Assessment of Microbial Health Hazards Associated With Wastewater Application to Willow Coppice, Coniferous Forest and Wetland Systems

Anneli Carlander
Faculty of Natural Resources and Agricultural Sciences
Department of Crop Production Ecology
Uppsala

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Abstract

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Treatment and reuse of wastewater by irrigation of willow coppice, forest or wetlands may create new exposure routes for pathogens. This thesis summarises results from a series of field and laboratory studies aimed at identifying and quantifying the microbial health hazards associated with such alternative wastewater treatment systems.

Leaching and retention of viruses in the soil-plant system were studied in a lysimeter experiment using a bacteriophage as model organism. The presence and die-off of pathogens was studied in three full-scale systems with wastewater irrigation of willow in southern Sweden. The reduction in pathogens was also studied in microcosms under controlled conditions. In addition, the presence and die-off of pathogens in two wetlands was studied. Finally, a risk assessment was made in order to identify and quantify the most important exposure routes of pathogens.

In the Swedish full-scale systems, the average reduction in microorganisms in the wastewater treatment plants was in the range 1.3-2.5 $\log_{10}$. Analyses of faeces collected in the irrigated area did not indicate an increase in pathogens in mammals and birds, whereas indicator organisms were detected in foliage and in some groundwater samples in the fields. The results of the lysimeter study showed very high retention of viruses in sandy soils, whereas leaching to groundwater was substantial and extremely rapid in the clay soil. In the microcosm study *Campylobacter* were rapidly reduced (<3 h) while *Salmonella* bacteria were highly resistant. No single factor (light, temperature or radiation) was found to govern the reduction. In the wetlands studied, the reduction in suspended particles seemed to be the main factor controlling bacterial elimination from the water phase. In the sediment, survival of microorganisms was prolonged. The theoretical microbial risk assessment indicated a substantial risk of viral infections caused by direct contact with the wastewater, with aerosols from irrigation, or by drinking contaminated groundwater.

Keywords: indicator organism, irrigation, leaching, lysimeters, pathogens, risk assessment, transmission, wastewater, wetlands, willow, zoonose.

Author's adress: Anneli Carlander, Swedish Institute for Infectious Disease Control, SE-171 82 Solna, Sweden

To my grandmother Elsa

♥ *13/8 1919*
† *16/3 2006*

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Appendix

Paper I-V

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Carlander, A., Aronsson, P., Allestam, G., Stenström, T.A. & Perttu, K. 2000. Transport and retention of bacteriophages in two types of willow-cropped zero-tension lysimeters. *Journal of Environmental Science and Health A35:*8, 1477-1492.

II. Carlander, A. & Stenström, T.A. 2001. *Irrigation with pretreated wastewater on short rotation willow coppice – a sanitary study in Sweden*. International Ecological Engineering Conference, Christchurch, New Zealand, 25-29 November, 2001.

III. Åström, J., **Carlander, A**., Sahlén, K. & Stenström, T.A. Faecal indicator and pathogen reduction in vegetation microcosm (manuscript submitted to *Water, Air & Soil Pollution.*

IV. Stenström, T.A. & **Carlander, A**. 2000. Occurrence and die-off of indicator organisms in the sediment and in two constructed wetlands. *Water Science and Technology 44* (11-12), 223-230.

V. Carlander, A., Schönning, C. & Stenström, T.A. Comparative microbial risk assessment – short rotation willow coppice irrigated with wastewater in Greece, Northern Ireland and Sweden (manuscript)

Introduction

Reuse of wastewater –history

During recent years, growing concern and interest has been directed towards alternative or complementary solutions to the existing sanitary systems for recirculating plant nutrients in wastewater and sludge back to arable soils. This is based on the realisation that wastewaters and sludge can pollute water bodies and lead to eutrophication, while at the same time they constitute a valuable fertiliser resource for plants.

The reuse of wastewater for irrigation, with land as a recipient, includes both forest and agricultural crops and has historically been practised in many countries (Crites, 1984; Asano & Levine, 1996). However, rather than arising from tradition and history, the growing interest in wastewater irrigation in many parts of the world today is a response to pressures of increased population and overexploitation of valuable resources, *e.g*. use of groundwater for food production and commercial fertilisers for plant nutrients. In addition, environmental problems such as climate change indicate the need for alternative fuel sources. During the past decade, discussions regarding the reuse of sludge and wastewater on agricultural land have appeared on the agenda due to additional problems, *e.g*. with disposal of sewage, as well as leaching of nutrients from landfills.

However, if not managed properly, the reuse of wastewater can cause negative side-effects in relation to both humans and the environment. Risks relate to the content of heavy metals and persistent organic contaminants emanating from industrial or household sources, which can accumulate in the soil, with potential negative effects on both plant growth and the soil biota. Risks can also arise from the content of pathogenic microorganisms in wastewater, reflecting the disease prevalence in the population served, where transmission may occur to exposed individuals through different routes from the recirculated wastewater. The safe use and management of wastewater in agriculture have recently been discussed in new WHO Guidelines (WHO, 2006). These provide suggestions for treatment and exposure barriers, in order to limit the effects on humans to an acceptable risk level.

One alternative way of reducing the negative effects of different types of waste products while at the same time taking advantage of the positive effects of the water and plant nutrients is their utilisation and treatment in different types of crops, *e.g*. utilising fast-growing willow plantations for purification of wastewater and sludge (Perttu, 1993; Perttu & Kowalik, 1997). The cultivation of such short rotation willow coppice started in Sweden during the oil crisis in the 1970s, with the aim of cultivating fast-growing crops for bio-fuels (Perttu, 1998). In addition to a potentially very high growth, willow showed efficient nutrient uptake (Ericsson, 1981), high evapotranspiration (Persson & Lindroth, 1994), and for some clones a capacity for taking up heavy metals, especially cadmium (Cd)

(Landberg & Greger, 1994; Klang-Westin & Perttu, 2002; Lundström & Hasselgren, 2003) thereby giving a possible remediation of contaminated soils (Perttu, 1998). Using wastewater, partly treated or untreated, as irrigation water on willow coppice sanitises the wastewater while at the same time using both the nutrients and the water as a resource for biomass production. In addition to wastewater, sludge from municipal treatment plants has also been used for fertilisation of willow coppice and today, approximately 10% of the sludge produced in Sweden (100000 dewatered tonnes) is used as fertiliser in short rotation willow coppice (Alvén *et al.*, 2003).

