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Hygiene Aspects of Greywater and Greywater Reuse

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Licentiate Thesis

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ABSTRACT

Greywater is domestic household wastewater without input from the toilet, i.e. wastewater from sinks, the shower, washing machine and dishwasher in a home. Source separation of greywater can be a strategy to enhance recirculation of plant nutrients and/or improve water use. The risk for transmission of disease when reusing greywater is largely dependent on the cross-contamination by faeces. High levels of faecal indicators, mainly thermotolerant coliform bacteria, have been reported in greywater, indicating substantial faecal pollution. However, growth of indicator bacteria within the system leads to an overestimation of the faecal input and thus the hygiene risk. The faecal input of the greywater in Vibyåsen, Sollentuna, North of Stockholm, was estimated to be 0.04 ± 0.02 g faeces person⁻¹ day⁻¹ from the quantification of the faecal sterol coprostanol, compared to 65 g, 5.2 g and 0.22 g p⁻¹ d⁻¹ using *E. coli*, enterococci and cholesterol respectively.

Prevalence of pathogens in the population and the faecal load based on coprostanol concentrations were used to form the basis of a screening-level quantitative microbial risk assessment (QMRA) that was undertaken for rotavirus, *Salmonella typhimurium*, *Campylobacter jejuni*, *Giardia intestinalis* and *Cryptosporidium parvum*, looking at the treatment required to be below an acceptable level of risk (10^{-3}) for reuse or discharge of the greywater. The different exposure scenarios simulated – groundwater recharge, direct contact, irrigation and recreational water – showed that a reduction of 0.7 – 3.7 log was needed for rotavirus, with the measured level of faecal load in Vibyåsen. The other pathogen of concern was *Campylobacter*, where a 2.2 log reduction was needed for groundwater recharge. The infectious dose of *Salmonella* is high and the excretion numbers of *Giardia* cysts and *Cryptosporidium* oocysts low, resulting in no treatment requirements for these organisms under these circumstances. Pathogen input from contaminated food via the kitchen sink had a minor effect on the microbiological quality of the greywater. Studies on virus occurrence in greywater as well as validation of the faecal load of greywater at another site would give valuable input for future QMRAs.

Greywater treatment efficiency studies, especially on virus removal, are scarce and more investigations are warranted. Active sludge may not be a suitable technique for greywater due to the low carbon content in this flow. Chemical precipitation has the advantage of removing phosphorus as well as viruses efficiently and it is suggested as one possible method for treating greywater. Otherwise the most common practice for greywater treatment in Sweden is soil infiltration. However, it is suggested that the recommendations for wastewater infiltration also be observed for greywater, despite the low faecal load, due to the simulated results on virus reduction needed.

Key words: greywater, greywater reuse, greywater treatment, microbial risk assessment, groundwater recharge, irrigation, recreational water, faecal contamination, indicator bacteria, index organisms, faecal sterols, bacteriophages, enteric pathogens, rotavirus, *Salmonella*, *Campylobacter*, *Giardia*, *Cryptosporidium*, *Legionella*

LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. Ottosson, J., Stenström, T.A. (2003) Faecal contamination of greywater and associated microbial risks. *Water Research* **37**(3), 645-55
- II. Ottosson, J., Stenström, T.A. (2002) Growth/reduction of micro-organisms in sediments collected from a greywater treatment system. Accepted for publication in *Letters in Applied Microbiology*
- III. Ottosson, J. (2002) Faecal contamination of greywater – a microbial risk assessment. Accepted for oral presentation at the *2nd International Symposium on Ecological Sanitation*, 7th-11th April, 2003 Lübeck, Germany.

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1. INTRODUCTION

1.1. Background

The MISTRA programme Sustainable Urban Water Management has been designed to develop tools and knowledge to guide the planning and management of the future water and wastewater systems in Swedish cities, ultimately answering the central question: How should urban water and wastewater systems be designed and operated in the “Sustainable Sweden” of the future (Urban Water, 2001).

The water and wastewater system consists of its water users, its technical infrastructure and institutions involved and it can be judged by how well it meets chosen aspects of sustainability: health, environment, economy, socio-culture and technical function (Figure 1.1).

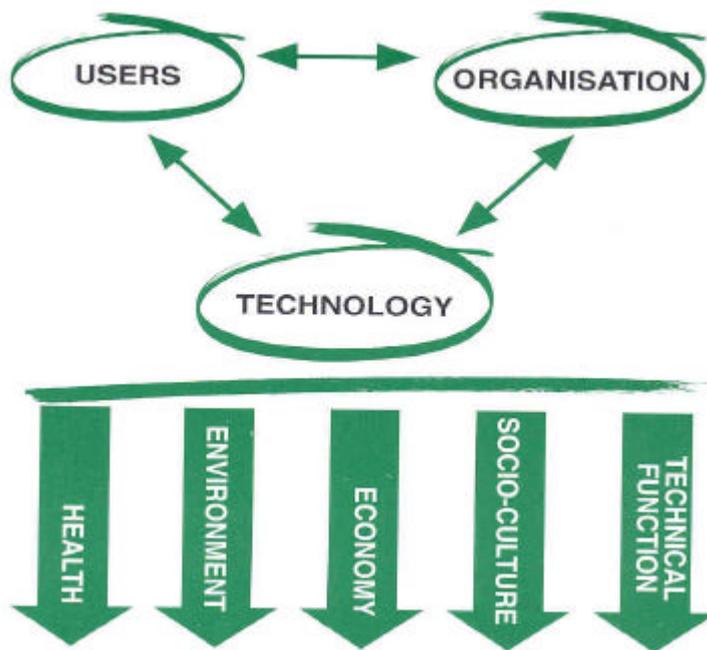


Figure 1.1. The urban water conceptual framework has two parts. At the top is the system with its subsystems: technology, water users and organisation. Below are the aspects of sustainability that will be analysed: health, environment, economy, socio-culture and technical function (Urban Water, 2001).

Sustainability criteria for these five aspects have been defined and a toolbox intended for urban planners, water utilities and other actors involved in the planning of future systems is under development (Urban Water, 2001). This licentiate thesis deals with source separation of greywater as a strategy of sustainable wastewater management providing input to the health criterion.

1.2. Health and hygiene

The overall criterion for the water and wastewater system from a health point of view is that the risk of infection from environmental sources should never exceed a minimal background level. However, this background level might differ for various regions of the world and over time (Urban Water, 2001). In Sweden and other developed areas of the world, wastewater from cities is most often treated at a municipal treatment plant. All water is collected – stormwater from roofs, streets and other paved areas, blackwater from toilets, greywater from kitchen sinks, bathrooms and washing facilities and industrial wastewater - and transported to the treatment plant. The treated water is then released into a receiving water body with enough diluting capacity to take care of the harmful substances and organisms that have not been separated in the wastewater treatment plant. Among these, pathogens are of great concern. In a community or larger city, some citizens are almost certainly suffering from infections and will shed bacteria, parasites and/or viruses of health concern. However, the types of etiological agents and their concentrations will vary due to the epidemiological situation and the input of pathogens from different sources. If there are no technical failures within the system, it is in the receiving water body that potential human exposure first occurs, maintenance workers excluded. Wastewater treatment reduces the number of pathogens, thereby functioning as a barrier. The effluent most often still contains high numbers of harmful organisms. Recreational water is an important exposure point. Cabelli et al. (1982) found appreciable attack rates despite low densities of indicator organisms in the water in an epidemiological study on bathing water exposure. When comparing the disease frequency within the groups swimmers and non-swimmers, the results indicated that swimming in even marginally polluted bathing water is a significant route of transmission. The frequency of gastrointestinal symptoms also had a high degree of association with distance from known sources of municipal wastewater. Several other studies have come to the same conclusion (EPA, 1984; Ferley et al., 1989; Kay et al., 1994), which has been the background for the proposed EU bathing water directive (EU, 2002). An indirect exposure route from a wastewater outlet is shellfish. Filter feeders such as molluscs concentrate contaminated water by filtration and may infect the consumer (Tamburrini & Pozio, 1999). At a mussel-farm used for bioremediation, enteric viruses were found in 50 – 60 % of the mussel samples (Hernroth, 2002). Wastewater may also contaminate surface and ground waters used for drinking water production, an important transmission route but with many potential barriers. When wastewater is treated, plant nutrients (phosphorus) are concentrated in the sludge that is formed in the process. The sludge fraction has been widely used for soil improvement. Sludge handling and run-off are other transmission routes for infectious diseases, although poorly documented (Stenström & Carlander, 1999).

During recent years, growing concern and interest have been directed towards alternative or supplementary solutions to the existing sanitary systems to enhance the recirculation of plant nutrients to arable land and to reduce the demand for water. This is a response to the increased population and overexploitation of valuable resources. One person produces about 500 L of urine and 50 L of faeces per year (= blackwater). The same person uses 20,000 to over 100,000 L of water to flush toilets and for other household activities. Blackwater and

greywater (wastewater excluding toilet flush-water) have very different characteristics. If blackwater is collected separately with low dilution, it can be converted to a safe natural fertiliser at the same time as contamination of receiving waters from pathogens and other pollutants is prevented (Otterpohl, 2002). The main chemical pollutants are generally found in wastewater from industries, road run-off (Balmer, 2001) and in the greywater from households (Naturvårdsverket, 1995). Still, the interest in reusing greywater has increased dramatically in recent years, especially in arid areas. In some densely populated areas such as Singapore and Tokyo, greywater reuse is already a common practice (Asano & Levine, 1996; Jeppesen, 1996; Trujillo et al., 1998; Dixon et al., 1999; Shrestha et al., 2001).

Source-separation of wastewater has benefits but it also leads to new pathways of disease transmission to humans and domestic animals depending on the solutions. The desire for recirculation of waste streams to arable land raises questions about the relative impact of different transmission routes for pathogens in the environment, such as contamination of groundwater or inhalation of contaminated aerosols formed during wastewater (greywater) irrigation. Quantitative information related to the factors that influence survival and transmission of pathogens is limited and thus the hygiene effects of the new application areas of different waste streams need to be estimated. The limitation in sensitivity or the lack of epidemiological studies on these transmission routes has led to the adoption of microbial risk assessment (MRA) as a supplementary method to assess the microbial risks associated with urban water systems.

1.3. Greywater

Greywater is defined as household wastewater not including toilet waste, i.e. wastewater from sinks, showers, washing machine and dishwasher in a home. The average flow is 150 L person⁻¹ day⁻¹ (Swedish design value (Naturvårdsverket, 1995)). As a consequence of changed habits and water-saving equipment, recent measurements at three different sites have led to a proposed new design value of 100 L p⁻¹ d⁻¹ (Vinnerås, 2002). The separation of blackwater (toilet waste) results in a greywater with low faecal contamination and with less eutrophying substances, thus simplifying local treatment and reuse. In Sweden, greywater is seen as a contaminated waste flow due to the high metal content compared to plant nutrients (Vinnerås, 2002). The load of other adverse chemical compounds is also significant. Eriksson et al. (2002) identified 900 xenobiotic organic compounds (XOCs) as potentially present in greywater. Despite the fact that greywater has a rather low nutrient status, it is considered to be a valuable resource, sometimes in combination with rainwater (Albrechtsen, 1998; Dixon et al., 1999). Potential reuse options are listed in Table 1.1. However, to meet proposed guidelines extensive treatment is needed and in some places greywater reuse is still illegal (Trujillo et al., 1998). Whether a system is built to discharge the water or to reuse it, hygienic risks have to be considered.

Table 1.1. Considered options for greywater reuse

Reuse	Reference
Groundwater recharge	(Asano & Levine, 1996)
Toilet flushing	(Bingley, 1996; Christova-Boal et al., 1996; Dixon et al., 1999; Hills et al., 2000; Nolde, 1999; Santala et al., 1998; Shrestha et al., 2001)
Irrigation (of gardens, crops, parks, golf courses etc.)	(Al Jayyousi, 2002; Asano & Levine, 1996; Christova-Boal et al., 1996; Jeppesen, 1996; Okun, 1997; Shrestha et al., 2001; Trujillo et al., 1998)

1.3.1. Pathogens in greywater

In the hot-water system in a building, some opportunistic pathogenic bacteria may grow and thus create a risk of infection. However, this individual risk is probably higher from direct exposure to the tapwater than from exposure to the reused water. The main hazard of greywater emanates from the faecal cross-contamination. However, since toilet waste is not included in greywater, faecal contamination is limited to activities such as washing faecally contaminated laundry (i.e. diapers), childcare, anal cleansing and showering. Faecal contamination has historically been measured by the use of the common indicator organisms such as Coliforms (1.5.1) and Enterococci (1.5.2). These have also been applied for assessing faecal contamination of greywater. Some studies have reported high numbers of these organism groups, which would indicate substantial faecal contamination of the greywater (Table 1.2).

