonella (MPN/100ML) [c]	3-930	316	25 - 55	1120 - 1943	Zoonosis
Protozoa					
Cryptosporidium (oocyst/L) [b]	4000 - 24000	6781	NA	NA	Zoonosis
Giardia (cyst/L) [b]	4000 - 28000	7595	1 - 12	179 - 427	Potentially Zoonosis
Viruses					
Norovirus (genome copies / L) [d]	2.6×10^5 - 2.1×10^7	5.1×10^6	NA	NA	Potentially Zoonosis

NA means data is not available
MSIS: Norwegian surveillance system for communicable disease

[a] (MSIS)
[b] (Robertson et al., 2006)
[c] (Langeland, 1982)
[d] (Myrmel et al, 2015)
[e] (Nygård, 2008)

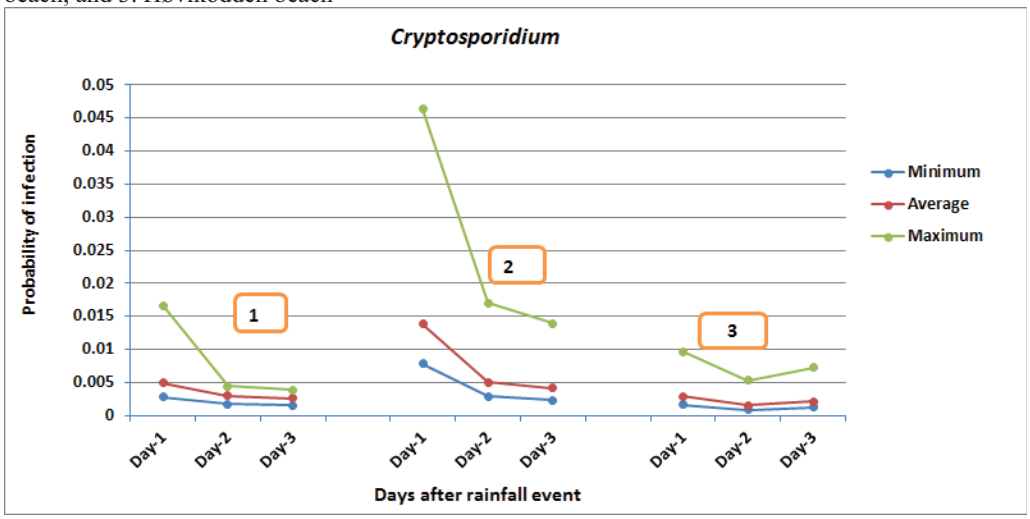
Appendix B.

The effect of first order decay coefficient on the estimated risk at Kadettangen beach: 1. First day, 2. Second day, 3. Third day



Appendix C.

Probability of infection of non-adult person using minimum, average and maximum Cryptosporidium concentration in the sewer system at 1. Kadettangen beach, 2. Kalvøya small beach, and 3. Høvikodden beach



Paper IV

Eregno, F. E., Tryland, I., T., Myrmel, Wennberg, A., Oliinyk, A., Khatri, M., & Heistad, A. (2017). Decay rate of virus and faecal indicator bacteria (FIB) in seawater and the concentration of FIBs in different wastewater systems. *Microbial risk Analysis*. (Revised version submitted)

Decay rate of virus and faecal indicator bacteria (FIB) in seawater and the concentration of FIBs in different wastewater systems

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Abstract

Information about the concentration, growth and decay rates of enteric microorganisms in different wastewater systems and water bodies is crucial for quantitative microbial risk assessment (QMRA). In general, there is little information about this and in particular about the decay rates of faecal bacteria and viruses at different depths of the marine environment where many wastewater treatment plants discharge their effluent. This study was designed to investigate the concentration of faecal indicator bacteria (FIB) in different types of wastewater, the potential for growth of FIB in greywater, and the decay rates of FIB and pathogenic viruses in seawater samples from different depths. Average concentrations of total coliforms (TC), *Escherichia coli* (*E. coli*) and intestinal enterococci (IE) (log_{10} per 100 ml) were 7.2, 6.7 and 6.1 in blackwater; 6.7, 6.1 and 3.8 in greywater; and 6.8, 6.6 and 5.2 in municipal wastewater, respectively. The numbers of TC and *E. coli* increased when greywater was stored for 24 hours at 20 ^{o}C but decreased after 96 hours. No growth of IE was observed in the greywater. The decay rate experiment showed that both the viruses and FIB were inactivated relatively rapidly at 20 ^{o}C in seawater collected from 1 m depth, while a slow inactivation was observed at 4 ^{o}C in seawater collected from 60 m depth, with a significant difference in decay rates ($p<0.05$). Our results suggest that temperature and biological activity are important factors for the self-purification capacity when wastewater containing faecal bacteria and viruses is discharged to coastal water, and the varying decay rates must be taken into account in risk assessments.

Keywords: Adenovirus; decay rate; faecal indicator bacteria; MS2; seawater; wastewater

1. Introduction

Leakages, overflows from sewer systems and discharges of partially treated wastewater from wastewater treatment plants (WWTP) release a complex array of microorganisms into the environment, which potentially includes pathogenic bacteria, protozoa, viruses, fungi, and helminths that could create a serious public health risk (Brombach et al., 2005; Gasperi et al., 2008). The abundance and density of pathogenic organisms released to the environment will vary depending on the prevalence of infections in the population, the efficiency of the treatment system and the dilution and decay rate in the receiving water body.

Isolation, detection and quantification of pathogenic microorganisms in aquatic environments are difficult, expensive and time-consuming (Fong and Lipp, 2005). As an alternative, total coliforms (TC), *Escherichia coli* (*E. coli*) and intestinal enterococci (IE), which are found in the intestinal tracts of warm blooded animals, are used as indicators of faecal contamination (Savichtcheva and Okabe, 2006). In the absence of data for specific pathogens, the concentration of these faecal indicator bacteria (FIB) is often used for quantitative microbial risk assessment (QMRA), but this requires several assumptions regarding the relationship between pathogens and FIB (Haas et al., 1999; Schijven et al., 2011; Smeets et al., 2010). A number of studies have revealed that FIB and pathogens are weakly correlated or uncorrelated (Harwood et al., 2005; O'Toole et al., 2014). Nevertheless, QMRA approaches based on FIB will still be essential because of the difficulty to monitor all known waterborne pathogens directly.

The aim of wastewater treatment is to obtain an effluent that can be reused or returned to the water cycle with minimal impact on environment and health. Small on-site treatment plants are often based on soil infiltration with physical, chemical and biological treatment processes and discharge to groundwater, or compact biological/chemical package plants that discharge their effluent into local creeks/rivers or groundwater (Scandura and Sobsey, 1997). An emerging sanitation approach is the use of source separation, which promotes energy recovery and closed-loop material flow through the recovery of nutrients, water, and organic matter. In this context source separation means separate collection and treatment of blackwater (urine and faeces), and greywater (from shower, bath, and kitchen) (Larsen et al., 2009). Reuse of greywater is of emerging interest, but the sometimes high numbers of FIB, that may be caused by multiplication in the collection system (Ottoson and Stenström, 2003), requires local investigations to estimate the relationship between FIB and enteric viruses.

Centralized, municipal WWTPs typically discharge effluents directly to a water body (river, lake, estuary or ocean) where natural physical, chemical and biological processes are expected to provide additional treatment (Brombach et al., 2005; Loos et al., 2013). The fate of enteric microorganisms in a water body is influenced by type of microorganism (species, strain and physiological status), physical and chemical characteristics encountered (temperature, salinity, organic matter, oxygenation, pH), atmospheric conditions (sunlight irradiation) and biotic factors (predation and competition) (Carlucci and Pramer, 1959; Cornax et al., 1990; Rozen and Belkin, 2001; Suttle and Chen, 1992; Wu et al., 2016). In coastal areas, treated wastewater and untreated wastewater overflow from combined sewer systems, are often discharged directly into the ocean (Griffith et al., 2009; Wood et al., 1993). There are a number of studies on microbial decay rates at different marine discharge depths and conditions in seawater (Bae and Wuertz, 2009; Jeanneau et al., 2012; Lee et al., 2011; Mattioli et al., 2017; Noble et al., 2004), only a few studies deal with the effect of seawater sample from different depth (Wilhelm et al., 1998). In deep seawater, the

temperature and biological activity are assumed to be lower than in the surface water and therefore decay rates are expected to vary by water depth (Wei et al., 2010).

A four-step QMRA framework is usually applied to investigate microbial pathogen concentration, behaviour, exposure, and pathways to identify where and when they can become a danger and estimate the risk that they pose to human health (Haas et al., 1999). The exposure assessment step of QMRA focused on estimating the magnitude and frequency of exposure to a specific reference pathogen. In this step, the concentration of microbial pathogens from the source to the point of exposure is investigated based on the initial concentration, the transport, decay rate in a specific condition, and the volume of water consumed per exposure (Medema, 2012; WHO, 2016). Microbial pathogens are usually exposed to different environmental factors, for instance, different temperature, sunlight etc. and as a result, they are susceptible to inactivation or die off (Rogers et al., 2011). For QMRA study, understanding of microbial decay rate due to various environmental factors enables us to compute the concentration of microbial pathogens in a particular environmental condition. Therefore, microbial decay rate study in different environmental conditions improve QMRA approach. On the other hand, pieces of evidence showed that pathogenic bacteria could grow in aquatic environments, such as surface waters, dams, ponds, water treatment systems, and wastewater systems (Camper et al., 1985; Juhna et al., 2007; Vital et al., 2010). Understanding and quantifying the growth potential of bacteria in water is essential for a holistic approach to microbial risk assessment. However, including pathogen growth into QMRA framework is not widely practiced (Vital et al., 2010).

Microbial water quality monitoring has been conducted by quantifying indicator organisms that are used as a pointer of the presence of microbial pathogens found in water intend to irrigation, bathing, drinking and other non-potable purposes. Commonly used indicators are coliforms (total coliforms), faecal coliforms, *Escherichia coli*, intestinal enterococci and bacteriophages (Fewtrell and Bartram, 2001). Due to the absence of information on microbial water quality based on pathogenic microorganisms, a number of QMRA have been carried out using indicator organisms (Eregno et al., 2016; Hamilton et al., 2006; Mara et al., 2007). The concentration of reference pathogens was estimated by extrapolation and the procedure was based on the predefined ratio of indicator organisms and pathogens. However, the weak correlation between numbers of indicator organisms and enteric pathogens are a challenge for QMRA approaches using indicator organisms. Although weak correlation of indicator organisms and pathogens raise a question on the estimated risk level (Harwood et al., 2005; Williams and Ebel, 2014), it is still valuable in an area where information on microbial pathogens are hardly found and also depend on the purpose of risk assessment.

The work reported herein had three objectives: (1) estimate the decay rates of viruses and FIB in seawater collected from different depths and incubated at different temperatures, (2) provide information about the concentration of FIB in different types of wastewater, (3) investigate the growth potential of FIB in the greywater system.

2. Materials and Methods

2.1. Sampling points for wastewater and seawater

Source separation of wastewater was established in 1997 at a dormitory serving 48 students at the Norwegian University of Life Sciences (NMBU) and collects greywater and blackwater separately. The greywater system collects wastewater from washbasins, showers, kitchen sinks,

and laundry whereas the blackwater system collects toilet waste separately. Wastewater from both systems was pumped into the laboratory for sampling as earlier described (Todt et al., 2015). A local combined wastewater system collects a mixture of greywater, blackwater and storm water in a municipal wastewater pipe (with approximately 500 houses connected). Sampling from this sewage pipe was performed through a manhole near the Faculty of Science and Technology laboratory. The concentration of FIB was monitored from different wastewater systems in order to evaluate the impact of source separation system. Samples of seawater were collected from the Norwegian Institute of Water Research (NIVA) research facility at Solbergstrand in Drøbak (59º36'56.7''N and 10º39'10.1''E), while untreated wastewater was concurrently collected from the inlet of municipal WWTP in Drøbak (12 000 persons connected) for decay rate experiment.

2.2. Collection of water samples

Greywater, blackwater and combined municipal wastewater were collected in 1-liter sterile bottles and analysed within 1 hour, while seawater was sampled in 30-liter sterile plastic containers stored on ice and analysed within 3 hours. All wastewater samples were stored on ice until analysis. The seawater samples were taken from tubes that pump water from depths of 1 m (surface seawater) and 60 m (deep seawater) in October 2015. Since the water collected from 1 m, contained lower amounts of plankton than observed during the bathing season, it was enriched 10 times using a 50 µm sieve, i.e. the plankton from 30 l was suspended in 3 l surface seawater.

2.3. Characterization of seawater samples

Seawater samples were analysed for physicochemical parameters using standard methods (pH: potentiometric measurement using probe according to NS-EN ISO 10523:2012; Conductivity: electrometric measurement using platina probe according to NS-ISO 7888:1993; Salinity: electrometric measurement using platina probe; Total organic carbon: catalytic combustion and infrared spectroscopy using the instrument Tekmar Dohrmann Apollo 9000HS according to modified NS 1484:1997; Turbidity: light scattering measurement at 860 nm). Zooplankton and algae were analysed by microscopy.

2.4. Production of adenovirus 40 and bacteriophage MS2 stock solutions

The cell line PLC/PRF/5 (ATCC®CRL-8024) was used for propagation of adenovirus 40 (adv40) (ATCC®VR-931). Cells were grown in minimal essential medium (MEM) with Earle`s salts and glutamine, 10% foetal calf serum (FCS) and 1% penicillin/streptomycin (P/S) and incubated at 37 °C with 5% CO_2. Monolayers of 3-4 days old cells were inoculated with adv40 for 60 min, the inoculum was removed and MEM containing 2% FCS and 1% P/S was added. After 4 days of incubation, the cells were freeze-thawed twice and the virus containing supernatant collected after centrifugation at 2500 x g for 8 min. In order to prepare a high titer purified virus stock, adv40 was precipitated using ammonium sulphate and finally desalted. In short, $(NH_4)_2SO_4$ was added to 240 ml supernatant (final concentration of 35%), incubated at room temperature (RT) for 2 h, re-centrifuged at RT for 15 min at 1600 x g and the pellet dissolved in 30 ml PBS. For desalting, the virus solution was filtered through a 10 K Amicon Ultra-15 Centrifugal Filter Unit (Millipore) by centrifugation at RT for 15 min at 4000 x g and PBS was added to the retentate that was re centrifuged as described. The adv40 titer in the stock solution was about 7 $\log_{10}$ FFU/ml (focus forming units/ml).

MS2 was propagated using Salmonella Typhimurium WG49 (NCTC 12484) as host (ISO 10705-1:1995). A virus stock was prepared following a similar method, with a titer of about 8 $\log_{10}$ PFU/ml (plaque forming units/ml), without precipitate with $(NH_4)_2SO_4$.

2.5. Quantification of infective adv40 and MS2

Culturable adv40 was quantified using PLC/PRF/5 cells and immunofluorescence foci assay (IFA). Samples (stock and water) were inoculated onto 3-4 days old cells and incubated for 2 days prior to immunostaining. The Intra Cellular Fixation & Permeabilization Buffer Set (eBioscience, CA, USA) was used to fix the cells, as described by the manufacturer. A 1:800 dilution of Mouse anti-adenovirus monoclonal Ab (MAB8051, Merck Millipore), was used as primary antibodies and Alexa 488 goat anti-mouse IgG 1:800 dilution (Life Technologies, CA, USA) as secondary antibodies. FFU were counted using an Olympus fluorescence microscope.

Infective MS2 was enumerated by a double agar layer PFU assay, using bacterial host *E.coli* Famp, as described by (Debartolomeis and Cabelli, 1991).

2.6. Molecular quantification of adv40 and MS2

DNA and RNA were extracted from 1 ml water samples using the MiniMag system (BioMerieux, France), eluted in 100 µl buffer and stored at -80 °C until use (ISO/TS 15216-1). The (RT)-qPCR reactions contained 2 µl of RNA/DNA in a total volume of 20 µl and were run in the Stratagene AriaMx Real-Time PCR System (Agilent Technologies, CA, USA). Positive and negative controls were included in each run and each sample was run in technical duplicates.

Adv40 was detected using primers and probe as described (Jothikumar et al., 2005) and Brilliant III Ultra-Fast qPCR Master Mix (Agilent Technologies), according to the manufacturer. In order to detect MS2, RT-qPCR was performed using the RNA UltraSense™ One-Step Quantitative RT-PCR System kit (Invitrogen, USA) and primers, probe and RT-qPCR conditions as described (Dreier et al., 2005).

Relative quantification was performed using individual standard curves, prepared from 10-fold serial dilutions of homologous viral DNA (adv40) or RNA (MS2), run in (RT)-PCR triplicates. The amount of viral DNA and RNA was expressed in PCR units (PCRU) per mL sample. One PCRU was defined as the amount of viral DNA or RNA in the highest dilution of the standard curve that gave a positive result for adv40 or MS2. The number of PCRU in each sample was estimated according to the standard curves using the AriaMx Real-Time PCR software.

2.7. Microbial analysis

TC and *E. coli* were quantified using a most probable number method (MPN) with Colilert-18 (IDEXX) and Quantitray 2000 (IDEXX) according to ISO 9308-2:2012. IE was quantified after membrane filtration using the ISO 7899-2 method. The results are given as colony forming units (cfu). Heterotrophic plate count (HPC) was determined by the spread plate method and marine agar using 5 days incubation at 20 °C.

To detect bacteroides, waters samples (1-10 ml) were filtered using a 0.2 µm pore size nitrocellulose filter (Millipore) and DNA extracted from the filter using the PowerWater DNA extraction kit (MoBio) per manufacturer's instruction. Real time PCR was performed with SsoFast EvaGreen super mix (BioRad) and a final reaction volume of 20 µl, containing, 0.5 µM of each primer and 2 µl template DNA using a BioRad CFX96. Primers used were HF 183 Fw (Bernhard and Field, 2000) and HF183 Re (Seurinck et al., 2005). A 10-fold dilution series of a linearized

plasmid (pCR2.1) containing the HF183 PCR product was used as a standard curve for quantification.

2.8. Analysis of coprostanol

The concentration of coprostanol in greywater, blackwater and municipal wastewater was analysed by GC-MS (Agilent Technologies). Ten ml of the sample was added to d12 Chrysene and d12 Perylene as an internal standard, filtered through pre-washed glass wool and passed through an activated and pre-washed 200 mg SPE column (HLB, Oasis, and Waters). The SPE column was dried and then washed with 6 ml of methylene chloride. The extract was concentrated to 0.1-2 ml by evaporation under a gentle flow of nitrogen before identification and quantification by GC-MS.

2.9. Growth potential of indicator bacteria in greywater

Greywater samples were incubated in the dark at 4 ^{o}C and 20 ^{o}C (three replicates of each) and TC, *E. coli*, and IE were enumerated after 0, 24, 48, and 98 h of incubation.

2.10. Decay rate for indicator bacteria and viruses in seawater

A spike-mixture was prepared by mixing 4.5 ml municipal wastewater, 4.5 ml adv40, and 225 µl MS2. From this mixture, 4.1 ml was immediately added to samples A) 2 L surface seawater and B) 2 L deep seawater. Spiked A and B were then divided into six aliquots of 250 ml and incubated in the dark at 4 ^{o}C and 20 ^{o}C. Sub-samples were collected after 0, 2 and 5 days and analysed for TC, *E. coli*, IE, adv40 (qPCR and FFU), and MS2 (RT-qPCR and PFU). Samples were kept at -20 °C and analysed for the virus at the end of the experiment. A positive control of the spike-mixture in virus growth medium (three replicates), was prepared to detect any immediate drop in virus titer caused by seawater.

2.11. Decay rate modelling

The decay rate for MS2, adv40 and indicator organisms was calculated from linear regression of log-transformed data, which is currently being used to model microorganism's decay coefficient in the aquatic environment. The model describes inactivation with time and the concentration time relationship is:

$$C = C_o\, e^{-kt} \tag{1}$$

where C is the concentration of organism at time t, C_o is initial concentration of organisms at time zero, t is the independent variable time (day) and k the decay rate coefficient.

2.12. Statistical analysis

The concentrations of adv40, MS2, IE, *E. coli*, and TC were transformed into the logarithmic scale for statistical analysis. Paired t-tests were performed at 0.05 level of significance to determine the potential difference of the mean between the initial microbial concentrations with the concentration after the 5th day. The Minitab statistical package program Version 17 was used for all statistical analyses.

3. Results

3.1. The concentration of FIB in different wastewater systems

TC, *E. coli* and IE were high in blackwater relative to greywater and municipal wastewater samples, however, the concentration of TC and *E. coli* in greywater was slightly lower than blackwater, and IE was substantially lower than blackwater and municipal wastewater (Figure 1).

The three-wastewater types were analysed for the faecal sterol coprostanol and gene markers of human related *Bacteroides*, in order to investigate whether the high numbers of *E. coli* and TC in the greywater were a result of multiplication in the collection system rather than actual faecal pollution. The level of coprostanol and gene markers of human related *Bacteroides* in greywater was less than or equal to 0.02% of the level in blackwater (Table 1). Thus, the higher greywater to blackwater ratio of *E. coli* than of *Bacteroides* may indicate growth of *E. coli* in the greywater collection system.

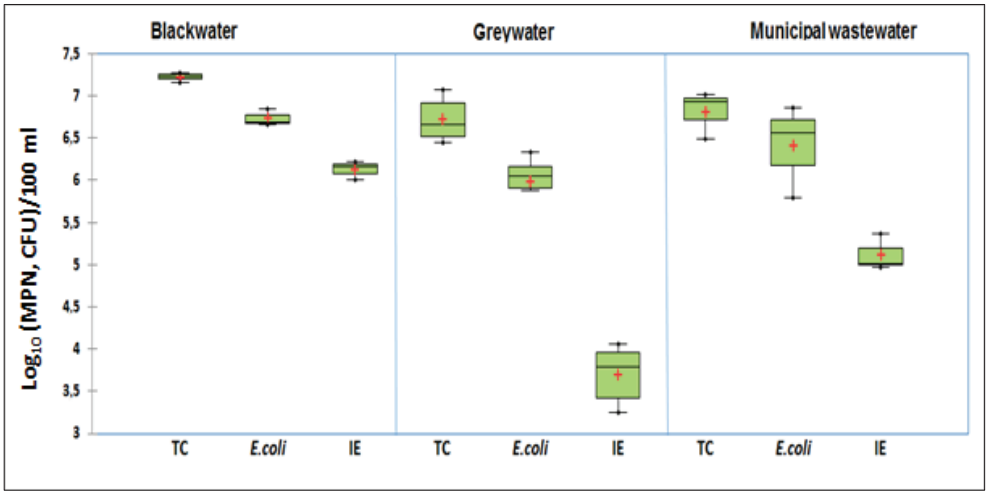

Figure 1. The concentration of TC (MPN/100 ml), *E. coli* (MPN/100 ml), and IE (CFU/100 ml) in blackwater, greywater, and municipal wastewater (combined municipal wastewater). The box shows the range from first to third quartiles, the median line divides the box, the red cross marks the mean, and the line extending vertically from the box indicates maximum and minimum values.

Table 1. Concentrations of the faecal indicators (*E. coli*, human related *Bacteroides* and coprostanol) in greywater, combined municipal wastewater and blackwater, reported as mean (± standard deviation).

Parameters	Greywater	Combined municipal wastewater	Blackwater	Greywater as % of blackwater
E. coli (Log_{10} cfu/ml)	5.9 (±0.3)	6.4 (±0.5)	6.7 (±0.9)	16
Coprostanol (mg/l)	0.013 (±0.007)	15 (±8)	59 (±7)	0.02
Bacteroides (Log_{10} GC/ml)[1]	4.1 (±0.2)	8.9 (±0.4	9.1 (±0.1	0.001
Ratio coprostanol to *E. coli* (mg/cfu)	1.64×10^{-11}	5.97×10^{-9}	1.18×10^{-8}	-

[1] GC is gene copy

3.2. Growth and decay of microorganisms in greywater

The experimental study for the growth potential of FIB on greywater shown in Figure 2. TC and *E. coli* showed a tendency of further growth in the greywater for the first 24 h at 20 ºC, thereafter the concentration abruptly reduced, whereas no growth was observed in the case of IE at 20 ºC. On the other hand, none of the FIB showed any further growth at 4 ºC.

3.3. Seawater characteristics and decay rates of viruses and FIB

The seawater samples collected from the surface had lower salinity, but higher turbidity and total organic carbon (TOC) than seawater collected from the deep (Table 2). The surface seawater also contained zooplankton like rotifers (178 per L) and ciliates (267 per L) while in the deep seawater no zooplankton was detected. Moreover, the algae concentration and heterotrophic plate count were also much higher in the surface than in the deep seawater. This indicates that surface seawater had high biological activity compared to the deep seawater. After the virus and municipal wastewater spike-mixture were added to the seawater, TOC increased by 0.4 mg/l, but the other physicochemical water quality parameters were not changed.

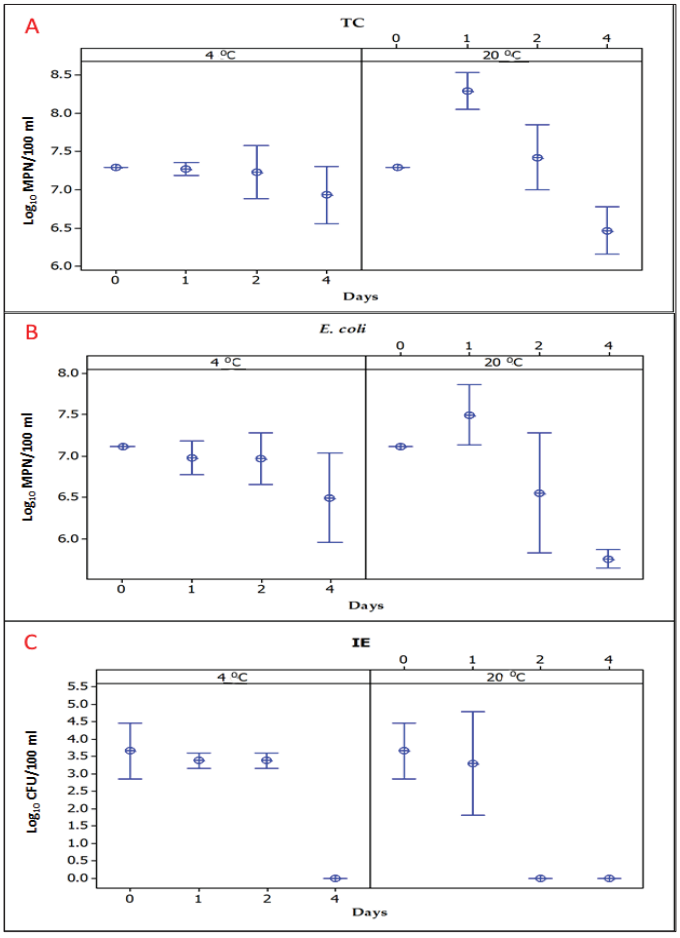

Figure 2. Growth and decay rate of TC, E. coli and IE in greywater, with 4 days of incubation in the dark. The dot marks the mean, and the line extending vertically from the dot indicates 95% confidence interval of the mean.

Table 2. Biological activities and physicochemical characteristics of surface seawater sample (1 m) and deep seawater sample (60 m).

Parameters	Unit	Deep seawater (60 m)	Surface seawater (1 m)	Deep to surface seawater ratio (%)
Zooplankton	Copepods/l	1	4	25
	Nauplii/l	0	13	0
	Rotifers/l	0	178	0
	Oikopleura/l	0	13	0
	Ciliates/l	0	267	0
Algae	Cell/ml	< 10	129	< 8
	species	0	Dinoflagellates, flagellates	0
Heterotrophic plate count (HPC)	cfu/ml	380	7600	5
Salinity	PSU	33	23	143
pH		7.8	7.8	100
Conductivity	mS/m	7000	4800	145
Total organic carbon	mg C/l	1.1	3.8	29
Turbidity	FNU	0.4	0.6	67

Results from the decay experiment shown in Tables S1 and S2. The reduction in the number of virus genomes and FIB was in general highest in surface seawater at 20 ºC (Figure 3 and 4). During the five days experiment, adenovirus was reduced by > 2 Log_{10} PCR U/ml at 20 °C, but nearly no reduction was observed at 4 °C (Figure 3A). Infective adenovirus showed the same tendency of reduction as adenovirus concentration detected by PCR except for the magnitude change (Figure 3). The concentration of MS2 was not reduced as similar to adenovirus at 20 ºC (Figure 3C). The number of infective MS2 at 4 ºC in both seawaters (surface and deep) showed an increasing trend, however, at 20 ºC a decreasing trend was seen (Figure 3D). In the case of FIB in surface seawater, during the incubation period of 5 days, TC, *E. coli*, and IE decreased by 1.5, 2.5, and 2.3 log_{10} at 20 ºC, respectively and a small reduction in deep seawater. A low reduction of FIB was observed at 4 °C (Figure 4). The experiment also showed that at 20 ºC, the concentration of FIB was slightly reduced the first 2 days, followed by 1-2 log_{10} reduction the next 3 days (Table S1).