Wastewater irrigation and sludge application have also been performed in conventional forestry (K. Sahlén, pers. comm). In order to increase wood production, conventional forests in Sweden have been fertilised with mineral nitrogen fertilisers (*e.g*. ammonium nitrate and urea) since the 1960s (Pettersson & Högblom, 2004). Since 2005, land filling of organic waste is no longer allowed in the EU, which has resulted in a need for new and alternative applications for the sludge produced, and fertilisation of forest could potentially be such an alternative way of recycling the sludge.

Besides treatment by application to arable- or forest land, wastewater could also be treated in wetlands constructed specifically for treatment of various type of polluted water. Natural wetlands have been used for wastewater discharge as long as sewage has been collected, but were not monitored regarding treatment efficiency until the 1960-1970s (Kadlec & Knight, 1996). When monitoring was initiated, an awareness of the water purification potential of wetland emerged. In Sweden, interest in wetlands increased during the 1980s due to the discussion regarding eutrophication of recipient waters in general and the Baltic Sea in particular (Lundberg, 2005). Several Swedish municipalities have constructed wetlands as a complementary treatment step for municipal wastewater and/or stormwater.

Reuse in willow coppice, coniferous forest and wetlands

Willow coppice

Today, approximately 14000 ha of agricultural land (*i.e.* approximately 0.5% of Swedish farmland) are planted with short rotation willow coppice for use as fuel in district heating plants (SJV, 2005). Of the total area of willow coppice, approximately 150-200 ha are irrigated with municipal wastewater, and a further 50 ha irrigated with other types of contaminated water such as industrial wastewater or leachate water (Aronsson, pers. comm. 2006). The dominant species in short rotation forestry is willow (*Salix* spp.) but grey alder and poplar have also been tested in Sweden (Perttu, 1998).

When establishing a willow coppice, between 12000 and 14000 stem cuttings per hectare (Aronsson, 2000) are planted during spring in a double-row pattern with 1.50/0.75 m spacings between rows and 0.6-0.7 m between plants within rows (Hasselgren, 2003) The double-row system is adopted to facilitate fully mechanised harvest. If wastewater irrigated, the irrigation system (tubes or sprinklers) is placed within the double rows. The willow coppice is harvested during winter every 3-5 years, and after harvest the plants resprout from the stumps. The economic lifespan of a willow coppice is estimated to be around 25 years. If sludge is used as a fertiliser, it is applied before planting or after harvest. The average annual production of wood chips from a well-established willow coppice is in the range 5-10 tonnes of dry matter per hectare, which is equivalent to 2.5-5 tonnes of oil per hectare and year. Willow coppice responds strongly to fertilisation, with approximately 100% increases in growth when N is applied according to recommendations (*i.e.* in the order of 80 kg N per ha and yr (Nordh, 2005)).

When willow coppice is used for wastewater treatment, the treatment can be achieved at a reasonably low cost (Rosenqvist *et al.*, 1997). Other benefits include the cost saving for avoiding treatment in the conventional treatment plant, the access to plant nutrients for free and increased biomass productivity. In many instances, the prospect of reducing the treatment costs is the most important motive for alternative treatment approaches, combined with enhanced reuse of resources in the wastewater (Wittgren & Hasselgren, 1992).

Coniferous forest

The dominating tree species in the Nordic countries are spruce and pine, with rotation periods often exceeding 100 years (Skogsstyrelsen, 2005). Nitrogen is the most important limiting nutrient for tree growth of the boreal conifers, and considerable growth increases are generally found after application of mineral N-fertilizers, commonly in doses of 150 kg N/ha (Pettersson & Högblom, 2004). The use of sewage sludge for fertilization of coniferous forests has mainly been tested in North America, but also in the Nordic countries. In the rest of Europe sludge fertilization of conventional forests has not been practiced in operational scale. Results from sludge fertilization trials in the Nordic countries are still limited, but from the existing experiments it has been shown that the use of sludge as fertilizer in coniferous forests can result in an increased tree growth of some 50% when applying 20 tonnes (DW) sludge per hectare (Bramryd, 2001). Sludge can be applied in various ways *e.g.* as sludge pellets, ash/sludge pellets, dewatered, aerobically stabilized sludge, or as mechanically treated wastewater.

One of the Swedish study areas is situated in Vindeln in Northern Sweden. Raw wastewater is applied during summertime (June to August) with sprinklers to a 60-year old Scots pine forest in amounts corresponding to approximately 100 kg N/ha and year. During the study period 1997-2002, the stem growth was around 70% higher for the irrigated trees compared with unirrigated trees (Sahlén, pers. comm.).

Wetlands

In the beginning of the 1990s, constructed wetlands attracted increasing interest as an alternative or complementary treatment step for municipal wastewater or stormwater, mainly due to their potential reduction of nitrogen (Kim, Seagren & Davis, 2003), but also to their removal of chemicals (Hsieh & Davis, 2005), as well as phosphorus and suspended matter (Tonderski, Arheimer & Pers, 2005). To reduce the load of nutrients to the Baltic Sea, coastal treatment plants in southern Sweden that were larger than 10000 pe, were forced to reduce discharge of nitrogen to the sea (Naturvårdsverket, 1993). The first full-scale wetland for treatment of municipal wastewater was established in Oxelösund in 1993 (Andersson, Wittgren & Ridderstolpe, 2000). In addition to nutrients, chemicals, particulate matter and faecal organisms present in the wastewater also need to be removed. The processes responsible for their removal include filtration, solar irradiation, sedimentation, predation and competition (Gersberg *et al.*, 1987). Wetlands for treatment of stormwater have been constructed as well, this in order to control floods, reduce the amount of nutrients, pollutants and particles, reaching the recipient (Livingstone, 1989). The stormwater contains a wide range of pollutants, dependent on the run off area, *e.g.* oil, litters, nutrients, sediments, organic matter and microorganisms (Davies & Bavor, 2000). Current knowledge regarding the microbial quality of stormwater wetlands is limited both in Sweden and internationally, and the risks with pathogens occurring in this type of water are often not considered when planning and constructing wetlands for this purpose.