Table 1.2. Reported numbers of indicator bacteria in grey wastewater [$\log_{10}/100\text{mL}$]

Wastewater origin	Total		Thermotolerant		Reference
	Coliforms	Coliforms	<i>E. coli</i>	Enterococci	
Bath, hand basin			4.4	1.0-5.4	(Albrechtsen, 1998)
Laundry	3.4-5.5	2.0-3.0		1.4-3.4	(Christova-Boal et al., 1996)
Shower, hand basin	2.7-7.4	2.2-3.5		1.9-3.4	(Christova-Boal et al., 1996)
Greywater	7.9	5.8		2.4	(Casanova et al., 2001)
Shower, bath	1.8-3.9	0-3.7		0-4.8	(Faechem et al., 1983)
Laundry, wash	1.9-5.9	1.0-4.2		1.5-3.9	(Faechem et al., 1983)
Laundry, rinse	2.3-5.2	0-5.4		0-6.1	(Faechem et al., 1983)
Greywater	7.2-8.8				(Gerba et al., 1995)
Hand basin, kitchen sink		5.0		4.6	(Gunther, 2000)
Greywater		5.2-7.0	3.2-5.1		(Lindgren & Grette, 1998)
Greywater, 79% shower	7.4	4.3-6.9			(Rose et al., 1991)
Kitchen sink		7.6	7.4	7.7	(Naturvårdsverket, 1995)
Greywater		5.8	5.4	4.6	(Naturvårdsverket, 1995)

However, greywater may have an elevated load of easily degradable organic compounds, which may favour growth of enteric bacteria such as the faecal indicators. Such growth has been reported in wastewater systems (Manville et al., 2001). Hence, a focus on bacterial indicator numbers may lead to an overestimation of faecal loads and thus risk. Occasionally enteric pathogenic bacteria, such as *Salmonella* and *Campylobacter*, can be introduced by

food handling in the kitchen (Cogan et al., 1999). Again the individual risk is higher from the direct handling of the contaminated food, but limited to only one or a few exposed persons in the individual household, whereas a number of people will be exposed to the reused water. There is also a risk of regrowth of pathogenic bacteria within the greywater system.

1.3.2. Greywater treatment

A range of treatment alternatives, mainly small-scale, has been used for greywater. The most common is to have a settling tank followed by an infiltration unit. Other techniques that are developed for water and wastewater treatment can also be adopted for greywater treatment.

Settling tanks

In settling tanks the water is mechanically treated. Larger particles are allowed to settle due to gravity. Pathogens can be attached to these particles and thereby be removed. The pathogen removal in settling tanks is, however, generally poor (Stenström, 1986). To enhance phosphorus removal, chemicals can be added forming flocks that settle more easily. This also improves the microorganism removal and the reduction was 93-99.6% for indicator organisms in Swedish wastewater treatment plants in the chemical-mechanical process (Stenström, 1986).

Active sludge treatment

In active sludge treatment, bacteria digest organic material in the wastewater, reducing the organic material, measured as BOD and COD, significantly. In the process, nitrogen, phosphorus, inorganic substances and pathogens are also reduced. The pathogen reduction is due to competition, digestion and sedimentation. In Swedish wastewater treatment plants, a 53-98% reduction in different microorganisms was observed in the active sludge treatment process (Stenström, 1986). The greywater treatment system in Vibyåsen has active sludge (Puracomb®) as part of the treatment (I). However the low content of carbon in Swedish greywater (Naturvårdsverket, 1995; Gunther, 2000) does not favour active sludge treatment.

Infiltration units

The most common way to treat greywater in Sweden is by infiltration of the water after it has been allowed to settle. After leaving the settling tank, the effluent is distributed evenly over a subsurface disposal system. Sand filters, consisting of 60-150 cm of sand (or other media) in a bed, improve the quality further. Intermittent dosing of the grey wastewater is mainly applied (Stevik et al., 1999a). However, there are sites where infiltration systems may endanger groundwater quality. Suitable sites for infiltration have deep, well-drained, well-developed, medium-textured soils (such as silty loam and loam). At least 3 m unsaturated soil beneath the disposal system is recommended to adequately remove pathogens and organic matter from wastewater (Naturvårdsverket, 1985). Disposal systems that are downslope and far from water wells will better protect possible water supplies from contamination. At least 2 months retention time in the saturated zone is recommended (Naturvårdsverket, 1985). Impermeable soils, shallow rock, shallow watertables, or very permeable soils such as coarse sand or

gravelly soil are considered unsuitable sites. Normally, soil infiltration is an adequate wastewater treatment method (Stenström, 1985).

Sand filter trenches, biofilters and constructed wetlands

If the water from the infiltration unit is collected for further treatment, reuse or released into a receiving water body, the terms sand filter trenches, biofilters or constructed wetlands are used. The latter are planted, forming a root-zone for direct uptake of water and plant nutrients (Figure 1.2).

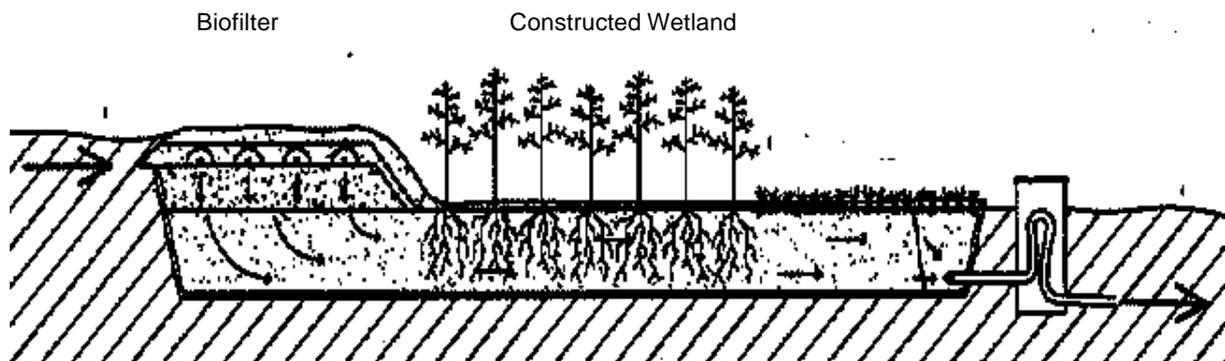


Figure 1.2. A subsurface flow wetland with integrated biofilter (Jenssen, 2001).

Biofilters and constructed wetlands using light-weight aggregates were pioneered in Norway (Jenssen, 2001). High removals of indicators have been observed during intermittent filtration with hydraulic loading rate, media grain size and retention time being the most important factors (Stevik et al., 1999a; Stevik et al., 1999b; Ausland et al., 2002). Pretreatment in a biofilter aerates the wastewater and reduces BOD and bacteria, so higher loading rates can be obtained for the subsequent wetland or infiltration system (Jenssen, 2001).

Membranes

Membrane processes use a semipermeable membrane and osmotic or other pressure differential to force water across the membrane as permeate, with dissolved solids or other constituents captured as retentate or concentrate. Membranes are often made of organic polymers, but new types of inorganic polymers as well as ceramic and metallic membranes are under development. The basic membrane systems include microfiltration, ultrafiltration, nanofiltration and reverse osmosis (RO), each of which retains a different range of particle sizes (Figure 1.3) (EPA, 2001).

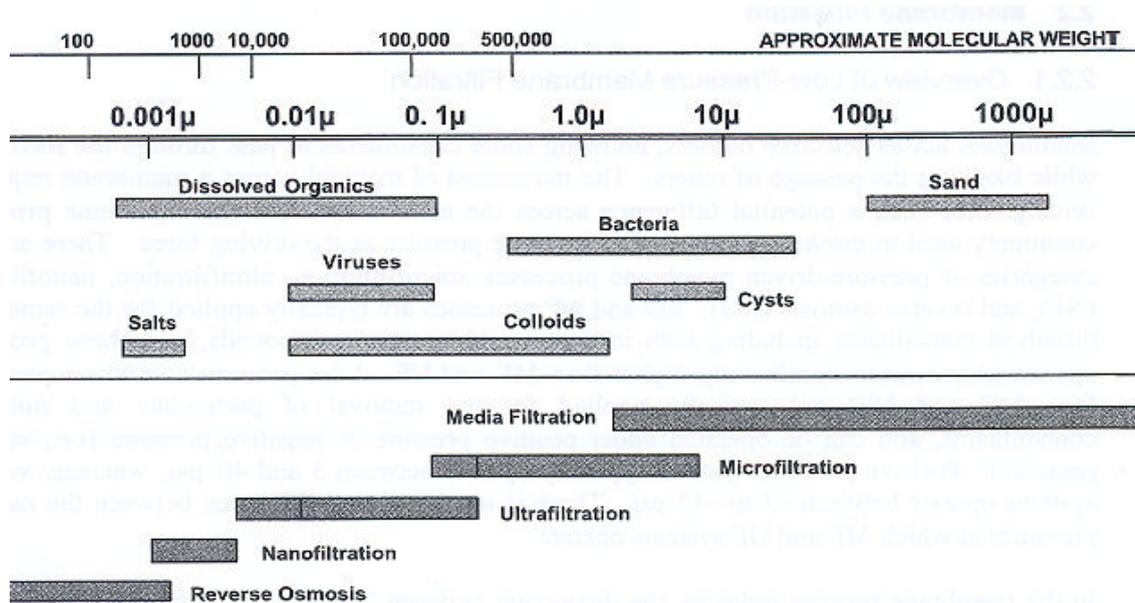


Figure 1.3. Membrane process classification (EPA, 2001).

Membrane bioreactors (MBRs) have several advantages over other conventional wastewater treatment technologies, including lower energy use, smaller space requirements, better control of microbes and organic matter in the process effluent, giving an improved water quality (Jefferson et al., 2001). Madaeni (1999) reported 7 log removal of polioviruses and 5 log removal of coliphages in an RO process. One major disadvantage with membrane treatment is fouling as a result of material build-up, blocking fluid flow across the membrane. Reverse osmosis is particularly susceptible to blockage and therefore requires pre-treatment of the wastewater before the RO-process (EPA, 2001).

Ponds

Ponds can be used either to treat the water or to polish it after other types of prior treatment. The reduction in pathogens is dependent on system design, retention time and dilution (Mezrioui et al., 1995; Raangeby et al., 1996). In Sweden, the climate is not favourable for pond treatment. Since virus particles do not seem to settle as easily as bacteria and protozoa (Walker et al., 1980; Westrell, 1997; Stenström & Carlander, 2001), UV and/or temperature mediated inactivation is important (Gantzer et al., 1998). In a greywater separating system in Kalmar, the water is treated in three consecutive ponds and with a total retention time of about one year, including at least one summer period (Gunther, 2000). In the investigated area Vibyåsen, the pond reduced somatic coliphages (bacterial virus) and *E. coli* by 1.2 log and 3 log respectively. This was more than the settling tanks, active sludge treatment and biofilter together (I).

Disinfection

Disinfection is used to eliminate many types of pathogens in sewage effluent. Chlorination has traditionally been the most common disinfection method. It is effective against most, but not all, pathogens. The protozoan oocysts especially have been shown to be resistant to

disinfection by chlorine (Robertson et al., 1994). However, chlorine can be toxic to aquatic life. Ozonation works in a manner similar to chlorine disinfection (a reactive gas is introduced into the wastewater stream to chemically disinfect it), but avoids the concern of introducing chlorinated organic compounds into the wastewater stream. Ozonation is further more effective in inactivating protozoan (oo)cysts than chlorine compounds (Robertson et al., 1994). However, disinfection is not a method practised to treat greywater in Sweden. One exception is ultraviolet disinfection, which can be used especially in low-concentration wastewater streams to permit reuse of the water. It is of particular interest in water recycling applications, where pathogenic contamination is the primary obstacle to recycling of the water stream. The AquatronTM separates the liquid from the solid part of the toilet waste by centrifugal forces. The liquid flow then enters the greywater stream after UV disinfection. This system is used in Ekoporten, Norrköping, with a subsequent treatment in a settling tank (1.6.1) and a constructed wetland (1.6.4). However, the UV unit did not disinfect the toilet flushwater properly due to the amount of suspended solids. The suggestion is therefore to move it further down the system, after the constructed wetland (Lindgren & Grette, 1998). Similar problems with UV-disinfection have been reported from other sites (Westrell, 1997).