Calculated exponential decay rate coefficient (day^{-1}) for FIB and viruses in different seawaters and at different temperatures are shown in Table 3. The decay rate coefficients were higher at 20 °C than at 4 °C. In addition, an effect of water quality was observed at 20 °C, with generally higher decay rate coefficients in surface seawater than in deep seawater, although due to high variations between the replicates, the differences were not always statistically significant. A t-test was included to investigate if the mean decay rates observed in the different seawaters (effect of water quality) and temperatures (effect of temperature) were different at 0.05 significance level (Table S3).

The control experiment was conducted to investigate if the seawater caused an immediate drop in virus concentration. The result showed that adenovirus (PCR U/ml) and MS2 (PCR U/ml) were

not significantly reduced when the spike-mixture was added to surface seawater (Figure 5), and a drop of adenovirus (PCR U/ml) about 0.5 $\log_{10}$ was observed in deep seawater. The concentration of infective adenovirus (FFU/ml) was about 2.5 $\log_{10}$ lower than the concentration of adenovirus (PCR U/ml) in the virus growth medium and this difference increased in seawater, in particular in the deep seawater (Figure 5A). Similar results were seen for MS2 (Figure 5B).

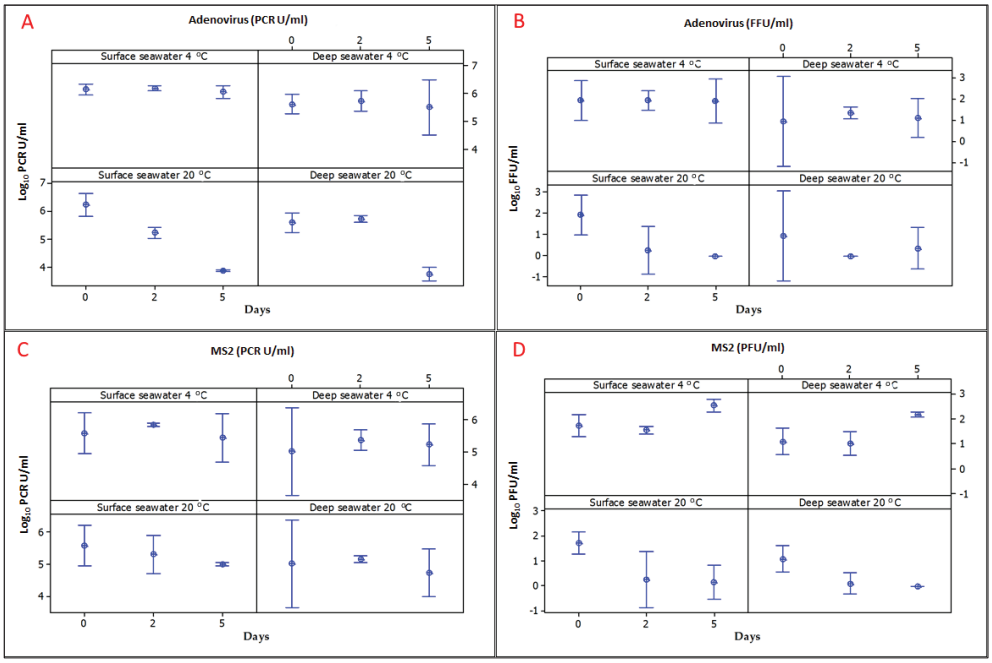

Figure 3. The decay of viruses analyzed by different methods A) Adenovirus PCR U/ml, B) Adenovirus FFU/ml, C) MS2 PCRU/ml, and D) MS2 PFU/ml in spiked deep seawater (60 m) and surface seawater (1 m) incubated at 4 °C and 20 °C, the dot marks the mean, and the line extending vertically from the dot indicates 95% confidence interval of the mean

4. Discussion

Data on concentration and decay of enteric pathogens and their indicators in different wastewater systems and water bodies is crucial for QMRA. FIB are often monitored to assess the faecal load in different wastewater systems and water bodies. The concentrations of FIB in the greywater, blackwater and municipal wastewater in the present study, were similar to levels reported in other studies (Grøndahl-Rosado et al., 2014; Ottoson and Stenström, 2003). Based on the observed levels of coprostanol and gene markers of human related *Bacteroides* in source separated greywater and blackwater, we confirmed earlier findings that TC and *E. coli*, due to multiplication, are inadequate indicators for the faecal load in greywater (Ottoson and Stenström, 2003). In this study, IE did not show a tendency of growth in greywater. In other studies, IE showed a tendency to multiply within greywater systems, but in general less than coliform bacteria and *E. coli*, they are therefore

considered IE to be a better indicator of a faecal load in greywater than coliform bacteria and *E. coli* (Ottoson and Stenström, 2003).

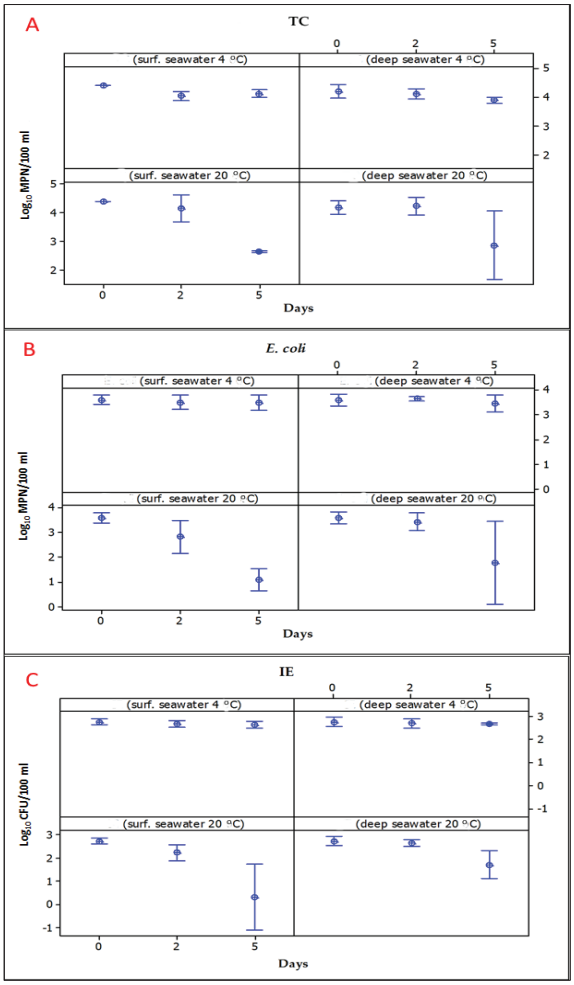

Figure 4. The decay of indicator bacteria A) TC (Log_{10} MPN/100 ml), B) *E. coli* (Log_{10} MPN/100 ml), and C) IE (Log_{10} CFU/100 ml), in spiked deep seawater (60 m) and surface seawater (1 m) incubated at 4 °C and 20 °C, the dot marks the mean, and the line extending vertically from the dot indicates 95% confidence interval of the mean.

There is a wide range of pathogens that may be present in wastewater and different water bodies. However, not all these pathogens can be accounted for in QMRA, hence the need for reference pathogens to be used. On the other hand, it is difficult to find data on the concentration of reference pathogens, as an alternative, QMRA is often conducted using indicator organisms by transforming the concentration of indicators into reference pathogen. The weak correlation between indicator

organisms and enteric pathogens have usually weakened the trustworthiness of the computed health risk (Harwood et al., 2005). However, in the absence of data on reference pathogens, faecal indicators organisms is still useful to estimate health risk. Therefore, the information on FIB concentration in different wastewater system could be utilized to estimate reference pathogen concentrations.

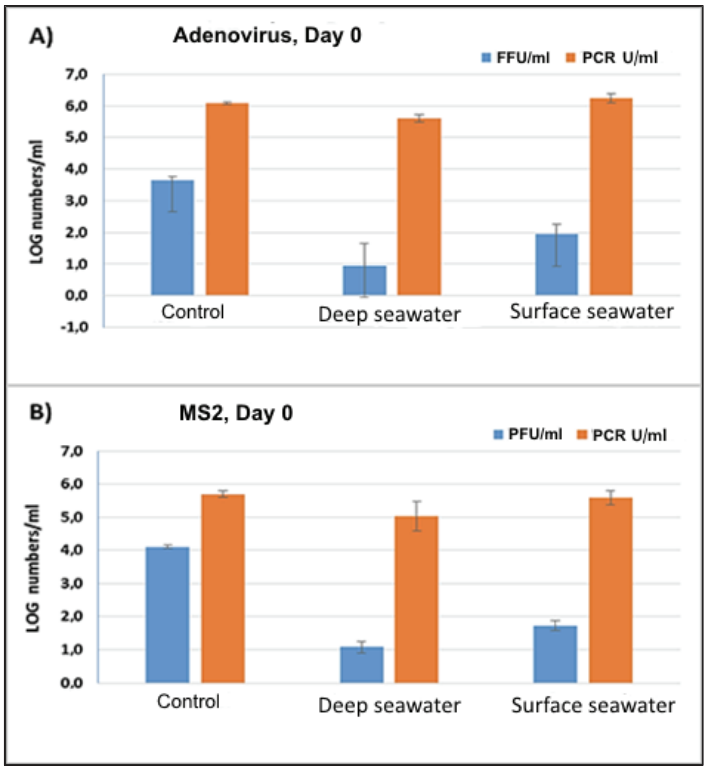

Figure 5. Concentrations of A) Adenovirus and B) MS2 after addition of spike-mixture to deep seawater (60 m) and surface seawater (1 m) compared with medium control. Analyzed by using cultivation and PCR method. Error bars represent standard deviation from triplicate flasks.

The decay rate experiment was performed to address the scarcity of information regarding decay rates of FIB and viruses at different depths of the marine environment. There was a large variation between the number of infectious virus and total virus particles, which was also observed in other studies and recognized as a huge challenge for risk assessment (Rames et al., 2016). Virus analysis based on (RT)-qPCR may significantly overestimate the concentration of infectious virus (Lodder et al., 2015), whereas virus analysis based on cell culture may potentially underestimate the actual number of infectious virus due to aggregation. The immediate drop in infective viruses after addition to seawater, which was significant in the deep seawater with higher salinity than the surface seawater, and it can be explained by virus inactivation, aggregation or attachment to other particles (Da Silva et al., 2010). The unexpected increase of infectious MS2 from day 2 to day 5

at 4°C (Figure 3D) may be explained by detachment from particles, which potentially may occur due to changes in the water chemistry (Mattle et al., 2011).

Table 3. Exponential decay rate coefficient (day^{-1}) for indicator bacteria and viruses in seawater (from 1 m and 60 m depths) incubated at 4 °C and 20 °C.

Microorganism	Unit	Seawater sample depth (meter)	Temperature (°C)	Method of analysis	Decay rate coefficient (k)
Adenovirus	PCR U/ml	1	4	qPCR	0.10
			20		1.10
		60	4		0.00
			20		0.85
Adenovirus	FFU/ml	1	4	Cultivation	0.00
			20		0.94
		60	4		0.04
			20		0.60
MS2	PCR U/ml	1	4	RT-qPCR	0.06
			20		0.30
		60	4		0.01
			20		0.14
MS2	PFU/ml	1	4	Cultivation	0.38
			20		0.79
		60	4		-0.53
			20		0.80
Intestinal enterococci	CFU/100 ml	1	4	Membrane filtration with incubation on selective media	0.05
			20		1.05
		60	4		0.03
			20		0.46
E. coli	MPN/100 ml	1	4	IDEXX Colilert 18 Quanti-Tray/2000	0.05
			20		1.13
		60	4		0.06
			20		0.73
Total coliforms	MPN/100 ml	1	4	IDEXX Colilert 18 Quanti-Tray/2000	0.12
			20		0.79
		60	4		0.15
			20		0.54

$C_t = C_o * e^{-(kt)}$ Where C_t is the concentration at any time t, C_o is the initial concentration on day 0, k is first-order decay rate coefficients, and t is the transition time

In spite of the uncertainties related to enumeration of infective virus particles, the experiment showed a significant reduction of total adenovirus particles in seawater at 20 °C, and the decay rate was higher in the seawater collected from the surface water than from the deep water. This

may be explained by higher biological activity, microbial diversity, and abundance of organisms in the surface seawater that may promote grazing and competition. Under natural conditions, inactivation caused by exposure to sunlight during the summer season may further increase the decay rate in the surface seawater (Love et al., 2010; Mattioli et al., 2017; Sinton et al., 2002; Yukselen et al., 2003).

The decay rate of FIB is relatively high in the case of surface seawater at 20 ºC as compared to infective adenovirus and MS2. Which is very important for QMRA. This is, therefore; the utilization of FIB decay rate for virus could lead unreliable estimate of health risk. i.e. underestimate the health risk. Moreover, for both FIB and virus, the observed decay rate coefficients were low at 4 °C. The results from the experiment suggest that the discharge from combined sewer overflows and effluents from WWTP into the cold deep layers of the Oslofjord or the surface layers during the cold winter season is expected to provide lower decay rates of FIB and viruses than in the surface layers during a warm summer. Discharge based hydrodynamic models that make use of different decay rates at different temperatures, water qualities and metrological conditions may give a better estimate of pathogen concentration for specific sites and can be utilized as an input for QMRA. Published results from such modelling are based on many assumptions (Eregno et al., 2016; Sokolova et al., 2013) and the present study provides additional information concerning decay rates of FIB and viruses in seawater.

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Author Contributions
The study was initiated and lead by Ingun Tryland. Mette Myrmel and Mamata Khatri were responsible for the analysis of viruses and drafted the method of virus analysis. Fasil Ejigu Eregno, Anastasiia Oliinyk, Ingun Tryland, and Aina Charlotte Wennberg performed the analysis of FIB in all experiments. Fasil Ejigu Eregno performed the statistical analysis, decay rate modelling, and drafted the manuscript, which was critically revised by Mette Myrmel, Ingun Tryland, and Arve Heistad. All authors read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest

References

Bae, S., Wuertz, S., 2009. Rapid decay of host-specific faecal Bacteroidales cells in seawater as measured by quantitative PCR with propidium monoazide. Water research. 43**,** 4850-4859.

Bernhard, A. E., Field, K. G., 2000. A PCR assay to discriminate human and ruminant feces on the basis of host differences in Bacteroides-Prevotella genes encoding 16S rRNA. Applied and Environmental Microbiology. 66**,** 4571-4574.

Brombach, H., et al., 2005. A new database on urban runoff pollution: comparison of separate and combined sewer systems. Water science and technology. 51**,** 119-128.

Camper, A. K., et al., 1985. Growth and persistence of pathogens on granular activated carbon filters. Applied and environmental microbiology. 50**,** 1378-1382.

Carlucci, A., Pramer, D., 1959. Factors affecting the survival of bacteria in sea water. Applied microbiology. 7**,** 388-392.

Cornax, R., et al., 1990. Survival of pathogenic microorganisms in seawater. Current microbiology. 20**,** 293-298.

Da Silva, A. K., et al., 2010. Adsorption and aggregation properties of norovirus GI and GII virus-like particles demonstrate differing responses to solution chemistry. Environmental science & technology. 45**,** 520-526.

Debartolomeis, J., Cabelli, V. J., 1991. Evaluation of an Escherichia coli host strain for enumeration of F male-specific bacteriophages. Applied and Environmental Microbiology. 57**,** 1301-1305.

Dreier, L., et al., 2005. LIN-23-Mediated Degradation of β-Catenin Regulates the Abundance of GLR-1 Glutamate Receptors in the Ventral Nerve Cord of C. elegans. Neuron. 46**,** 51-64.

Eregno, F. E., et al., 2016. Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. Science of the Total Environment. 548-549**,** 270-279.

Fewtrell, L., Bartram, J., 2001. Water quality: WHO guidelines, standards and health: assessment of risk and risk management for water-related infectious diseases.

Fong, T.-T., Lipp, E. K., 2005. Enteric viruses of humans and animals in aquatic environments: health risks, detection, and potential water quality assessment tools. Microbiology and molecular biology reviews. 69**,** 357-371.

Gasperi, J., et al., 2008. Priority pollutants in wastewater and combined sewer overflow. Science of the total environment. 407**,** 263-272.

Griffith, D. R., et al., 2009. Inputs of fossil carbon from wastewater treatment plants to US rivers and oceans. Environmental science & technology. 43**,** 5647-5651.

Haas, C. N., et al., 1999. Quantitative microbial risk assessment. John Wiley & Sons.

Hamilton, A. J., et al., 2006. Quantitative microbial risk assessment models for consumption of raw vegetables irrigated with reclaimed water. Applied and environmental microbiology. 72**,** 3284-3290.

Harwood, V. J., et al., 2005. Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. Applied and Environmental Microbiology. 71**,** 3163-3170.

Jeanneau, L., et al., 2012. Relative decay of fecal indicator bacteria and human-associated markers: a microcosm study simulating wastewater input into seawater and freshwater. Environmental science & technology. 46**,** 2375-2382.

Jothikumar, N., et al., 2005. Quantitative real-time PCR assays for detection of human adenoviruses and identification of serotypes 40 and 41. Applied and environmental microbiology. 71, 3131-3136.
Juhna, T., et al., 2007. Detection of Escherichia coli in biofilms from pipe samples and coupons in drinking water distribution networks. Applied and environmental microbiology. 73, 7456-7464.
Larsen, T. A., et al., 2009. Source separation: Will we see a paradigm shift in wastewater handling? 1. Environmental science & technology. 43, 6121-6125.
Lee, C. W., et al., 2011. Investigating the decay rates of Escherichia coli relative to Vibrio parahemolyticus and Salmonella Typhi in tropical coastal waters. water research. 45, 1561-1570.
Lodder, W. J., et al., 2015. Entero- and parechovirus distributions in surface water and probabilities of exposure to these viruses during water recreation. Water Research. 75, 25-32.
Loos, R., et al., 2013. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. Water research. 47, 6475-6487.
Love, D. C., et al., 2010. Human Virus and Bacteriophage Inactivation in Clear Water by Simulated Sunlight Compared to Bacteriophage Inactivation at a Southern California Beach. Environmental Science & Technology. 44, 6965-6970.
Mara, D., et al., 2007. Health risks in wastewater irrigation: comparing estimates from quantitative microbial risk analyses and epidemiological studies. Journal of water and health. 5, 39-50.
Mattioli, M. C., et al., 2017. Decay of sewage-sourced microbial source tracking markers and fecal indicator bacteria in marine waters. Water Research. 108, 106-114.
Mattle, M. J., et al., 2011. Impact of virus aggregation on inactivation by peracetic acid and implications for other disinfectants. Environmental science & technology. 45, 7710-7717.
Medema, G., Microbial Risk Assessment of Pathogens pathogen in Water pathogen in water. Encyclopedia of Sustainability Science and Technology. Springer, 2012, pp. 6605-6633.
Noble, R. T., et al., 2004. Inactivation of indicator micro-organisms from various sources of faecal contamination in seawater and freshwater. Journal of applied microbiology. 96, 464-472.
O'Toole, J., et al., 2014. Advice to Risk Assessors Modeling Viral Health Risk Associated with Household Graywater. Risk Analysis. 34, 797-802.
Ottoson, J., Stenström, T. A., 2003. Faecal contamination of greywater and associated microbial risks. Water Research. 37, 645-655.
Rames, E., et al., 2016. Technical aspects of using human adenovirus as a viral water quality indicator. Water research. 96, 308-326.
Rogers, S. W., et al., 2011. Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure-amended soils. Applied and environmental microbiology. 77, 4839-4848.
Rozen, Y., Belkin, S., 2001. Survival of enteric bacteria in seawater. FEMS microbiology reviews. 25, 513-529.
Savichtcheva, O., Okabe, S., 2006. Alternative indicators of fecal pollution: relations with pathogens and conventional indicators, current methodologies for direct pathogen monitoring and future application perspectives. Water research. 40, 2463-2476.
Scandura, J., Sobsey, M. D., 1997. Viral and bacterial contamination of groundwater from on-site sewage treatment systems. Water Science and Technology. 35, 141-146.
Schijven, J. F., et al., 2011. QMRAspot: a tool for quantitative microbial risk assessment from surface water to potable water. Water research. 45, 5564-5576.

Seurinck, S., et al., 2005. Detection and quantification of the human-specific HF183 Bacteroides 16S rRNA genetic marker with real-time PCR for assessment of human faecal pollution in freshwater. Environmental Microbiology. 7**,** 249-259.
Sinton, L. W., et al., 2002. Sunlight inactivation of fecal indicator bacteria and bacteriophages from waste stabilization pond effluent in fresh and saline waters. Applied and Environmental Microbiology. 68**,** 1122-1131.
Smeets, P., et al., 2010. Practical applications of quantitative microbial risk assessment (QMRA) for water safety plans. Water Science and Technology. 61**,** 1561-1568.
Sokolova, E., et al., 2013. Hydrodynamic modelling of the microbial water quality in a drinking water source as input for risk reduction management. Journal of Hydrology. 497**,** 15-23.
Suttle, C. A., Chen, F., 1992. Mechanisms and rates of decay of marine viruses in seawater. Applied and Environmental Microbiology. 58**,** 3721-3729.
Todt, D., et al., 2015. Load and distribution of organic matter and nutrients in a separated household wastewater stream. Environmental technology. 36**,** 1584-1593.
Vital, M., et al., 2010. Evaluating the growth potential of pathogenic bacteria in water. Applied and environmental microbiology. 76**,** 6477-6484.
Wei, C.-L., et al., 2010. Global patterns and predictions of seafloor biomass using random forests. PLoS One. 5**,** e15323.
WHO, 2016. Quantitative microbial risk assessment: application for water safety management. Quantitative microbial risk assessment: application for water safety management.
Wilhelm, S. W., et al., 1998. The role of sunlight in the removal and repair of viruses in the sea. Limnology and Oceanography. 43**,** 586-592.
Williams, M. S., Ebel, E. D., 2014. Estimating the correlation between concentrations of two species of bacteria with censored microbial testing data. International journal of food microbiology. 175**,** 1-5.
Wood, I. R., et al., 1993. Ocean disposal of wastewater. World Scientific.
Wu, J. Y., et al., 2016. Decay of Coliphages in Sewage-Contaminated Freshwater: Uncertainty and Seasonal Effects. Environmental Science & Technology. 50**,** 11593-11601.
Yukselen, M. A., et al., 2003. Inactivation of coliform bacteria in Black Sea waters due to solar radiation. Environment International. 29**,** 45-50.

Supplementary Materials

Table S1. Statistical summary of the results from the microbial decay experiment (3 replicates)

Microorganisms	Unit	Seawater	Temperature (°C)	Statistical summary (Log_{10})															
				Day 0					Day 2					Day 5					
				Mean	Med	Sd	Min	Max	Mean	Med	Sd	Min	Max	Mean	Med	Sd	Min	Max
TC	MPN/100 ml	Surface	4	4.38	4.38	0.01	4.38	4.38	4.03	4.05	0.06	3.96	4.08	4.12	4.15	0.06	4.05	4.15
			20						4.16	4.05	0.19	4.05	4.38	2.67	2.67	0.01	2.65	2.68
		Deep	4	4.20	4.19	0.09	4.11	4.30	4.11	4.08	0.07	4.05	4.19	3.89	3.91	0.04	3.84	3.91
			20						4.24	4.19	0.12	4.15	4.38	2.88	2.92	0.48	2.38	3.33
E. coli	MPN/100 ml	Surface	4	3.58	3.56	0.08	3.51	3.66	3.48	3.46	0.12	3.38	3.61	3.47	3.54	0.12	3.32	3.54
			20						2.82	2.70	0.26	2.63	3.12	1.10	1.00	0.17	1.00	1.30
		Deep	4	3.58	3.56	0.10	3.49	3.69	3.63	3.64	0.04	3.59	3.66	3.44	3.49	0.13	3.29	3.54
			20						3.42	3.36	0.14	3.32	3.59	1.77	2.09	0.68	1.00	2.24
IE	CFU/100 ml	Surface	4	2.76	2.74	0.05	2.72	2.81	2.68	2.69	0.06	2.61	2.72	2.65	2.66	0.05	2.59	2.70
			20						2.26	2.23	0.14	2.15	2.41	0.33	0.00	0.58	0.00	1.00
		Deep	4	2.76	2.77	0.08	2.67	2.84	2.71	2.71	0.08	2.63	2.79	2.69	2.69	0.01	2.68	2.71
			20						2.68	2.71	0.06	2.61	2.73	1.74	1.78	0.24	1.48	1.95
MS2	PCR U/ml	Surface	4	5.60	5.67	0.26	5.31	5.81	5.87	5.86	0.02	5.86	5.90	5.45	5.61	0.30	5.10	5.64
			20						5.32	5.20	0.24	5.17	5.60	5.02	5.02	0.02	4.99	5.04
		Deep	4	5.03	5.12	0.55	4.44	5.54	5.38	5.38	0.13	5.25	5.50	5.24	5.21	0.26	4.99	5.52
			20						5.17	5.16	0.05	5.13	5.22	4.75	4.90	0.30	4.40	4.93
	PFU/ml	Surface	4	1.73	1.83	0.18	1.52	1.83	1.54	1.56	0.06	1.48	1.59	2.53	2.57	0.10	2.41	2.60
			20						0.26	0.00	0.45	0.00	0.78	0.16	0.00	0.28	0.00	0.48
		Deep	4	1.08	1.15	0.21	0.85	1.26	1.00	1.08	0.20	0.78	1.15	2.16	2.16	0.04	2.12	2.20
			20						0.10	0.00	0.17	0.00	0.30	0.00	0.00	0.00	0.00	0.00
Adenovirus	PCR U/ml	Surface	4	6.24	6.33	0.17	6.05	6.34	6.17	6.15	0.03	6.15	6.21	6.04	6.05	0.09	5.94	6.13
			20						5.25	5.27	0.08	5.16	5.31	3.88	3.88	0.01	3.88	3.89
		Deep	4	5.61	5.54	0.14	5.52	5.77	5.72	5.65	0.14	5.61	5.88	5.50	5.42	0.40	5.15	5.93
			20						5.74	5.76	0.05	5.69	5.77	3.77	3.80	0.10	3.66	3.85
	FFU/ml	Surface	4	1.94	2.04	0.38	1.52	2.26	1.94	1.94	0.19	1.74	2.12	1.91	2.02	0.42	1.45	2.27
			20						0.26	0.00	0.45	0.00	0.78	0.00	0.00	0.00	0.00	0.00
		Deep	4	0.96	1.23	0.85	0.00	1.64	1.34	1.34	0.11	1.23	1.45	1.11	1.04	0.38	0.78	1.52
			20						0.00	0.00	0.00	0.00	0.00	0.36	0.30	0.39	0.00	0.78

Med: median; Sd: standard deviation; Min: minimum; Max: maximum

Table S2. Paired t-test to evaluate if the mean concentrations observed at day 0 and day 5 were statistically different at significance level 0.05

Microorganism and method of analysis	Experimental set up	T-value	p-value
Adenovirus (Log_{10} PCR U/ml)	DS4	0.42	0.717
	DS20	22.42	0.002
	SS4	1.39	0.299
	SS20	23.61	0.002
Adenovirus (Log_{10} FFU/ml)	DS4	-0.28	0.806
	DS20	0.83	0.493
	SS4	1.10	0.387
	SS20	8.82	0.013
MS2 (Log_{10} PCR U/ml)	DS4	-0.47	0.682
	DS20	1.58	0.255
	SS4	0.56	0.630
	SS20	3.77	0.064
MS2 (Log_{10} PFU/ml	DS4	-8.28	0.014
	DS20	8.82	0.013
	SS4	-17.29	0.003
	SS20	5.95	0.027
Intestinal enterococci (Log_{10} CFU/100ml)	DS4	1.25	0.339
	DS20	7.59	0.017
	SS4	1.81	0.212
	SS20	6.84	0.021
E. coli (Log_{10} MPN/100ml)	DS4	1.06	0.401
	DS20	4.08	0.055
	SS4	1.48	0.277
	SS20	41.27	0.001
Total coliforms (Log_{10} MPN/100ml)	DS4	4.09	0.055
	DS20	4.03	0.056
	SS4	7.92	0.016
	SS20	198.54	0.000