In 1999, the Swedish Parliament adopted 15 environmental goals, and a 16th was adopted in November 2005. The goals define the state of the environment which environmental policy aims to achieve and provide a coherent framework for environmental programmes and initiatives at national, regional and local level (Naturvårdsverket, 2006). Reuse of wastewater, with the aim of returning nutrients to arable soil as well as using wetlands for reducing the amounts of nutrients reaching recipient waters, fits well into several of these goals.

Reuse of wastewater – new transmission routes?

One main risk regarding the reuse of wastewater to the occurrence of pathogenic organisms in the wastewater or sludge applied to land or water and their potential further transmission to humans. In conventional systems, the faeces containing the excreted pathogens end up in a wastewater treatment plant, where both the nutrients and the organisms are reduced, the latter normally by 1-3 $\log_{10}$, depending on treatment level and organism, before the wastewater is discharged to a recipient. A large fraction of the faecal organisms are attached to solid particles and are thus concentrated in the sludge. Potential transmission of pathogenic organisms to humans can occur directly from those remaining in the water and indirectly when the recipient water is used, *e.g.* for recreational activities. Correspondingly, the sludge produced, if used as a fertiliser in agriculture, can also transmit pathogens to humans upon exposure, where the risks relate to the treatment and other applied barriers.

The use of treated wastewater or sludge in energy forest or conventional forestry or the further treatment of municipal wastewater or stormwater in wetlands can function as a barrier against transmission. On the other hand, new transmission routes can also be created where people or animals come into contact with the wastewater or sludge. According to WHO (2006), the most important exposure routes when reusing wastewater and sludge in agriculture are human contact with wastewater or contaminated crops, inhalation of aerosols, consumption of wastewater-irrigated crops, consumption of contaminated drinking water, consumption of animals or animal products that have been contaminated through wastewater exposure, and vector-borne disease transmission. The magnitude of the risk is dependent on the storage or pre-treatment of the wastewater, as well as on the methods of application.

Direct contact

Direct contact with the contaminated wastewater or sludge can occur if humans have access to wastewater/sludge-supplied areas. For a willow coppice, staff working in the fields would probably be the group potentially exposed. For forest and wetland areas, people could accidentally come into contact with the wastewater or sludge if the areas are also used as recreational sites.

Humans and animals could also come into contact with contaminated soil or crops. Short rotation forest, conventional forest and wetlands are not used for food or fodder crops, which is a benefit, but forests are used for recreational activities with people picking wild berries and mushrooms.

Groundwater

Groundwater is generally used as an untreated drinking water source. Viruses, bacteria and protozoa are of concern for potential groundwater pollution. Several waterborne outbreaks have been reported, caused by contaminated groundwater, in for example Sweden, the USA and Great Britain (Stenström *et al.*, 1994; Furtado *et al.*, 1998; Barwick *et al.*, 2000). High levels of wastewater applied to the field could increase the risk for transport of microorganisms down to the groundwater.

Viruses are of prime interest due to the potentially high numbers excreted and the low infection dose (Oron *et al.*, 1995), as well as their small size and consequently their easy transport through the soil to the groundwater. Viruses also have increased persistence due to the low temperature in the groundwater (Yates, Gerba & Kelley, 1985). Therefore, the transport behaviour of viruses in soils where wastewater irrigation has been applied is of major importance when evaluating the sanitary risks. As stated, the transport of viruses in the soil-water matrix can be rapid, but varies depending on, for example, soil characteristics, types of virus and climate (Keswick & Gerba, 1980; Yates, Gerba & Kelley, 1985). The risk is lower if the water is evenly distributed over the soil surface and does not exceed plant requirements for water. In permeable soils with high groundwater levels, the application of water can give locally high loads, which may lead to a higher penetration of the microorganisms. In addition to viruses, larger organisms like *Cryptosporidium* have also been found in groundwater (Foster, 2000; Morris & Foster, 2000).

Aerosols

Dependent on the irrigation method, aerosols can be created, when the wastewater is applied to the fields. Microorganisms, especially viruses, can be transported with aerosol droplets and spread by the wind (Carducci, Arrighi & Ruschi, 1995; Carducci *et al.*, 1999; Brandi, Sisti & Amagliani, 2000) and infect exposed humans and animals. Sprinkler irrigation should consequently be restricted close to human settlements or gathering places. Infections can occur either through swallowing or inhaling contaminated water drops, or by exposure through the eyes.

Various distances that aerosols can be transported over are reported in the literature, *e.g.* 40 m (Teltsch & Katzenelson, 1978) to 730 m downwind irrigated fields (Shuval *et al.*, 1989) referred in Schwartzbrod (1995). Airborne transmission was also exemplified by Bausum *et al.* (1982) who analysed the occurrence of bacteria and bacteriophages at an irrigation site in Arizona, USA. Bacteria were found in the air in concentrations of 500 colony-forming units per cubic metre (cfu/m^3) up to 150 metres from the irrigation source and coliphages up to 560 metres from source. The creation of aerosols is dependent on the type of irrigation system used. Their placement above ground and the radius of water emission are factors governing the further spreading. The risk is limited if the sprinkler system used creates large drops, the sprinklers are placed close to the ground and the water is distributed for short distances under low pressure. Most sprinkler irrigation systems require a safety buffer zones regarding distance to houses, roads, and edible crops or pasture land. To prevent disease transmission through aerosols from low-emitting sprinklers, the outer zone of large fields should not be irrigated in order to function as a shield against transmission. With surface or sub-surface irrigation, the risks of creating aerosols are minimised.