1.4. Microbial Risk Assessment (MRA)

Risk assessment is a tool that has been used to assess chemical risks for some time and it is now becoming more frequent as a tool for measuring microbial health effects on a population. MRAs were first developed for drinking water (Regli et al., 1991) but have lately been applied to other practices such as reuse of human urine (Höglund et al., 2002) and discharge to recreational waters (Ashbolt et al., 1997). At a screening level, MRA is a valuable tool to initially estimate risks even when the set of data is poor, giving the potential to make rational decisions at far less cost than epidemiological studies. Sensitivity analyses are made in order to obtain information on knowledge gaps and critical control points. Besides giving information on where to focus preventive measures within the risk management strategies, they also help to direct research in order to fill identified information gaps (Ashbolt, 1999). Whether the risk assessment is of a chemical or microbiological nature, the procedure contains four primary elements: 1) Hazard identification, 2) Exposure assessment, 3) Dose-response assessment and 4) Risk characterisation.

1.4.1. Hazard identification

In the hazard identification step, background information on the pathogens in a specific system is described. It also includes the spectra of human illness and disease associated with the identified microorganisms (Haas et al., 1999). For the greywater system, the hazard emanates from faecal cross-contamination, for example from contaminated laundry and other sources. Pathogens can also be introduced via the kitchen sink from contaminated food. Opportunistic bacteria known to grow in hot water systems, e.g. *Pseudomonas* spp., *Mycobacteria* or *Legionella* spp., could pose a threat depending on reuse options and technical solutions. There is also a risk of introducing pathogenic bacteria from contaminated food via the kitchen sink (1.3.1).

1.4.2. Exposure assessment

In the exposure assessment step, the size and nature of the population exposed and the routes, concentrations and distribution of the microorganisms are determined. If greywater is used for groundwater recharge, drinking water from the tap will be the primary transmission route and the total population exposed to about 1 L day⁻¹ (Roseberry & Burmaster, 1992). Greywater used for irrigation may expose people directly via inhalation of aerosols as well as through ingestion of irrigated crops. The dose of a pathogen is calculated from the density of the organism in the water times the volume ingested. Densities are preferably based on occurrence data from direct measurements, but most often on index organisms* or via indirect estimation (density in untreated greywater – expected reduction). Reuse options for greywater are listed in Table 1.1.

1.4.3. Dose-Response relationship

To establish a relationship between the dose of a microbial agent and the rate of infection in a population, human volunteer studies have been performed. The resulting infected and unaffected individuals were used to create a mathematical relationship between the dose administered and the probability of infection in the exposed population. The current baseline information from such studies has been compiled by Haas et al. (1999) and Teunis et al. (1996). Two main equations have been used to describe the relationship; exponential (1) and Beta-Poisson (2):

When organisms are distributed randomly (Poisson) and the probability of infection for any organism equals r , then:

$$P_{\text{inf}} = 1 - e^{-r\text{Dose}} \quad (1)$$

When the probability r is not constant, but has a probability distribution in itself (β -distribution) due to either the nature of the organism or the exposed population, two parameters, α and β , describe the relation as:

$$P_{\text{inf}} \sim 1 - (1 + \text{Dose}/\beta)^{-\alpha} \quad (2)$$

The Beta-Poisson model fits well with many dose-response datasets, adds plausibility to the assumption that ingestion of a single organism is sufficient to cause infection and is conservative when extrapolating to low doses (Teunis et al., 1996). However it can be misused. Equation (3) is a simplified relation that is only valid for certain parameter values, e.g. $\beta \gg \alpha$, $\beta \gg 1$ (Furumoto & Mickey, 1967). Furthermore, the upper 95% confidence region of the Beta-Poisson model exceeds the limiting exponential curve at low doses for some organisms since β is a scale parameter just moving the dose-response curve across the dose axis without limits in low doses (Teunis & Havelaar, 2000). The suggestion is to use a hypergeometric model which has a good fit of data and for which the risk of infection is never

* An index organism is a group or species indicative of pathogen presence (Ashbolt et al. 2001).

higher than the probability of exposure to at least one organism (Teunis & Havelaar, 2000). However, the constants for different pathogens have yet to be published.

Another approach, applicable when dealing with pathogens for which no dose-response studies have been made, vulnerable populations and worst-case scenarios, is to use the exponential relationship with $r = 1$. This is a generic single hit model where the ingestion, inhalation or contact with one organism will lead to $P_{inf} = 0.63$ (Figure 1.2).

Drinking water is something people do every day. To assess repeated exposures, equation (3) is used so that the risk can be measured on a yearly basis ($n = 365$).

$$P_{n(inf)} = 1 - (1 - P_{inf})^n \quad (3)$$

Equation (3) can be simplified at low P_{inf} to:

$$P_{n(inf)} = n \cdot P_{inf} \quad (4)$$

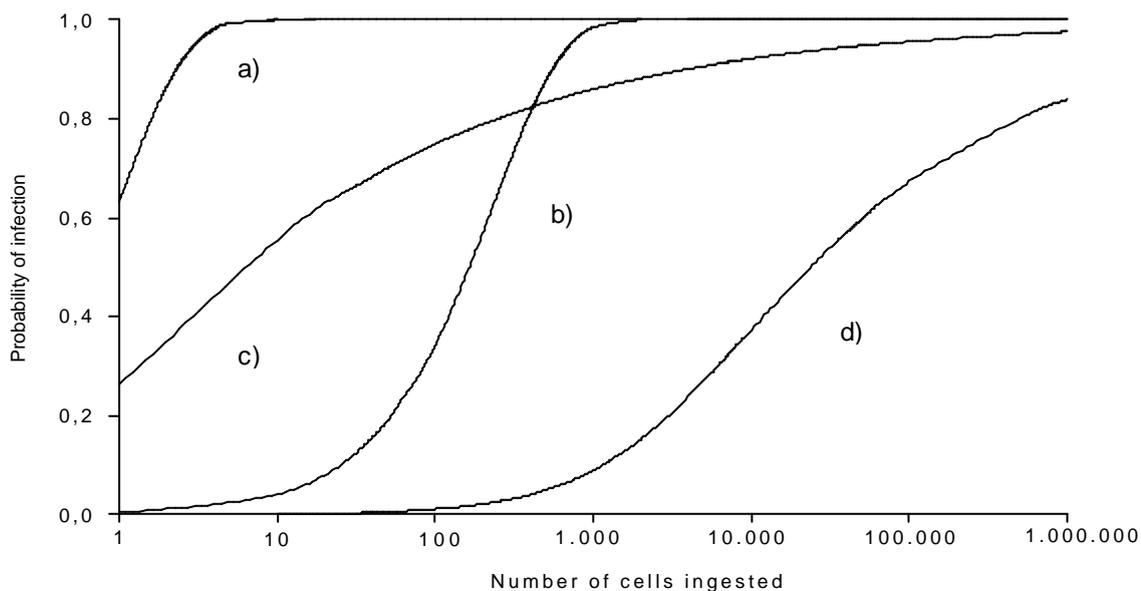


Figure 1.2. The probability of infection from the ingestion of pathogenic cells in different dose-response relationships: Exponential models for (a) a worst-case scenario, (b) *Cryptosporidium*, (c) Beta-Poisson models for rotavirus and (d) *Salmonella*.

1.4.4. Risk characterisation

The information from the hazard identification, exposure assessment and dose-response relationship steps is integrated in the risk characterisation in order to estimate the magnitude of the public health problem. Since the information is often incomplete and since the densities of pathogens fluctuate, probability density functions (PDFs) are often used instead of point estimates or constant values. Monte-Carlo simulations are then used to sample the PDFs in

risk calculations (Höglund, 2001). Most often the microbial risk is presented as the quotient infected/exposed number of people = probability of infection, P_{inf} . The risk can also be presented as total number of infections per annum or system lifetime (Fane et al., 2002). The US EPA has proposed a level of 1 infection per 10,000 people and year as the limit for an acceptable risk for the consumption of drinking water (Regli et al., 1991). This limit has been debated and it is argued that it should be lowered to 1:1,000 (Haas, 1996), which was the acceptable level used in (III). In the overall Sustainable Urban Water Management programme, MRAs are used to compare different systems to each other and from a management point of view, the performance and reliability of a system might be more important than the absolute number of infections.

1.5. Microbiological methods

Gastrointestinal pathogenic microorganisms do not occur as a natural part of the normal intestinal microbiota. Their presence and density in the wastewater and wastewater systems is dependent on the number of infected people in the population connected to the system. Furthermore, the enumeration of pathogens is often difficult. Therefore indicators of faecal pollution play an important role in water management (Stenström, 1985). A good indicator of faecal pollution should occur naturally in relatively high doses in faeces from humans and warm-blooded animals, but not elsewhere. It should have the same growth/reduction pattern in the environment and different water treatment processes as the pathogenic microorganisms that are indicated. Finally, it should be easy to analyse (Stenström, 1985). The most widely used indicators are members of the coliform group (1.5.1), but enterococci (1.5.2), bacteriophages (1.5.3) and spores of sulphite reducing anaerobic bacteria (1.5.4) are becoming more frequently used. There are also a number of chemical biomarkers that can be used to track faecal contamination, of which faecal sterols (1.5.5) are the most frequently used. Most bacteria in human and animal faeces are anaerobic, such as *Bacteroides fragilis* and Bifidobacteria. However, their rapid die-off in the environment makes them unsuitable as indicators of faecal pollution (Stenström, 1985), but their phages can be used (1.5.3.).

1.5.1. Coliform bacteria

Coliform bacteria are the most widely used faecal indicators and play an important role in water management, especially drinking water. They are a heterogeneous group that can originate from several environments. Thermotolerant coliform bacteria have a direct correlation to sewage pollution but some *Klebsiella* spp. can also originate from plant degradation. *E. coli* are almost exclusively of faecal origin and thus a more reliable faecal indicator than the other coliform groups (Stenström, 1985). *E. coli* are excreted in densities of $10^5 - 10^8$ c.f.u. g^{-1} (Geldreich, 1978). However, regrowth in the environment as well as different sensitivity in relation to many pathogens to treatment processes or environmental stress make members of the coliform group unsuitable as a sole indicator of the hygiene quality of reuse products (Bitton, 1994). Coliform bacteria were analysed with the spread plate method according to (ISO 9308-1, 2000), e.g. for Coliforms, m-Endo agar LES (Difco), 35 °C, 24 h and *E. coli*, MFC agar (Difco) 44 °C, 24 h with further verification of faecal coliforms in LTL5B, 44 °C, 24 h and testing for indole production.

1.5.2. Enterococci

Enterococci (faecal streptococci) are present in faeces in densities between $10^5 - 10^7$ c.f.u. g^{-1} (Geldreich, 1978). They are considered to be a good supplement to coliform bacteria since they are more tolerant to environmental stress. Enterococci have also been suggested as an indicator of the presence of enteric viruses, particularly in sludge and seawater (Bitton et al., 1981). Health risks from exposures to recreational water have been reported to correlate to enterococci densities (Kay et al., 1994). Enterococci may however adsorb more strongly to soil particles and are more sensitive to detergents than many pathogens (Stenström, 1985). Enterococci were analysed according to (ISO 7899-2, 2000) e.g. mEnterococcus agar (Difco), 35 °C, 44 h, with further verification on bile esculine agar, 44 °C, 2 h.

1.5.3. Bacteriophages

Bacteriophages are viruses that infect bacterial host cells and are harmless to humans. Many enteric viruses are more resistant in the environment, as well as to different treatments, than bacteria. They are also smaller, which assigns them different transport features. Therefore bacteriophages have been suggested as indicator organisms to predict the presence and behaviour of enteric viruses in the environment (Havelaar et al., 1991). Bacteriophages used as indicators for faecal pollution have been divided into three groups: 1) Somatic coliphages, infecting various *E. coli* and related strains by attachment to the cell wall as the first step in the infection process, are used as an indicator of faecal contamination as well as an index organism for virus reduction over treatment and in sediments. Somatic coliphages were analysed by the double agar method (Adams, 1959) according to (ISO 10705-2, 2000). 2) F-specific RNA bacteriophages are capable of infecting bacteria possessing the F-plasmid (sex plasmid) by adsorption to the F-pili as the first step in the infection process. Their presence indicates pollution by wastewater contaminated by human or animal faeces (Havelaar et al., 1986). F-specific RNA bacteriophages were analysed by the double agar method (Adams, 1959) according to (ISO 10705-1, 1995). 3) Phages infecting anaerobic bacterium *Bacteroides fragilis* attach to molecules in the cell wall of host bacteria as the first step in the infection process. Phages infecting *B. fragilis* RYC2056 in a water sample indicate human or animal faecal pollution, while phages infecting *B. fragilis* HSP40 in a sample preferably indicate faecal pollution of human origin (Puig et al., 1999). However, this group was not used in the investigations.