DS4: deep seawater at 4 ºC, DS20: deep seawater at 20 ºC, SS4: surface seawater at 4 ºC, and SS20: surface seawater at 20 ºC

Table S3. Paired t-test to evaluate if the mean decay rates observed in different seawaters (seawater sample effect) or temperatures (temperature effect) were statistically significant

Microorganism and method of analysis	Comparisons	Parameter evaluated	T-value	p-value
Adenovirus (Log10 PCR U/ml)	SS4 and DS4	Seawater sample effect	8.23	0.000
	SS20 and DS20	Seawater sample effect	0.50	0.633
	SS4 and SS20	Temperature effect	3.01	0.017
	DS4 and DS20	Temperature effect	1.88	0.098
Adenovirus (Log10 FFU/ml)	SS4 and DS4	Seawater sample effect	6.08	0.000
	SS20 and DS20	Seawater sample effect	1.24	0.250
	SS4 and SS20	Temperature effect	3.82	0.005
	DS4 and DS20	Temperature effect	3.32	0.011
MS2 (Log10 PCR U/ml)	SS4 and DS4	Seawater sample effect	5.29	0.001
	SS20 and DS20	Seawater sample effect	3.33	0.010
	SS4 and SS20	Temperature effect	3.11	0.015
	DS4 and DS20	Temperature effect	1.98	0.083
MS2 (Log10 PFU/ml	SS4 and DS4	Seawater sample effect	6.59	0.000
	SS20 and DS20	Seawater sample effect	2.27	0.053
	SS4 and SS20	Temperature effect	3.43	0.009
	DS4 and DS20	Temperature effect	3.23	0.012
Intestinal enterococci (Log10 CFU/100ml)	SS4 and DS4	Seawater sample effect	-0.86	0.413
	SS20 and DS20	Seawater sample effect	-2.76	0.025
	SS4 and SS20	Temperature effect	2.46	0.039
	DS4 and DS20	Temperature effect	2.02	0.078
E. coli (Log10 MPN/100ml)	SS4 and DS4	Seawater sample effect	-0.76	0.471
	SS20 and DS20	Seawater sample effect	-2.29	0.051
	SS4 and SS20	Temperature effect	2.83	0.022
	DS4 and DS20	Temperature effect	2.24	0.056
Total coliforms (Log10 MPN/100ml)	SS4 and DS4	Seawater sample effect	1.98	0.083
	SS20 and DS20	Seawater sample effect	-0.33	0.752
	SS4 and SS20	Temperature effect	1.71	0.125
	DS4 and DS20	Temperature effect	1.48	0.177

DS4: deep seawater at 4 ^{o}C, DS20: deep seawater at 20 ^{o}C, SS4: surface seawater at 4 ^{o}C, and SS20: surface seawater at 20 ^{o}C

Paper V

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Treated Greywater Reuse for Hydroponic Lettuce Production in a Green Wall System: Quantitative Health Risk Assessment

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Abstract: The scarcity and pollution of freshwater are extremely crucial issues today, and the expansion of water reuse has been considered as an option to reduce its impact. This study aims to assess the efficiency of an integrated greywater treatment system and hydroponic lettuce production as a part of a green wall structure, and to evaluate the health risk associated with the production and consumption of lettuce through a quantitative microbial risk assessment (QMRA) and a chemical health risk assessment. The study was conducted based on the unique configuration of a source separation system; an on-site greywater treatment system; a green wall structure as a polishing step; and hydroponic lettuce production in the green wall structure. The final effluent from the system was used to grow three lettuce varieties by adding urine as a nutrient solution. Both water samples and plant biomass were collected and tested for *Escherichia coli* (*E. coli*) and heavy metals contamination. The system has gained a cumulative 5.1 $\log_{10}$ reduction of *E. coli* in the final effluent and no *E. coli* found in the plant biomass. The estimated annual infection risk for *Cryptosporidium*, *Campylobacter*, and Norovirus was 10^{-6}–10^{-8}, 10^{-8}–10^{-10}, and 10^{-10}–10^{-11} respectively. These results indicate that the system attained the health-based targets, 10^{-6} disability adjusted life years (DALYs) per person per year. Similarly, the health risk index (*HRI*) and targeted hazard quotient (*THQ*) results did not exceed the permissible level, thus the chemical health risk concern was insignificant.

Keywords: source separation system; greywater treatment; water reuse; hydroponic system; green wall; heavy metals bioaccumulation; QMRA; health risk assessment

1. Introduction

Water scarcity and water pollution are causing serious health and environmental challenges around the world for a large proportion of the world's population, either because the proper infrastructure is absent, or wastewater is discharged untreated to its recipients. The water, energy, and nutrients in wastewater also represent valuable resources, which are needed to supply a growing population. Future wastewater infrastructure should therefore serve as combined resource recovery factories and wastewater treatment facilities, established as decentralized, semi-centralized, centralized, or combined systems, depending on local needs and constraints. Some regions are facing water stress and groundwater depletion because of population growth, frequent drought occurrence (low rainfall), or in combination with the over exploitation of local groundwater and wastewater being transported far away from the point of water extraction/use. In those areas, local groundwater recharge and reduced water consumption is crucial, and this calls for decentralized or semi-centralized treatment and recovery systems. Systems that are based on source separation have been suggested as an efficient strategy for nutrient recovery and water reuse [1,2].

Source separation of domestic wastewater is a system that provides an opportunity to collect the toilet waste separately, which contains the majority of the nutrients and carbon, but also waterborne pathogens that may constitute a major risk factor unless handled properly. Simultaneously, the system collects greywater, which has a much lower concentration of pathogens than combined domestic wastewater, and constitutes most of the wastewater quantity in households' wastewater. The source of greywater is kitchen and bathroom sinks, showers, and laundry; whereas blackwater consists of urine, faecal material, toilet paper, and flushing water from the toilet. In addition to the two broad classes, urine or urine with minimal flush water can be collected separately as yellow water [3–6]. Approximately 90% of the total nitrogen and 80–90% of the total phosphorus in domestic wastewater originates from the urine fraction, which constitutes only 2% of the wastewater volume. Greywater separation from blackwater offers chances to treat most of the wastewater easily using on-site treatment systems to a quality that can be discharged to local water recipients or reused for a non-potable purpose without negative effects on health and the environment if it is treated properly.

The major concern associated with water reuse is the quality of the wastewater in terms of microbial pathogens, heavy metals, organic pollutants, components in pharmaceutical residues, and personal care products, which threaten the public's health when reused directly with insufficient treatment. This potential threat can be reduced through proper wastewater treatment technologies as well as through efficient utilization systems. One of the most promising strategies to raise the coverage of domestic wastewater reuse as well as to reduce the associated public health risk is the integration of a source separation system with appropriate wastewater treatment technologies and growth systems that are effective, simple to operate, able to consume less energy, environmentally friendly, and low cost (in terms of investment, operation, and maintenance) [7]. The system becomes more effective and robust when the regular treatment system is further integrated with polishing steps like granular filtration. Moreover, the selection and use of less risky irrigation methods for plant growth further reduces public health and environmental risks.

Treated greywater can be utilized for non-potable purposes such as agriculture, flushing toilets, landscaping, and aquifer recharge, thereby addressing the issue of an imbalance between water supply and demand in a given region [8]. Treated wastewater reuse for agriculture is widely applied in the arid and semi-arid areas around the world. Likewise, treated domestic wastewater reuse in urban areas is increasing, especially in large cities [9]. However, a health risk is one of the limitations of utilizing treated greywater for plant production. The health risk associated with treated wastewater reuse for vegetable production and non-potable consumption depends on factors such as the quality of the treated wastewater, the irrigation method used, the time interval between irrigation–harvest–consumption, and producer and consumer habits [10]. Treated domestic wastewater may contain limited amounts of essential nutrients for plant growth, and crop production using such treated wastewater has been challenged by an inadequate supply of nutrients, particularly nitrogen [9]. This could potentially be supplied by the use of source-separated urine as a nutrient solution. The application of an integrated system between treated greywater and source-separated urine for hydroponic crop production could increase the efficiency of the system in terms of utilizing nutrients from the wastewater, maximizing the water's reuse potential, increasing control over the quality of the water, and reducing the risk of pathogen contamination.

A green wall, also known as a vertical garden, is a plant growth system attached to the walls of buildings that refers to all forms of vegetated wall surfaces. The advancement of green wall technologies provides a broad range of options for designers to realize multiple objectives, and to bring freestanding design features on the interior and exterior of buildings [11]. One of the options is to integrate a building's infrastructure as a component of on-site greywater treatment, so at the same time green wall plants obtain water and nutrients from the system. The integration of such a treatment system with green wall technology provides many environmental and financial benefits. The green wall provides an additional layer with dual effects, as it acts as an insulator reducing the need for cooling energy during summer and heating energy during winter, respectively. It is also aesthetically

appealing, and improves air quality by reducing the CO_2 level and increasing oxygen. Moreover, a green wall designed for urban agriculture can bring various benefits, such as providing the basis for healthier community interaction (community gardening), and improving access to fresh food [11–13].

Greywater, however, may contain various microbial pathogens and hazardous chemicals depending on the nature of the raw greywater and the treatment's efficiency. Irrigation with wastewater for vegetables and food crops may result in the bioaccumulation of heavy metals, and at the same time it may cause the contamination of plant products with microbial pathogens. Various health problems can occur and develop due to the consumption of contaminated vegetables and the consumption of food contaminated with heavy metals, and this may cause the disruption of various biological processes in the body, leading to a decreased immunological defence, growth retardation, disability associated with malnutrition, and cardiovascular, neurological, kidney, and bone diseases [14,15]. Quantitative microbial risk assessment (QMRA) models and chemical health risk assessment (CHRA) approaches will enable us to evaluate the adverse health effects of operational activities and the consumption of vegetables, and support risk management decisions.

Quantitative microbial risk assessment (QMRA) models have been used to evaluate the health risk associated with the treated wastewater irrigation of vegetables and food crops [10,16–21]. On the other hand, the health risk of heavy metals bioaccumulation in vegetables and food crops irrigated by untreated and treated wastewater has been evaluated in different studies [22–26]. This study was conducted in a unique configuration of an on-site greywater treatment system, granular filtration as part of a green wall structure, and a hydroponic lettuce production system using urine as a nutrient solution. The aim of this study is to assess the efficiency of an integrated system, and to evaluate the health risk associated with the production and consumption of lettuce through a quantitative microbial risk assessment (QMRA) and a chemical health risk assessment (CHRA) approach.

2. Materials and Methods

2.1. System Configuration

The source separation for wastewater management system was established in 1997 at the Norwegian University of Life Sciences' (NMBU) student dormitory, which serves 48 students at Kaya, Ås, Norway. The greywater system collects wastewater from washbasins, showers, kitchen sink, and laundry, whereas the blackwater system collects toilet waste separately. Both systems are pumped into the laboratory (fløy 4) through a separate pipeline, for different experiments. This source separation system is described in detail in [27]. In this study, the greywater was first treated with a package greywater treatment plant (biofilter system), which encompasses a sequence of a primary settler, an unsaturated fixed-film biofilter, and a secondary clarifier. Furthermore, the effluent from the biofilter system was polished by an infiltration system. Three filtration columns (2.5 m in height and 31.5 cm in diameter) as a part of a green wall were constructed in order to polish the effluent discharged from the greywater treatment plant (GWTP). The filtration columns were constructed with three layers: the 1 m bottom layer is 0.8–1.6 mm diameter Filtralite, the 0.3 m in the middle consists of granular activated carbon, and the 1.1 m on top of the activated carbon is 2–4 mm diameter Filtralite. The top 10 cm is air space used to feed the water uniformly from the top of the column by using nozzles. The columns were run in parallel with similar intermittent loading rates of 2 min followed by a rest period of 8 h, and with a daily loading rate of 0.58 $m^3 \cdot m^{-2} \cdot d^{-1}$. The effluent from the filtration columns collected in the bucket at the bottom of the column was used to grow lettuce hydroponically by adding human urine—which was stored for three months—as a nutrient solution. The plantation pots were mounted on the green wall's shelves, and irrigated with the treated greywater from the buckets by using small submersible pumps for circulation (Figure 1).

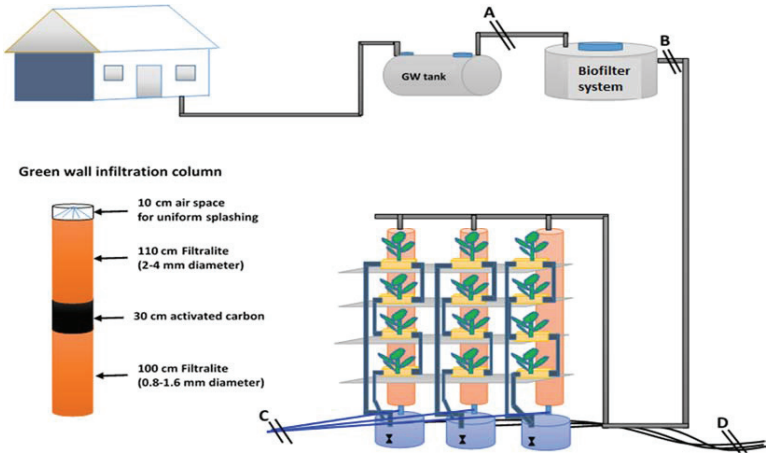

Figure 1. Greywater treatment steps, green wall, and lettuce production configuration (GW tank: greywater tank).

2.2. Lettuce Pots Alignment and Hydroponic System

A flush and drain hydroponic system was designed with perlite as a growth medium. Perlite is a volcanic porous lightweight and inert material, which is commonly used for hydroponic plant growth. Three lettuce varieties, namely: (a) *Lactuca sativa 'Lobjoits Green Cos'*; (b) *Lactuca sativa 'Red Salad Bowl'* and (c) *Lactuca sativa 'Australische Gele'*, were used for this study. Each plantation container holds eight pots, and each pot contained three lettuce plants. The three lettuce varieties were mounted on the top, middle, and bottom shelves respectively. The first green wall column's effluent mixed with urine with the proportion of 0.3% for the first three weeks, and then increased to 0.6% until harvesting time. The second column's effluent mixed with 0.15% of urine for the first three weeks, and then increased to 0.3% until harvesting time. The third column's effluent directly irrigated from the treated greywater without urine. Each mix batch was circulated every 30 min using small-submerged pumps controlled by programmable logic controller (PLC). The desired urine concentration was added into the two columns every three days.

2.3. Water Sample Collection and Lab Analysis

Water samples were collected every two weeks from the raw greywater, the biofilter system's effluent, the filtration column's effluent (green wall), and the circulated irrigation water. The samples were collected in 1 L bottles and analyzed within an hour. The water samples were analyzed for total phosphorous (P) and total nitrogen (N) using spectrophotometric test kits (Hach-Lange, Düsseldorf, Germany); total coliforms (TC) and *Escherichia coli* were quantified using the most probable number method (MPN) with Colilert-18 (IDEXX, Westbrook, ME, USA) and Quantitray 2000 (IDEXX) according to ISO 9308-2:2012. In addition, grab samples were collected from the same position to analyze the heavy metals by using inductively coupled plasma mass spectrometry (ICP-MS, Oban, UK).

2.4. Plant Sample Collection and Lab Analysis

Seven to ten replicates of the lettuce plants, from each of the treatment plots, were collected for the plant growth examination and the heavy metal bioaccumulation analysis. For microbial assay, 25 g of composite lettuce samples from each of the treatment plots were collected and put into stomacher plastic bags containing 225 mL of sterile buffered peptone water (0.1%), homogenized by using a stomacher

for 1 min. *E. coli* was enumerated from the homogenised supernatant using the most probable number method (MPN) with Colilert-18 (IDEXX) and Quantitray 2000 (IDEXX) according to ISO 9308-2:2012.

2.5. Hydroponic Nutrient Uptake and Lettuce Growth Analysis

Nitrogen (N), phosphorus (P), and potassium (K) are essential elements in plant nutrition and their presence in the plant tissue was analyzed using inductively coupled plasma (ICP) spectrometry. Moreover, the lettuce growth analysis was performed using a plant growth index to describe the performance of the lettuce plant grown under this experimental set up. These plant growth indexes are:

(a) Specific leaf area (SLA): the SLA is the surface area of a fresh leaf divided by its oven dry mass. It reflects an essential trade-off in plant functioning between a rapid production of biomass in the case of high SLA and an efficient conservation of nutrients in low SLA. Moreover, species in permanently or temporarily resource-rich environments tend to have a higher SLA than do those in resource-poor environments [28,29].

(b) Leaf weight ratio (LWR): the LWR is the ratio of total leaf dry weight to the total dry weight of the plant. It describes the leafiness of the plant on a dry weight base, and measures the distribution of dry materials between the leaves and the rest of the plant [30].

(c) Leaf area ratio (LAR): the LAR is computed from the photosynthetic surface area per unit dry weight of a plant. It is a measure of the efficiency with which a plant deploys its photosynthesis and respiration per unit of its biomass [30].

(d) Root-shoot ratio: the root-shoot ratio is the ratio of the dry weights of the root system and aerial part of a plant. It is an index of plants' response to their environment through a growth balance between the root and the shoot of the plant. When nutrient availability increases, plants allocate relatively less to their roots, which means that less effort is required to acquire this resource. An alternative view is that relatively greater root growth in response to shortages of nutrients or water could maximize a plant's probability of capturing those resources [30,31].

2.6. Statstical Analysis

Two-way analysis of variance ANOVA was conducted to compare the means of shoot fresh biomass (as a growth performance indicator) at different levels of urine content in irrigation water and lettuce variety. Factors were considered significant when their p-values were below 0.05.

2.7. Health Risk Assessment

Both quantitative microbial risk assessment (QMRA) and chemical health risk assessment (CHRA) approaches were applied to evaluate the health risk of the microbial contamination and heavy metal bioaccumulation associated with reusing treated greywater for lettuce production (Figure 2).

2.7.1. Quantitative Microbial Risk Assessment (QMRA)

Given the probability of having a pathogen-infected person in the system, the study included a single reference pathogen from each group of enteric bacteria, viruses, and protozoa, and followed the water harmonized QMRA approach, which includes problem formulation, exposure assessment, health effect assessment, and risk characterizations.

Problem formulation: the main purpose of this study is to evaluate the health risk associated with the operational activities for lettuce production and consumption in terms of achieving health-based targets, which require that 10^{-6} disability adjusted life years (DALYs) per person per year is not exceeded. The study intended to address enteric pathogens that may be present in greywater. For this purpose, reference pathogens were selected from each of the three pathogen groups. For protozoa, *Cryptosporidium* was selected, as it has high infectivity, is resistant to disinfection units, and is one of the most important waterborne human pathogens. For bacteria, *Campylobacter* was selected, as it is the

most common cause of bacterial gastroenteritis. For viruses, Norovirus was selected, which is a very contagious virus that can infect anyone and is found in abundance in sewage systems.

Exposure assessment: the main exposure pathway considered in this study is operational activities in relation to lettuce production and raw lettuce consumption. The operational activities in relation to lettuce production that can potentially expose the operator to microbial pathogens are routine ingestion and accidental ingestion, assumed to be 0.0001 liter per event and 0.001 liter per event, respectively. The exposure dose, on the other hand, depends on the microbial quality of the circulated irrigation water. Exposure from lettuce consumption was quantified using the equation:

$$Exposure\ D = C \times v \times q \quad (1)$$

where exposure D is the mean dose per event; C is the concentration of pathogens in the circulated water applied to the plant through the hydroponic irrigation system (organism·L^{-1}); v is the volume of irrigation water in contact with the lettuce ($L \cdot g^{-1}$), and the assumed value is based on observation during harvesting; and q is the quantity of lettuce consumed per event.

Health effects assessment: the two important pathogen-specific factors for the risk assessment are the dose-response relationship and the illness per infection, assuming that the health end-point in this study is illness. Therefore, the dose-response models recommended for the reference pathogens to assess the probability of infection are shown in Table 1. In addition, since the probability of illness is often viewed as independent of dose, given that infection has occurred the estimated values of the probability of illness for a given infection of *Cryptosporidium*, *Campylobacter*, and Norovirus are 0.39, 0.33, and 0.73, respectively [32–34].

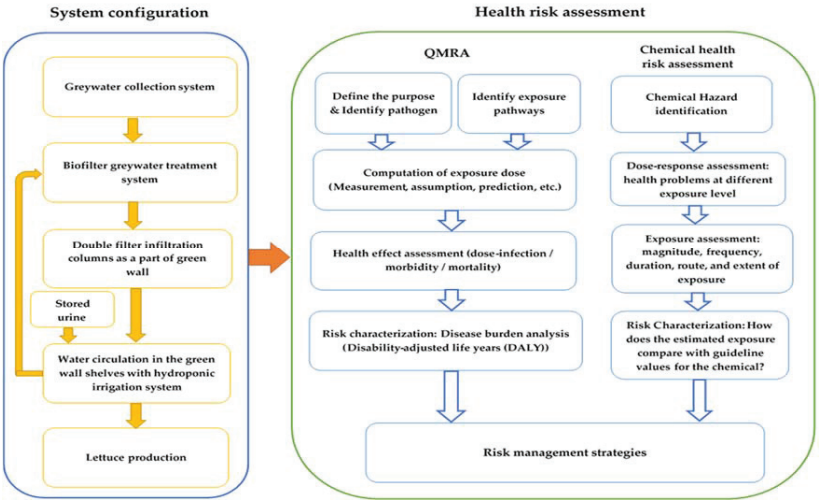

Figure 2. System configuration and health risk assessment procedures.

Risk characterization: the final step in this risk assessment approach is to determine the magnitude of the risk by integrating information from the problem formulation, exposure assessment, and health effect assessment. In this study, the computed health risk was based on the cumulative greywater treatment efficiency, the volume of routine and accidental ingestion of treated greywater during operational activities, and the volume of consumed lettuce. The probability of infection per exposure event was taken from the dose-response relation and adjusted to reflect a yearly risk of infection and illness by estimating the frequency of exposure per year. The equations used for risk characterization in this study are listed in Table 2.

Table 1. Dose-response relationships for reference organisms.

Organism Type	Distribution	Model	Parameters
Norovirus	Beta-Poisson	$P_{\text{inf}} = 1 - (1 + D/\beta)^{-\alpha}$	$\alpha = 0.04$ $\beta = 0.055$
Campylobacter	Beta-Poisson	$P_{\text{inf}} = 1 - (1 + D/\beta)^{-\alpha}$	$\alpha = 0.145$ $\beta = 7.58$
Cryptosporidium	Exponential	$P_{\text{inf}} = 1 - \exp^{(-rD)}$	$r = 0.059$

Table 2. Health risk characterizing computational equations.

Risk Characteristics	Computational Equations
Yearly probability of infection (assuming frequency per year)	$\mathrm{P}_{\text{inff/year}} = 1 - (1 - \mathrm{P}_{\text{inff/event}})^{\text{fr}}$
Risk of illness from a single exposure	$P_{\text{ill/ exp}} = P_{\text{ill/inff}} * P_{\text{inff}}$
Yearly risk of illness (assuming x exposure frequency per year)	$\mathrm{P}_{\text{ill/year}} = 1 - (1 - \mathrm{P}_{\text{ill/ exp}})^{\text{fr}}$

Note: $^{\text{fr}}$ is frequency per year.

2.7.2. Heavy Metals Health Risk Assessment

Metal pollution: heavy metals bioaccumulation may differ in different crops depending on the environment they are produced in. In order to measure the combined effect of all of the expected heavy metals, the Metal Pollution Index (MPI) is commonly applied. In addition, the MPI was used to normalize and compare the total metal content between the different plant varieties and treatment levels as proposed by [35].

$$MPI = \left[M_1 \times M_2 \times M_3 \ldots . M_j\right]^{1/n} \quad (2)$$

where M is the mean concentration of metal j (mg/kg dry wt); and n represents the number of heavy metals in the examined crop.

Plant uptake rate of heavy metals: a number of factors can affect a plant's uptake mechanism for heavy metals. These factors are the plant's species, properties of the plant's growth medium, root growth, vegetative growth, the bioavailability of the metal in the water phase (which depends on the retention time of the metal), and the interaction with other elements and substances in the water [36]. The daily heavy metals uptake rate across the lettuce varieties for each treatment level was described by the equation:

$$DUR_i = \frac{C_m}{T \times BM_p} \quad (3)$$

where DUR_i is the average daily uptake rate of heavy metals, normalized by the dry biomass of lettuce variety i (µg/day); C_m is the concentration of heavy metals in the lettuce tissue (µg/g); T is the total growth time (days); and BM_p the dry plant biomass (g).

Daily intake rate (DIR): one of the exposure pathways of heavy metals is through the ingestion of vegetables, which was determined by using a daily intake rate (DIR) (mg/kg·day). The DIR estimates the average daily loading of metal into the body system of a specified body weight of a consumer. The daily intake of metals depends on both the heavy metals' concentration and the amount of vegetables consumed. Moreover, its effect depends on the body weight of the consumer. The DIR is computed using the equation:

$$DIR = \frac{C_m \times C_{f\ x}\ IR}{BW} \quad (4)$$

where C_m is the heavy metal concentration in the vegetables (g/kg); C_f is the conversion factor that converts fresh lettuce weight to dry weight (our conversion factor is 0.065); IR is the daily intake of vegetables (g/day), assumed to be 0.05 kg/day; and BW is the average body weight, assumed to be 70 kg for this study.

Health risk index (HRI) is the ratio between the daily intake rate and the reference dose (R_fD) (mg/(kg·day) that expresses the health risk of non-carcinogenic effects [37], and is described by the equation:

$$HRI = \frac{DIR}{RfD} \tag{5}$$

where *DIR* is the daily intake rate; and R_fD is the reference dose expressed as an oral dose per kilogram of body weight, which is an estimate of the lowest daily human exposure that is likely to occur without an appreciable risk of toxicity for non-cancerous effects during a lifetime [38]. $HRI < 1$ indicates that the exposed population is safe from the health risk that comes from heavy metal consumption.

Targeted hazard quotient (THQ) is a ratio between heavy metal concentration and the oral reference dose, weighted by the duration and frequency of exposure, intake rate, and body weight [37]. The *THQ* parameter is a dimensionless index, and indicates a level of concern but does not measure the risk. $THQ < 1$ indicates that the exposed population to heavy metals through lettuce consumption is unlikely to experience visible adverse health effects. *THQ* values between 1 and 5 consider the exposed population to be at a certain level of health risk concern. The *THQ* is computed by the formula:

$$THQ = \frac{EF \times ED \times IR \times C \times 10^{-3}}{RfD \times BW \times TA} \tag{6}$$

where *EF* is exposure frequency (days per year); *ED* is the exposure duration (years, equivalent to an average lifetime); *IR* is the vegetable ingestion rate (g per person per day); *C* is the metal concentration in lettuce ($mg \cdot Kg^{-1}$); *BW* is the average body weight (kg); and *TA* is the average exposure time for non-carcinogens (days per year × exposure duration). The average body weight of a human is different from region to region, and for this study the average body weight of an adult was assumed to be 70 kg based on the literature [39], and also the daily lettuce consumption was assumed to be 50 g. In addition, the exposure frequency and duration were assumed to be 104 days per year and 70 years life expectancy, respectively.

3. Results

3.1. Irrigation Water Quality and Greywater Treatment Efficiency

The raw and treated greywater's quality was monitored every other week, from the first day of the planting of the lettuce until harvesting, and the results are shown in Figure 3. Based on the microbial water quality monitoring, the greywater treatment efficiency of the barrier structures in the system varied. The reduction of *E. coli* in Log_{10} MPN/100 mL was 1.6, 1.9, and 1.6 for the biofilter system, green wall filtration column, and circulated irrigation water, respectively. Therefore, the system has gained a cumulative reduction of *E. coli* of about 5.1 log_{10} MPN/100 mL in the final effluent. In addition, the reduction of total coliform bacteria was 1.4, 2.1, and 0.2 Log_{10} MPN/100 mL for the biofilter system, green wall filtration column, and circulated irrigation water respectively, resulting in a 3.7 log_{10} reduction in the final effluent.