Transmission to animals

Animals can act as secondary carriers of pathogens. Secondary transmission can be established from creatures living in the irrigated area to domestic animals or house pets and further to humans. Animals in an irrigated area live in a special environment, more or less continuously exposed to pathogenic microorganisms, and will probably also use the wastewater as drinking water. Birds and rodents may move to other localities and can thus transport pathogens. One example in the literature concerns a water reservoir in Norway, which became contaminated with *Campylobacter* transported by sea gulls which resulted in approximately 2000 infected persons (Stenström*, et al.*, 1994). In a study by Palmgren *et al.* (1997), migratory birds in Sweden were found to be carrying both *Salmonella* and *Campylobacter*.

Pathogens in wastewater

Untreated wastewater contains a large range of pathogenic microorganisms, where the type of pathogens varies with region and time. Faeces from a healthy individual contain large numbers of bacteria that do not cause any diseases, while infected persons may excrete large amounts of pathogens, the numbers depending on the etiological agents in question. Pathogens in the wastewater are excreted in the faeces from infected individuals and consist of bacteria, viruses, parasitic protozoa and/or helminths. Examples from these groups and the corresponding diseases and symptoms are summarised in Table 1.

The waterborne outbreaks that have occurred in Sweden during recent years have mainly been attributed to *Campylobacter*, *Giardia intestinalis* and noroviruses. Toxin-producing *E. coli*, *Entamoeba histolytica* and *Cryptosporidium* have also been found, but for the majority of the outbreaks the causative agents have not been identified (SMI, 2006).

Bacteria

Among the etiological agents identified to have caused waterborne outbreaks, *Campylobacter* is the most frequently identified bacterium in Sweden (SMI, 2005). The genus *Campylobacter* contains 16 species, including several that may be pathogenic to both humans and animals (Fricker, 1999; Schroeder & Wuertz, 2003). Of greatest concern for human infection are *C. jejuni* and *C. coli* (SMI, 2006) and approximately 6000 cases were reported in Sweden in 2004 (SMI, 2005). In addition to humans, *Campylobacter* has been found in a wide variety of domestic and wild animals, particularly birds, where almost all bird species tested have been found to carry *Campylobacter* (Fricker, 1999). The infective dose for *Campylobacter* is normally low, *i.e.* 500-1000 bacteria can cause infection (CDC, 2006a; WHO, 2004).

Internationally, *Salmonella* is an important environmental and food pathogen and it has been known for more than 100 years that it causes illness (CDC, 2006b). *Salmonella* is the second most common bacterial cause, after *Campylobacter,* of enteric disease in Sweden (SMI, 2005). *Salmonella* is a group of intestinal bacterial species belonging to the *Enterobacteriaceae* family and includes more than 2000 serotypes, of which approx. 20 are relatively common in Sweden (SMI, 2006). About 3500 cases were reported in Sweden in 2004, of which approximately 85% were infected abroad (SMI, 2005). *Salmonella*, as a zoonotic agent, can be transmitted between humans and animals. Some of the serotypes are host-specific, *e.g*. *S. dublin* - cattle, *S. typhi* and *S. paratyphi* - humans, but most are not host-specific and can infect several different species. The infective dose for *Salmonella* is normally high, at least 10^5 bacteria for causing symptoms (SMI, 2006).

Parasitic protozoa

Giardia is a protozoan parasite belonging to the flagellates. *Giardia* can infect several mammal species like dogs, cats and beavers, as well as humans (Schaefer, 1999). The infective stage is a resting stage, cysts, excreted in the faeces. *Giardia* is also present in a vegetative form in the gut (trophozoite). The infective dose for *Giardia* is considered low, normally less than 100 cysts (SMI, 2006). In Sweden, approximately 1500 cases per year are reported, among which the majority are infected abroad (SMI, 2006).

Cryptosporidium was first described in 1912 on the basis of its morphology and life cycle, but was not identified as a human pathogen until 1976 (Sterling & Marshall, 1999). *Cryptosporidium* is a unicellular eucaryotic organism and is present in several animal species, for example cattle and sheep. The transmissible stage is thick-walled oocysts, 4 to 6 µm in size, excreted in faeces. This can survive for months in cold, moist environments like lakes or streams (Sterling & Marshall, 1999).

Enteric viruses

Viruses are the most common cause of gastrointestinal infection worldwide (Heritage, 2003) and more than 140 types of pathogenic viruses can be excreted in the faeces and further transmitted (Schwartzbrod, 1995). Viruses are often excreted in high numbers and are not able to replicate outside the host. Often a low infective dose suffices. Of the viruses that could be excreted in the faeces, enteroviruses, including hepatitis A virus, adenovirus and rotaviruses, are frequently present in domestic wastewater (Oragui, 2003). Rotaviruses are the most common cause of severe diarrhoea among children (CDC, 2006c).

Helminths

Infections with helminths are not common in Sweden and are mostly associated with areas in developing countries with poor sanitation. The helminth eggs, *e.g. Ascaris,* are very resistant and can survive for long periods, months to years, in the environment (Feachem *et al.*, 1983). Since they are very resistant to different treatments, such as heat, desiccation, chemical and biological degradation, and thus very persistent in the environment, actual elimination of *Ascaris* eggs would also result in the elimination of most other pathogens. This makes *Ascaris* eggs a good process indicator for hygiene testing of faecal material that is to be reused as a fertiliser (Feachem*, et al.*, 1983).