1.5.4. Spores of sulphite reducing anaerobic bacteria

These are dominated by *Clostridium perfringens* spores, which are present in human and animal faecal matter. The spores survive for long periods in water and are more resistant than vegetative forms to chemical and physical factors and may give an indication of remote or intermittent faecal pollution (ISO 6461/2, 1986). They are resistant to chlorination at levels that are normally used for the treatment of water and are suggested as index organisms for parasitic protozoan reduction in water treatment processes (Payment & Franco, 1993; Hijnen et al., 2000). Spores of sulphite-reducing anaerobes were analysed by the pour plate method

according to (ISO 6461/2, 1986), e.g. anaerobic on perfringens agar base (Oxoid), 37 °C, 24 h after inactivation of vegetative cells, 75 °C, 15 min.

1.5.5. Faecal sterols

Faecal sterols is the collective name for the sterols and stanols excreted in faeces. The composition of faecal sterols in faeces depends on diet, age and endogenous synthesis and biohydrogenation in the digestive tract (Midtvedt et al., 1988; Leeming et al., 1996; Midtvedt & Midtvedt, 1993). Coprostanol (5 β -cholestan-3 β -ol) is the principal faecal sterol in human faeces, constituting about 40-60% of the total sterol content (Walker et al., 1982). It is produced from cholesterol by anaerobic bacteria in the digestive tract (Midtvedt, 1999). Analyses of faecal sterols, especially coprostanol, have been used as an alternative to indicator bacteria to determine faecal contamination (Walker et al., 1982; Leeming et al., 1996). Coprostanol can also be used to quantify the faecal load under specific circumstances (Schönning et al., 2002). Faecal sterols were concentrated by filtration. The filters were treated by saponification in 95% ethanol and 10 M NaOH (2:1) and the faecal sterols captured by extraction in hexane as described in Midtvedt et al. (1988). After evaporation, the samples were silylated with bis(trimethylsilyl)trifluoroacetamide and analysed as in Leeming et al. (1996) with 5 α -cholestane as internal standard. The GC-MS (Hewlett Packard 5890 and 5970 mass-selective detector fitted with a direct capillary inlet, split/splitless injector and a 50 m fused-silica capillary column) was operated in scan acquisition mode described in Nichols et al. (1992).

2. OBJECTIVES AND SCOPE

The overall aim of this thesis was to investigate and evaluate health risks from infectious diseases related to the reuse of source-separated greywater and to give input to the urban water toolbox.

The specific objectives of the investigations were:

- o To determine the faecal contamination in the greywater flow
- o To measure the growth/reduction of selected pathogenic bacteria in sediment formed in a greywater system
- o To quantify microbial health risks from reusing greywater by Quantitative Microbial Risk Assessment (QMRA)
- o To determine the level of treatment needed to be inside a 10^{-3} risk of infection for different exposure scenarios

Samples were collected from Vibyåsen, an estate in Sollentuna, north of Stockholm. Faecal contamination was determined by the quantification of faecal indicator bacteria and biomarkers coprostanol and cholesterol (I). To assess the risk from pathogens from the kitchen, a laboratory study was conducted on sediment collected from a settling tank in a greywater treatment system to look at the effect of three of the most important factors for the survival/growth of microorganisms: (1) competing microbiota, (2) temperature and (3) nutrient availability (II). The treatment efficiencies required to be below a P_{inf} of 10^{-3} for different exposures to reused greywater were calculated in (III). A more thorough QMRA was performed for a specific system in (I), which was the basis for the risk characterisation in this thesis (Chapter 6).

3. HAZARD IDENTIFICATION

Since this study was concerned with water as a transmission route, the focus was on the faecal-oral transmission of gastrointestinal pathogens and the main hazard emanated from faecal contamination of the greywater and enteric disease. In Table 3.1, some of the most important waterborne agents, the disease they cause and the type of reporting in the Swedish surveillance system are listed.

3.1. Bacteria

There is a range of bacteria that can cause infection, but *Salmonella* and *Campylobacter* were chosen as index organisms for the bacteria group due to their high endemic infection level. *Shigella* and EHEC are other important water and food-borne pathogens that may be subjects for future MRAs. For exposure to aerosols during irrigation, *Legionella pneumophila* may be a hazard.

3.1.1. *Campylobacter*

Campylobacter is the most common cause of bacterial gastroenteritis in Sweden. In 2000, there were more than 8,000 reported cases, of which one third were acquired in Sweden (SMI, 2001). *Campylobacter* are harboured in the intestines of a wide range of domestic and wild animals and have been found in almost all bird species that have been examined. They are particularly prevalent in poultry, which is a likely source of human infection (AWWA, 1999). At present the genus contains 16 species and six subspecies. *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis* and *C. helveticus* form a genetically close group of species which are the most commonly isolated from human and animal diarrhoea (On, 2001). Of greatest concern for human infections are *C. jejuni* (approx. 85% of the nationally acquired cases), *C. coli* and *C. upsaliensis*. Clinical symptoms are: severe and often bloody diarrhoea, stomach pain and fever. Malaise and vomiting can also occur (SLV, 2002). The disease is, however, often self-limiting but complications such as arthritis and Guillain-Barré syndrome have been documented (Smith, 2002). In a survey by the Swedish Food Administration (SLV, 2002) on the occurrence of *Campylobacter* in Sweden, 9.5% of the chickens tested were positive. Furthermore, *Campylobacter* could be detected in the raw water used in 38% (n = 102) of the surface water treatment plants included in the study. The endemic level of *Campylobacter* was based on SMI, (2001) and corrected for underreporting (Mead et al., 1999) and disease level (Havelaar et al., 2000) giving a yearly incidence of infection of 15.6% in Sweden (I).

Table 3.1. Etiological agents that can be transmitted through water and reporting of these in Sweden (Stenström et al., 1994; AWWA, 1999; SMI, 2002)(www.smittskyddsinstitutet.se)

Etiological agent	Disease	Reporting
Virus		
Adenovirus	Unspecified	3
"	Encephalitis	
" (Ead 40 and 41)	Enteritis	
Astrovirus	Enteritis	3
Calicivirus, including Norwalk	Enteritis	3
Like Viruses		
Coxsackievirus	Meningitis	3
Echovirus	Meningitis	3
Enterovirus, types 68-71	Meningoencephalitis	3
Hepatitis A virus (HAV)	Hepatitis	1
Hepatitis E virus (HEV)	Hepatitis	1
Poliovirus	Poliomyelitis	1
Rotavirus	Enteritis	3
"	Encephalitis	3
Small round viruses (SRV)	Enteritis	3
Bacteria		
<i>Aeromonas</i> spp.	Enteritis	
<i>Campylobacter jejuni/coli</i>	Campylobacteriosis	2
<i>Escherichia coli</i>	Enteritis	2
(EIEC, EPEC, ETEC, EHEC)		
<i>Legionella</i> spp.	Legionellosis	2
<i>Mycobacterium avium</i> complex	Respiratory symptoms	2
<i>Plesiomonas shigelloides</i>	Enteritis	
<i>Pseudomonas aeruginosa</i>	Varying	
<i>Salmonella typhi/paratyphi</i>	Typhoid/paratyphoid fever	1
<i>Salmonella</i> spp.	Salmonellosis	1
<i>Shigella</i> spp.	Shigellosis	1
<i>Vibrio cholera</i>	Cholera	1
<i>Yersinia</i>	Yersiniosis	2
Parasites		
<i>Acanthamoeba</i> spp.	Varying	2
<i>Ascaris lumbricoides</i>	Loeffler's syndrome, enteritis	
<i>Cryptosporidium parvum</i>	Cryptosporidiosis	3
<i>Cyclospora cayetanensis</i>	Enteritis	
<i>Entamoeba histolytica</i>	Amoebiasis	2
<i>Giardia intestinalis</i>	Giardiasis	2

1. Diseases dangerous to the community, obligatory reporting

2. Other notifiable diseases, obligatory reporting

3. Voluntary laboratory reporting

3.1.2. *Salmonella*

Salmonella spp. are gram negative, aerobic, rod-shaped, zoonotic* bacteria that can infect people, birds, reptiles, and other animals. The occurrence of chronic carriers is rare in humans* but common in birds and animals (AWWA, 1999). The genus includes approximately 2000 serogroups divided into five subgenera. Salmonellosis has different disease outcomes. Typhoid fever, caused by *S. typhi* or *paratyphi* (human specific), is rare in Sweden (Stenström et al., 1980). A total of 23 cases were reported in 2000, of which 2 were acquired in Sweden (SMI, 2001). *Salmonella* is the second most common cause of gastroenteritis in Sweden. Almost 5,000 cases were reported in the year 2000, of which one seventh was nationally acquired. *S. typhimurium* and *S. enteritidis* caused most of the clinical cases in Sweden in 2000 (SMI, 2001). The endemic level of *Salmonella* was taken from SMI (2001) and corrected for underreporting and disease level as above, giving a yearly incidence of infection of 9.0% in Sweden.

3.1.3. *Legionella*

Bacteria of the *Legionella* species are ubiquitous in aquatic environments and as biofilm organisms. They are able to colonise hot-water tanks, cooling towers, distribution systems and other water sources. They are also able to survive and regrow inside free-living amoeba. All *Legionella* spp. can probably cause illness but *L. pneumophila* causes most infections. There are two forms of legionellosis, Legionnaires' disease and Pontiac fever. Legionnaires' disease is a severe respiratory illness characterised by pneumonia, which most often affects individuals with an underlying disease or immunosuppression. Pontiac fever is a self-limiting, non-pneumonic influenza-like illness (AWWA, 1999). The natural reservoir for *Legionella* is aquatic habitats. The bacteria amplify in water distribution systems and are transmitted by the inhalation of contaminated aerosols. There are documented outbreaks from cooling towers, potable water, condensers and whirlpools (Muraca et al., 1988). In a survey on the occurrence in Swedish water systems, *Legionella* was detected in all hot-water tanks without circulation and with a temperature of < 50° C and in 25% of all hot water samples tested in densities of $10^{3 \pm 0.6}$ cfu 100 mL⁻¹ (Szewzyk & Stenström, 1993). These figures formed the basis for the background level of *Legionella*, assuming half of the water entering the greywater system to be hot water from showers. Furthermore, the risk of regrowth of *Legionella* in the tank was assessed.

3.2. Viruses

As for bacteria, several gastrointestinal viruses are of health concern. Since viruses are usually excreted in high numbers, persistent in the environment and have low infectious doses, they are important waterborne agents and it is believed that a major part of the waterborne outbreaks with unknown etiological agent are viral (Schwartzbrod, 1995). The main reason why they are not diagnosed is due to methodological problems and the fact that gastrointestinal viral diseases usually have a shorter duration than bacterial and parasitic ones

* Only non-typhoid *Salmonella* strains.

(Hedberg & Osterholm, 1993). Not all viruses are culturable, but new and improved detection methods will improve surveillance. Rotavirus was chosen as an index virus in this study, but enteric adenoviruses 40 and 41, Caliciviruses and Hepatitis A virus (HAV) are examples of other gastrointestinal viruses of great importance for greywater reuse as a transmission route.

3.2.1. Rotavirus

Rotaviruses are the most common cause of diarrhoea in children but can also infect adults. The incubation period of rotavirus infection is less than 48 hours, with duration of illness for 5 to 8 days. Symptoms usually include vomiting, diarrhoea and dehydration. Fever and respiratory problems can occur, but these symptoms are mainly associated with infection in children. Excretion of as much as 10^{11} rotavirus particles g^{-1} faeces has been reported (AWWA, 1999). Mead et al. (1999) estimated the incidence of disease to be 0.71% in the U.S. With a disease rate of 75% (Gerba et al., 1996), the incidence of infection was thus 0.95%.

3.3. Protozoa

Protozoa are single or unicellular, eucaryotic organisms divided into four main groups: flagellated, amoeboid, ciliated and sporozoids. All four groups contain intestinal parasites, of which many are zoonotic, i.e. can be transmitted directly from animals to humans. This is the case for both *Giardia* (3.3.1) and *Cryptosporidium* (3.3.2) (Marshall et al., 1997).