The average total phosphorus and total nitrogen in the raw greywater during this experimental period was 0.91 mg/L and 8.52 mg/L, respectively. It was reduced to 0.53 mg/L and 4.37 mg/L by the biofilter system and 0.08 mg/L and 1.73 mg/L by the green wall filtration column, respectively. When 3–6% urine was added to the effluent of infiltration column 1, and 1.5–3% urine was added to the effluent of infiltration column 2, the average total phosphorus and total nitrogen concentration rose to 0.85 mg/L and 34.97 mg/L in the first circulated water and 0.68 mg/L and 24.27 mg/L in the second circulated water, respectively.

On the other hand, the heavy metal analysis result based on the grab samples shows that the concentration of Zn and Cr was 87.3 µg/L and 20 µg/L, respectively, and was relatively higher in the raw greywater as compared to the concentration of other heavy metals. The heavy metals removal efficiency of the greywater treatment steps was different for each heavy metal element, and it was

up to 82% for Cr in the case of the biofilter system. However, the concentration of some of the heavy metal elements increased in the treated gray water, such as Mn in the biofilter system effluent, and the levels of Cu, As, Cd, and Pb were increased in the effluent of the filtration columns (Table 3).

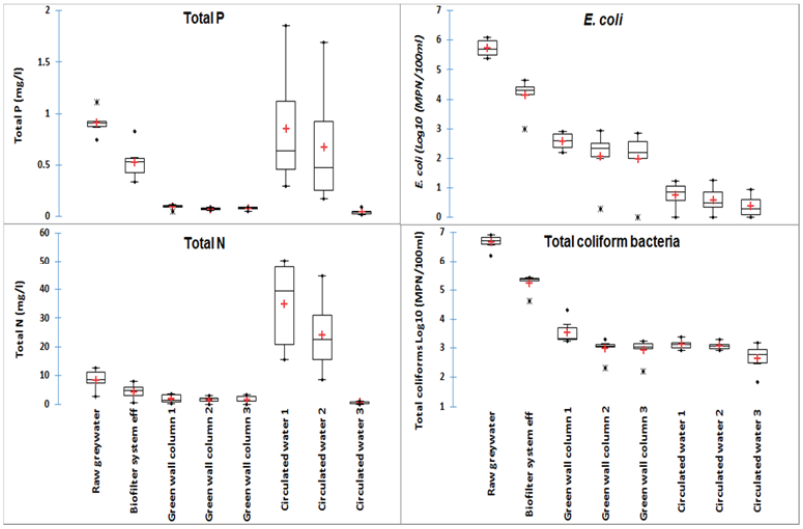

Figure 3. Water quality at different treatment steps of the system.

Table 3. Heavy metal concentration (μg/L) in greywater and different treatment steps.

Sampling Points	Cr	Mn	Ni	Cu	Zn	As	Cd	Hg	Pb
Raw greywater	20.0	12.7	17.0	10.7	87.3	<0.26	<0.01	<0.02	0.6
Biofilter system effluent	3.6	15.7	9.6	5.6	34.7	<0.26	<0.01	<0.02	0.2
Filtration column effluent	<2.5	3.4	3.6	8.0	<21	0.67	0.036	<0.02	0.25
Human Urine	8	17	3.9	14	600	30	0.2	<0.02	0.4

3.2. The Level of Microbial Contamination and Heavy Metal Bioaccumulation in the Lettuce

The lettuce biomass was subjected to an *E. coli* test, and the result shows that there was no positive sample in the case of all of the plots, and this indicates that the contamination of the lettuce from the irrigation water is very limited due to the hydroponic irrigation system. On the other hand, the heavy metal analysis result shows that the bioaccumulation of Zn, Mn, and Cu in the plant tissue was relatively high as compared with the other elements (Table 4).

Cu and As appear to have the highest concentrations in plant tissue when irrigated with urine-free irrigation water, despite the fact that urine contains higher concentrations of these metals. Moreover, the Zn and Mn concentrations in the plant tissue appear highest in the lower urine concentration (0.15–0.3%). The presence of heavy metals in irrigation water does not imply that they are available to plants. One of the most important factors governing metal speciation, solubility, transport, and the eventual bioavailability of metals is the pH of the irrigation water and the growth media. In addition, the abundance of other elements may reduce the bioavailability of the heavy metals. Therefore, the presence or absence of urine in the irrigation water may affect the bioavailability of heavy metals through changing the chemical properties of irrigation water [40,41].

Table 4. Heavy metal concentration in three varieties of lettuce for different treatment.

Urine in Irrigation Water (%)	Lettuce Type	Heavy Metals Concentration (mg/kg)								
		As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3–0.6	a	0.13	0.02	1.43	11.00	27.00	3.30	0.38	51.25	0.02
	b	0.40	0.02	2.50	13.00	32.00	3.50	0.96	59.00	0.02
	c	0.11	0.02	2.50	12.33	30.33	3.73	0.66	55.00	0.01
0.15–0.3	a	0.09	0.02	1.00	12.00	36.00	4.10	0.26	78.00	0.02
	b	0.11	0.03	1.20	10.00	43.00	2.70	0.30	75.00	0.02
	c	0.13	0.04	0.86	12.00	49.00	3.50	0.39	74.00	0.02
0	a	0.22	0.02	0.76	9.90	13.00	2.20	0.42	38.00	0.01
	b	0.22	0.03	1.20	43.00	27.00	3.10	0.63	55.00	0.01
	c	0.27	0.02	1.80	20.00	18.00	4.00	0.73	63.00	0.01

Notes: a—*Lactuca sativa 'Lobjoits Green Cos'*; b—*Lactuca sativa 'Red Salad Bowl'*; c—*Lactuca sativa 'Australische Gele'*.

3.3. Hydroponic Nutrient Uptake and Lettuce Growth Analysis

The two-way ANOVA gives F statistics 76.56, $p < 0.001$; 11.19, $p < 0.001$; and 5.00, $p = 0.003$ for urine content in irrigation water, lettuce variety, and their interaction, respectively. Therefore, both urine content in irrigation water and lettuce variety, as well as their interaction, explains the shoot fresh biomass.

The growth analysis of the lettuce was evaluated using four plant growth indexes and is presented in Figure 4. As we can see from the figure, the value of the specific leaf area (SLA) for each of the three lettuce species and the associated treatment level was different. The SLA value reduced with the reduction of urine in the irrigation water, and it was clearly observed in the case of *Lactuca sativa 'Red Salad Bowl'*, which had an SLA value of 0.014 $m^2 \cdot g^{-1}$ in the case of 0.3–0.6% urine as compared to 0.003 $m^2 \cdot g^{-1}$ in the absence of urine. The average SLA value of all three lettuce varieties with the application of 0.3–0.6% urine and in the absence of urine as a treatment was 0.012 $m^2 \cdot g^{-1}$ and 0.005 $m^2 \cdot g^{-1}$, respectively. The computed leaf weight ratio (LWR) and leaf area ratio (LAR) for the three species is 11.57 and 0.13 in the application of 0.3–0.6% urine with the treated greywater irrigation and 3.93 and 0.02 in the absence of urine, respectively. The average root-shoot ratio of the lettuce irrigated with the 0.3–0.6% urine mix was 0.35, and was lowest (0.22) in the case of *Lactuca sativa 'Lobjoits Green Cos'*, while that of the lettuce irrigated without urine was 0.96, and was highest (1.15) in the case of *Lactuca sativa 'Australische Gele'*.

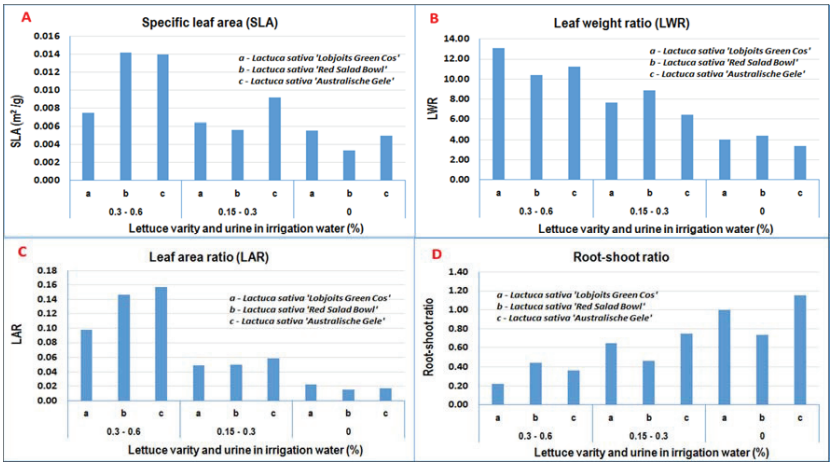

Figure 4. Growth indexes result for different lettuce varieties and treatment levels (**A**) specific leaf area; (**B**) leaf weight ratio; (**C**) leaf area ratio; and (**D**) root-shoot ratio.

3.4. Quantitative Microbial Risk Assessment

3.4.1. Estimation of Reference Pathogens in Irrigation Water and Lettuce Biomass

The estimation of the reference pathogens at different greywater treatment steps and in a produced lettuce was based on *E. coli* concentration as an indication of microbial contamination. As shown in Table 5, the *E .coli* concentration decreased because of the different greywater treatment steps. The base for the estimate of reference pathogen concentration was the combination of information about *E. coli* at each treatment step with the concentration of the reference pathogens in the sewage system, which has been published in different studies. Moreover, with 1% of the sewage assumed to be mixed with the greywater system (Table S2), from the previous studies the average concentration of *Cryptosporidium*, *Campylobacter*, and Norovirus in the sewage system was estimated to be 678.1 oocysts/100 mL, 118 MPN/100 mL, and 5.1×10^4 gene copies/100 mL, respectively [42–44]. The concentration of reference pathogens in the irrigation water was dependent on the efficiency of the greywater treatment steps of the system, which was based on *E. coli* removal efficiency. The final concentration of *Cryptosporidium*, *Campylobacter*, and Norovirus in the circulated irrigation water was 4.7×10^{-4} oocysts/100 mL, 8.2×10^{-6} MPN/100 mL, and 3.5×10^{-8} gene copies/100 mL, respectively (Table 5). The microbial contamination of the lettuce was assumed to be unintentional contact with the irrigation water during harvesting time.

Table 5. Estimated reference pathogens' concentration at different treatment steps.

Pathogens	Variables	An Average Value
Cryptosporidium	Concentration (C) in raw greywater (L^{-1})	6.8×10^1
	Biofilter system $\log_{10}$ reduction	1.59
	Concentration in biofilter system effluent (L^{-1})	1.7×10
	Infiltration column $\log_{10}$ reduction	1.93
	Concentration in infiltration column effluent (L^{-1})	2.1×10^{-2}
	Circulated irrigation water $\log_{10}$ reduction	1.64
	Concentration in circulated irrigation water (L^{-1})	4.7×10^{-4}
Campylobacter	Concentration (C) in raw greywater (L^{-1})	1.2×10
	Biofilter system $\log_{10}$ reduction	1.59
	Concentration in biofilter system effluent (L^{-1})	3.0×10^{-2}
	Infiltration column $\log_{10}$ reduction	1.93
	Concentration in infiltration column effluent (L^{-1})	3.6×10^{-4}
	Circulated irrigation water $\log_{10}$ reduction	1.64
	Concentration in circulated irrigation water (L^{-1})	8.2×10^{-6}
Norovirus	Concentration (C) in raw greywater (L^{-1})	5.1×10
	Biofilter system $\log_{10}$ reduction	1.59
	Concentration in biofilter system effluent (L^{-1})	1.3×10^{-1}
	Infiltration column $\log_{10}$ reduction	1.93
	Concentration in infiltration column effluent (L^{-1})	1.5×10^{-3}
	Circulated irrigation water $\log_{10}$ reduction	1.64
	Concentration in circulated irrigation water (L^{-1})	3.5×10^{-5}

Treatment efficiency based on *E. coli* Log_{10} reduction in the system.

3.4.2. Health Risk Assessment Computation

Routine ingestion and accidental ingestion are the two routes to exposure in the operation of lettuce production during irrigation and harvesting practices. Based on practical observation, routine ingestion was assumed to be 0.0001 L per event and occurred more frequently during the irrigation practices, whereas accidental ingestion was estimated to be about 0.001 L per event and occurred less frequently for both operational activities. The exposure dose during the operational activities of lettuce production was estimated to be from 4.7×10^{-7} to 4.7×10^{-8} for *Cryptosporidium*, 8.2×10^{-9} to 8.2×10^{-10} for *Campylobacter*, and 3.5×10^{-11} to 3.5×10^{-12} for Norovirus. The exposure dose due

to lettuce consumption is dependent on the volume of irrigation water accidentally contaminating the lettuce and the amount of lettuce consumption. Moreover, the estimated exposure dose due to contaminated lettuce consumption was 2.35×10^{-9}, 1.75×10^{-10}, and 4.1×10^{-11} in the case of *Cryptosporidium*, *Campylobacter*, and Norovirus, respectively (Table 6).

The computed health risk that accounts for lettuce production (irrigation and harvesting) and consumption, expressed in terms of the probability of infection for a single exposure, ranges from 2.8×10^{-8} in the case of the accidental ingestion of *Cryptosporidium* to 2.5×10^{-12} in the case of the routine ingestion of Norovirus during the lettuce production process. On the other hand, the probability of infection due to lettuce consumption per single exposure was estimated to be 1.4×10^{-10}, 7.8×10^{-13}, and 1.3×10^{-10} in the case of *Cryptosporidium*, *Campylobacter*, and Norovirus, respectively (Table 7).

Table 6. Estimation of exposure dose (D) and exposure frequency for reference pathogens during lettuce production and consumption.

Pathogens	Activities	Route of Exposure	Concentration (*C*)	Volume (*L*) Per Event	Exposure Dose (*D*) Per Event	Frequency/ Person/Year
Cryptosporidium	Hydroponic irrigation	Routine ingestion	4.7×10^{-4}	1.0×10^{-4}	4.7×10^{-8}	365
		Accidental ingestion	4.7×10^{-4}	1.0×10^{-3}	4.7×10^{-7}	10
	Lettuce harvest	Routine ingestion	4.7×10^{-4}	1.0×10^{-4}	4.7×10^{-8}	30
		Accidental ingestion	4.7×10^{-4}	1.0×10^{-3}	4.7×10^{-7}	5
	Lettuce consumption	Deliberate ingestion	4.7×10^{-4}	5.0×10^{-6}	2.35×10^{-9}	104
Campylobacter	Hydroponic irrigation	Routine ingestion	8.2×10^{-6}	1.0×10^{-4}	8.2×10^{-10}	365
		Accidental ingestion	8.2×10^{-6}	1.0×10^{-3}	8.2×10^{-9}	10
	Lettuce harvest	Routine ingestion	8.2×10^{-6}	1.0×10^{-4}	8.2×10^{-10}	30
		Accidental ingestion	8.2×10^{-6}	1.0×10^{-3}	8.2×10^{-9}	5
	Lettuce consumption	Deliberate ingestion	8.2×10^{-6}	5.0×10^{-6}	4.1×10^{-11}	104
Norovirus	Hydroponic irrigation	Routine ingestion	3.5×10^{-8}	1.0×10^{-4}	3.5×10^{-12}	365
		Accidental ingestion	3.5×10^{-8}	1.0×10^{-3}	3.5×10^{-11}	10
	Lettuce harvest	Routine ingestion	3.5×10^{-8}	1.0×10^{-4}	3.5×10^{-12}	30
		Accidental ingestion	3.5×10^{-8}	1.0×10^{-3}	3.5×10^{-11}	5
	Lettuce consumption	Deliberate ingestion	3.5×10^{-8}	5.0×10^{-6}	1.8×10^{-10}	104

Table 7. The health risk of lettuce production and consumption.

Pathogens	Route of Exposure	Activities	$P_{inf/event}$	$P_{inff/year}$	$P_{ill/expo}$	$P_{ill/year}$
Cryptosporidium	Hydroponic irrigation	Routine ingestion	2.8×10^{-9}	1.0×10^{-6}	1.1×10^{-9}	3.9×10^{-7}
		Accidental ingestion	2.8×10^{-8}	2.8×10^{-7}	1.1×10^{-8}	1.1×10^{-7}
	Lettuce harvest	Routine ingestion	2.8×10^{-9}	8.3×10^{-8}	1.1×10^{-9}	3.2×10^{-8}
		Accidental ingestion	2.8×10^{-8}	1.4×10^{-7}	1.1×10^{-8}	5.4×10^{-8}
	Lettuce consumption	Deliberate ingestion	1.4×10^{-10}	1.4×10^{-8}	5.4×10^{-11}	5.6×10^{-9}
Campylobacter	Hydroponic irrigation	Routine ingestion	1.6×10^{-11}	5.7×10^{-9}	5.2×10^{-12}	1.9×10^{-9}
		Accidental ingestion	1.6×10^{-10}	1.6×10^{-9}	5.2×10^{-11}	5.2×10^{-10}
	Lettuce harvest	Routine ingestion	1.6×10^{-11}	4.7×10^{-10}	5.2×10^{-12}	1.6×10^{-10}
		Accidental ingestion	1.6×10^{-10}	7.8×10^{-10}	5.2×10^{-11}	2.6×10^{-10}
	Lettuce consumption	Deliberate ingestion	7.8×10^{-13}	8.2×10^{-11}	2.6×10^{-13}	2.7×10^{-11}
Norovirus	Hydroponic irrigation	Routine ingestion	2.5×10^{-12}	9.3×10^{-10}	1.9×10^{-12}	6.8×10^{-10}
		Accidental ingestion	2.5×10^{-11}	2.5×10^{-10}	1.9×10^{-11}	1.9×10^{-10}
	Lettuce harvest	Routine ingestion	2.5×10^{-12}	7.6×10^{-11}	1.9×10^{-12}	5.6×10^{-11}
		Accidental ingestion	2.5×10^{-11}	1.3×10^{-10}	1.9×10^{-11}	9.3×10^{-11}
	Lettuce consumption	Deliberate ingestion	1.3×10^{-10}	1.3×10^{-8}	9.3×10^{-11}	9.7×10^{-9}

3.5. Heavy Metals Health Risk Assessment

The relative daily uptake rate of heavy metals between the lettuce varieties was different for different heavy metal elements (Figure 5). For example, the daily uptake rate of arsenic by *Lactuca sativa 'Red Salad Bowl'* was highest as compared to the other two varieties, whereas the daily uptake rate of nickel was relatively lowest. On the other hand, the relative daily uptake rate of heavy metals is also varied depending on the volume of urine mix in the irrigation water. For example, the relative

daily uptake rate of chromium increases as the urine mix in the irrigation water increases, and is the same for all other cases.

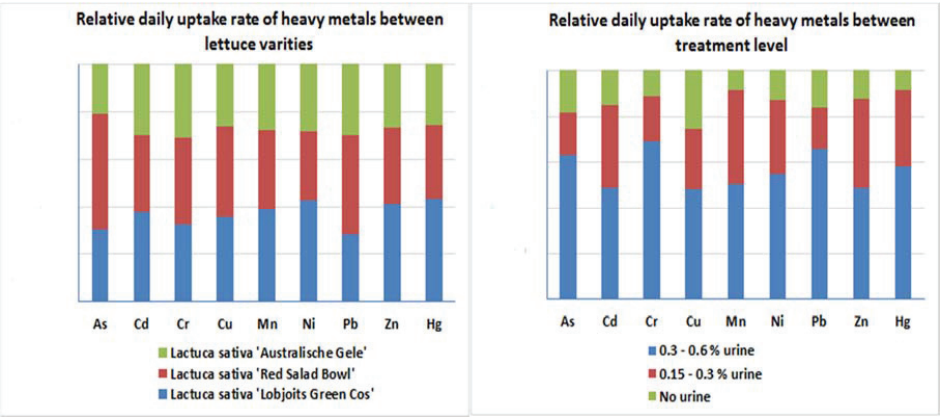

Figure 5. Relative daily uptake rate of heavy metals for the lettuce plants.

The Metal Pollution Index (MPI) values for the three lettuce varieties and the associated treatments of urine mix with irrigation water are shown in Table 8. The MPI value of *Lactuca sativa 'Lobjoits Green Cos'* was lowest in all treatment cases as compared to the other varieties, and it was 0.91, 0.83, and 0.67 for the 0.3–0.6% urine mix, the 0.15–0.3% urine mix, and without urine, respectively. The MPI value of *Lactuca sativa 'Red Salad Bowl'* was highest: it was 1.26 in the case of the 0.3–0.6% urine mix treatment level (Table 8).

Table 8. Metal pollution index for lettuce varieties.

Urine in Irrigation Water (%)	Lettuce Type	Metal Pollution Index (MPI)
0.3–0.6	*a—Lactuca sativa 'Lobjoits Green Cos'*	0.91
	b—Lactuca sativa 'Red Salad Bowl'	1.26
	c—Lactuca sativa 'Australische Gele'	0.99
0.15–0.3	*a—Lactuca sativa 'Lobjoits Green Cos'*	0.83
	b—Lactuca sativa 'Red Salad Bowl'	0.87
	c—Lactuca sativa 'Australische Gele'	1.02
0	*a—Lactuca sativa 'Lobjoits Green Cos'*	0.67
	b—Lactuca sativa 'Red Salad Bowl'	1.13
	c—Lactuca sativa 'Australische Gele'	1.10

The health risks of heavy metal through lettuce consumption were evaluated based on the health risk index (*HRI*) and the target hazard quotient (*THQ*). For the computation of the health risk indexes, the daily intake rate is very crucial to estimating the level of exposure through a food chain (lettuce consumption). The daily intake rate of the heavy metals from the different varieties of lettuce along with the treatment level is presented in Table S3. The daily intake of Zn was found to be greater than the other heavy metals: it ranged from 3.5×10^{-3} to 7.2×10^{-3} μg/kg·day. In addition, the daily intake of Mn and Cu was also higher: it ranged from 1.2×10^{-3} to 4.6×10^{-3} μg/kg·day and 1.1×10^{-3} to 9.3×10^{-4} μg/kg·day, respectively.

The health risk index and the target hazard quotient are an indication of the health risk level of consuming lettuce that is produced in the system, and are computed using the reference dose (R_fD). The estimated values of the reference dose (R_fD) of the heavy metals, which is an oral dose per km

of body weight, were taken from the United States Environmental Protection Agency (US-EPA) web page and shown in Table 9. The computational result of the *HRI* shows that the highest value was obtained in the case of As: it was 0.062 in the case of the *Lactuca sativa 'Red Salad Bowl'* lettuce variety grown with 0.3–0.6% urine mix in the irrigation water. However, the value of the *HRI* for all of the lettuces and treatment levels was below 1, and it shows that the population is not at risk and the public health risk concern due to the lettuce's consumption is very low for the given assumptions (Table 10). The *THQ* value for As and Cr are relatively higher, but still <1 in all cases (Table 11), and it implies that the concern level of a health risk is low for the given assumptions. The overall evaluation result shows that the lowest value of *THQ* was obtained in the case of *Lactuca sativa 'Lobjoits Green Cos'*, and the highest value was obtained in the case of *Lactuca sativa 'Red Salad Bowl'*.

Table 9. Reference dose (R_fD) of heavy metals (mg/kg/day).

Parameter	Heavy Metals								
	As	**Cd**	**Cr**	**Cu**	**Mn**	**Ni**	**Pb**	**Zn**	**Hg**
Reference dose (R_fD)	0.0003	0.001	0.003	0.04	0.14	0.02	0.0035	0.3	0.0003

Table 10. Health risk index (*HRI*) of heavy metals caused by the consumption of lettuce.

Urine in Irrigation Water (%)	Lettuce Type	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3–0.6	a	0.021	0.001	0.022	0.013	0.009	0.008	0.005	0.008	0.003
	b	0.062	0.001	0.039	0.015	0.011	0.008	0.013	0.009	0.003
	c	0.017	0.001	0.039	0.014	0.010	0.009	0.009	0.009	0.002
0.15–0.3	a	0.014	0.001	0.015	0.014	0.012	0.010	0.003	0.012	0.002
	b	0.017	0.001	0.019	0.012	0.014	0.006	0.004	0.012	0.003
	c	0.020	0.002	0.013	0.014	0.016	0.008	0.005	0.011	0.003
0	a	0.034	0.001	0.012	0.011	0.004	0.005	0.006	0.006	0.001
	b	0.034	0.001	0.019	0.050	0.009	0.007	0.008	0.009	0.002
	c	0.042	0.001	0.028	0.023	0.006	0.009	0.010	0.010	0.002

Notes: a—*Lactuca sativa 'Lobjoits Green Cos'*; b—*Lactuca sativa 'Red Salad Bowl'*; c—*Lactuca sativa 'Australische Gele'*.

Table 11. Calculated target hazard quotient (*THQ*) for heavy metals in in different varieties of lettuce.

Urine in Irrigation Water (%)	Lettuce Type	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3–0.6	a	0.32	0.01	0.34	0.20	0.14	0.12	0.08	0.12	0.05
	b	0.95	0.01	0.60	0.23	0.16	0.13	0.20	0.14	0.04
	c	0.26	0.02	0.60	0.22	0.15	0.13	0.14	0.13	0.03
0.15-0.3	a	0.21	0.01	0.24	0.21	0.18	0.15	0.05	0.19	0.04
	b	0.26	0.02	0.29	0.18	0.22	0.10	0.06	0.18	0.04
	c	0.31	0.03	0.20	0.21	0.25	0.13	0.08	0.18	0.05
0	a	0.52	0.01	0.18	0.18	0.07	0.08	0.09	0.09	0.02
	b	0.52	0.02	0.29	0.77	0.14	0.11	0.13	0.13	0.03
	c	0.64	0.02	0.43	0.36	0.09	0.14	0.15	0.15	0.03

Notes: a—*Lactuca sativa 'Lobjoits Green Cos'*; b—*Lactuca sativa 'Red Salad Bowl'*; c—*Lactuca sativa 'Australische Gele'*.

The variation of *THQ* value due to the change in the input variable of lettuce intake rate per body weight was referred as the sensitivity of *THQ* for this particular input variable. The effect of the input on *THQ* were investigated by varying only this variable across its range of plausible values, while keeping all other inputs at their nominal values as it is described in the assumption for the other computations.

The consumption rate of vegetables varies from country to country depending on the culture, religion, availability, etc. Therefore, a sensitivity analysis based on intake rate versus *THQ* value for

the different heavy metals provides an opportunity to notice the effect of a wide range of heavy metals intake rate per body weight (Figure 6). The results were presented graphically to visualize how an output was sensitive to the change in inputs with respect to its critical value. As we can see from the graph, the *THQ* value was above the critical value when the value of lettuce intake rate per body weight of As, Cr, and Cu was above 1.65 g/kg, 2.05 g/kg, and 2.55 g/kg, respectively. It must be noted that the THQ value computed here only signifies the contribution from the specific lettuce produced in our system, whereas the contribution of heavy metals from other daily diets will potentially increase the risk.

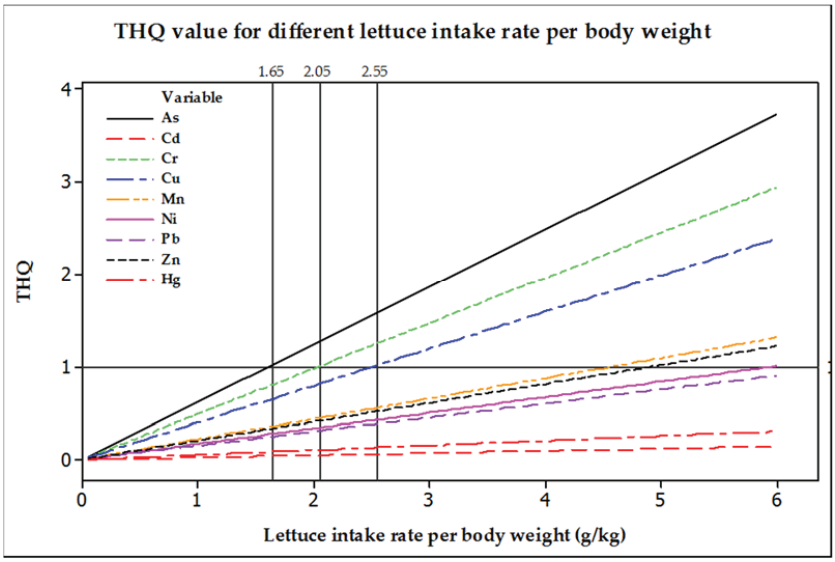

Figure 6. The sensitivity of calculated *THQ* for lettuce intake rate for a given body weight.