Table 1. *Examples of pathogens that may be excreted in faeces and related diseases, including examples of symptoms (adopted from Schönning & Stenström (2004) and Ottoson (2005))*

Pathogen species	Disease; symptoms
Bacteria	
Aeromonas spp.	Enteritis
Campylobacter jejuni/coli	Campylobacteriosis; diarrhoea, cramping, abdominal pain, fever, nausea, arthritis; Guillain-Barré syndrome
Escherichia coli (EIEC, EPEC, ETEC, EHEC)	Enteritis
Salmonella typhi/paratyphi	Typhoid/paratyphoid fever; headache, fever, malaise, anorexia, bradycardia, splenomegaly, cough
Salmonella spp.	Salmonellosis; diarrhoea, fever, abdominal cramps
Shigella spp.	Shigellosis; dysentery (bloody diarrhoea), vomiting, cramps, fever, Reiter´s syndrome
Vibrio cholerae	Cholera; watery diarrhoea, lethal if severe and untreated
Yersinia spp.	Yersiniosis; fever, abdominal pain, diarrhoea, joint pains, rash
Viruses	
Adenovirus	Various; respiratory illness. Included here due to the enteric types (see below)
Enteric adenovirus 40 and 41	Enteritis
Astrovirus	Enteritis
Calicivirus (Noro- and sapovirus)	Enteritis
Coxsackie virus	Various, respiratory illness, enteritis, viral meningitis
Echovirus	Aseptic meningitis, encephalitis, often asymptomatic
Enterovirus types 68-71	Meningitis, encephalitis, paralysis
Hepatitis A	Hepatitis; fever, malaise, anorexia, nausea, abdominal discomfort, jaundice
Hepatitis E	Hepatitis
Poliovirus	Poliomyelitis; often asymptomatic, fever, nausea, vomiting, headache, paralysis
Rotavirus	Enteritis
Parasitic protozoa	
Cryptosporidium parvum/hominis	Cryptosporidiosis; watery diarrhoea, abdominal cramps and pain
Cyclospora cayatanensis	Often asymptomatic; diarrhoea, abdominal pain
Entamoeba histolytica	Amoebiasis; often asymptomatic, dysentery, abdominal discomfort, fever, chills
Giardia intestinalis	Giardiasis; diarrhoea, abdominal cramps, malaise, weight loss
Helminths	
Ascaris lumbricoides	Generally few or no symptoms, wheezing, coughing, fever, enteritis, pulmonary eosinophilia

Treatment

The pathogenic organisms are excreted in the faeces in various concentrations resulting in varying levels also in wastewater and sludge due to the prevalence in the population connected to the wastewater system (Table 2). The pathogens end up in the sewage and, if connected to a treatment plant, are reduced in concentration due to the treatment in question before being discharged to recipients.

Table 2. *Concentrations of excreted pathogenic organisms in raw wastewater (Ottoson, 2001; Ottoson et al., 2006; WHO, 2006)*

Organism	Concentration in raw wastewater (log_{10} per litre)
Pathogenic bacteria	
Salmonella spp	1-4
Campylobacter spp	1-4
Protozoa	
Giardia spp	1-5
Cryptosporidium spp	<1-4
Viruses	
Enteric viruses	5-6
Rotavirus	2-5
Noroviruses	<2.9-3.6
Enteroviruses	3.6-5.9

In Sweden, almost all population centres are connected to a treatment plant and approximately 95% of the wastewater undergoes biological and chemical treatment before being discharged to recipient waters (Naturvårdsverket, 2004). The removal of organisms varies between the treatment steps used, where the primary treatment, *i.e.* primary sedimentation, has a low removal, 0-1 log_{10} for most organisms (Feachem *et al.*, 1983; Yates & Gerba, 1998; WHO, 2006). Secondary treatment, a biological process (*e.g.* activated sludge, trickling filters) follows the sedimentation, and further reduces the concentrations of organisms, 0-2 log_{10} units of viruses, bacteria and protozoans (oo)cysts and 1-2 log_{10} for helminths (Feachem *et al.*, 1983; Rose *et al.*, 1996; Yates & Gerba, 1998; WHO, 2006). Additional removal occurs if the wastewater is further treated with *e.g.* chemical flocculation, sedimentation or filtration, giving 0-1 log_{10} removal for the bacteria and 1-3 log_{10} removal for viruses and protozoans (WHO, 2006).

Barriers

In the reuse of 'products' such as sludge and wastewater from society, the aim must be to reduce the number of potential pathogens by adequate treatment and thus to create barriers against further transmission resulting in an insignificant level of risk. If a biological or biological-chemical treatment is applied before irrigation with wastewater, a major proportion of the organisms present are generally reduced by 1-3 log_{10}. Storage ponds are one alternative way of pre-treatment, where the water is stored before irrigation. This method can efficiently reduce the amount of organisms further, but also results in loss of nutrients (especially nitrogen).

In addition to pre-treatment of the wastewater or sludge, crop restrictions, wastewater application techniques and human exposure controls can be important barriers (WHO, 2006). If the crop is used for human consumption, additional management actions, such as imposing a time period between the last irrigation and harvest can be effective in reducing the risks, together with food preparation measures, *e.g*. washing, peeling and cooking. The interval between the last irrigation and 'consumption' could be of interest when discussing the time of forest fertilisation and subsequent access of humans to the area to pick berries and mushrooms.

Survival in the environment

A willow plantation, forest or wetland area is probably a good environment for prolonged survival for several of the pathogens that may be transmitted with wastewater or sludge. The persistence of the pathogens that may reach the irrigated area depends on several factors, such as the temperature (Badawy, Rose & Gerba, 1990; Kudva, Blanch & Hovde, 1998; Jenkins *et al.*, 2002), the moisture content (Gantzer *et al.*, 2001) and the sunlight (Chang *et al.*, 1985). However, the survival is also related to other factors, such as microbial competition (Edmonds, 1976; Davies *et al.*, 1995;). Conditions that generally favour survival are high humidity, low temperature, no or low sunlight, and neutral to slightly alkaline pH (Roszak & Colwell, 1987). High humidity and low UV-irradiation are conditions occurring in a developed willow coppice stand or forest area in the Nordic climate, while low temperatures also occur during long periods of the year. Pond systems as treatment for wastewater are used in many parts of the world but often in areas with high temperature and sun intensity and often with long retention periods.