3.3.1. *Giardia*

Giardia is a flagellate belonging to the order *Diplomanidida*. The genus comprises several species, all intestinal parasites, distinguished from each other on morphological criteria and host range. *G. intestinalis* is probably the only species causing disease in humans and is considered to be the most common intestinal parasite in the world (Marshall et al., 1997). With over 1,500 human cases, *Giardia* was the third most reported cause of gastrointestinal infection in Sweden in 2000, (SMI, 2001). The infectious life stage of *Giardia* is the cyst, which is a 10-12 μm long and 5-8 μm wide oval highly resistant stage of its life-cycle that can survive and remain infectious for several months in water (Wolfe, 1992). The manifestation of giardiasis in humans varies from asymptomatic to chronic diarrhoea and is very much dependent on the immunological status of the host. First symptoms are often nausea, anorexia, malaise, fever and chills, followed by an explosive, watery and foul-smelling diarrhoea (Marshall et al., 1997). The endemic level of *Giardia* was calculated from reported cases in Sweden (SMI, 2001), adjusted for underreporting and disease rate according to Mead et al. (1999) and Haas et al. (1999), giving a yearly incidence of infection of 0.84% in Sweden (I).

3.3.2. *Cryptosporidium*

Cryptosporidium is divided into 23 species, of which 3 have been shown to be able to infect humans. It is, however, only the human and bovine genotypes of *C. parvum* that seem to be of any health significance (Kosek et al., 2001). *Cryptosporidium* is present in the environment as an oocyst, which is a 4.5-6 μm in diameter spherical, thick-walled stage in its life-cycle. In aqueous solutions, oocysts remain infectious for up to 6 months and are viable for 9 months

(Medema et al., 1997). Oocysts are also tolerant to high doses of disinfectants (Robertson et al., 1994). *Cryptosporidium* caused the largest waterborne outbreak in the industrialised world during the past decade in Milwaukee, where more than 400,000 people were assumed to be infected by contaminated municipal drinking water (Addiss et al., 1995). A *Cryptosporidium* infection can give cholera-like diarrhoea in immunocompromised individuals, up to 71 stools and 17 L water loss per day having been reported as being the ultimate cause of death for AIDS patients. Immunocompetent people usually have self-limiting watery diarrhoea with a flu-like low-grade fever. There is still no medication for cryptosporidiosis but paromomycin has been shown to decrease stool frequency and oocyst excretion in humans (Kosek et al., 2001). *C. parvum* is currently reported voluntarily in the Swedish surveillance system (Table 3.1), but it is suggested to be notifiable. Together with viral agents, *Cryptosporidium* is thought to be a main cause of outbreaks with unknown etiological agent (Stenström et al., 1994). The incidence of infection level, 0.31%, was calculated from Mead et al. (1999) and Haas et al. (1999).

4. EXPOSURE ASSESSMENT

Exposure assessments are preferably made from direct measurement of pathogens at different exposure points. However, this is difficult, especially in greywater, which is less faecally contaminated than wastewater. An attempt was therefore made to measure the faecal load (4.2), using epidemiological data (Chapter 3) and index organisms or literature data on environmental decay (4.3) to assess the probable number of pathogens at the exposure points.

4.1. Transmission pathways

In the modelling of microbial risks, a system has been built up of several so-called health-related modelling units (HMUs), which are defined as physical units that in some ways alter the concentration of pathogens (Fane & Ashbolt, 2000). In this respect they are most often some kind of microbiological barrier, such as a treatment unit, but they also include reduction and/or growth in the environment. The most important HMUs in water and wastewater management - whether it concerns drinking, grey, storm or wastewater - are in most cases different steps in the water treatment. That is why the focus in the present work was on greywater treatment efficiency and reliability. The pathways assessed in (I) were accidental ingestion of greywater, drinking recharged groundwater after different retention times and direct exposure at a sports field irrigated with greywater. This study was later extended to include more pathways (Table 4.1) and with updated figures on environmental reduction of pathogens (Table 4.3), looking at the treatment efficiency needed in the black box (Figure 4.1) to be inside an acceptable level of infection (10^{-3}). For exposure to sink trap sediments, the P_{inf} was assessed.

Table 4.1. Transmission pathways for exposures to reused or discharged greywater and health-related modelling units (HMUs), except treatment, involved. (II) refers to Paper II in this thesis

Exposure	HMUs involved	Volume ingested	References
1) Drinking recharged groundwater (yearly risk from 365 exposures).	Dilution ^a , unsaturated zone ^a and saturated zone*	$e^{(6.87 \pm 0.53)}$ mL day ⁻¹ ^b	^a (Asano et al., 1992), ^b (Roseberry & Burmaster, 1992)
2) Accidental ingestion to treated greywater (one time exposure)		1 mL exposure ⁻¹	
3) Ingestion from a field irrigated with treated greywater (yearly risk from 26 exposures)	Survival on grass*	1 mL exposure ⁻¹	
4) Ingestion/inhalation of aerosols	Tank*	$e^{(-4.2 \pm 2.2)}$ mL ^{c, d}	^c (Dowd et al., 2000), ^d (Kincaid et al., 1996)
5) Swimming in recreational water receiving treated greywater.	Dilution	$e^{(3.9 \pm 0.3)}$ mL	
6) Untreated greywater, <i>Salmonella</i> regrowth	Sink trap* (growth) ^e	0.1 g	^e (II)

* Table 4.3.

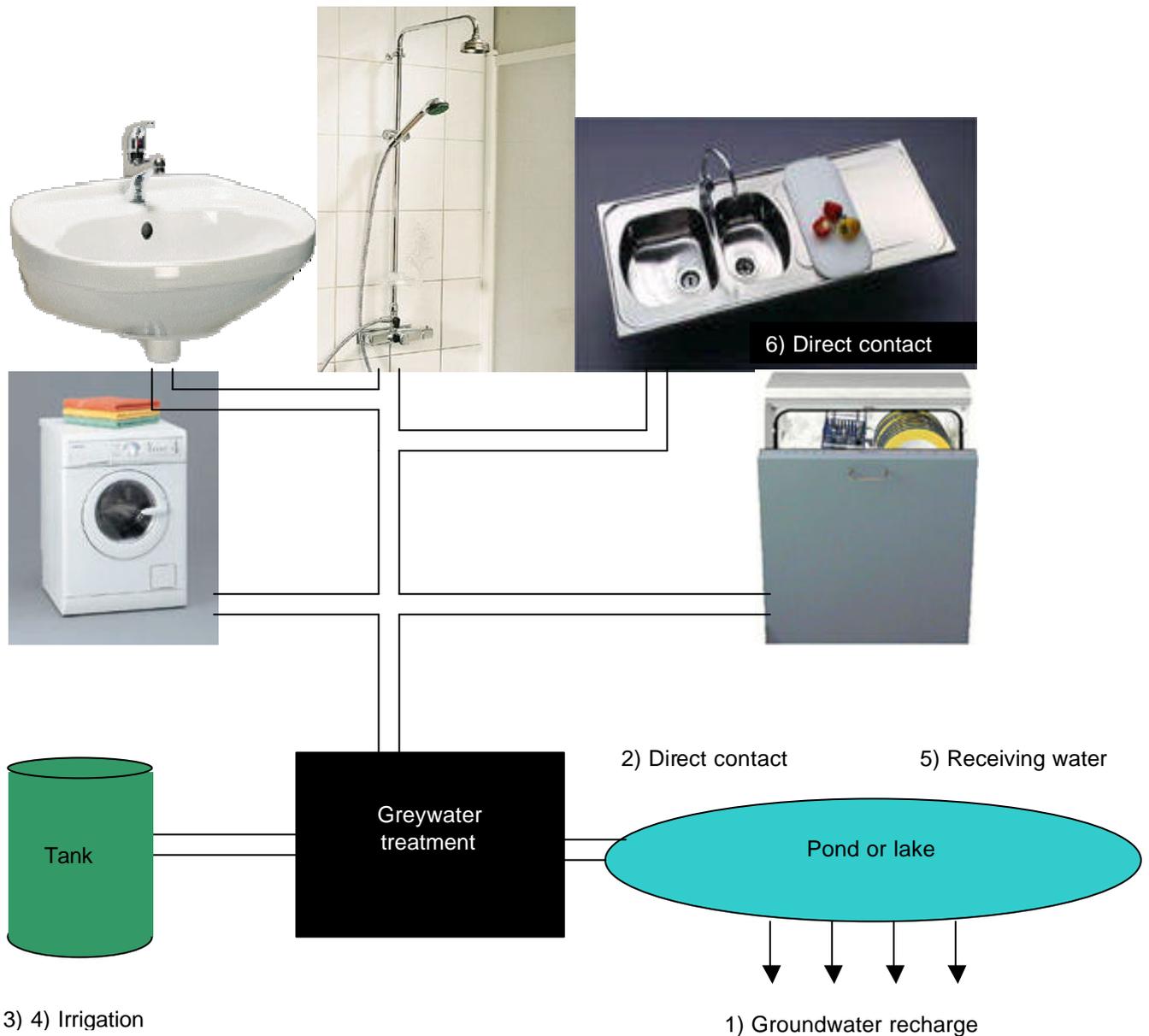


Figure 4.1. Greywater input and exposure points to greywater: 1) drinking recharged groundwater, 2) direct contact, 3) playing on a field irrigated with greywater, 4) ingestion/inhalation of aerosols when irrigating, 5) swimming in a lake receiving greywater and 6) ingestion of sink trap sediments (Table 4.1).

4.1.1. Groundwater

For exposure to groundwater, the height of the unsaturated zone was assumed to be three metres and the retention time in the saturated zone two months, based on the recommendation in the report “Wastewater Infiltration: Conditions, Function, Environmental Consequences” (Naturvårdsverket, 1985). The dilution factor was defined as a triangular PDF with min 1 (no dilution), max 30 and most probable 2 (Asano et al., 1992), i.e. most often half of the water from the tap will be reclaimed greywater. The water intake was log normally distributed with a medium daily intake of $963 \text{ mL p}^{-1} \text{ d}^{-1}$ (Figure 4.2) (Roseberry & Burmaster, 1992).

4.1.2. Direct contact

If the treated greywater is used for an aesthetic landscaping approach, there is a risk for accidental contact with the water and the assumed water intake is a conservative 1 mL event⁻¹ (Ashbolt, 1999). No dilution of the treated greywater was considered.

4.1.3. Irrigation

Treated greywater can be collected in a tank to be used for irrigation. If the water was used for irrigation of a public sports field (Exposure 3), the irrigation was assumed to take place the day before access and with a negligible holding time in the tank. The water intake was assumed to be a conservative 1 mL 26 times per year based on weekly activities during the summer season in Sweden. For private use of greywater in the garden (Exposure 4), the greywater was assumed to be applied once a week, thus with an average holding time in the tank of 3.5 days. The water intake from inhalation of aerosols is time dependent based on average aerosol ingestion (Dowd et al., 2000) and droplet size distribution (Kincaid et al., 1996), giving a log normal distribution (Figure 4.2). Furthermore, 70% of the inhaled volume of aerosols was assumed to be ingested. Exposure 4 can, with some modifications, be applied to toilet flushing. However, the water intake is smaller since the time a person is exposed to aerosols is shorter when flushing the toilet than when irrigating.

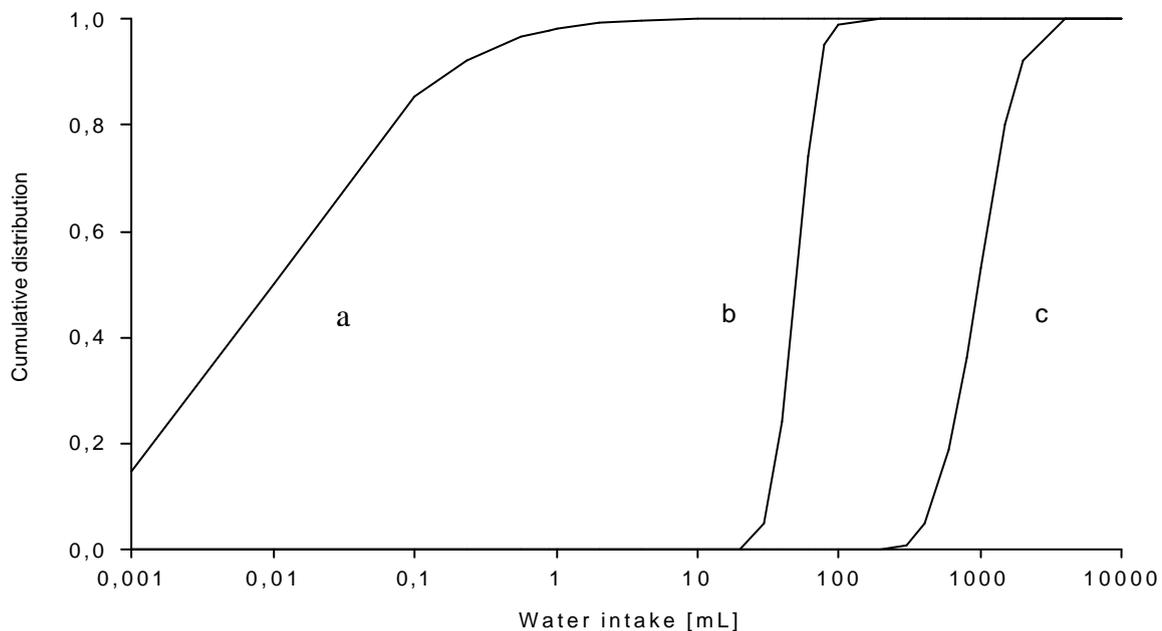


Figure 4.2. Cumulative distributions for the water intake from inhalation of aerosols (a) and ingestion of recreational (b) and drinking water (c) used in the quantitative microbial risk assessment.