4. Discussion

The lettuce growth analysis showed that the performance of urine treated lettuce (0.3 to 0.6%) was better than a lettuce plant produced with little urine (0.15 to 0.3%) and without urine in terms of appearance, height, and number of leaves (Figure S1). The overall better performance of urine treated lettuce was attributed to the adequate provision of essential nutrients for plant growth, and it confirms the potential of urine for fertilizing in a hydroponic system. Different plants may require different proportions of urine in the irrigation water, depending on the plant's type and its growth stage. It has been observed that the application of 0.15 to 0.3% urine mix in the irrigation water was not optimal, as lettuce is a leafy plant that requires a higher nitrogen concentration for its vegetative growth; however, 0.3 to 0.6% gives the impression that it is the optimal level, but further assessment is required.

A two-way ANOVA found significant difference between the mean values of shoot fresh biomass (an indicator for growth performance) with the factor of urine content in irrigation water and lettuce variety ($p < 0.001$). A significance in the interaction of the variables ($p = 0.003$) also shows shoot fresh biomass changes for urine content in irrigation water depending on lettuce variety. This indicates the synergistic effect of applied urine and lettuce variety on lettuce growth.

The lettuce growth analysis within the natural development cycle may enable us to evaluate the adaptive feature of the plant. In order to evaluate lettuce growth in quantitative terms, the specific leaf area (SLA), leaf weight ratio (LWR), leaf area ratio (LAR), and root-shoot ratio were assessed. SLA is a measure of the thickness of leaves relative to area, which is associated with the availability of

sufficient plant nutrients.The high specific leaf area (SLA) associated with the application of 0.3 to 0.6% urine in treated greywater irrigation, and low SLA in the absence of urine, demonstrates the challenge of the reuse of treated greywater alone for plant biomass production. The insufficient plant nutrient in the case of irrigation without urine significantly constrained plant growth in all of the three varieties. LWR is a measure of biomass allocation to leaves, and the highest LWR in the case of 0.3 to 0.6% urine mix in the irrigation water was associated with the highest leaf biomass production, whereas the lowest LWR in the absence of urine linked with the lowest leaf biomass production. Leaf area ratio is a measure of leafiness or photosynthetic area relative to respiratory mass of the plant that characterizes the plant–atmosphere interaction where most of the energy fluxes exchange through photosynthesis and respiration. The highest LAR (0.16) in the urine treated irrigation compared to the lowest LAR (0.01) in the absence of urine was reflected the value of urine as a nutrient solution for the growth performance of the lettuce in this system. The average root-shoot ratio varied widely between lettuce varieties of the same treatment. A relatively high root-shoot ratio was found in the case of lettuce grown without urine as a response to the shortage of nutrients.

In order to utilize treated greywater for an irrigation purpose, it is important to select greywater treatment steps that reduce microbial pathogens and at the same time retain the nutrients. However, it is often difficult to find treatment processes that perform the two demands simultaneously. Therefore, stored human urine was mixed with the final effluent of the greywater treatment system as a nutrient solution for this system. A significant reduction of *E. coli* was observed in the final effluent of the greywater treatment step. No *E. coli* were observed in any of the plant samples collected from each of the treatment plots. The results of this study point out that the greywater treatment system efficiently removed *E. coli*. The integrated hydroponic system has shown to produce lettuce using recycled greywater and urine without exceeding target risk thresholds. Therefore, this study confirmed that the type of irrigation plays an important role in terms of reducing the risk of contamination.

Quantitative microbiological risk assessment (QMRA) is the process of estimating the risk from exposure to microorganisms. With the intention of determining whether the production and consumption of treated greywater irrigated lettuce has a health risk, a quantitative microbial risk assessment (QMRA) model was developed. The *E. coli* concentration in the final effluent of the greywater treatment system has been considered as a base for the level of microbial contamination of the irrigation water. The average concentration of reference pathogens (*Cryptosporidium*, *Campylobacter*, and Norovirus) in our system was derived from the pathogen load of the municipal sewer system. The volume of water retained by the lettuce leaves and ingested with the lettuce is assumed to be very low (1.0×10^{-7} L/g), because of the limited accidental contact with irrigation water during harvesting. On the other hand, the volume of water ingestion either accidentally or routinely in production activities during irrigation and harvesting was determined from practical observation and expert opinion; we assumed that it was very little in a closed hydroponic system.

The two major activities for lettuce production that expose the operator to microbial contamination are irrigation and harvesting. Most of these activities are often carried out manually, and the probability of hand contamination is high. Pathogen transmission through the consumption of food with contaminated hands and the accidental splashing of irrigation water into the mouth were considered the most likely routes of exposure for this study. Considering the routes of exposure and other assumptions, the computed QMRA result showed that the infection risk of *Cryptosporidium*, *Campylobacter*, and Norovirus due to a lettuce consumption event were very low: 1.4×10^{-10}, 7.8×10^{-13}, and 1.3×10^{-10}, respectively. The health risk of both lettuce consumption and production activities based on the corresponding assumptions and scenarios were below World Health Organisation (WHO) health-based targets, which is 10^{-6} DALYs per person per year. Most of the pathogen load studies in the sewage system as well as in the influent of wastewater treatment plants cannot capture the peak concentration of a pathogen, but rather capture the average load, and such information is important for QMRA studies. In the case of a household greywater treatment system, the peak load could appear when one of the family members becomes sick, and the expected risk

could be much higher as compared with the average load in the municipal sewer system. Therefore, the conversion of *E. coli* concentration into reference pathogens based on the average pathogen load of the sewer system could give us a clue about the average risk of our system, but it will undermine the peak risk when the incidence of pathogens is elevated at a household level, and eventually in the greywater system.

The health risk of lettuce production as well as consumption in our system was relatively very low as compared with other studies using raw or partially treated wastewater for irrigating. For example, a rotavirus infection estimation from consuming crops that have been irrigated with effluents from stabilization ponds was 10^{-3} and 10^{-4} [45], and the median annual probability of infection from the consumption of vegetables that have been irrigated with wastewater using an overhead sprinkler also ranged from 10^{-3} to 10^{-4} per year [46]. The daily probability of illness from eating raw unwashed vegetables irrigated with untreated wastewater the Bogotá river receives from the city and towns ranged between 0.62 and 0.85 [47]. Compared with these studies, the treatment level in combination with the hydroponic irrigation scheme of our system reduced the health risk substantially.

The chemical health risk due to lettuce consumption was expressed in terms of a health risk index (*HRI*) and targeted hazard quotient (HQ), which are commonly used to evaluate non-carcinogenic health effects. The exposed population will experience a risk if the value of both indexes are greater than one, which means if the exposed dose is greater than the reference dose. The major risk contributor elements due to lettuce consumption were As and Cr, whereas the lowest risk contributor element in the system was Cd. However, the value of both the *HRI* and *THQ* indexes was less than one for all of the lettuce varieties and treatment levels, for the given assumptions. This result shows that the heavy metals health risk from the consumption of lettuce produced in this system was not significant. On the other hand, the heavy metals' bioaccumulation potential varied substantially among the different lettuce varieties. Thus, *Lactuca sativa 'Australische Gele'* has a relatively lower *THQ* value as compared with the other lettuce varieties, and this indicates the opportunity to reduce the health risk caused by heavy metals through the proper selection of plant varieties.

5. Conclusions

The configuration of greywater treatment systems and hydroponic lettuce production as a part of a green wall structure that makes use of urine as a nutrient solution was the unique feature of this study. Considering its distinctive arrangement, this study provides key information about the health risk associated with treated greywater reuse for lettuce production. The integration of a greywater treatment system with green wall technology provides additional environmental benefits through aesthetic appeal and an improved air quality by increasing the oxygen level. Moreover, a green wall may also act as an urban and semi-urban agriculture that can bring various economic and social benefits, including the generation of additional household income, the provision of a good opportunity for healthier community interaction, and the improvement of access to fresh food.

Performing both a microbial and heavy metal health risk assessment on the same subject enable us to observe the most critical risks to prioritize mitigation measures, and at the same time to perceive the health risk in different directions. The results of the QMRA demonstrate the importance of microbial removal to the efficiency of an integrated greywater treatment system and hydroponic irrigation scheme to minimize the health risk below the health-based targets, 10^{-6} DALYs per person per year. Different studies have linked wastewater reuse with the excessive bioaccumulation of heavy metals in the produced crops that could potentially pose both short- and long-term health risks. Contrary to these studies, the heavy metals risk assessment based on the *HRI* and *THQ* indexes did not exceed the permissible level (one), and as a result the health risk concern of consuming lettuce was insignificant. The heavy metal uptake rate of the plant varieties is different: this study reveals that the heavy metal uptake rate of *Lactuca sativa 'Australische Gele'* was relatively low, and the result highlights the importance of having selected plant varieties that uptake a minimum amount of heavy metals in

order to reduce the health risk. Therefore, the selection of potential varieties should be considered in future studies.

By considering all of the the benefits that may arise from this scheme, this study points out some vital health risk minimizing strategies that may potentially further reduce health risks, and these include: (1) improving the microbial and heavy metal removal efficiency of greywater treatment systems through appropriate research approaches; (2) growing plant varieties that have the potential for reduced heavy metal bioaccumulation; and (3) taking regulatory measures on consumable goods that can potentially release heavy metals at a household level.

Supplementary Materials: The following are available online at www.mdpi.com/2073-4441/9/7/454/s1, Figure S1: The appearance of lettuce at different growth stages, A. after three weeks of planting, B. after six weeks of planting, and C. after eight weeks of planting. The left column is treated greywater with (0.3–0.6% urine), the middle column is with (0.15–0.3% urine), and the right column is without urine for each growth stage, Table S1: The average concentration of pathogens in the sewage system assuming 1% of sewage present in the greywater considering *E. coli* as a surrogate for faecal contamination, Table S2: The proportion of essential plant nutrient uptake by lettuce variety, Table S3: Daily Intake rate (DIR) (mg/Kg·day) of heavy metals in different varieties of lettuce.

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References

1. Haruvy, N. Agricultural reuse of wastewater: Nation-wide cost-benefit analysis. *Agric. Ecosyst. Environ.* **1997**, *66*, 113–119. [CrossRef]
2. Angelakis, A.N.; Marecos Do Monte, M.H.F.; Bontoux, L.; Asano, T. The status of wastewater reuse practice in the mediterranean basin: Need for guidelines. *Water Res.* **1999**, *33*, 2201–2217. [CrossRef]
3. Larsen, T.A.; Alder, A.C.; Eggen, R.I.L.; Maurer, M.; Lienert, J. Source separation: Will we see a paradigm shift in wastewater handling? *Environ. Sci. Technol.* **2009**, *43*, 6121–6125. [CrossRef] [PubMed]
4. Jönsson, H. Urine separation—Swedish experiences. *EcoEng Newsl.* **2001**, *1*, 1–7.
5. Otterpohl, R.; Grottker, M.; Lange, J. Sustainable water and waste management in urban areas. *Water Sci. Technol.* **1997**, *35*, 121–133. [CrossRef]
6. Nelson, K.L.; Murray, A. Sanitation for unserved populations: Technologies, implementation challenges, and opportunities. *Annu. Rev. Environ. Resour.* **2008**, *33*, 119–151. [CrossRef]
7. Jhansi, S.C.; Mishra, S.K. Wastewater treatment and reuse: Sustainability options. *Cons. J. Sustain. Dev.* **2013**, *10*, 1–15.
8. Allen, L.; Christian-Smith, J.; Palaniappan, M. *Overview of Greywater Reuse: The Potential of Greywater Systems to Aid Sustainable Water Management*; Pacific Institute: Oakland, CA, USA, 2010; Volume 654.
9. Oyama, N. *Hydroponics System for Wastewater Treatment and Reuse in Horticulture*; Murdoch University: Perth, Australia, 2008.
10. Ginneken, M.; Oron, G. Risk assessment of consuming agricultural products irrigated with reclaimed wastewater: An exposure model. *Water Resour. Res.* **2000**, *36*, 2691–2699. [CrossRef]
11. Timur, Ö.B.; Karaca, E. Vertical gardens. In *Advances in Landscape Architecture*; Intech: Rijeka, Croatia, 2013.
12. Pearen, B.; Wilson, C. Feasibility Study for Green Roof Application on Queen's University Campus. *Queen's Phys. Plant Serv.* **2006**.
13. Feng, H. Lifecycle Based Energy Assessment of Green Roofs and Walls. Ph.D. Thesis, University of British Columbia, Vancouver, BC, Canada, 2013.
14. Järup, L. Hazards of heavy metal contamination. *Br. Med. Bull.* **2003**, *68*, 167–182. [CrossRef] [PubMed]

15. Orisakwe, O.E.; Nduka, J.K.; Amadi, C.N.; Dike, D.O.; Bede, O. Heavy metals health risk assessment for population via consumption of food crops and fruits in Owerri, South Eastern, Nigeria. *Chem. Cent. J.* **2012**, *6*, 77. [CrossRef] [PubMed]
16. Shuval, H.; Lampert, Y.; Fattal, B. Development of a risk assessment approach for evaluating wastewater reuse standards for agriculture. *Water Sci. Technol.* **1997**, *35*, 15–20. [CrossRef]
17. Tanaka, H.; Asano, T.; Schroeder, E.D.; Tchobanoglous, G. Estimating the safety of wastewater reclamation and reuse using enteric virus monitoring data. *Water Environ. Res.* **1998**, *70*, 39–51. [CrossRef]
18. Petterson, S.R.; Ashbolt, N.J.; Sharma, A. Discussion of: Microbial risks from wastewater irrigation of salad crops: A screening-level risk assessment. *Water Environ. Res.* **2002**, *74*, 411. [CrossRef] [PubMed]
19. Petterson, S.R.; Ashbolt, N.J.; Sharma, A. Microbial risks from wastewater irrigation of salad crops: A screening-level risk assessment. *Water Environ. Res.* **2001**, *73*, 667–672. [CrossRef] [PubMed]
20. Oyama, N.; Nair, J.; Ho, G. Recycling of treated domestic effluent from an on-site wastewater treatment system for hydroponics. *Water Sci. Technol.* **2005**, *51*, 211–219. [PubMed]
21. Jackson, S.; Rodda, N.; Salukazana, L. Microbiological assessment of food crops irrigated with domestic greywater. *Water SA* **2006**, *32*, 700–704. [CrossRef]
22. Drechsel, P.; Keraita, B.; Seidu, R.; Abaidoo, R. *Human Health Risks from Wastewater-Irrigated Vegetable Farming*; International Water Management Institute (IWMI): Colombo, Sri Lanka, 2014; pp. 104–115.
23. Gupta, N.; Khan, D.; Santra, S. An assessment of heavy metal contamination in vegetables grown in wastewater-irrigated areas of titagarh, west bengal, india. *Bull. Environ. Contam. Toxicol.* **2008**, *80*, 115–118. [CrossRef] [PubMed]
24. Likuku, A.S.; Obuseng, G. Health Risk Assessment of Heavy Metals Via Dietary Intake of Vegetables Irrigated with Treated Wastewater around Gaborone, Botswana. In Proceedings of the International Conference on Plant, Marine and Environmental Sciences (PMES-2015), Kuala Lumpur, Malaysia, 1–2 January 2015; pp. 32–37.
25. Hossain, M.S.; Ahmed, F.; Abdullah, A.T.M.; Akbor, M.A.; Ahsan, M.A. Public health risk assessment of heavy metal uptake by vegetables grown at a waste-water-irrigated site in dhaka, bangladesh. *J. Health Pollut.* **2015**, *5*, 78–85. [CrossRef]
26. Khan, S.A.; Liu, X.; Shah, B.R.; Fan, W.; Li, H.; Khan, S.B.; Ahmad, Z. Metals uptake by wastewater irrigated vegetables and their daily dietary intake in peshawar, pakistan/pobieranie metali przez warzywa nawadniane ściekami i ich dzienne stężenie w diecie ludności peszawaru, pakistan. *Ecol. Chem. Eng. S* **2015**, *22*, 125–139. [CrossRef]
27. Todt, D.; Heistad, A.; Jenssen, P.D. Load and distribution of organic matter and nutrients in a separated household wastewater stream. *Environ. Technol.* **2015**, *36*, 1584–1593. [CrossRef] [PubMed]
28. Garnier, E.; Shipley, B.; Roumet, C.; Laurent, G. A standardized protocol for the determination of specific leaf area and leaf dry matter content. *Funct. Ecol.* **2001**, *15*, 688–695. [CrossRef]
29. Westoby, M.; Falster, D.S.; Moles, A.T.; Vesk, P.A.; Wright, I.J. Plant ecological strategies: Some leading dimensions of variation between species. *Annu. Rev. Ecol. Syst.* **2002**, 125–159. [CrossRef]
30. Benjamin, L.R. Growth and development | growth analysis, crops a2—Thomas, brian. In *Encyclopedia of Applied Plant Sciences*; Elsevier: Oxford, UK, 2003; pp. 588–595.
31. Ågren, G.I.; Franklin, O. Root: Shoot ratios, optimization and nitrogen productivity. *Ann. Bot.* **2003**, *92*, 795–800. [CrossRef] [PubMed]
32. DuPont, H.L.; Chappell, C.L.; Sterling, C.R.; Okhuysen, P.C.; Rose, J.B.; Jakubowski, W. The infectivity of cryptosporidium parvum in healthy volunteers. *N. Engl. J. Med.* **1995**, *332*, 855–859. [CrossRef] [PubMed]
33. Megraud, F.; Brassens-Rabbe, M.; Denis, F.; Belbouri, A.; Hoa, D.Q. Seroepidemiology of campylobacter pylori infection in various populations. *J. Clin. Microbiol.* **1989**, *27*, 1870–1873. [PubMed]
34. Hall, A.J.; Lopman, B.A.; Payne, D.C.; Patel, M.M.; Gastañaduy, P.A.; Vinjé, J.; Parashar, U.D. Norovirus disease in the united states. *Emerg. Infect. Dis.* **2013**, *19*, 1198–1205. [CrossRef] [PubMed]
35. Usero, J.; Gonzalez-Regalado, E.; Gracia, I. Trace metals in the bivalve molluscs ruditapes decussatus and ruditapes philippinarum from the atlantic coast of southern spain. *Environ. Int.* **1997**, *23*, 291–298. [CrossRef]
36. Tangahu, B.V.; Sheikh Abdullah, S.R.; Basri, H.; Idris, M.; Anuar, N.; Mukhlisin, M. A review on heavy metals (As, Pb, and Hg) uptake by plants through phytoremediation. *Int. J. Chem. Eng.* **2011**, *2011*, 939161. [CrossRef]

37. Hope, B.; Stock, M. *Guidance for Use of Probabilistic Analysis in Human Health Risk Assessments*; Oregon Department of Environmental Quality: Portland, OR, USA, 1998.
38. United States Environmental Protection Agency (US-EPA). Integrated Risk Information System. Available online: https://www.epa.gov/iris (accessed on 14 January 2017).
39. Walpole, S.C.; Prieto-Merino, D.; Edwards, P.; Cleland, J.; Stevens, G.; Roberts, I. The weight of nations: An estimation of adult human biomass. *BMC Public Health* **2012**, *12*, 439. [CrossRef] [PubMed]
40. Chojnacka, K.; Chojnacki, A.; Gorecka, H.; Górecki, H. Bioavailability of heavy metals from polluted soils to plants. *Sci. Total Environ.* **2005**, *337*, 175–182. [CrossRef] [PubMed]
41. Agrawal, S.; Singh, A.; Sharma, R.; Agrawal, M. Bioaccumulation of heavy metals in vegetables: A threat to human health. *Terr. Aquat. Environ. Toxicol.* **2007**, *1*, 13–23.
42. Eregno, F.E.; Tryland, I.; Tjomsland, T.; Myrmel, M.; Robertson, L.; Heistad, A. Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. *Sci. Total Environ.* **2016**, *548–549*, 270–279. [CrossRef] [PubMed]
43. Robertson, L.J.; Hermansen, L.; Gjerde, B.K. Occurrence of cryptosporidium oocysts and giardia cysts in sewage in norway. *Appl. Environ. Microbiol.* **2006**, *72*, 5297–5303. [CrossRef] [PubMed]
44. Myrmel, M.; Lange, H.; Rimstad, E. A 1-year quantitative survey of noro-, adeno-, human boca-, and hepatitis E viruses in raw and secondarily treated sewage from two plants in norway. *Food Environ. Virol.* **2015**, *7*, 213–223. [CrossRef] [PubMed]
45. Pavione, D.; Bastos, R.; Bevilacqua, P. Quantitative microbial risk assessment applied to irrigation of salad crops with waste stabilization pond effluents. *Water Sci. Technol.* **2013**, *67*, 1208–1215. [CrossRef] [PubMed]
46. Mok, H.F.; Hamilton, A.J. Exposure factors for wastewater-irrigated asian vegetables and a probabilistic rotavirus disease burden model for their consumption. *Risk Anal.* **2014**, *34*, 602–613. [CrossRef] [PubMed]
47. Henao-Herreno, L.X.; Lopez-Tamayo, A.M.; Ramos-Bonilla, J.P.; Haas, C.N.; Husserl, J. Risk of illness with salmonella due to consumption of raw unwashed vegetables irrigated with water from the bogota river. *Risk Anal.* **2017**, *37*, 733–743. [CrossRef] [PubMed]

Supplementary Materials

Figure S1. The appearance of lettuce at different growth stage, A. after three weeks of planting, B. after six weeks of planting, and C. after eight weeks of planting, the left column is treated greywater with (0.3% - 0.6% urine), the middle column is with (0.15% - 0.3% urine), and the right column is without urine for each growth stage

Table S1. The average concentration of pathogens in the sewage system assuming 1% of sewage present in the greywater considering *E. coli* as a surrogate for faecal contamination

Microbial pathogens and indicator	Unit	Average concentration in the sewage system [a]	Pathogen concentration in graywater
Cryptosporidium	oocysts/100ml	678.1	6.8
Campylobacter	MPN/100ml	118	1.18
Norovirus	gene copies/100ml	5.1×10^4	5100 (Assume 0.1 % infective virus = 5.1)

[a] Pathogen concentration in the sewage depend on literature value [40-42].

Table S2. The proportion of essential plant nutrient uptake by lettuce variety

Urine in irrigation water (%)	Lettuce Varity	Total C (%)	Total N (%)	Total P (%)	Total K (%)	C-N ratio
0.3 - 0.6	*Lactuca sativa 'Lobjoits Green Cos'*	42,20	4,27	0,33	3,10	9,87
	Lactuca sativa 'Red Salad Bowl'	41,40	4,59	0,36	3,20	9,02
	Lactuca sativa 'Australische Gele'	39,90	4,94	0,39	4,20	8,08
0.15 - 0.3	*Lactuca sativa 'Lobjoits Green Cos'*	41,90	4,55	0,35	3,70	9,21
	Lactuca sativa 'Red Salad Bowl'	42,50	3,74	0,26	2,80	11,37
	Lactuca sativa 'Australische Gele'	40,50	3,91	0,36	3,80	10,37
0	*Lactuca sativa 'Lobjoits Green Cos'*	43,10	1,00	0,12	0,83	43,28
	Lactuca sativa 'Red Salad Bowl'	42,90	0,99	0,18	1,10	43,15
	Lactuca sativa 'Australische Gele'	43,10	1,37	0,25	1,20	31,53

Table S3. Daily Intake rate (DIR) (mg/Kg*day) of heavy metals in different varieties of lettuce

Urine in irrigation water (%)	Lettuce type	As	Cd	Cr	Cu	Mn	Ni	Pb	Zn	Hg
0.3-0.6	a	1.2E-05	1.8E-06	1.3E-04	1.0E-03	2.5E-03	3.1E-04	3.6E-05	4.8E-03	1.8E-06
	b	3.7E-05	1.9E-06	2.3E-04	1.2E-03	3.0E-03	3.3E-04	8.9E-05	5.5E-03	1.7E-06
	c	1.0E-05	2.0E-06	2.3E-04	1.1E-03	2.8E-03	3.5E-04	6.2E-05	5.1E-03	1.4E-06
0.15-0.3	a	8.4E-06	1.9E-06	9.3E-05	1.1E-03	3.3E-03	3.8E-04	2.4E-05	7.2E-03	1.5E-06
	b	1.0E-05	2.4E-06	1.1E-04	9.3E-04	4.0E-03	2.5E-04	2.8E-05	7.0E-03	1.6E-06
	c	1.2E-05	3.3E-06	8.0E-05	1.1E-03	4.6E-03	3.3E-04	3.6E-05	6.9E-03	2.0E-06
0	a	2.0E-05	1.7E-06	7.1E-05	9.2E-04	1.2E-03	2.0E-04	3.9E-05	3.5E-03	7.2E-07
	b	2.0E-05	2.8E-06	1.1E-04	4.0E-03	2.5E-03	2.9E-04	5.9E-05	5.1E-03	1.0E-06
	c	2.5E-05	2.0E-06	1.7E-04	1.9E-03	1.7E-03	3.7E-04	6.8E-05	5.9E-03	1.1E-06

a - Lactuca sativa 'Lobjoits Green Cos' b - Lactuca sativa 'Red Salad Bowl' c - Lactuca sativa 'Australische Gele'

Paper VI

Eregno, F. E., & Heistad, A. (2017). Nutrients, Bacteria and Virus Removal Efficiency of On-Site Treated Greywater Disposal System – Infiltration and Saturated Flow. (Submitted to Water Research)

Nutrients, Bacteria and Virus Removal Efficiency of On-Site Treated Greywater Disposal System – Infiltration and Saturated Flow

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Abstract

The transmission of microbial pathogens from the discharge of partially or fully treated wastewater to different water sources are a pervasive risk to public health. In order to reduce the risk, the integration of source separation, on-site greywater treatment system, and efficient disposal scheme is the major alternative option. Thus, the purpose of this study was to examine the removal efficiency of nutrient and microbial suspension (total coliform bacteria, *Escherichia coli*, and *Salmonella typhimurium* phage 28B) in four different unsaturated infiltration systems and saturated flow condition. For the range of infiltration system tested, column-B (15 cm layer of each, Filtralite, fine sand, and till soil) indicated the highest removal of total coliforms and *E. coli*, 3-4 $\log_{10}$ reduction, whereas, the poorest removal observed in column-C (a layer of 25 cm crushed stone and 50 cm till soil), 2-3 $\log_{10}$ reduction. The average *Salmonella typhimurium* phage 28B removal efficiency also ranges from 1.06 to 1.92 $\log_{10}$ reduction in column-C and column-D respectively. The experiment on virus removal efficiency of infiltration systems during rainfall revealed that the efficiency of the columns reduced from 19 % to 70 %. The overall evaluation indicated that column with 30 cm filtralite at the top and 50 cm quarry waste "subbus" at the bottom (column-D), and filtralite-fine sand-till soil stratified filtration system (column-B) provided better treatment performance without clogging problem within the experimental period. Saturated flow experiment using compacted till soil deposit (5m) with a water retention time of 23 days can significantly eliminate total coliforms and *E. coli*. However, only 1.96 $\log_{10}$ reductions of virus observed and this indicates that careful setback distance should be set to rely on saturated flow condition as efficient treatment barriers for viruses.

Keywords: Filter media; Infiltration system; Non-linear exponential model; on-site greywater treatment

1. Introduction

Centralized wastewater treatment systems for sparsely populated rural communities and recreational cabins are not economically and technically feasible. As an alternative, decentralised on-site wastewater treatment systems are commonly utilized with the intention to treat relatively small volumes of wastewater originated from individual dwellings, a cluster of homes or businesses, institutional facilities and dispose of the effluents in the vicinity close to its source of generation (Wood et al., 2016). There are different types of on-site wastewater treatment systems designed to treat wastewater to various levels before it is disposed into the soil infiltration system (Ho and Anda, 2006; Meinzinger, 2010; Wood et al., 2015). Frequently, the effluent from on-site treatment system is infiltrated through unsaturated soil media and further treated by adsorption, chemical reaction and biodegradation prior to recharge to ground water (Gill et al., 2009; Van Cuyk et al., 2001). On-site treated wastewater disposal systems are usually constructed as a soil infiltration trench with a variety of configurations, receive effluent through the perforated piping system. The type of configurations depends on the level of treatment, the sensitivity of the recipient, the regulation of the country, availability of materials, and hydrogeological setting of the area (Kaseva, 2004; Stevik et al., 2004; Stevik et al., 1999; Van Cuyk and Siegrist, 2007).