After applications of sludge and wastewater to land, the time to reach a total microbial die-off may range between days and months for viruses, bacteria and parasitic protozoa (Brown, Jones & Donnelly 1980; Badawy, Rose & Gerba, 1990), while helminth eggs may persist for years (O´Donnell *et al.*, 1984). Together with improved survival conditions for microorganisms, animals and birds living in the irrigated area may lead to an increased risk for transmission.

Aims, rationale and approaches

Aims

The reuse of wastewater for irrigation may result in transmission to exposed humans of pathogenic microorganisms present in the wastewater. The main focus of this thesis was to investigate factors relating to this when wastewater irrigation is practised in short rotation willow coppice, with comparisons to other systems of reuse such as irrigation in coniferous forest and the use of constructed wetlands for treatment of the wastewater.

The general aim of this thesis was thus to investigate the pathogen reduction potential on the basis of field studies and controlled field and laboratory experiments, and to relate this to the potential health risk when using wastewater for irrigation of short rotation willow coppice or coniferous forest or when treated in constructed wetlands. This was achieved by studying the pathogen load in the wastewater and its removal in the treatments, by conducting groundwater sampling, tracer studies, by analyses of animal stools and organs, and by determining the occurrence and survival of microorganisms in wetland sediments. It was further exemplified by a risk assessment approach for some of the selected systems.

Each of the papers presented addressed specific questions:

To what extent does the soil type affect the transport and retention of microorganisms present in the irrigation wastewater, and what factors facilitate the transport of microorganisms to groundwater level? (**Paper I**).

What is the occurrence and removal of indicator organisms and selected pathogens in three Swedish treatment plants and does the use of pre-treated wastewater for irrigation lead to groundwater contamination, creation of aerosols, or other transmission risks? (**Paper II**).

Do temperature, light exposure and type of vegetation influence the survival of pathogenic microorganisms in ground forest vegetation? (**Paper III**).

What are the occurrence, reduction and survival of indicator organisms in the sediments in two constructed wetlands used for wastewater and stormwater treatment? (**Paper IV**).

What is the pathogenic load that enters the fields with the irrigation water and what is the risk with this type of reuse in subsequent exposures? (**Paper V**).

Rationale

Paper I. When wastewater is applied on land, the transport of microorganisms down to the groundwater is crucial for potential exposure through drinking water from wells. The group of organisms of main concern is pathogenic viruses present in the wastewater due to their small size, high excreted numbers and low infective dose. If transported to the groundwater, virus survival can be prolonged due to the protected environment with low temperature and no sunlight. In order to study the transport and retention of viruses, bacteriophages can be used as a tracer. The advantage of using bacteriophages is that the assay allows high dilution, the phages are harmless to humans and to the environment, and they are easily detected and quantified (Havelaar *et al*., 1991).

Paper II. Irrigation with municipal wastewater on willow coppice is a new way of reusing wastewater in Sweden. The application has several advantages. The wastewater is used as a valuable water and nutrient resource and willow is a crop that is not used as food or fodder, thus excluding an essential transmission route. However, the irrigation can create new routes of exposure compared with the conventional treatment and these new routes need to be evaluated.

Paper III. In 2005, a prohibition on landfilling sludge was introduced in the EU. Applying wastewater and sludge in forest areas as fertilisers can be an alternative but could create new exposure situations for pathogens occurring in the wastewater and sludge. Transmission of pathogenic organisms to humans can occur when an irrigated or sludge-fertilised area is used for recreation. Animals living in the area could also potentially be exposed and infected.

Paper IV. Most studies in wetlands for wastewater treatment concern the water phase and its nutrient and particle removal. Less is understood regarding the removal process and fate of faecal pathogens present in the wastewater. Exposure from constructed wetland for wastewater treatments may, for example, occur if wetland areas are used as recreational areas for people. The limited knowledge related to survival and reduction of pathogens in these systems in cold climate raises questions regarding potential secondary transmission.

Paper V. Wastewater irrigation of willow coppice has also been conducted in other countries than Sweden, *e.g.* Greece and Northern Ireland**.** Baseline information regarding treatment levels, occurrence of faecal organisms in groundwater in irrigated areas, irrigation methods, potential exposure situations and barriers was obtained at six different field sites and it would be logical to use this background information in an assessment of subsequent risks.

Approaches

Paper I. A lysimeter study was conducted with the bacteriophage *Salmonella typhimurium* type 28B as a model virus applied on lysimeters of two different sizes filled with two types of soil, cropped with willow plants and non-cropped. The percolated water was collected and analysed for the presence and concentrations of the added bacteriophage.

Paper II. A baseline study was conducted in three Swedish full-scale field sites with characterisation of the wastewater used and removal of pathogens and indicator organisms, as well as sampling of the groundwater and faecal stools from animals living in the irrigated area.

Paper III. A microcosm study was set up in the laboratory with added selected organisms, both pathogens and indicator organisms, applied to two different types of vegetation, under controlled light exposure and temperature regimes. The persistence of the selected organisms was analysed.

Paper IV. Sediment traps were placed in transects in two wetlands and the amount of particulate matter and faecal organisms analysed in order to assess the occurrence and removal of faecal organisms. A supplementary laboratory study was performed with sediment columns from the wetlands to which faecal organisms were added. The columns were placed in different temperatures in order to study the organism survival.

Paper V. A baseline study was conducted at six study sites with wastewater irrigation of willow coppice in Greece, Sweden and Northern Ireland. The occurrence and reduction in pathogens and indicator organisms in wastewater and the potential occurrence of faecal organisms in groundwater were evaluated and used in a comparative risk assessment.

Materials and methods

Study sites

Treatment plants and irrigation areas (Papers II and V)

Two linked field studies were carried out in Sweden, Greece and Northern Ireland, regarding the sanitary aspects of wastewater irrigation of energy forests. In total, six field sites were included with large variation in size, level of pre-treatment of the irrigation water, climate conditions, *etc*. The occurrence and removal of organisms in the wastewater treatment plants (Papers II, V), occurrence in groundwater (Papers II, V) and in foliage and in faecal stools from animals living in the area (Paper II) were analysed. A comparative microbial risk assessment was performed with selected scenarios regarding risk for transmission and infection during wastewater irrigation (Paper V).