4.1.4. Recreational water

Usually the common practice for exposure to recreational water is a conservative 100 mL water intake based on 50 mL intake hour⁻¹, mean bathing time 2 hours day⁻¹ (Ashbolt et al., 1997). This ingested volume was changed for the present simulation to a time dependent distribution with the mean time of bathing being 1 hour (log normally distributed) (Figure 4.2). The dilution in the receiving water was assumed to be 1,000 times.

4.1.4. Exposure to sediment

The P_{inf} to sediment exposure was based on eating chicken once a week and cleaning the sink trap once a month. A worst-case scenario with growth of *Salmonella* (II) was simulated, whilst *Campylobacter* was assumed to die-off in a rate of 0.11 log day⁻¹ (Table 4.2). From every contaminated chicken, 10^{3 ± 1} cells were assumed to end up in the sediment and the accidental intake was assumed to be 0.1 g.

4.2. Faecal load

A number of faecal indicator organisms and biomarkers were used as the basis for a quantification of the faecal load in greywater (Table 4.2). The faecal load was calculated from equation (5):

$$\frac{(\text{Microorganism density [numbers mL}^{-1}] * \text{Flow [mL person}^{-1} \text{ day}^{-1}])}{\text{Excretion density [numbers g faeces}^{-1}]} \quad (5)$$

Table 4.2. Faecal indicators in Vibyåsen greywater system [log 10 100 mL⁻¹] and the corresponding faecal load [g person⁻¹ day⁻¹] with an average flow of 64.9 L person⁻¹ day⁻¹ (Palmqvist, 2001)

Organism/biomarker	Mean	Min	Max	Excretion density [numbers or mg g ⁻¹ faeces]	Mean faecal load (min – max) [g person ⁻¹ day ⁻¹]
Coliform	8.1	5.5	8.7		
<i>E. coli</i>	6.0	4.3	6.8	10E7 ^a	65 (1.3 – 410)
Enterococci	4.4	3.0	5.1	10E6.5 ^a	5.2 (0.2 – 26)
Sulphite reducing anaerobes	3.3	2.3	4.8		
Somatic coliphages	3.3	1.4	4.0		
Coprostanol [µg L ⁻¹]	8.6	3.1	14.9	12.74 ^b	0.04 (0.016 – 0.076)
Cholesterol [µg L ⁻¹]	17.3	7.4	31.6	5.08 ^b	0.22 (0.094 – 0.40)

^a (Geldreich, 1978)

^b (Leeming et al., 1998)

Using *E. coli* to estimate the faecal load would lead to an overestimation of more than 1,000 times, enterococci 100 times, compared to the use of coprostanol. In the QMRA, coprostanol was used as a conservative biomarker. The pathogen density in the untreated greywater was thus:

$$\frac{(0.04 \text{ [g p}^{-1} \text{ d}^{-1}] * \text{excretion density [numbers g faeces}^{-1}] * \text{excretion time [d]} * \text{yearly incidence})}{(64900 \text{ [mL d}^{-1}] * 365 \text{ [d]})} \quad (6)$$

with the faecal load, excretion density and excretion time expressed as PDFs (I).

4.3. Growth/reduction of microorganisms

Sediment is formed in several in-house piping installations. These can provide good growth niches for bacteria. Growth of indicator bacteria has been suggested, but there is also a risk for growth of bacterial pathogens, especially in the sink trap where pathogens such as *Salmonella* and *Campylobacter* can be introduced from poor food handling. In (II), the effects of temperature, competition from commensal microbiota and nutrient availability were measured for *Salmonella*, *Campylobacter*, spores of sulphite-reducing anaerobes and two bacteriophages. *Campylobacter* died rapidly in all regimes, or entered a viable but non-culturable (VBNC) state and subsequent results not provided. However, *Campylobacter* isolates of clinical importance are thermophilic and thus not likely to grow in temperatures below 30°C (Hazeleger et al., 1998) Therefore they will not regrow under conditions most often prevailing in greywater treatment systems. *Salmonella* can grow at 20 °C and below, but is likely to be suppressed by the indigenous microorganisms (II), also shown by Sidhu et al. (2001). The growth rate of *Salmonella* in a tyndallised regime at 20 °C, $0.022 \pm 0.02 \text{ log day}^{-1}$ (II), was used in the worst-case scenario at exposure point 6 (Table 4.1). However, in most situations pathogens are likely to decline outside their host. Decay rates in sediment and other matrices used in quantitative MRA are listed in Table 4.3. If decay rates were available for the organism in question, these were used in the QMRA. Otherwise enterococci were used as the index organism for *Salmonella* and *Campylobacter*, somatic coliphages and F-specific RNA bacteriophages for rotavirus and spores of sulphite reducing anaerobic bacteria for *Giardia* and *Cryptosporidium* (oo)cysts.

Table 4.3. Decay rate of selected microorganisms in different matrices and at different temperatures. (II) refers to Paper II in this thesis

Microorganism	Decay rate [log ₁₀ dag ⁻¹]	Matrix	Temp. [°C]	Method	Reference
Bacteria <i>Salmonella typhimurium</i>	- 0.048 ± 0.0092 - 0.12 ± 0.0011 - 0.048 ± 0.0056 - 0.074 ± 0.014 - 0.031 ± 0.0019 - 0.056 ± 0.011 - 0.36	Greywater sediment Sand Silt Greywater	4 20 4 20 4 20 ambient	Culture	(II) (Stenström & Blomén, 1981) (Nolde, 1999)
<i>Campylobacter jejuni</i>	- 1.30 ± 0.16 - 0.11 ± < 0.01 - 0.02 ± < 0.01	Riverwater with sediment	25 15 5	Culture	(Thomas et al., 1999)
Enterococci (bacterial indicator)	- 0.032 ± 0.016 - 0.078 ± 0.038 - 0.0049 ± 0.0022 - 0.10 ± 0.014 - 0.0062 ± 0.0024 - 0.079 ± 0.0060	Greywater sediment Sand Silt	4 20 4 20 4 20	ISO 7899-2	(II) (Stenström & Blomén, 1981)
<i>Legionella pneumophila</i>	- 0.12 - 0.03	Brackish water	20 4	Culture	(Heller et al., 1998)
Virus Rotavirus	- 0.016 ± 0.010 - 0.119 ± 0.00835 ^a	Liquid waste Grass	12 – 17 4 – 16	Cell culture	(Pesaro et al., 1995) (Badawy et al., 1990)
?X174 bacteriophage (viral indicator)	- 0.018 ± 0.0048 - 0.11 ± 0.031	Sediment	4 20	ISO 10705-2	(II)
MS2 bacteriophage (viral indicator)	- 0.021 ± 0.0069 - 0.029 ± 0.024	Sediment Groundwater	4 - 20 4	ISO 10705-1 Plaque assay	(II) (Yates et al., 1985)
Parasites <i>Cryptosporidium parvum</i> oocysts	- 0.006 ± 0.031 - 0.010 ± 0.032 - 0.011 ± 0.008 - 0.010 ± 0.016	River water	15 5 15 5	Excystation Dye exclusion	(Medema et al., 1997)
<i>Giardia intestinalis</i> cysts	- 0.042	Water	25	Dye exclusion	(Romig, 1990)
Spores of sulphite reducing anaerobes (parasite indicator)	- 0.00045 ± 0.0027 - 0.027 ± 0.0043 - 0.012 ± 0.0031	Sediment River water	4 – 20 15 5	ISO 6461/2 Culture	(II) (Medema et al., 1997)

^a [h⁻¹]

4.4. Treatment efficiency

It is important to look at the available techniques for treatment and their efficiency in the hygienisation of greywater. New techniques must also be taken into consideration. A study on small-scale solutions for greywater treatment was made. Most results were for the coliform group, which may not be relevant for virus reduction. Since data in this field are scarce, this study assessed the treatment efficiency and reliability needed to be inside an acceptable level (0.001) of risk which can be compared to the greywater treatment efficiencies reported (Table 4.4).

Table 4.4. Treatment efficiencies, expressed as log reduction, of indicator organism in greywater. 1 log reduction is equal to 90% reduction, 2 log to 99% etc.

Place	Treatment	Total coliforms	Thermo-tolerant coliforms	<i>E. coli</i>	Enterococci	<i>Clostridium perfringens</i> spores	Bacteriophages	Reference
Swedish Ekoporten, Norrköping	Aquatron, UV	-	1.2	1.1	-	-	-	(Lindgren & Grette, 1998)
Ekoporten, Norrköping	Constructed wetland	-	-	1.5	-	-	-	(Lindgren & Grette, 1998)
Vibyåsen, Sollentuna	Settling tank, active sludge, biofilter	0.86	-	1.0	0.66	0.31	0.24 ^a	(Ottosson & Stenström, 2002)
Vibyåsen, Sollentuna	Pond	3.1	-	3.0	2.3	1.0	1.2 ^a	(Ottosson & Stenström, 2002)
KTH, Stockholm	Sand column	-	-	2.4	2.1	-	1.5 ^b	(Anderson et al., 1980)
Abisko	Biofilters April July	- - -	- - -	1.8 1.8 0 0.92	1.7 1.8 0 0.32	- - - -	1.4 1.7 0.10 0.8	(Stenström et al., 1982)
Toarp	Constructed wetland	-	2.9	-	-	-	-	(Fittschen & Niemczynowicz, 1997)
Kalmar	Wetpark, pond system	-	2.9	-	2.7	-	-	(Gunther, 2000)
International Nyawita, Kenya	Constructed wetland Biofilter	- -	3.5 4.0	- -	1.7 2.5	- -	- -	(Emanuelsson & Linderholm, 2001)
Dunga, Kenya	Constructed wetland Biofilter	- -	2.7 1.6	- -	2.2 1.5	- -	1.7 ^c 0.47 ^c	(Emanuelsson & Linderholm, 2001)
Nepal	Constructed wetland	5	-	-	-	-	-	(Shrestha et al., 2001)

^a Somatic coliphages

^b *Salmonella paratyphi* F0 phage (Lilleengen, 1948)

^c *Salmonella typhimurium* phage 28B (Lilleengen, 1948)

5. DOSE-RESPONSE RELATIONSHIP

Despite implications (see 1.4.3) with the Beta-Poisson model (eq. 2) it was used for rotavirus, *Campylobacter* and *Salmonella*. For the other organisms for which quantitative risk assessments were performed, the exponential model (eq. 1) was used (Table 5.1). Confidence intervals for the dose-response relationships were assessed for *Giardia* and *Cryptosporidium*. The worst-case model was used for *Legionella* and for a worst-case scenario from exposure to sink trap sediment contaminated with *Salmonella*.

Table 5.1. Dose-response models and constants used in the risk calculations

Organism	Model	Constants	Reference
Rotavirus	Beta-Poisson	$a = 0.253$; $\beta = 0.422$	(Teunis et al., 1996)
<i>Salmonella</i> (multiple strains, non-typhoid)	Beta-Poisson	$a = 0.3126$; $\beta = 2884$	(Haas et al., 1999)
<i>Campylobacter jejuni</i>	Beta-Poisson	$a = 0.145$; $\beta = 7.589$	(Teunis et al., 1996)
<i>Giardia intestinalis</i>	Exponential	$r = 0.0199$ 95% ci (0.0044 – 0.0566)	(Teunis et al., 1996)
<i>Cryptosporidium parvum</i>	Exponential	$r = 0.00405$; 95% ci (0.00205 – 0.00723)	(Teunis et al., 1996)
Worst-case (generic)	Exponential	$r = 1$	(Teunis & Havelaar, 2000)

6. RISK CHARACTERISATION

Results from the simulations are presented in Tables 6.1 and 6.2 and in Figure 6.1a-f. Explanations and discussion are further included under each subheading. The largest risk emanated from rotavirus in all exposure scenarios simulated. All the selected pathogens are common in the population. However, the treatment required for rotavirus was higher, since rotavirus is excreted in high numbers compared to the other pathogens (I). The other pathogen of main concern was *Campylobacter*. *Salmonella* has a high infectious dose ($ID_{50} = 23,600$ (Haas et al. 1996)). That is why it is a less important organism to these exposures. *Giardia* cysts and *Cryptosporidium* oocysts have low infectious doses but they are not excreted in sufficient amounts to constitute health problems of concern with the low faecal load registered in Vibyåsen. The average number of (oo)cysts in untreated greywater was simulated to approximately 0.002 (oo)cysts mL^{-1} compared to 1.7 rotavirus particles mL^{-1} . The high infectious dose for *Salmonella* and low excretion numbers of parasitic protozoa are the main reasons for the negative values in Table 6.1. However, treatment of the greywater is needed to be within the acceptable risk with a 95 % confidence interval for exposure to groundwater for these organisms.