Filtration of treated wastewater through a natural soil profile or constructed filter media is the most frequently used post treatment system and it is more often constructed from different materials such as crushed stones, different soil types, and marine sediments (Levine et al., 2008). When treated wastewater filtered through the filter media, the effluent receives further treatment attributed to the interaction of different processes including infiltration and percolation processes coupled with physical, chemical, and biological processes. The removal of microbial pathogens during infiltration can be attributed to straining, adsorption, and inactivation (McCray et al., 2005; Stevik et al., 2004). Generally, the retention and transport of microorganisms in porous media are a function of various factors. These factors include physicochemical property of filter media, clogging, biofilm, microbial cell size and shape, hydraulic loading, moisture content, organic matter content, temperature, pH, ionic strength and species, electrostatic charge on the cell surface, hydrophobicity, chemotaxis, concentration of microbial cell, microbial species and the presence of other microorganisms (Bradford et al., 2013; Stevik et al., 2004). However, there is a large variation in pollutant removal efficiency of filter materials depending on its origin, mineralogy, chemical composition, and physical properties (Chen, 2012; Cucarella and Renman, 2009; Lamy et al., 2008; Seelsaen et al., 2006). These properties include particle size and shape, the porosity of the grains or aggregates define their specific surface area, and surface property (charge). The smaller the particle size, the larger the surface area to undergo high sorption of nutrient and viruses (Reddy et al., 1999). Also, the chemical property of filter media with the pH of infiltrated water determine the affinity or reactivity and the strength of the interaction (Khadhraoui et al., 2002).

When the treated or partially treated wastewater discharge into the unsaturated infiltration system, it deep percolates into sub surface media (unsaturated or saturated depending on the position of water table) and joins the ground water zone. These discharges are the major source of microbial and nutrient contaminants entering into different water bodies that are used as a source for drinking water, irrigation, and fishing (Abdel-Raouf et al., 2012; Geary and Whitehead, 2001; Naidoo and Olaniran, 2013; Tanner et al., 2012). Consequently, increased the concern about the treatment level of on-site treated wastewater disposal system, and saturated sub surface media. This information helps us to establish safe setback distances between on-site disposal fields and drinking water supply sources (e.g., wells, springs, reservoirs), food-growing waters (e.g., shellfish and salmon

farms), irrigation water (e.g., reservoir, ponds, rivers) and recreational water bodies (e.g., lakes, bathing beaches) (Blaschke et al., 2016; Charles et al., 2004; Yates and Yates, 1989). The purpose of defining setback distance is to provide adequate travel time and distance to ensure sustainable removal of contaminants from treated or partially treated wastewater by natural attenuation processes in sub surface media so that the quality of the receiving water is acceptable for specific purposes. However, the current knowledge gap limits the performance of the established setback distance (Blaschke et al., 2016). Therefore, detailed studies are required to address these problems in order to set the safest setback distance and minimize the risk of contamination.

Different infiltration media have a complex ecosystem having heterogeneous physical, chemical, and biological properties, and containing different species of virus, bacteria, protozoa, fungi, micro- and macro- fauna (Birch et al., 2007). Microbes and nutrient removal efficiency of the infiltration media have been studied at laboratory bench scale and such experiments of infiltration media allow a detailed investigation since it is based on well-controlled boundary conditions and its small size allows testing repeating measurements easily. However, it is difficult to extrapolate the significance of results from small laboratory scale to field scale studies (Oxarango et al., 2011). On the other hand, field scale studies are rare (Ausland et al., 2002; Martin et al., 1996) and the experiments are challenged by the interference of unknown factors and diffused boundary conditions. Because of the complexity and high degree of spatial variations as one moves from laboratory scale experiment to field scale experiment, the evaluation of infiltration media efficiency could potentially be affected by different ecological processes (Fellner et al., 2009; Oxarango et al., 2011). Considering the challenges of the two experimental scales (laboratory and field), this study conducted on "medium scale experiment", for both unsaturated and saturated flows. The medium scale experiment is expected to generate comparable results to that of a controlled experimental setup, despite the fact that it is costly, space and time-consuming.

The objectives of this study were: 1) to identify an efficient treated greywater disposal system in removing nutrients, virus and bacteria under different environmental conditions as a post treatment step. 2) to evaluate nutrient, virus and bacteria removal efficiency of saturated flow conditions that could potentially utilize for the computation of safest setback distance between treated greywater disposal site and water sources. The indicator microbial pollutants considered in the study are bacteria (total coliforms and *Escherichia coli),* and model virus (*Salmonella typhimurium* phage 28B). Four different infiltration systems were evaluated for their efficiency in removing nutrients, and microbial pollutants. The results of this study will provide insights about the removal efficiency of different infiltration systems that help to improve treated greywater disposal system design and lead to better assessment of microbial pollution risk of source water from treated greywater discharge.

2. Material and Method

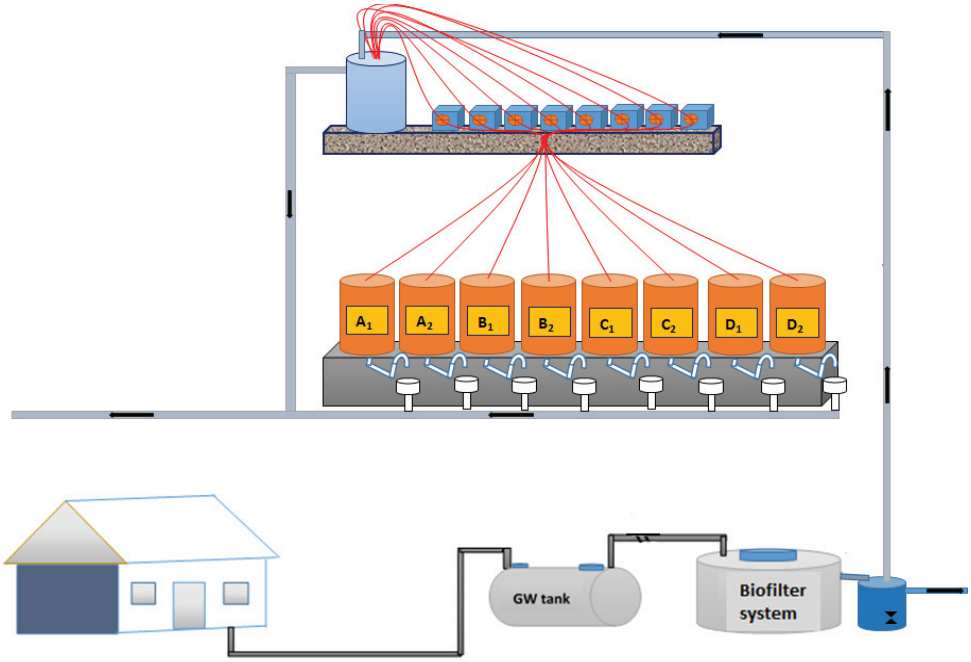
2.1. Unsaturated and saturated flow experimental set up

The source separation wastewater treatment system at Kaya student dormitory in Norwegian University of Life Sciences (NMBU), Ås, collect greywater and blackwater separately and pumped into the Faculty of Science and Technology laboratory for different experiment and the detail of system is described in (Todt et al., 2015). In this experiment, source separated greywater was treated with on-site greywater treatment plant (biofilter system), which consists of a sequence of a primary settler, an unsaturated fixed-film biofilter and a secondary clarifier and then the effluent pumped into unsaturated infiltration column representing infiltration trench in the actual disposal

system. Furthermore, the effluent from the A-columns (A1 & A2) pumped into the saturated trench, which was constructed with compacted till soil (Commonly found in Norway) and intend to mimic saturated groundwater flow conditions. The focus of this study was to investigate microbial pathogens and nutrient removal efficiency of different infiltration systems under unsaturated flow conditions and further evaluate the removal efficiency of saturated flow condition.

2.1.1. Unsaturated flow experimental set up

Unsaturated soil infiltration systems are regularly used for the disposal of on-site treated greywater in order to further treat the effluent through a variety of physical, chemical, and biochemical processes until it percolates into the groundwater. With the intention to investigate the treatment efficiency of soil infiltration systems, which is used as a post-treatment step for on-site greywater treatment system, four different stratified infiltration columns (Column- A, B, C, and D) with two replicate were constructed (Figure 1).



Figures 1. The schematic of source separation, greywater treatment system, and unsaturated flow experimental setup

Each column represents a single hole in the actual perforated disposal pipe that placed on the top of the infiltration trench. A cylindrical polypropylene opaque pipe (length: 100 cm; internal diameter: 30 cm) was used for the construction of infiltration column and the bottom was sealed with the same material. The infiltration media used for the construction of columns in this experiment include till soil (glacial till deposit), crushed granite stone, fine sand, lightweight clay aggregates (filtralite), and quarry waste “subbus”. The crushed stone (11-22 mm) and the quarry

waste “Subbus” (0.2-16 mm) were originated from gneiss and amphibolies, obtained from Vinterbro quarry, Norway. The till soil was excavated from Nordby area, Norway and contain Iren oxide (9.2 g/kg) and Aluminum oxide (2.4 g/kg). Fine sand (0.2-1.0 mm) dominated by silicon dioxide in the form of quartz, and the lightweight aggregate LWA (2-4 mm) (Filtralite, Saint-Gobain Byggevarer AS, Alnabru, Norway) were utilized to form stratification in the infiltration columns (Figure 2). Nonwoven geotextile fabrics were used to separate the layer. The columns were packed up to different height depending up on the specification of each column and care was taken to ensure uniform packing, and at the same time, preferential flow paths were avoided by pouring the media in small quantities during packing.

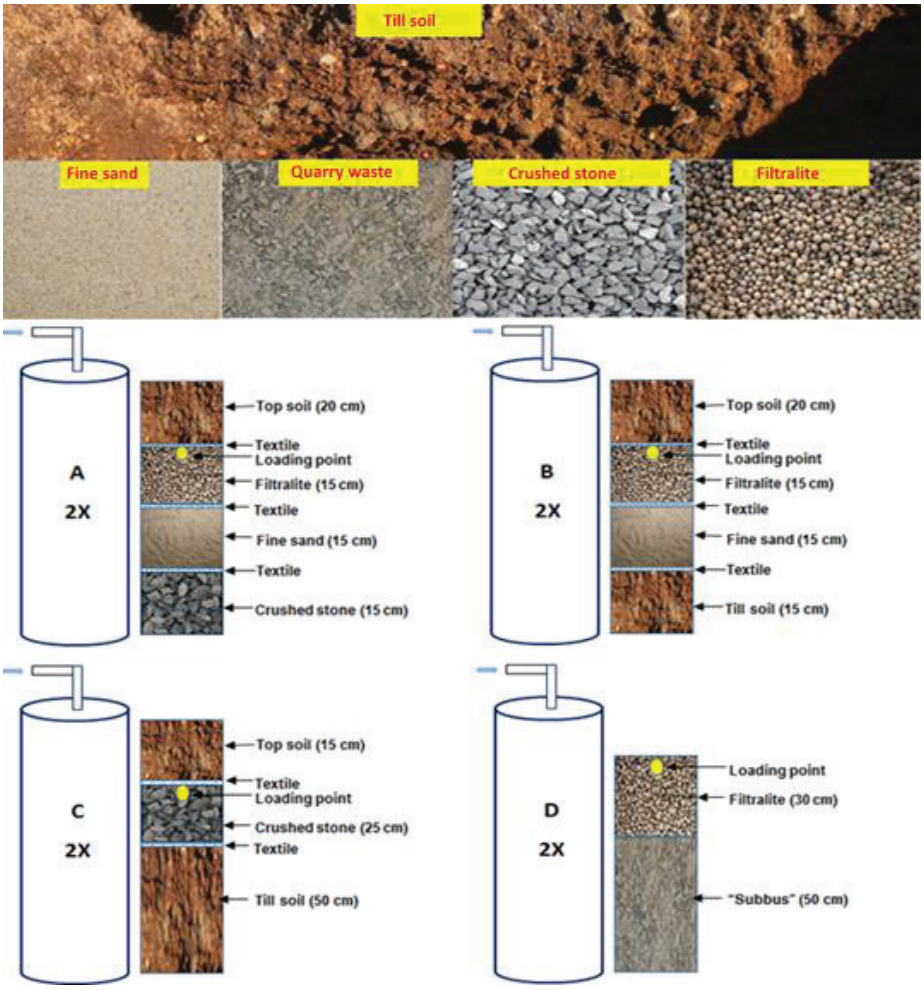


Figure 2. The infiltration media used to pack and the cross-sectional view of the columns

The packing was carried out by compacting with a 3.5 kg flat wooden pole that dropped from 60 cm height. Each column has unique stratification of infiltration media. Column-A comprises three layers, 15 cm of crushed stone at the bottom, an intermediate layer of 15 cm fine sand, and the upper 15 cm of filtralite, which is used to uniformly distribute the hydraulic load. Each layer was separated by geotextile. Column-B contains the same layer as column-A except for the bottom layer, which encompasses till soil, instead of crushed stone. Column-C represents the national standard for the construction of treated wastewater disposal system that consists of 50 cm till soil at the bottom and 25 cm of crushed stone at the top. Column-D is the new trial column constructed by 50 cm bottom layer with quarry waste "subbus" and the top 30 cm pack with Filtralite. The cross-sectional view of infiltration columns and the picture of infiltration media shown in Figure 2.

The effluent from on-site greywater treatment plant (biofilter system) was influent for the infiltration columns and delivered using peristaltic pumps, which was synchronized with the biofilter system-dosing pump that was controlled by the level switch in the primary settling tank and the timer gives the plus interval. The actual flow rate of the peristaltic pumps were 2.5 l h^{-1} with the daily load variation from 37.5 l d^{-1} to 44.8 l d^{-1} depending on the resting time of the treatment plant, which was fluctuating from 6 to 9 hours a day, and resulting 132 to 158 l m^{-2} d^{-2} surface loading rate. The columns were loaded using a plastic tube with inner diameter 6 mm and placed at the centre of the column 7.5 cm deep from the top layer of either Filtralite in the case of column A, B, and D or crushed stone in the case of column C.

The columns were placed in the laboratory room and exposed to a room temperature of 16 to 22 °C during summer and 10 to 15 °C during winter with minimum variation during day and night time. The raw greywater temperature has only minor variations between 18 to 22 ^{o}C due to a heating cable installed with the pipe to avoid freezing during winter. However, the water temperature reduced to 13-15 ^{o}C when it passed through the columns with minimum variation during winter and summer.

2.1.1.1.Characterization of filter media

The particle size distribution of the filter media on a weighted base was analysed in triplicate from each media by standard operating procedure, LS 13 320 Laser Diffraction Particle Size Analyser (Fraunhofer.rf780d optical model, Beckman Coulter, Inc. USA). The grain-size distribution plots were used to estimate D_{10} and D_{60}, which is used to calculate the uniformity of the particle-size distribution (uniformity coefficient, Cu), as the ratio of D_{60} to D_{10}, and the effective grain size of filter media, which is D_{10} strongly correlated with permeability (Figure 3). The higher uniformity coefficient, the larger the range of particle size in the filter media (Alyamani and Şen, 1993). In addition, to secure an adequate hydraulic conductivity and to minimize the risk of clogging, the effective grain size D_{10} should be in the range of 0.3 to 2.0 mm, D_{60} in the range of 0.5 to 8.0 mm, and the uniformity coefficient should be less than 4 (Brix et al., 2001) (Table 1).

As we can see from Figure 3, steep curves, in the case of Filtralite indicate the filter material with a narrow range of particle sizes, poorly graded filter. On the other hand, gentle slope curves, such as till soil, contains a wide range of particle sizes, well-graded filter. The treatment efficiency of filter media increases with decreasing the particle size of the filter media, which indicates the importance of small interstices between particles and larger surface area that allows straining more adsorption to take place (Jenkins et al., 2011). However, having a filter media with too fine grain size will lead to rapid clogging (Nam et al., 2000). Therefore, proper filter size selection is crucial.

Table 1. Particle size distribution measurement for filter material

Particle Size Distribution	Particle size (mm)			
	Filtralite	Quarry waste "subbus"	Till deposit	Fine sand
D_{90}	1.90	5.42	13.30	0.50
D_{60}	1.61	3.47	2.90	0.17
D_{50}	1.48	2.82	1.75	0.15
D_{10}	1.06	0.15	0.15	0.01
Coefficient of uniformity (Cu)	1.5	23.2	18.8	19.0

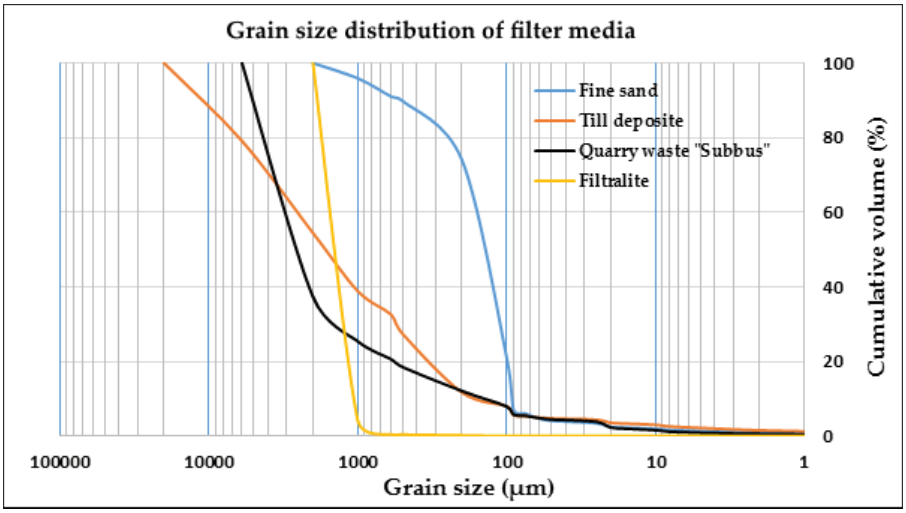


Figure 3. Particle size distribution curve of filter media

2.1.1.2.Tracer test using NaCl for filtration columns

The transport of conservative tracer in the unsaturated infiltration system was studied using sodium chloride (NaCl), which was added only on the initial pulse in the influent (electric conductivity of 950 µS/cm) for an hour. Breakthrough of NaCl was monitored every 20 minutes in the form of electrical conductivity (EC) using EC meter. The tracer transport was characterized by their breakthrough curve, plotting the NaCl concentration at the outlet (µS/cm) against the time taken to travel (Figure 4).

Consistent with the difference in the grain size distribution of infiltrations system and travelling distance (depth of columns), column-C had longer time (360 minutes) to the peak tracer appearance and a lower peak concentration as compared with the other columns. Column-A exhibited a time to peak appearance had shorter (60 minutes) as compared with the other columns but the peak concentration was almost the same as column-B and column-D. The average residence time for the influent in the case of column-D had 160 minutes, relatively lower as compared with the other columns travelling distance (Table 2).

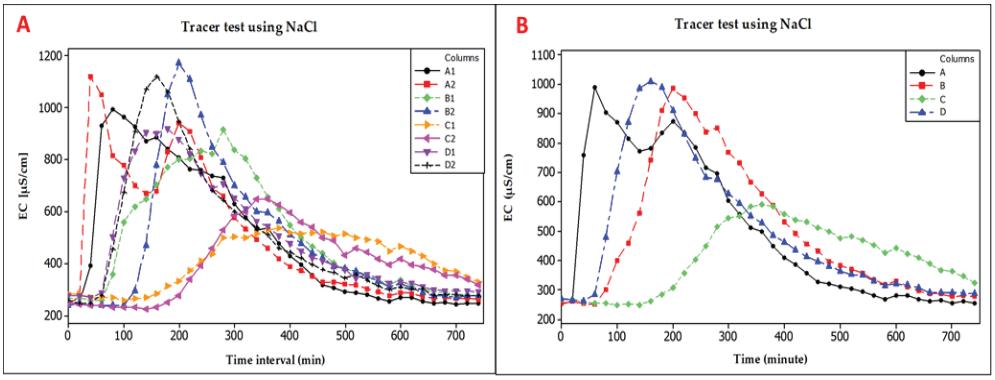


Figure 4. Breakthrough curve for NaCl tracer in columns with different stratified filter media. A) For each replicate column. B) The average value for the representative columns

Table 2. Peak breakthrough concentration and travel time relative to the columns depth

Column	Travel distance (cm)	Peak breakthrough concentration (µS/cm)	Peak breakthrough time (minute)
A1	37.5	995	80
A2	37.5	1120	40
A	37.5	991	60
B1	37.5	916	280
B2	37.5	1170	200
B	37.5	985	200
C1	62.5	538	380
C2	62.5	650	340
C	62.5	590	360
D1	72.5	920	180
D2	72.5	1120	160
D	72.5	1011	160

2.1.1.3.Experimental set up for rainfall impact assessment

The objective of rainfall simulation experiment in unsaturated columns were to test two conditions 1) to test the detachment of virus particles from the infiltration media when virus shedding followed by rainfall after three days. 2) Virus removal efficiency of the infiltration systems when both virus shedding and rainfall happened simultaneously at the same time (rainfall simulation run for 17 hours).

Rainfall simulation experiment was performed by collecting rainwater in a plastic tank. The experiment was carried out by mixing the rainwater with biofilter system effluent (1:3 rainwater to biofilter system effluent) and then pumped into the infiltration columns. The flow rate of the pump was increased from 2.5 lh^{-1} to 3.12 lh^{-1} in order to compensate the additional water that comes from rainfall, considering the same will happen in the actual fields during rainfall.

2.1.2. Saturated flow experimental set up

Medium-scale saturated flow experimental set up of glacial till deposit were constructed to investigate nutrients and microorganisms (total coliform bacteria, *E. coli*, and *Salmonella typhimurium* phage 28B) removal efficiency. The experimental setup mimicked the natural groundwater flow condition by compacting 0.4 m width, 0.4 m height, and 5 m length of the trench filled with glacial till soil and compacted by using a wooden pile uniformly (Figure 5). Three undisturbed core soil samples were taken from three different positions in the experimental trench to characterize the physical property of the soil, such as water retention curve, degree of compaction and saturated hydraulic conductivity. At the same time, disturbed soil samples were analysed for grain size distribution according to the standard procedures of dry sieve analysis using a standard operating procedure, LS 13 320 Laser Diffraction Particle Size Analyser (Fraunhofer.rf780d optical model, Beckman Coulter, Inc. USA).

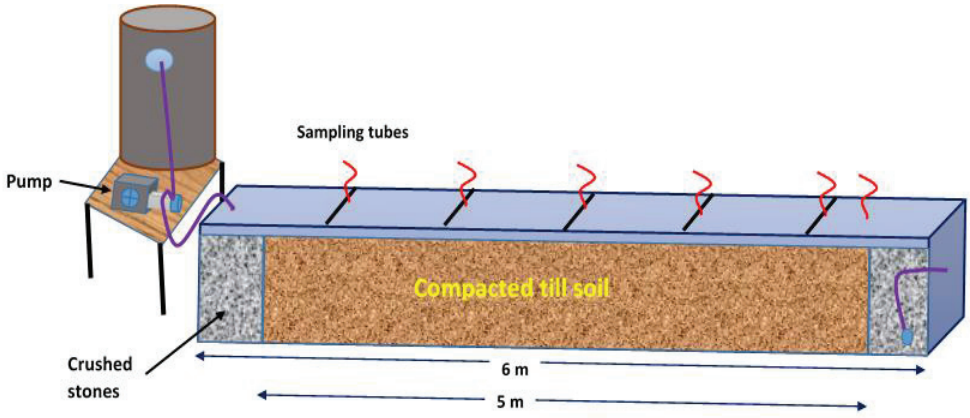


Figure 5. The sketch of saturated flow experimental trench

2.1.2.1.Characterization of the saturated flow media

The undisturbed core soil sample was saturated and then drained at different water suctions (0.2 to 15 bar) using the sand box, ceramic plates and pressure chamber apparatuses and when it reached equilibrium with the applied pressure, the gravimetric soil water content was determined. At the end, the soil sample dried in an oven at 105 °C for 16 hours to determine bulk density, particle density, and volumetric water content. Using suction pressure and volumetric water content, soil moisture retention curve (pF curve) were developed (Figure 6A). Based on the three samples taken from 1.5 m, 2.5 m, and 3.5 m of the saturated trench, the pF curves were not varied substantially and such uniformity confirms that the water flow along the trench was stable. The other important soil property is grain size distribution, which is one of the soil physical properties that affect the hydraulic conductivity of the soil. The curve represents an incremental series of particle sizes from the smallest to the largest in order to show the proportion of the dominant particle size (Figure 6B). If the soil contains a mixture of different grain size, the porosity will be less, and thus the hydraulic conductivity relatively lower.

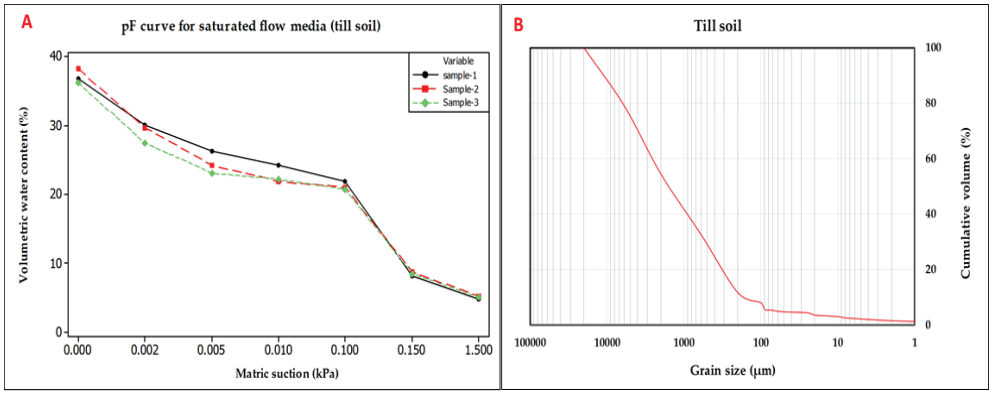


Figure 6. A) Water retention curve (pF curve) for samples1, 2, and 3 taken from 1.5 m, 2.5 m, and 3.5 m in saturated trench respectively. B) Grain size distribution curves of the till soil media.

The saturated flow trench was designed to receive an effluent from unsaturated experimental column-A with the hydraulic load of 11.5 l/day (80 l/m^2 day). An average saturated cross-sectional area of the saturated trench was 0.142 m^2 and the computed water flux was 0.08 m^3m^2/day. Saturated hydraulic conductivity based on Darcy's equation was 5.2 x 10^{-5} m/sec. The computed seepage velocity (v/n), which is commonly used to estimate pollutant transport in the subsoil, was resulted in 0.217 m/day (Table 3). Hence, the required time for microbial contaminants to pass through 5 m trench is approximately 23 days.

Table 3. Different parameter values of saturated flow experimental trench

Parameters	Unit	Average value
Length	m	5
Width	m	0.4
Height	m	0.4
Cros sectional area	m^2	0.16
Average wetted cross sectional area	m^2	0.142
Slope (i)		0.018
Bulk density	g/cm^3	1.71
Particle density	g/cm^3	2.7
Porosity (n)		0.368
Grain uniformity coefficient (Cu)		18.8
Discharge (Q)	m^3/day	0.0115
Flux (v)	m/day	0.08
Seepage velocity (v/n)	m/day	0.217
Hydraulic conductivity (k)	m/s	5.2 x 10^{-5}

2.1.2.2.Tracer test using NaCl for saturated trench

In order to estimate the hydraulic behaviour of the compacted till soil in a saturated flow experimental trench, tracer test experiment was carried out using NaCl by feeding continuously for 20 days. During tracer application, the electrical conductivity of the influent varied from 480 to 492 µS/cm. The tracer concentration as electrical conductivity was monitored at the middle of the trench (2.5 m of the till soil) using EC meter coupled with data logger and monitored continuously (Figure 7). In this experiment, the highest peak tracer concentration (298 µS/cm) occurred at 270 minutes after introduction of the tracer, which coincided with the estimated value based on the computation using seepage velocity (around 11.5 days).