Bromölla, Sweden (56º01'N, 14º11'E)

The treatment plant at Bromölla (which receives wastewater from approximately 10000 person equivalents (pe)) has a pre-sedimentation step followed by biological treatment in bio-beds and chemical treatment with phosphorus precipitation. Irrigation of willow coppice is conducted using perforated tubes placed on the ground, with a spacing of 11 m and perforations every 10 m (Carlander *et al.*, 2002). Approximately 10% of the total volume of wastewater produced is used for irrigation.

Kvidinge, Sweden (56º08'N, 13º04'E)

The treatment plant in Kvidinge receives wastewater from approximately 1400 pe and is a conventional active-sludge plant with after-sedimentation for separation of phosphorus. The water used for irrigation of willow coppice is extracted before the chemical treatment stage. The willow coppice of 10 ha was established in 1996 and receives the total amount of the wastewater produced during the vegetation period (Carlander*, et al.*, 2002). Low emitting sprinklers assembled on tubes placed on the ground are used for the irrigation.

Kågeröd, Sweden (56º00'N, 13º04'E)

The wastewater in Kågeröd (1500 pe) is biologically treated (active sludge) before being used as irrigation water in the planted field. During the winter season, the wastewater is chemically treated before being discharged to the recipient waters. The plantation of 11 ha willow coppice was established in 1995 and wastewater irrigation started in spring 1997 (Carlander*, et al.*, 2002). The irrigation system consists of perforated tubes placed on the ground at various spacings (4-18 m).

Roma, Sweden (57º30'N, 18º28'E)

The wastewater used for irrigation in Roma, Gotland, (1400 pe) is different from the other wastewaters described since it has been treated and stored in oxidation ponds. The willow cropped field in Roma has a size of 5 ha and the irrigation system consists of drip pipes placed on the ground, in every double row of willow.

Culmore, Northern Ireland, UK (55º03'N, 7º16'W)

The Culmore wastewater treatment plant receives approximately 60000 m^3 per day and the wastewater is mechanically treated before partly being used for irrigation. The willow coppice area is approximately 5 ha and is irrigated using low pressure sprinklers, at a height of approximately 25 cm above ground (for experimental plots) or tubes placed on the ground (surrounding willow coppice).

Larissa, Greece (39º39'N, 22º25'E)

The treatment plant in Larissa receives approximately 40000 m^3 per day and the wastewater is mechanically treated, taken from the outflow of primary clarifiers (Larsson, 2003) before being used for irrigation. The willow coppice consists of a 2 ha area and irrigation is conducted using drip irrigation pipes placed on the ground in every double row of willows.

Sampling

Samples of wastewater (both raw and pre-treated), groundwater, foliage, animal stools and organs were taken from the six field sites described above (Papers II and V) and the sampling schedule is summarised in Table 3. For the sites in Bromölla, Kvidinge and Kågeröd, raw and treated wastewater was sampled for 24 hours on each sampling occasion.

Table 3. *Type of samples taken in each of the six wastewater (WW) irrigated willow coppice sites studied in Papers II and V*

Site	Raw WW	Treated WW	Groundwater	Foliage	Stools	Organs
Bromölla	x	x	x			x
Kvidinge	x	x	x	x	x	x
Kågeröd	x	x	x	x	x	x
Roma	x	x	x			
Culmore	x	x	x			
Larissa	x	x				

Lysimeter study (Paper I)

The bacteriophage *Salmonella typhimurium* type 28B was mixed with artificial wastewater and applied to lysimeters cropped with willow plants on two occasions during two consecutive years (late autumn and spring, respectively). The lysimeters used in the study were of two sizes, 4 large (1200 litre) and 6 small (68 litre), and with arable clay (2 large) or sand (2 large and 6 small) soil. The lysimeters were placed outdoors at a lysimeter station at Uppsala, Sweden (59º49'N, 17º40'E). Two of the small sand lysimeters were not cropped and were used as controls. The drainage water from the lysimeters was collected and analysed for the occurrence of the added bacteriophage, giving information regarding the transport and retention of the phages in the soil-water matrix.

Coniferous forest

Microcosm study (Paper III)

Microcosm studies were carried out in the laboratory with two different vegetation types, lichen and moss, at two different temperatures and two light exposure regimes. Organisms, both indicator and pathogens, were added to the microcosms and thereafter sampled and analysed for the presence and survival time in this environment.

Vindeln, Sweden (64º12'N, 19º42'E) - Field study (unpublished results)

In Vindeln, northern Sweden, a field study was started in 1997 with wastewater irrigation of coniferous forest. The wastewater treatment plant receives wastewater from approximately 2500 persons and the wastewater is mechanically treated (grids and sand filter) before being used for irrigation. The 2 ha forest site was fenced and the main tree species was Scots pine (*Pinus sylvestris*), approx. 60-years old. Irrigation was conducted using sprinklers, approx. 85 cm high.

Mechanically treated wastewater, vegetation samples and water from suction cups (tension lysimeters) installed in the soil at approx 0.5 m depth within the irrigated area were sampled and analysed for the presence of indicator organisms and selected pathogens (unpublished results). Vegetation samples were taken on five occasions: before irrigation started, on two occasions during the irrigation period, and two and four weeks after irrigation had ceased.

Wetlands (Paper IV)

In two constructed wetlands for treatment of wastewater and stormwater, respectively, the occurrence and survival of pathogens was studied. Water was sampled at the inlet and the outlet, and sedimentary material collected in traps was analysed for the occurrence of indicator organisms. In addition to the analyses for occurrence, a survival study of selected organisms in sediment cores was carried out in the laboratory.