Table 6.1. Treatment required, expressed as log reduction (mean, StD), for different organisms at different exposure points, to be within an acceptable level of risk (0.001). Negative values indicate that no reduction is required or for bacteria that growth is tolerated

Exposure	Rotavirus	<i>Campylobacter</i>	<i>Salmonella</i>	<i>Giardia</i>	<i>Cryptosporidium</i>
Groundwater ^a	3.7 ± 2.1	2.2 ± 1.7	- 0.3 ± 1.7	- 1.4 ± 2.0	- 0.4 ± 2.5
Direct contact	3.0 ± 1.1	0.9 ± 1.0	- 1.6 ± 1.1	- 1.4 ± 1.1	- 2.2 ± 1.0
Sports field ^b	3.0 ± 1.1	0.9 ± 1.1	- 1.6 ± 1.1	- 1.4 ± 1.1	- 2.2 ± 1.0
Aerosol ^c	0.7 ± 1.4	- 1.3 ± 1.4	- 3.8 ± 1.4	- 3.7 ± 1.5	- 4.4 ± 1.4
Recipient	1.7 ± 1.1	- 0.4 ± 1.1	- 2.9 ± 1.1	- 2.7 ± 1.1	- 3.5 ± 1.1

^a Yearly risk from 365 exposures

^b Yearly risk from 26 exposures

^c Treatment required for *Legionella* = -1.2 ± 1.8 (Figure 6.1d).

The simulated standard deviations (StDs) originate from the PDFs used in the Monte Carlo simulation. The StD is higher for exposure to groundwater than the other scenarios, since more HMUs are included in that transmission route, giving additional uncertainties to the assessment compared to other exposures. The relatively higher StD compared to the mean from exposure to aerosol is due to the higher uncertainty in the water intake (Figure 4.2). The faecal load did not have a large impact on the result (Figure 6.2). However, the faecal contamination has only been measured in Vibyåsen, without further validation at other sites. With a faecal load of 1 $g p^{-1} d^{-1}$, the treatment need would increase 1.4 log and for a simulated wastewater 3.3 log.

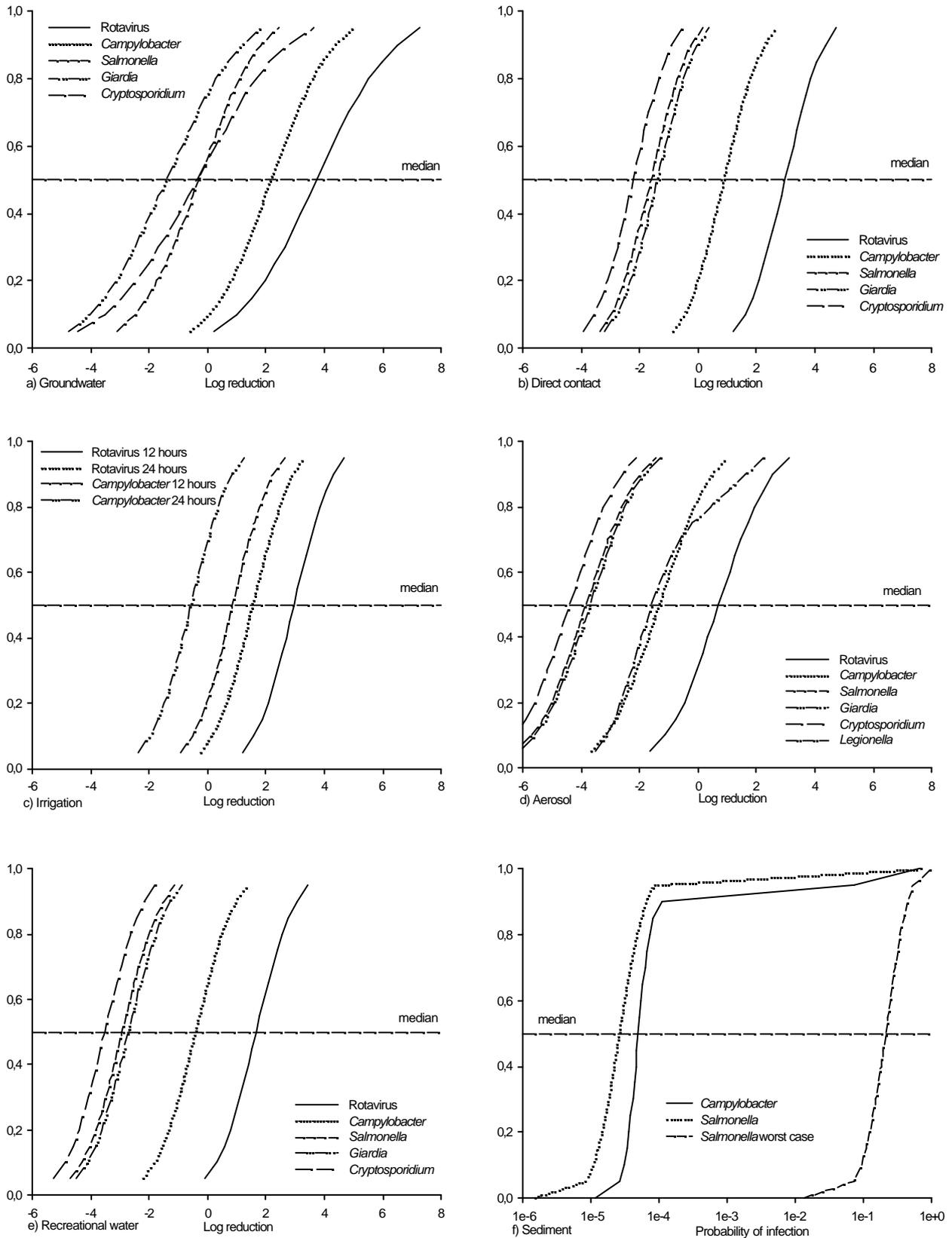


Figure 6.1. Greywater treatment needed (5 – 95%) to be within the acceptable risk level for exposure to (a) groundwater, (b) direct contact, (c) sports field (different retention times between irrigation and access), (d) aerosols and (e) recreational water; and (f) the P_{inf} (0 – 100%) from ingestion of sink trap sediment (f).

6.1. Groundwater exposure

The largest risk emanated from rotavirus and a mean 3.7 log reduction was needed according to the simulation. Rotavirus was used as an index for viruses as a group. The treatment requirement would be about the same for adenovirus and calicivirus, which are also common in the population, excreted in high numbers and have low infectious doses. Slightly less treatment is required for enteroviruses (AWWA, 1999). The other pathogen of concern in this study was *Campylobacter*, for which a 2.2 log reduction was required (Table 6.1). This scenario simulates a superficial groundwater, while the treated greywater at most places is more diluted in an aquifer than was assumed here. However, to be able to release the water untreated into the ground, a 60,000-fold dilution or an extension of the retention time to 190 days is needed to be within the 0.001 risk for rotavirus. The corresponding figures for *Campylobacter* are 1,700 dilution and 170 days retention. Sensitivity analyses showed that retention time in the saturated zone was the most important factor for the result for all organisms, followed by the reduction in the unsaturated zone, excretion densities, excretion days, dilution in aquifer, water intake and finally the faecal load. For *Giardia* and *Cryptosporidium*, where the dose-response constant had a distribution, this had the least effect of all parameters (Figure 6.2).

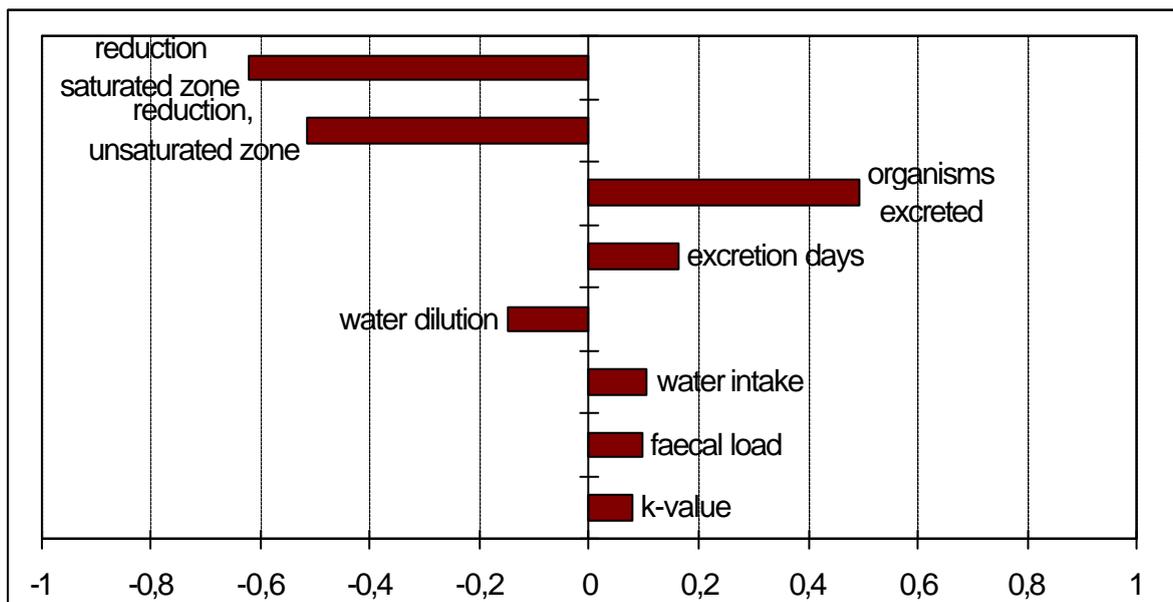


Figure 6.2. Regression sensitivity for different parameters affecting the treatment need for *Giardia*, groundwater exposure, from a Monte-Carlo simulation in @Risk 3.2 (Palisade Corporation, Newfield). The different treatment requirements correlated best to the simulated values from the probability density function (PDF) used to describe the reduction in the saturated zone, followed by the (PDF) describing the reduction in the unsaturated zone etc.

6.2. Direct contact with treated greywater

If the treated water is released locally into a pond such as in Vibyåsen (I), a mean 3 log reduction is desired for rotavirus and 0.9 for *Campylobacter* (Table 6.1) to be below a 0.001 risk of infection to a single exposure of 1 mL of treated greywater. The span (5 – 95%) was narrower here (Figure 6.1b), since the main uncertainties, reduction in the saturated and unsaturated zones from exposure to groundwater, were not a part of the simulation. Instead, excretion density was the most important factor. A way to minimise risks for direct contact with the treated greywater would be to fence the pond, making it a part of the treatment system. For the other pathogens investigated, the concentration before treatment was low enough not to imply risks over the accepted level.

6.3. Exposure via irrigation of sports fields and gardens

Irrigation is suggested in several studies as a means of reusing greywater (Table 1.1). This can lead to health risks with contact with the irrigated area or ingestion of irrigated crops, as well as ingestion/inhalation of aerosols during irrigation. To be within a 0.001 yearly risk from 26 repeated exposures to a sports field, a 3 log reduction is desired (Table 6.1). Drying, UV-light and temperature-mediated inactivation reduced the treatment requirements partly (Badawy et al. 1990). Figure 6.1c shows the difference in treatment needed for rotavirus and *Campylobacter* with a prolonged retention time between irrigation and access to the field. After a retention time of 37 hours, no reduction of rotavirus was needed according to the simulation, which was based on an assumed decay of $0.11 \log h^{-1}$ (Badwy et al. 1990). A change in the external factors listed above affects the reduction and thus also the risk of infection. For exposure to aerosols during irrigation, the treatment requirements for different organisms are presented in Figure 6.1d. For this exposure scenario, *Legionella* spp. were assessed due to their proven growth possibilities and due to aerosols being their mode of transmission. The treatment need was lower than for rotavirus, however, - 1.2 log compared to 0.7 log (Table 6.1). There is a relatively higher uncertainty connected to the treatment required for this exposure, depending on the model used for water intake (Figure 4.2).