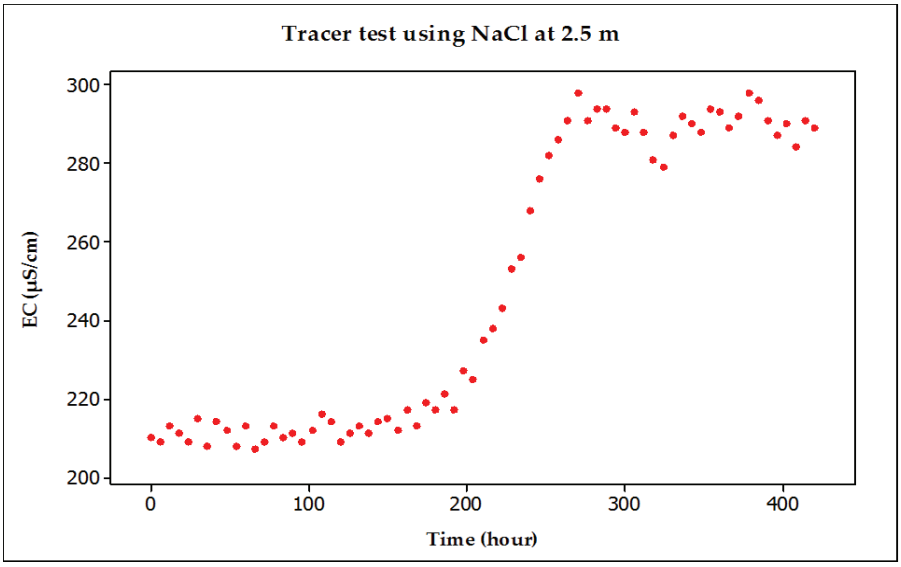


Figure 7. NaCl concentration profiles stated as EC at half way of the trench with elapsed time.

2.2. Microbial water quality analysis

2.2.1. *Salmonella typhimurium* phage 28B

The *Salmonella typhimurium* phage 28B (St28B) (Lilleengen, 1948) was propagated using a host culture of *S. typhimurium* type 5 in nutrient broth and analysed according (Görel Allestam and Carlander, 2000) and described in (Höglund et al., 2002). The growth medium was prepared with distilled water, nutrient broth (0.8 %), and yeast extract (0.05 %). Chloroform (10 ml/l) was added to kill and lyse the host cells after incubation and the suspension was then centrifuged for 10 minutes at 3000 rpm and filtered through a 0.45µm filter. The final concentration of the propagation was 9.8 x 10^9 PFU/ml and this stock kept at 4 ^oC until the time of usage.

The st28b enumeration was carried out using a double-layer agar plaque assay. First, petri dishes with 20 ml solid bottom-agar (growth medium with 1.5 % w/v agar) were prepared. Then, 0.5 ml sample (after serial dilution in 0.9 % NaCl, if needed), 0.5 ml exponential growth-phase host culture, and 4 ml molten top-agar (growth medium with 0.65 % w/v agar) were mixed and poured

over the solid agar in the petri dishes. Finally, samples were incubated at 37 ^{o}C for 18 hours and plaques were counted.

2.2.2. Total coliforms and *Escherichia coli*

Enumeration of total coliforms (TC) and *Escherichia coli* (*E. coli*) were performed using Colilert-18 with Quanti-Tray/2000 (IDEXX Laboratories, USA) using the most probable number method (MPN) according to ISO 9308-2:2012.

2.2.3. Physicochemical water quality analysis

The water samples were analysed for physicochemical parameters using standard methods: pH potentiometric measurement using probe according to NS-EN ISO 10523:2012; Conductivity: electrometric measurement using platina probe according to NS-ISO 7888:1993. For COD, total phosphorus (P), total nitrogen (N) spectrophotometric test kits (Hach-Lange, Berlin, Germany). Total suspended solids (TSS) were determined with 1.2 µm glass fibre filters (Whatman GF-C, GE Healthcare, and Little Chal-font, UK) and turbidity was measured with light scattering measurement at 860 nm.

2.3. Statistical Analysis

The $\log_{10}$ reduction of TC, *E. coli*, and St28B in the infiltration system, between the concentration of column influent and effluent, was calculated as Log_{10} reduction = - Log_{10} (C/C_o), where C is column effluent concentration, C_o is column influent concentration, and the negative sign is to make the reduction positive.

A one-way analysis of variance (ANOVA) with Tukey's post hoc test was used to examine whether $\log_{10}$ reduction of TC, *E. coli*, and St28B in the effluent of representative columns were a function of the infiltration system. The independent variable represented the four different type of infiltration system, columns- A, B, C, and D. The dependent variable was the $\log_{10}$ reduction of TC, *E. coli*, and St28B. An alpha level of 0.05 was used to determine statistical significance for all analyses. All statistical analyses were performed using Minitab 17 statistical software (State College, PA: Minitab, Inc.).

2.4. The removal rates of microbial suspension in saturated flow condition

The observed total coliforms, *E. coli* and St28B were decreasing with respect to a certain flow length, x, and over time, t, in a saturated filter media due to the filter effect, expressed as a filter coefficient, β (m^{-1} or day^{-1}). Removal of microorganisms through porous media can be described by exponential temporal/spatial reduction equation (Equation 1) (Merkli, 1975) and (Equation 2) (Iwasaki et al., 1937).

$$C/Co = \lambda_o \, e^{-\beta t} \quad \text{(eq1)}$$

$$C/Co = \lambda_o \, e^{-\beta x} \quad \text{(eq2)}$$

Where C is the concentration of a microbial suspension at time t and at flow length x, C_o is the initial concentration of a microbial suspension, β is filter removal rate coefficient, t is time and x is flow length. The model uses time-dependent removal coefficient of saturated filter media $\beta(t)$ and flows distance-dependent removal coefficient of saturated filter media or filters efficiency factor $\beta(x)$ (Matthess et al., 1988). The model fitted to the experimental data using nonlinear least squares algorithms to obtain λ_o, and β.

3. Result and Discussion

Nutrient removal efficiency of unsaturated columns

Water quality assessment (raw greywater, biofilter system effluent, and columns effluent) of the system presented in Table 4. The average total phosphorus (P) in the raw greywater was 0.95 ± 0.25 mg/l and the concentration in the effluent of the biofilter system effluent was 34.7 % lower than the influent concentration (raw grey water). Furthermore, P in the effluent of the columns were 73.7 % to 82.1 % lower than the raw greywater concentration. The average concentration of total nitrogen (N) at the columns effluent were 53.0 % to 61.3 % lower than the concentration in the raw greywater. On the other hand, the concentration of nitrate in the raw greywater was very low 0.33 mg/l and it increases to 3.08 mg/l in the biofilter system effluent and further increases from 4.68 to 6.20 mg/l in the columns effluent. The increasing trend of nitrate in the columns effluent could an indication of nitrification, which is the processes of converting ammonia into nitrate in the presence of oxygen and nitrifying bacteria in the unsaturated flow condition. The concentration of BOD in the raw greywater was 137 ± 38 mg O_2/L and 80 % removal was observed in the biofilter system effluent. Whereas, BOD was below the detection limit in all columns effluent. The concentration of COD in the raw greywater was 274 ± 87 mg O_2/L and 90.9 % to 96.1 % was reduced in the column effluent. Suspended solids and turbidity in the raw greywater were highly varied with time and the average suspended solids and turbidity were 32.47 ± 29 mg/l and 40.33 ± 39 FNU respectively. The infiltration column was effective in reducing both suspended solids and turbidity and the reduction reached up to 90.9 % in suspended solids and 87.9 % in turbidity. Pollutants removal efficiency of both on-site greywater treatment plant coupled with unsaturated infiltration systems for effluent disposal were substantial and it should be noticed that the variation in pollutant removal efficiency of columns was because of the difference in filter media; however, there was no single infiltration system universally efficient to remove all pollutants.

Bacteria removal efficiency of unsaturated columns

In this study, the concentration of indicator bacteria in the raw greywater was exhibited high variability and the average concentration of total coliforms and *E. coli* in the raw greywater were 5.4 x 10^6 (±2.8 x 10^6) and 1.0 x 10^6 (±9.2 x 10^6) respectively. The on-site greywater treatment plant (biofilter system) reduced total coliforms and *E. coli* to 3.8 x 10^5 (±5.8 x 10^5) and 9.2 x 10^4 (±1.4 x 10^5) MPN/100 ml respectively. The effluent from the on-site greywater treatment system was used as the influent for the filtration columns. Effluent from each column was collected on a daily basis for four to five days over five periods and the concentration of total coliforms and *E. coli* were assessed and compared with the concentration of the influent in order to determine the removal efficiency of the columns (Table 8). Actually, it is difficult to differentiate between die off, straining and sorption as reduction mechanisms. But, regardless of the mechanisms, the average $\log_{10}$ reduction of *E. coli* ranges from 2.6 to 3.0 in period 1 (T1) and *E. coli* removal efficiency of all columns improved through time and reached from 2.9 to 3.4 $\log_{10}$ reduction in the fourth period (T4), however, the reduction decline to 0.4 to 3.0 at period 5 (T5).

Table 4. Quantified water quality parameters in raw greywater, bio filter system effluent, and columns effluent ($n = 22$)

Water quality parameters	Unit	Statistical value	Raw greywater, Biofilter system effluent, and columns effluent									
			Raw greywater	Biofilter system effluent	A1	A2	B1	B2	C1	C2	D1	D2
P_{total}	mg/l	Mean	0.95	0.62	0.25	0.23	0.13	0.12	0.17	0.17	0.21	0.20
		StDev	0.25	0.23	0.20	0.16	0.06	0.05	0.04	0.09	0.16	0.15
N_{total}	mg/l	Mean	10.4	6.22	4.14	4.06	4.81	4.89	4.06	4.04	4.18	4.02
		StDev	4.38	2.81	2.25	2.34	3.40	3.54	2.37	2.23	2.18	2.21
NO_3-N (Nitrate)	mg/l	Mean	0.33	3.08	4.68	5.08	5.61	6.20	5.61	5.58	5.29	5.26
		StDev	0.03	0.55	0.61	0.63	0.38	0.46	0.27	0.09	0.37	0.30
COD	mg/l O_2	Mean	274	77.8	25.0	19.3	10.7	11.3	23.5	20.2	11.5	12.0
		StDev	87.5	24.4	15.7	7.75	0.59	1.37	11.0	4.30	4.34	3.90
Suspended solid	mg/l	Mean	32.47	14.53	6.63	5.58	3.51	2.94	5.01	5.44	6.78	5.98
		StDev	29.55	9.62	14.03	9.45	4.74	2.86	2.91	6.63	14.19	12.14
Turbidity	FNU	Mean	40.3	20.7	5.26	4.86	5.20	6.60	15.4	14.2	5.69	4.21
		StDev	39.0	20.5	3.73	3.33	2.58	2.76	10.8	7.08	8.60	5.26
EC	µS/cm	Mean	305	319	343	339	324	317	291	287	361	361
		StDev	76.1	90.1	101	88.6	81.1	79.2	83.6	80.0	110	110
pH		Mean	7.04	7.15	7.50	7.55	7.39	7.36	6.95	7.05	7.78	7.83
		StDev	0.20	0.16	0.18	0.21	0.20	0.22	0.25	0.33	0.22	0.16

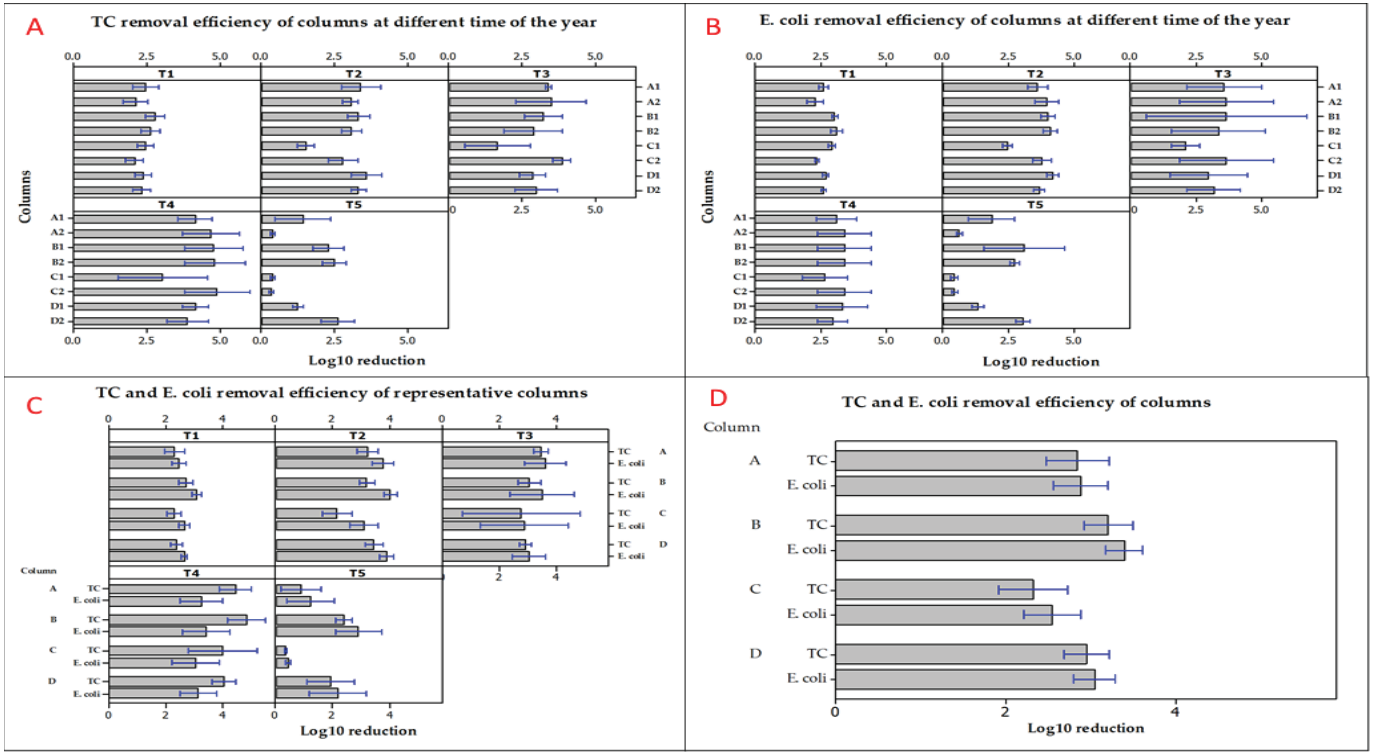


Figure 8. TC and *E. coli* removal efficiency of columns (T1-March, 2016, T2-April 2016, T3-June, 2016, T4-August, 2016, and T5-February, 2017), A) TC removal efficiency of replicate columns at different time of the year, B) *E. coli* removal efficiency of replicate columns at different time of the year, C) TC and *E. coli* removal efficiency of representative columns at different time of the year, and D) Average TC and *E. coli* removal efficiency of representative columns. Error bar represents 95% confidence interval of the mean.

Considerable improvement in total coliform and *E. coli* removal efficiency of columns were noted until the fourth period but at the fifth period after one year, the removal efficiency decline, specifically significant removal efficiency decline was observed in column-A and column-C. It could be explained by biofilm dispersion, which can be happened due to stress, conditions like alteration in nutrient availability, oxygen fluctuations, and increase in toxic products (Kostakioti et al., 2013; Rowe et al., 2010; Sauer et al., 2004), however, to confirm the real causes, further in depth study is required. Relatively, column-B (15 cm layer of each, Filtralite, fine sand, and till soil) showed the highest removal of total coliforms and *E. coli* [3-4 log reduction (99.9 - 99.99%)], whereas, the poorest removal observed in column-C (a layer of 25 cm crushed stone and 50 cm till soil) [2-3 log reduction (99 – 99.9%)]. Thus, the different grain size layer of filtralite fins sand, and till soil in the case of column-B could fever for the formation of biofilm and the order of stratified layer could resist biofilm stress conditions that favour for dispersion.

One-way analysis of variance for log reduction of total coliforms and *E. coli* revealed that at list one column log reduction was significantly different from the others ($p > 0.05$). Post hoc comparison using Tukey test indicates that total coliform and *E. coli* removal efficiency of column B and column C had significantly different.

Virus removal efficiency of unsaturated columns

The virus removal efficiency of columns was investigated under three conditions. 1) the removal efficiency of columns under normal condition, 2) the removal efficiency of columns when virus shedding simultaneously with rainfall, and 3) virus detachment from saturated filter media during the recession due to the effect of rainfall. In order to estimate the inactivation of virus in the infiltration system, St28B, were added to the column influent and the concentration in the effluent was analysed.

Virus removal efficiency under normal condition (without rainfall)

St28B was mixed with treated greywater and continuously feed into the columns for 15 hours and three samples with one-hour interval were taken from the columns effluent after 15 hours in order to calculate the removal efficiency of virus from each column. These experiments were repeated two times, T1-April 2016, and T2-February 2017, with an average inlet concentration of 1.0×10^5 and 4.5×10^5 PFU/ml respectively. The average $\log_{10}$ reduction of St28B during the two periods were 1.8, 1.4, 1.1, and 1.9 in the representative column-A, B, C, and D respectively. The initial (T1) virus removal efficiency of columns have been relatively higher with the variation from 1.4 to 2.4 $\log_{10}$ reduction in the case of column-C2 and column-A2 respectively. While during the second period (T2), the removal efficiency of columns ranged from 0.56 to 1.94 $\log_{10}$ reduction in the case of column-C2 and column-D1 respectively. The virus removal efficiency of all columns was declined during the second period (T2) (Figure 9) and the reduction of removal efficiency could be explained by the saturation of adsorption surface of filter media, due to biofilm dispersion, or a combination of different factors. The infiltration system using quarry west “subbus”, which was dominantly characterised by granite and amphibolite, have been relatively efficient in removing the virus, column-D. However, statistical analysis result showed that there was not a significant difference ($p < 0.05$) in the removal of virus in the columns.

Attachment and detachment of St28B during and after rainfall

This experiment was conducted by mixing rainwater, treated greywater with 1:3 ratio, and added St28B into the mix. At the same time, the pumping rate was increased by 25% in order to compensate the additional rainwater that can infiltrate in the column during the rainfall event.

During this experiment, the EC and the pH measurement of the influent water were 235 µS/cm and 7.02 respectively. In addition, the effluent water temperature ranges from 14.4 to 14.8 ^{o}C in both cases of with and without rainfall experiment. Figure 10 illustrated the St28B removal efficiencies of columns with and without rainfall condition.

The change in influent water chemistry due to rainwater and the higher loading rate due to rainfall resulted in lowering the St28B removal efficiency of the columns. The highest removal efficiency was 1.02 $\log_{10}$ reduction in column-A and the lowest reduction was 0.19 $\log_{10}$ reduction in column-C, however, clogging was observed in column-A1 and that increase the residence time of water as a result the removal efficiency increases. The overall St28B removal efficiency of column A, B, C and D reduced by 19.3 %, 57.7 %, 70.8 %, and 40.4 % respectively as compared with the removal efficiency without rainfall. Of the tested infiltration system, column-D packed with quarry waste "subbus" and Filtralite was the most effective in removing St28B.

Rainfall can adversely affect the performance of treated greywater disposal system by placing an additional hydraulic load on the infiltration scheme, changing active-solid water interface and changing the water chemistry. The effect of rainfall on the detachment of St28B from the infiltration columns was an extension of virus removal experiment. After virus loading ended during virus removal experiment, the concentration of St28B in the effluent of columns were monitored to develop the recession curve by loading treated greywater as the same as the normal influent. At the 79th hour, since the experiment started, rainwater was pumped with the same rate instead of treated greywater for one hour (EC 23 µS/cm and pH 6.94) and the concentration of St28B in the columns effluent were monitored and plotted with the recession and saturated effluent concentration on the same graph (Figure 11). The recession curve in the case of column A1 has gentle slope due to clogging and retarded infiltration, whereas the recession curve of the other columns has a similar pattern. The concentration of St28B after the application of rainfall increases and it could be the sign of detachment enhanced during the application of low ionic strength rainwater. As the EC of the influent water reduced, the surface potential of the adsorbed St28B most likely increased due to the expansion of electrostatic double layer surrounding the virus particle and collector surface, leading to an increase in virus detachment. Then the detached virus particles can be re-inter into the infiltrated water and leached out during rainfall event (Penrod et al., 1996; Quanrud et al., 2003).

Nutrient removal efficiency of saturated trench

The influent for saturated flow experiment was pumped from column-A effluent and raw greywater mixed with a proportion of 10:1 in order to increase the concentration of faecal indicator bacteria's and nutrients in the influent that enable us to detect at different distance in the saturated trench. The experiment was in a steady state flow condition with a continuous inlet flow rate of 11.5 l/day. The trench was placed indoors and exposed to a constant temperature with minor variation and it gives a temperature 14 ± 0.9 ^{o}C for the water sample from the trench. The mean concentration of total phosphorus (P), total nitrogen (N), and COD along with the value of pH, and EC at the inlet, 2.5 m, and 5 m of the experimental trench presented in Table 5. The inlet water was characterized by an average value of pH was 7.5, EC was 315 µS/cm, and the concentration of organic matter was about 58 ± 8 mg/l O_2. The result indicated that nutrient removal efficiency with saturated flow distance was higher in the first 2.5 m as compared with the second half (from 2.5 m to 5 m) and 92.7 %, 84.0 %, and 43.8 % removal of P, N, and COD respectively were observed at the end of the trench (5 m) as compared with the inlet.

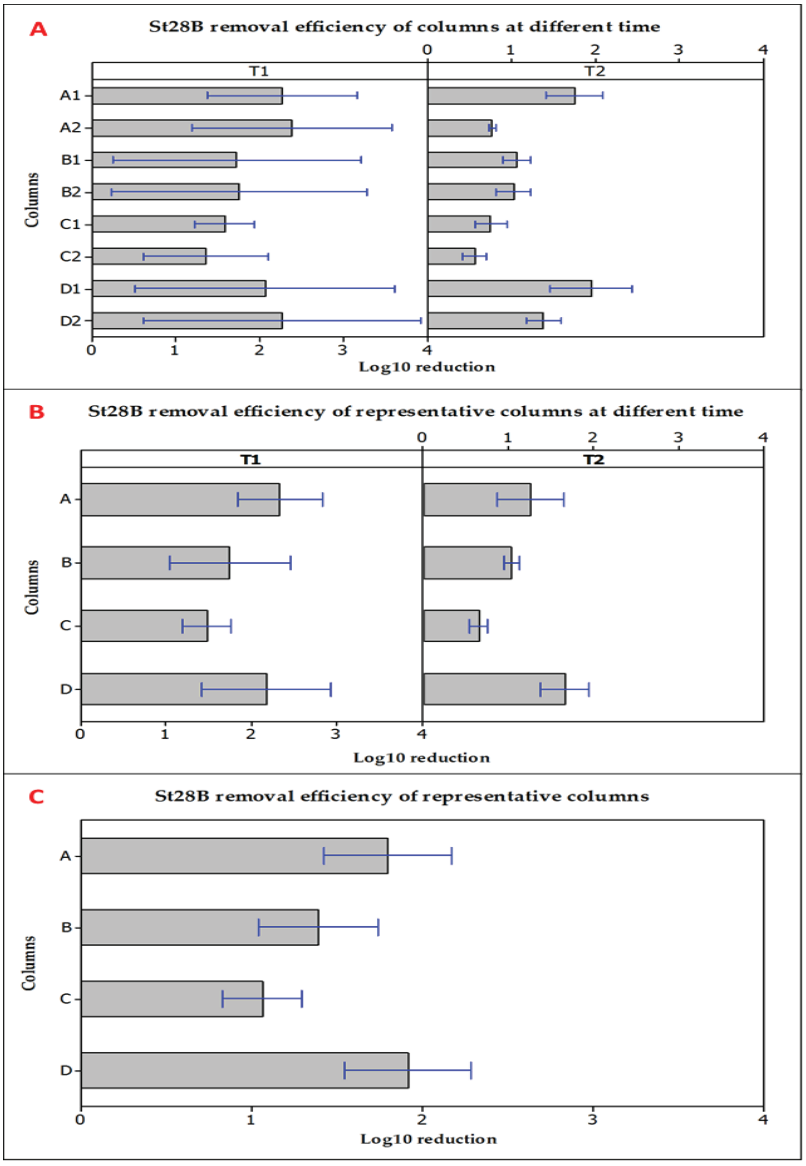


Figure 9. A) Variations in St28B removal efficiency of replicate columns at the different time (T1-April, 2016, T2-February, 2017), B) Variations in St28B removal efficiency of representative columns at the different time (T1-April, 2016, T2-February, 2017), C) Mean St28B removal efficiency of representative columns. Error bar represents 95% confidence interval of the mean.

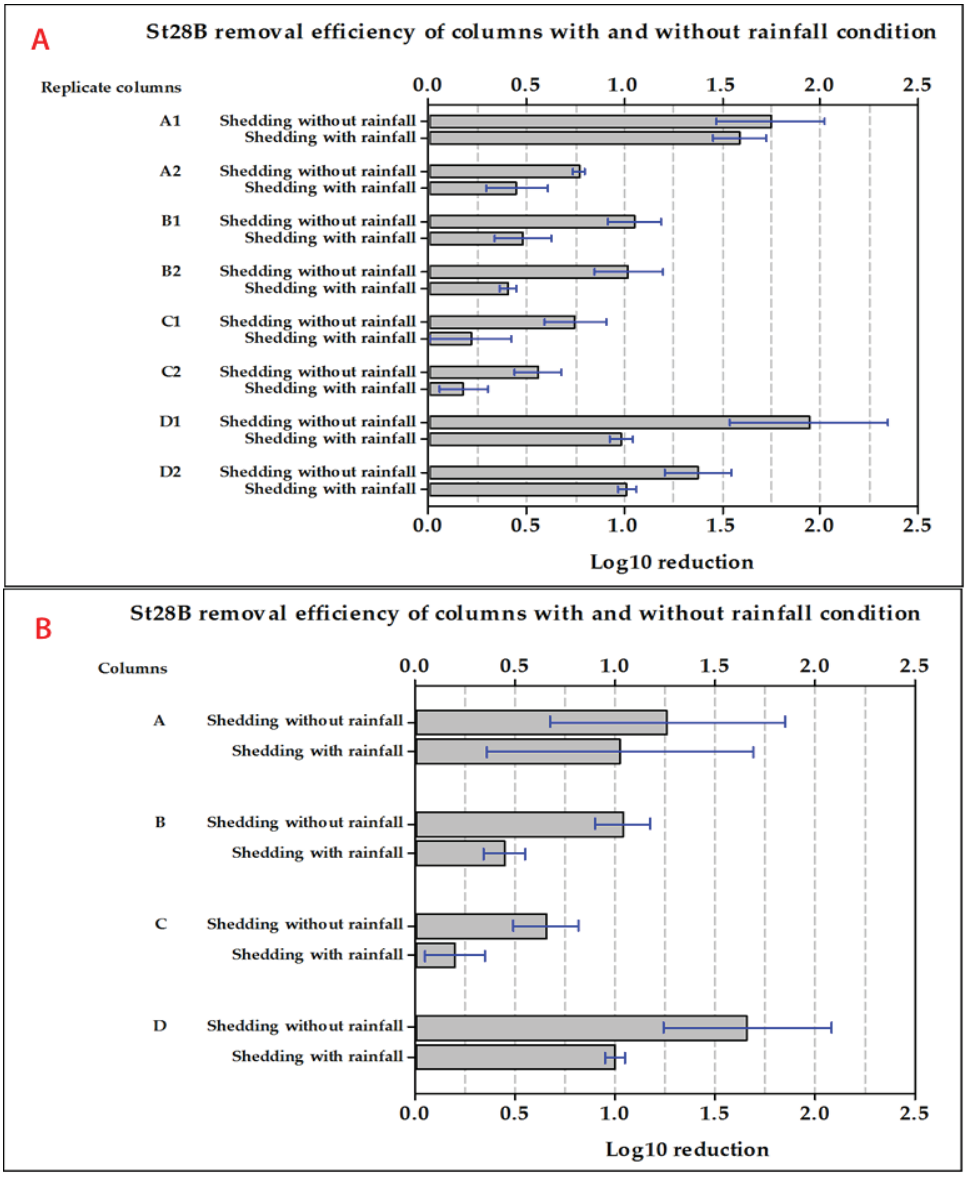


Table 10. Variations of St28B removal efficiency when shedding simultaneously with rainfall and without rainfall in A) replicate columns, and B) representative columns

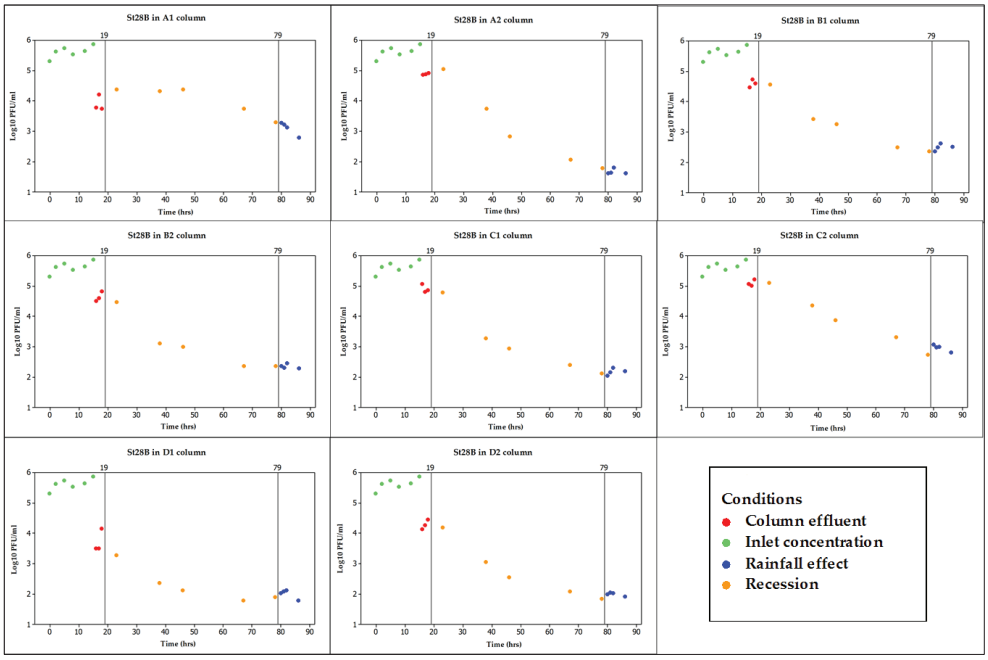


Figure 11. St28B concentration at the inlet of the columns for 15 hours (green dots), St28B concentration in the columns effluent after saturation (red dots), St28B concentration in the columns effluent at the time of recession between 19th to 79th hours (goldenrod dots), St28B concentration in the columns effluent after rainfall application at 79Th hour (blue dots).