6.4. Recreational water exposure

The mean treatment required for rotavirus was 1.7 log to be within the risk level of 0.001 with a 1,000-fold dilution in the receiving water of the treated greywater of Vibyåsen. To be able to release the water untreated, the dilution would have to be 56,000-fold. No other agent than viruses would pose any larger threat to public health according to the simulation based on the average values (Table 6.1). To be inside the acceptable level (95 % CI), some treatment of *Campylobacter* is required. As for direct contact and irrigation, no treatment is required for *Salmonella*, *Giardia* and *Cryptosporidium*, despite the median intake of 50 mL swim⁻¹. However, 50 mL recreational water corresponds to 0.5 mL greywater taking the dilution into consideration.

6.5. Exposure to sink trap sediment

The risk from exposure to sink trap sediments is shown in Table 6.2. The effect of the high infectious dose that is used for *Salmonella* in most MRAs is demonstrated by a 500-fold increased mean risk from the simulation with the generic single hit model. The table also shows the P_{inf} from accidental intake of 1 mL of untreated greywater. Here, the maximum risk model has been applied for all organisms to show the influence of the dose-response model on the outcome of the results.

Table 6.2. Probability of infection from ingestion of 1 mL untreated greywater and 0.1 g sink trap sediment, normal dose-response model and maximum risk dose response model ($P_{inf} = 1 - e^{-Dose^6}$)

Organism	Sediment	Max risk	Greywater	Max risk
Rotavirus	-	-	0.34 ± 0.25	0.64 ± 0.37
<i>Campylobacter</i>	0.013 ± 0.058	0.11 ± 0.28	0.036 ± 0.25	0.44 ± 0.38
<i>Salmonella</i>	0.0051 ± 0.013	0.25 ± 0.28	0.00043 ± 0.0034	0.36 ± 0.37
<i>Giardia</i>	-	-	0.00087 ± 0.0082	0.026 ± 0.092
<i>Cryptosporidium</i>	-	-	0.000091 ± 0.00081	0.018 ± 0.069

The sharp bends of the graphs in Figure 6.1f. arise from the final percentages being connected to exposure to sediments contaminated with infected chicken. The P_{inf} up to the bend is from exposure to a distribution between 0 – 0.04 c.f.u. g^{-1} (detection limit). Despite the high P_{inf} from direct exposure to sink trap sediment (last 10%) (Figure 6.1f), the influence of pathogenic bacteria from the kitchen sink to the total amount in the greywater was negligible. The medium input from the sink trap compared to that from faecal sources was less than 1/4,200 from a single household according to the simulations.

7. FINAL DISCUSSION AND CONCLUSIONS

7.1. Faecal load

If the investigated greywater system were to be judged according to the traditional indicator bacteria analyses, the conclusion would be that a substantial, although highly variable, faecal input had taken place. This is consistent with several other studies (Table 1.2). However, bacterial indicator growth, especially the coliform group, leads to an overestimation of the faecal load. The *E. coli* density in the greywater from Vibyåsen corresponded to a median faecal load of 65 g person⁻¹ day⁻¹ (min/max 1.3 – 410). This can be compared to the faecal load of 5.2 (0.2 – 26), 0.04 (0.016 – 0.076) and 0.22 (0.094 – 0.40) g p⁻¹ d⁻¹ from the measurement of enterococci, coprostanol and cholesterol respectively (Table 4.2). Of the bacterial indicators, enterococci seem to be the most appropriate to use. They do not grow as extensively in the greywater system as the coliform group. Furthermore, they are more tolerant to environmental stress and a better indicator of enteric viruses than the coliforms (Bitton et al., 1981). In (I), the assessment from exposure to water with different enterococci densities correlated well to the simulated risk from rotavirus exposure. One problem with enterococci in greywater, however, is their higher sensitivity to detergents than many pathogens (Stenström, 1985). Instead of bacterial indicators, chemical biomarkers can be used. The faecal load in the simulations was based on coprostanol as a conservative biomarker. There are some limitations with this measurement as well. Not all young children under two years excrete coprostanol, since they lack the microbiota needed to convert cholesterol to coprostanol (Midtvedt & Midtvedt, 1993). One major input of faeces in the greywater may come from washing diapers and soiled infant clothes, as well as from the babies themselves, and an underestimation of the faecal load is thus possible. That is why cholesterol concentrations were also measured, even though they may overestimate the faecal load since cholesterol may also emanate from the kitchen sink and other non-faecal sources. However, the measurement of the two different sterols gives a concentration span of potential faecal input that is more realistic than when taking into account the indicator bacteria. The detection of faecal sterols requires expensive equipment, however. Despite the low faecal load, the median input of rotavirus was simulated to be 1.7 particles mL⁻¹ and thus detectable. A follow-up study including analysis of enteric viruses would give substantial input to the risk models.

7.2. Risk characterisation

For the different exposure scenarios simulated, the treatment need for rotavirus was more than 3 log in many cases (Table 6.1). At the same time there is little information on the efficiency of virus removal in different treatment processes. Kayaalp (1996) stressed the need for site-specific considerations when planning for infiltration units, which is the traditional method of greywater treatment in Sweden, after pre-treatment in a settling tank. Of great importance is the height of the watertable. The longer the distance organisms have to travel through the unsaturated zone, the better the reduction effect (Stenström et al., 1980). In “Wastewater Infiltration: Conditions, Function, Environmental Consequences” (Naturvårdsverket, 1985),

there are recommendations for the planning of wastewater infiltration units depending on site-specific conditions such as topography, type of soil and height of the watertable. The suggestion is to follow these even though they have been prepared for wastewater, not greywater, infiltration. This is because the simulated risk (I) and treatment requirements for rotavirus were unacceptably high. Factors that influence virus movement in soil, and that are thus important to evaluate when preparing an infiltration site, are listed in Table 7.1. In addition to the microbial contamination of soil and groundwater, the impact from household chemicals as well as phosphorus and nitrogen contamination must be considered, which might be a problem of larger magnitude. There is a risk of a pH rise and zinc accumulation in the soil as well as excessive phosphorus leakage to groundwater in sandy soils (Christova-Boal et al., 1996).

Table 7.1. Factors that influence the movement of viruses in soil

Factor	Remark
Rainfall	Viruses retained near the soil surface may be eluted after a heavy rainfall because of the establishment of ionic gradients within the soil column
pH	Low pH favours virus adsorption; high pH results in elution of adsorbed virus.
Soil composition	Viruses are readily adsorbed to clays under appropriate conditions and the higher the clay content of the soil, the greater the expected removal of virus. Sandy loam soils and other soils containing organic matter are also favourable for virus removal. Soils with a low surface area do not achieve good virus removal.
Flow rate	As the flow rate increases, virus removal declines.
Soluble organics	Soluble organic matter competes with viruses for adsorption sites on the soil particles, resulting in decreased virus adsorption or even elution of already adsorbed virus.
Cations	The presence of cations usually enhances the retention of viruses by soil.

Irrigation is suggested in many studies as a means of reusing greywater (Table 1.1). The treatment demand for using greywater on a sports field was simulated to a mean of 3 log, after a retention time of 12 hours between irrigation and access. Prolonging this access time and exposing viruses to sunlight would decrease the treatment need significantly. Greywater can also be used on a local (household) scale. The risk of infection from this exposure is lower due to the lower volume ingested and a mean of 0.7 log reduction of rotavirus is suggested. Besides, the infection status may be known on a household level. Due to aerosols as the mode of transmission, *Legionella* was assessed for this scenario. Depending on the temperature, the presence of other microorganisms, available carbon sources and other factors (Muraca et al., 1988) *Legionella* may grow in the tank, increasing the risk of infection. However, the simulation indicated a low risk and it is probably more likely that an infection would be caught in the shower or from other sources of exposure. Greywater may also be used in combination with rainwater (Dixon et al. 1999; Albrechtsen 2001). This would dilute the greywater with roof-collected rainwater, which however may be contaminated with pathogens from birds or other animals.

Viruses were the only pathogens of concern from recreational water exposure based on median averages from the simulation. A mean treatment need of 1.7 log was simulated. However, this was based on an acceptable risk of infection of 0.1%. The guidelines for the proposed EU bathing water directive are based on a mean acceptable excess risk of illness due to bathing of 1% (EU, 2002), which is fulfilled with just a 0.6 log reduction of rotavirus. Jenssen (2001) considered no or primary treatment to be sufficient for greywater before discharge to the sea, while secondary treatment was recommended for discharge to inland lakes or rivers. This statement, referring to eutrophying substances, might however be valid for microorganisms as well. In order to discharge greywater to small local streams or use it for irrigation or groundwater recharge, reduction of the hygiene parameters is important and pre-treatment suggested (Jenssen, 2001), which is in line with the results from this study.

As stated in the hazard identification, pathogens can be introduced in the kitchen from contaminated food and from the sink trap functioning as a place for regrowth of pathogenic bacteria. Christova-Boal et al. (1996) suggest that the kitchen sink should not be included in the greywater. This would decrease the growth possibilities for pathogenic bacteria in the greywater system significantly (Naturvårdsverket, 1995). Vinnerås (2002) stressed the possibility of treating blackwater together with biosolids, for example by including kitchen waste directly from the sink with a grinder, which is also proposed by Otterpohl (2002). This creates possibilities for new technical solutions that could be beneficial from a health point of view. However, exposure to sink trap sediments did not result in an unacceptably high risk of infection. Even with simulated growth in the sediments, the input from the kitchen did little to the total input of pathogenic bacteria to the greywater.

For exposure to groundwater, reduction in the saturated zone had the most significant impact on the result, not surprisingly since the log-reduction day⁻¹ was amplified by a magnitude of 60 (2 months) to the final result. In the other scenarios, excretion density and excretion days had the most influence. One way to get better data on these distributions is to examine more faecal samples and to monitor infected people to see how long they continue to shed pathogens. However, this is time-consuming and may impinge on people's private lives. Since the amount of viruses estimated from the simulation could be detectable, it is better to enumerate viruses in the greywater and assess the microbial risks based on occurrence data. The sensitivity analyses in @Risk (Palisade Corporation, Newfield) are, however, limited to measuring the correlation between the input from a probability density function and the risk outcome, not to the impact of a change in the performance of an HMU on the outcome. The faecal load did for example not have such a large effect on the result in the simulation. However, the faecal load was only measured with coprostanol at one site. A higher faecal load would increase the treatment requirements significantly.

7.3. Greywater treatment

Despite the low faecal load of greywater the treatment demand, especially for rotavirus, was significant. Treatment required for several of the simulated scenarios was 3 log (99.9%). The reduction from the studies in Table 4.4 may be sufficient for separating bacteria but the mean

reduction of bacteriophages was only 1.0 log. Infiltration, the most common method, is generally an adequate wastewater treatment method (Stenström, 1985), but demands a thorough investigation when planning for the site (Naturvårdsverket, 1985; Kayaalp, 1996). Even under proper conditions there is a risk of macropore flow of viruses as shown in Carlander et al. (2000). Additional pre-treatment to settling tanks can be needed. Chemical precipitation is one option that efficiently removes both viruses (Stenström, 1986) and phosphorus (Hanaeus, 1987). An aerating biofilter or a constructed wetland is proposed by Jenssen (2001). A pond with an impermeable foundation is a third alternative, although the risk of exposure to the untreated water must be considered due to the unacceptable risk from direct contact with untreated greywater.

Active sludge or other types of biological treatment have been an important strategy in reducing nitrogen effluents to receiving waters in Sweden (Scharin, 2002). However, Swedish greywater has a low nitrogen content as well as available carbon sources (Naturvårdsverket, 1995), which is why this kind of treatment may not be so efficient under Swedish conditions (I). Membrane technology is an interesting alternative. In a study on the performance of two advanced biological unit processes for greywater recycling, a membrane bioreactor (MBR) and biologically aerated filters (BAF), the MBR performed better, especially in regard to microbiological quality (Jefferson et al., 2001). Due to fouling of the membranes, pre-treatment is necessary (EPA, 2001). Another uncertainty may be the cost of maintenance under local decentralised conditions. Looking at the different treatments required to meet an acceptable greywater quality (Table 6.1), disinfection could be a cost-effective alternative. However, the use of chemicals does not go hand in hand with a sustainable wastewater management. Furthermore, chemical disinfection does not reduce the phosphorus levels, which may be a larger problem than microbial contaminants (Christova-Boal et al., 1996). UV-disinfection has been tried but the current experience is that pre-treatment is needed to efficiently reduce pathogen levels (Westrell, 1997; Lindgren & Grette, 1998). Evaluations of different methods to treat greywater with Swedish characteristics and under Swedish conditions, including models for virus removal, are warranted.

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