Table 5. Water quality parameters values at the inlet, 2.5 m, and 5 m (n = 8)

		Inlet		2.5 m		5 m	
Parameters	Unit	Mean	StDev	Mean	StDev	Mean	StDev
P	mg/l	0.69	0.17	0.14	0.01	0.05	0.03
N	mg/l	3.19	0.99	0.57	0.37	0.51	0.08
COD	mg/l O_2	58.81	8.35	35.58	2.95	33.08	5.21
pH		7.49	0.09	6.63	0.10	6.94	0.14
EC	µS/cm	315	100	388	5.5	394	14.7

Bacteria and virus removal efficiency of saturated columns

The drop in the average concentration of total coliforms between 0 to 5 m in the saturated trench was from 5.16 $\log_{10}$ to 0.33 $\log_{10}$, and the drop in the average concentration of *E. coli* between 0 to 3.5 m was from 4.43 $\log_{10}$ to 0.05 $\log_{10}$ and *E. coli* did not observe after 3.5 m. Moreover, the removal of total coliforms and *E. coli* were more rapid at the beginning and slows down after 0.5 m (Figure 12). On the other hand, the drop in St28B concentration was rather constant over the saturated flow distance of the whole experiment (Figure 12C). The St28B concentration was reduced from an initial concentration of 4.7 $\log_{10}$ to 3.8 $\log_{10}$ at 2.5 m and further reduced to 2.74 $\log_{10}$ at 5 m distance on an average. Therefore, 1.96 $\log_{10}$ PFU/ml reduction of St28B was observed in 5 m saturated trench. For all bacteria and virus, the largest drop in concentration at the beginning of the trench was in the case of *E. coli* (Table 12D).

Spatial and temporal removal rates modelling

Equation 1 and 2 was fitted to the dimensionless concentration (*C/Co*) of total coliforms, *E. coli* and St28B at x distance and t time in the saturated flow condition to obtain λ_o, and β (Figure 13). The time was based on the computation using seepage velocity (v/n), which also coincided with the tracer test result. The estimation of parameters λ_o, $\beta(x)$, and $\beta(t)$ along the flow path and over time assumed steady-state condition and first-order die-off kinetics. The removal mechanisms such as straining, adsorption, and die-off along the length of the flow path and over time denoted by single coefficient β.

In each nonlinear exponential fittings with the data points, the corresponding exponential equation was recorded together with the plots. For total coliforms, *E. coli* and St28B, the computed $\beta(x)$ were 10.81, 10.99, and 1.11 and at the same time, $\beta(t)$ were 2.35, 2.39, and 0.24 respectively. The coefficient indicates the removal rate of total coliforms and *E. coli* was higher than St28B for the same flow distance and retention time. The computed $\beta(x)$ and $\beta(t)$ can be used to calculate the expected removal distance or required retention time between treated greywater disposal system and different water sources, considering the degree of similarity between this experimental setup and the area where we want to extrapolate in terms of hydrogeological settings and physicochemical property of filter media.

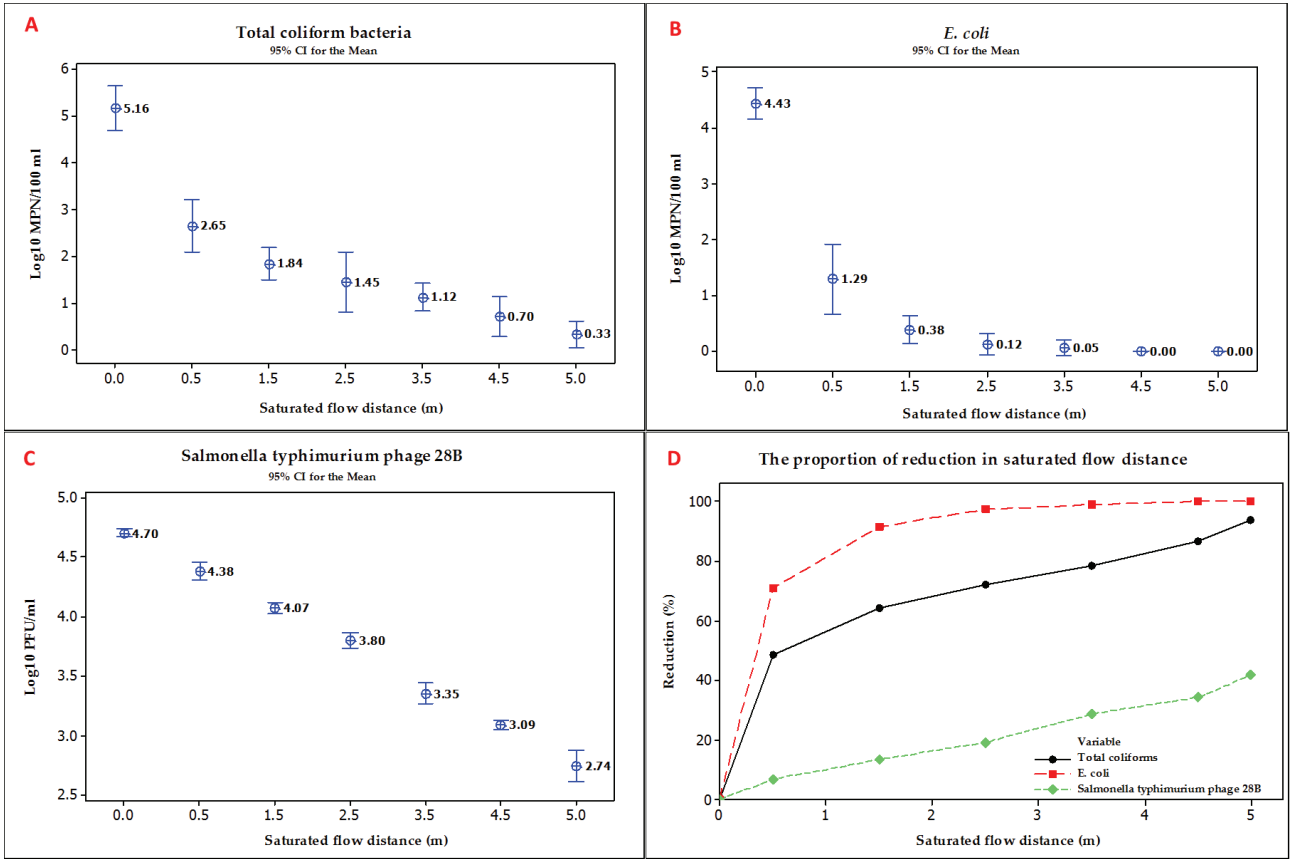


Figure 12. The removal efficiency along the saturated trench, A) total coliform, B) *E. coli*, C) St28B, and D) the relative removal efficiency of total coliforms, *E. coli*, and St28B (cumulative).

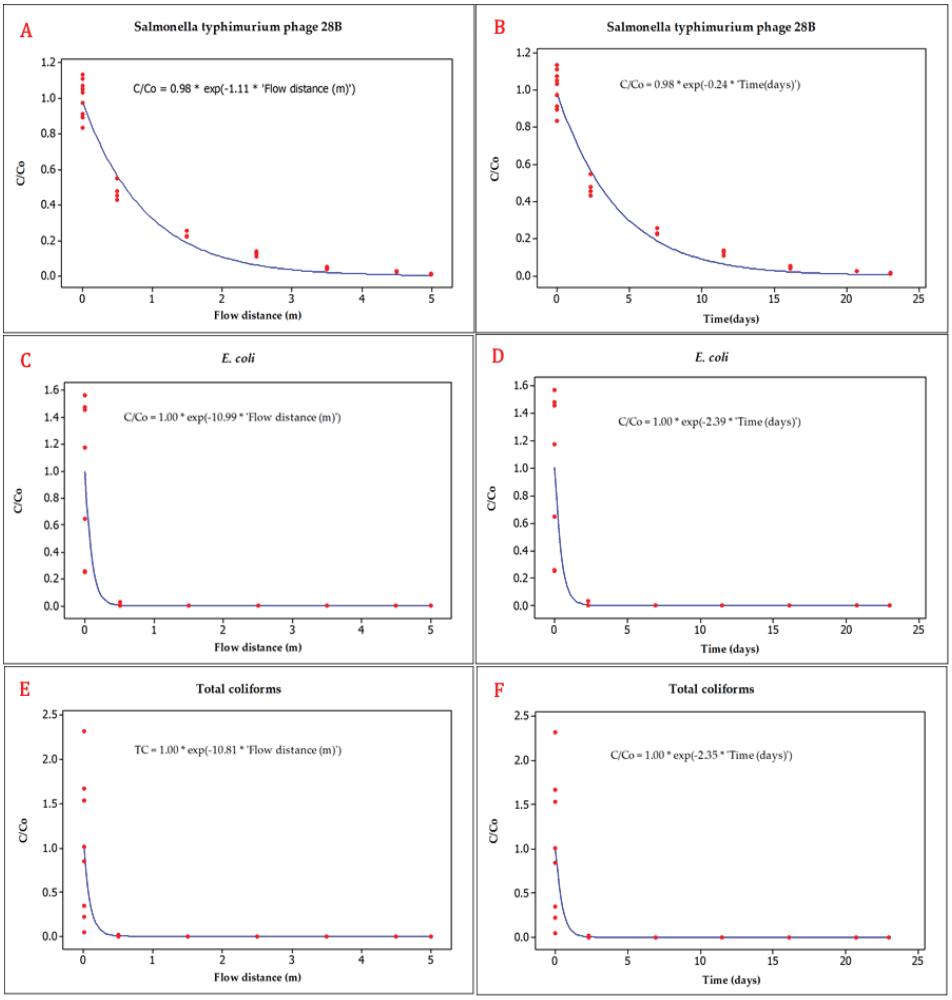


Figure 13. Data fitted 1st order exponential model A) St28B, C*) E. coli*, E) total coliforms, expressed in terms of flow distance, and B) St28B, D) *E. coli*, F) total coliform expressed in terms of retention time.

4. Conclusion

The construction of on-site treated greywater disposal system from selected filter media with different stratification operate as a post treatment step and greatly reduced microbial pathogens, nutrients, organic load and also brought a degree of denitrification. In order to estimate the removal of microbial pathogens and nutrients in unsaturated and saturated flow conditions, it is necessary to conduct the experiment in an appropriate design, scale, and test the performance of different filter media with different stratifications. This study intends to address this issue by considering the Norwegian guideline for the construction of treated wastewater disposal system. Hence, such quantitative information enabled to identify the more efficient treated

greywater disposal system and contribute for the computation of safest setback distance between greywater disposal system and different water sources.

The evidence from this study indicated that on-site treated greywater effluent received significant treatment after infiltrating through different unsaturated infiltration systems. Indicator bacteria removal efficiency of all infiltration systems were improved through time; however, the efficiency suddenly reduced after one-year and the causes for the poor performance of the infiltration systems will need more investigation in the future. Moreover, the removal of total coliforms and *E. coli* in all infiltration system were relatively higher as compare to St28B. The rainfall experiment result demonstrated that St28B removal efficiency of the infiltration systems significantly reduced when virus shedding simultaneously with the rainfall event and the magnitude of efficiency reduction ranges from 19 % to 70 % depending on the infiltration system. For the range of infiltration system tested, column with 30 cm filtralite at the top and 50 cm quarry waste "subbus" at the bottom (Column-D), and filtralite - fine sand - till soil stratified filtration system (Column-B) provided comparably better treatment performance with respect to total coliforms, *E. coli*, St28B, nutrients and organic load removal efficiency without clogging problem within the experimental period.

Total coliforms and *E. coli* removal efficiency in saturated flow trench were relatively high in the front 0.5 meter and then, the removal rate reduced and kept constant along the trench length. While the removal rate of St28B was decreased constantly along the flow length. Saturated flow experiment using compacted till soil deposit (5 m) with a water retention time of 23 days can significantly eliminate indicator bacteria (total coliforms and *E. coli*). However, only 1.96 $\log_{10}$ reduction of virus (St28B) after 23 days retention indicates that careful setup of setback distance will be essential to rely upon the saturated flow as efficient treatment barriers for viruses.

This study recommended properly designed infiltration system as a post treatment step (such as column-B and column-D) coupled with adequate setback distance between the disposal system and different water sources, considering the potential for public health problems that may arise from the discharge of on-site treated wastewater that may release pathogenic organisms into different water sources.

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References

Abdel-Raouf, N., et al., 2012. Microalgae and wastewater treatment. Saudi Journal of Biological Sciences. 19**,** 257-275.

Alyamani, M. S., Şen, Z., 1993. Determination of Hydraulic Conductivity from Complete Grain-Size Distribution Curves. Groundwater. 31**,** 551-555.

Ausland, G., et al., 2002. Intermittent filtration of wastewater—removal of fecal coliforms and fecal streptococci. Water Research. 36**,** 3507-3516.

Birch, A. N. E., et al., 2007. The role of laboratory, glasshouse and field scale experiments in understanding the interactions between genetically modified crops and soil ecosystems: A review of the ECOGEN project. Pedobiologia. 51**,** 251-260.

Blaschke, A. P., et al., 2016. Setback distances between small biological wastewater treatment systems and drinking water wells against virus contamination in alluvial aquifers. Science of the Total Environment. 573**,** 278-289.

Bradford, S. A., et al., 2013. Transport and fate of microbial pathogens in agricultural settings. Critical Reviews in Environmental Science and Technology. 43**,** 775-893.

Brix, H., et al., 2001. Media selection for sustainable phosphorus removal in subsurface flow constructed wetlands. Water science and technology. 44**,** 47-54.

Charles, K., et al., 2004. Quantitative microbial risk assessment: a catchment management tool to delineate setback distances for septic systems. Young Researchers. Water and Environment Management Series. 139-46.

Chen, J. M., Comparative experiment study on the total phosphorus removal efficiency of different infiltration media combinations in the CRI system. Advanced Materials Research, Vol. 415. Trans Tech Publ, 2012, pp. 1735-1739.

Cucarella, V., Renman, G., 2009. Phosphorus sorption capacity of filter materials used for on-site wastewater treatment determined in batch experiments–a comparative study. Journal of environmental quality. 38**,** 381-392.

Fellner, J., et al., 2009. Comparing field investigations with laboratory models to predict landfill leachate emissions. Waste Management. 29**,** 1844-1851.

Geary, P., Whitehead, J., Groundwater contamination from on-site domestic wastewater management systems in a coastal catchment. On-Site Wastewater Treatment. American Society of Agricultural and Biological Engineers, 2001, pp. 479.

Gill, L., et al., 2009. Nutrient loading on subsoils from on-site wastewater effluent, comparing septic tank and secondary treatment systems. Water Research. 43**,** 2739-2749.

Görel Allestam, Carlander, A., Bacteriophages as tracers and hygiene indicator Swedish institute for infectious disease control, Solana, Sweden, 2000, pp. 1-9.

Ho, G., Anda, M., 2006. Centralised versus decentralised wastewater systems in an urban context: the sustainability dimension. 2nd IWA leading edge on sustainability in water-limited environments. IWA Publishing. 81-89.

Höglund, C., et al., 2002. Viral persistence in source-separated human urine. Advances in Environmental Research. 6**,** 265-275.

Iwasaki, T., et al., 1937. SOME NOTES ON SAND FILTRATION [with Discussion]. Journal (American Water Works Association). 29**,** 1591-1602.

Jenkins, M. W., et al., 2011. Bacterial, viral and turbidity removal by intermittent slow sand filtration for household use in developing countries: Experimental investigation and modeling. Water research. 45**,** 6227-6239.

Kaseva, M., 2004. Performance of a sub-surface flow constructed wetland in polishing pre-treated wastewater—a tropical case study. Water research. 38**,** 681-687.

Khadhraoui, M., et al., 2002. The effect of the physical structure of a porous Ca-based sorbent on its phosphorus removal capacity. Water Research. 36**,** 3711-3718.

Kostakioti, M., et al., 2013. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harbor perspectives in medicine. 3, a010306.

Lamy, E., et al., Influence of porous media heterogeneity on flow and pollutant transfer in infiltration basin sub-soils. 11th International Conference on Urban Drainage, Edinburgh, Scotland, UK, 2008.

Levine, A. D., et al., 2008. Pathogen and Indicator Organism Reduction Through Secondary Effluent Filtration: Implications for Reclaimed Water Production. Water Environment Research. 80, 596-608.

Lilleengen, K., yping of Salmonella typhi murium by means of bacteriophage: an experimental bacteriologic study for the purpose of devising a phage typing method to be used as an aid in epidemiologic and epizootologic investigations in outbreaks of typhimurium infection. Bacteriological Hygenical Department, Vol. PhD. Stockholm: Royal Veterinary College, 1948.

Martin, M. J., et al., 1996. Scaling bacterial filtration rates in different sized porous media. Journal of Environmental Engineering. 122, 407-415.

Matthess, G., et al., 1988. Persistence and transport of bacteria and viruses in groundwater—a conceptual evaluation. Journal of Contaminant Hydrology. 2, 171-188.

McCray, J. E., et al., 2005. Model parameters for simulating fate and transport of on-site wastewater nutrients. Groundwater. 43, 628-639.

Meinzinger, F., 2010. Resource efficiency of urban sanitation systems: a comparative assessment using material and energy flow analysis. Technische Universität Hamburg.

Merkli, B., Untersuchungen über Mechanismen und Kinetik der Elimination von bakterien und Viren im Grundwasser. 1975.

Naidoo, S., Olaniran, A. O., 2013. Treated wastewater effluent as a source of microbial pollution of surface water resources. International journal of environmental research and public health. 11, 249-270.

Nam, T. K., et al., 2000. Biofilm characteristics as affected by sand size and location in fluidized bed vessels. Aquacultural engineering. 22, 213-224.

Oxarango, L., et al., Leachate Flows in Landfill: Laboratory and field scale experiments. HPM4 Workshop, Santander, Spain, April 27th-28th, 2011.

Penrod, S. L., et al., 1996. Deposition kinetics of two viruses in packed beds of quartz granular media. Langmuir. 12, 5576-5587.

Quanrud, D. M., et al., 2003. Virus removal during simulated soil-aquifer treatment. Water Research. 37, 753-762.

Reddy, K., et al., 1999. Phosphorus retention in streams and wetlands: a review. Critical reviews in environmental science and technology. 29, 83-146.

Rowe, M. C., et al., 2010. Uropathogenic Escherichia coli forms biofilm aggregates under iron restriction that disperse upon the supply of iron. FEMS microbiology letters. 307, 102-109.

Sauer, K., et al., 2004. Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. Journal of Bacteriology. 186, 7312-7326.

Seelsaen, N., et al., 2006. Pollutant removal efficiency of alternative filtration media in stormwater treatment. Water science and technology. 54, 299-305.

Stevik, T. K., et al., 2004. Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review. Water research. 38, 1355-1367.

Stevik, T. K., et al., 1999. Removal of E. coli during intermittent filtration of wastewater effluent as affected by dosing rate and media type. Water Research. 33, 2088-2098.

Tanner, C. C., et al., 2012. Constructed wetlands and denitrifying bioreactors for on-site and decentralised wastewater treatment: comparison of five alternative configurations. Ecological Engineering. 42**,** 112-123.
Todt, D., et al., 2015. Load and distribution of organic matter and nutrients in a separated household wastewater stream. Environmental technology. 36**,** 1584-1593.
Van Cuyk, S., et al., 2001. Hydraulic and purification behaviors and their interactions during wastewater treatment in soil infiltration systems. Water Research. 35**,** 953-964.
Van Cuyk, S., Siegrist, R. L., 2007. Virus removal within a soil infiltration zone as affected by effluent composition, application rate, and soil type. Water research. 41**,** 699-709.
Wood, A., et al., 2016. Incentivizing decentralized sanitation: The role of discount rates. Environ. Sci. Technol. 50**,** 6146-6153.
Wood, A., et al., 2015. Cost-effectiveness of nitrogen mitigation by alternative household wastewater management technologies. Journal of environmental management. 150**,** 344-354.
Yates, M. V., Yates, S., 1989. Septic tank setback distances: a way to minimize virus contamination of drinking water. Groundwater. 27**,** 202-208.

Thesis Errata Sheet

This sheet lists errors found in the submitted version of Fasil Ejigu Eregno's PhD thesis and the following changes have been included in the thesis.

Page number	Paragraph	Change from	Change to
iii	1st	One of the major challenges is the shortage of quantitative information about the microbial pathogens' concentrations and their inconsistency in water related pathways.	One of the major challenges are the shortage of quantitative data on the concentration of microbial pathogens and inconsistency in water-related pathways.
iv	1st	"….a boat at a 300-meter distance…"	"….a boat up to 300-meter distance…"
iv	2nd	"…risk contributor element due to lettuce consumption was As, Cr and Cu"	"…risk contributor elements due to lettuce consumption are As, Cr and Cu"
xv	1st	"Biological oxygen demand"	"Biochemical oxygen demand"
1	1st	"…in the development of world…"	"…in the development of the world…"
1	1st	"…harmful substances enter into the water bodies, being dissolved in them, lying suspended in the water or depositing on the bed."	"…harmful substances enter into receiving water bodies, being dissolved or suspended in the water or depositing to sediments."
2	2nd	"biological oxygen demand (BOD)"	"biochemical oxygen demand (BOD)"
3	3rd	"…water quality modelling tool depends..."	"…water quality modelling tools depends..."
3	3rd	"On order to understand the present..."	"In order to understand the present..."
5	2nd	"… in the different environment is normally carried out and tells us about …."	"…in different environments are normally carried out and tell us about …"
7	1st	"…to use "index pathogens" or "reference pathogen" that …"	"…to use "index pathogens" or "reference pathogens" that …"
10	2nd	"…microbial pathogens are considerably vary over time"	"…microbial pathogens vary considerably over time"
10	3rd	"…models needed detailed catchment…"	"…models need detailed catchment…"

11	2nd	"Some research conducted on the QMRA for a drinking water production chain from surface water to potable water (Schijven et al. 2011) and quantifying the impact …"	"Some research were conducted on the QMRA for a drinking water production chain from surface water to potable water (Schijven et al. 2011) and to quantify the impact…"
11	3rd	In those beach sites were impacted by point sources of human faecal pollution showed the correlation between increasing incidences of GI illness among bathers, who were exposed to waters with increasing concentrations of FIB.	The beach sites impacted by point sources of human faecal pollution showed increasing incidences of GI illness among bathers, who were exposed to waters with increasing concentrations of FIB.
11	3rd	"However, at beaches were contaminated with non-point sources of FIB, no correlation between FIB densities and GI illness"	"Whereas in beaches contaminated with non-point sources of FIB, no correlation exists between FIB densities and GI illness."
11	4th	"Frequently, the QMRA applied on the recreational coastal water as a complimentary…"	"QMRA can also be applied on recreational coastal water as a complimentary …."
12	3rd	"Compared with others, recycled water could potentially constant water supply throughout the year, acceptable infrastructure and energy consumption costs, and great human benefits"	"Compared to the other alternative water resources, recycled water could potentially provide constant water supply throughout the year, with great human benefits"
15	5th	"…QMRA approach combining with hydrodynamic modelling …"	"…QMRA approach that combines hydrodynamic modelling …"
17	2nd	"…common pathways, this study designed to focus on drinking water source (Paper I), recreational water (Paper II, III, and IV), and recycled water (Paper V and VI) with respect to QMRA framework."	"…common pathways, the study was designed to focus on applying a QMRA framework to a drinking water source (Paper I), recreational water (Paper II, III, and IV), and recycled water (Paper V and VI)."
17	3rd	"There are different opportunities need to be utilized for QMRA, and…)	"There are different opportunities for application of QMRA, and…)
18	2nd	"…treatment could not be sufficient…)	"…treatment may not be sufficient…)

18	3rd	"Overall, this study will be the significant endeavour to enrich QMRA approach …)	"Overall, the goal of this body of research was to enrich the QMRA approach …)
18	3rd	"…techniques coupled with QMRA…"	"…techniques coupled with the QMRA…"
21	2nd	"Paper II and III were…"	"Papers II and III were…"
22	2nd	"Paper V and Paper VI were based on experimental setup on greywater…"	"Papers V and VI were based on an experimental setup for greywater…"
22	3rd	"…namely, Heterotrophic plate count (HPC), *Clostridium perfringens* (*C.perfringens*), intestinal enterococci (IE), *Escherichia coli* (*E.coli*), and …"	"…namely, heterotrophic plate count (HPC), *Clostridium perfringens* (*C. perfringens*), intestinal enterococci (IE), *Escherichia coli* (*E. coli*), and …"
23	2nd	"The main discharge camas from Sandvikselva…"	"The main discharge came from the Sandvikselva…"
23	3rd	"…*E. coli* concentration in each beaches were …"	"…*E. coli* concentrations at each beach were …"
23	4th	"…total organic carbon, turbidity. Zooplankton and algae."	"…total organic carbon, turbidity, zooplankton and algae."
24	3rd	"…culture of *S. typhimurium* type 5."	"…culture of *S. enterica Typhimurium* type 5."
24	3rd	"First, petri dishes with…"	"First, Petri dishes with…"
25	2nd	"…need to conduct QMRA at the beach sites…"	"…need to conduct QMRA for beach sites…"
25	2nd	"…behaviour of the extreme microbial load."	"…behaviour of extreme microbial loads."
25	2nd	"…information gap about microbial water…"	"…information gaps about microbial water…"
25	2nd	"…integrate with the QMRA framework. With the overall intention of improving QMRA framework, the study focus on three different water uses (pathways), through which humans are exposed to agents of water related illness. The study gives special attention to QMRA framework in relation to drinking water source, recreation…"	"…integrate that data within the QMRA framework. The overall intention was to improve the QMRA framework, focussing on three different water uses (pathways), through which humans are exposed to waterborne pathogens. The study gives special attention to QMRA framework in relation to source drinking water, recreation…"