Wetland for wastewater treatment, Oxelösund, Sweden (58º40'N, 17º06'E)

The wetland receives municipal wastewater that is mechanically and chemically pre-treated before it enters the wetland. The wetland consists of two parallel pond systems (Fig. 1) with a daily load of approximately 6000 m^3 and with an average retention time of 8 days at the time of the study (Wittgren, Stenström & Sundblad, 1996; Andersson, Wittgren & Ridderstolpe, 2000;). After 2000, the retention time was reduced to 6 days (Andersson & Kallner, 2002). Sampling of sediments was conducted in one of the parallel systems, where each of the ponds holds 20000-25000 m^3 and is intermittently filled and emptied. Due to the intermittent operation and potential creation of channels/canals, the effective surface varies from 18 to 24 hectares. Large areas of the wetland are open ponds with sparse vegetation.

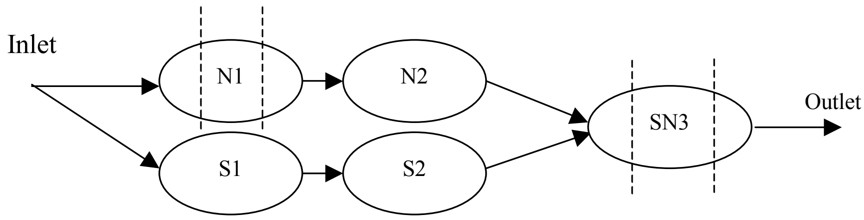

Fig. 1. Constructed wetlands for treatment of municipal wastewater in Oxelösund, Sweden. Dotted lines indicate transects with sediment traps (Paper IV).

Wetland for stormwater treatment

The 18 ha stormwater wetland (Fig. 2) receives run-off water from housing and industrial areas. The total drainage area is 7 km^2 producing approximately $1.8*10^6$ m^3 stormwater each year. The wetland area consists of a sedimentation pond, a surface overflow area and a denitrification pond, and has a total retention time of 3-5 days.

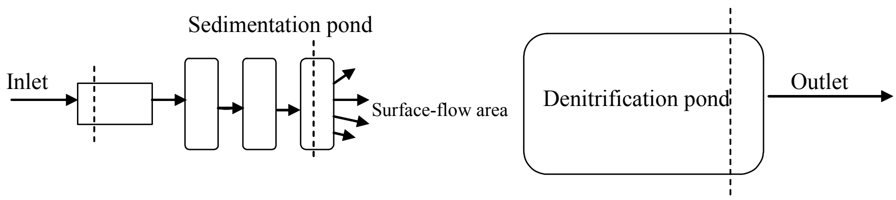

Fig. 2. Constructed stormwater wetland in Flemingsberg, Sweden. Dotted lines indicate transects with sediment traps (Paper IV).

Microbiological methods

Disease-causing microorganisms are often difficult and time-consuming to analyse, and are also risky to handle. As an alternative, indicator organisms are commonly used as substitutes. These are microorganisms that occur normally in the gut of humans and other warm-blooded animals but do not normally cause disease. Under normal conditions, the indicator organisms do not grow or propagate in the environment, are relatively easy and rapid to analyse and give information on the extent to which water or other types of samples are polluted by faeces (WHO, 1993). The main groups of indicator organisms that are analysed are total coliforms (with subgroups faecal coliforms and *E. coli*), faecal enterococci, sulphite-reducing, sporeforming anaerobic bacteria (*Clostridia*) and coliphages. All of the indicator organisms were analysed in the untreated and treated wastewater to validate the treatment efficiency, and in the groundwater to obtain information about the potential faecal pollution. The samples of wastewater, groundwater, foliage and faecal stools were analysed at the Swedish Institute for Infectious Disease Control (SMI), while the organs and some of the faecal stools were analysed at the National Veterinary Institute (SVA) in Uppsala. The indicator organisms analysed are further described below and concentrations in raw wastewater are summarised in Table 4.

Total coliforms is a large group of bacterial species belonging to the *Enterobacteriaceae* normally present in the gut in concentrations of around 10^8 cfu per gram (Stenström, 1996) and also found during the decomposition of organic matter in polluted water. This group was studied when characterising groundwater, to indicate trends of regrowth and faecal contamination, and also to establish a reference to the current drinking water guidelines, where no total coliforms should be detected in any 100 ml sample of treated water entering or present in the distribution system (WHO, 1993).

Faecal coliforms are a subgroup of the total coliforms and include mainly species within *Escherichia* and *Klebsiella* that normally occur in the gut. Presumptive *E. coli* is a subgroup within the faecal coliforms, mainly *Escherichia coli. E. coli* is a more specific indicator of faecal pollution, but the analytical method is not fully species-specific.

Faecal enterococci (intestinal enterococci) is the name for several species of related Gram positive bacteria. The survival in water varies, but *Enterococcus faecalis* and *E. faecium* mainly have the best indicative value. Faecal enterococci occur in the intestines of humans, animals and birds but some species also occur in association with plant material and its decomposition. *E. faecalis* and *E. faecium* may have a better survival than the faecal coliforms.

Sulphite-reducing, sporeforming anaerobic bacteria, are dominated by *Clostridium perfringens*, and occur in the gut in concentrations of approximately 10^4 per gram faeces (Stenström, 1996). The *Clostridium* bacterium is sporeforming and the spores are very resistant and can survive for long periods.

Coliphages is a group of viruses using *Escherichia coli* as a host. Bacteriophages can normally not replicate in water or soil but have a longer survival time and a greater resistance than coliforms. Coliphages are mainly used as an indicator for viruses and are harmless to humans and animals.

Table 4. *Concentrations of faecal indicator organisms in raw wastewater (Horan, 2003; Stenström, 1986)*

Organisms	Concentration (log_{10} 100 ml^{-1})
Total coliforms	5.8-7.8
Faecal coliforms	5.3-7.4
*E. col*i	6.2-6.4
Intestinal enterococci	5.4-6.7
Clostridia spp	4.3-4.7
Coliphages	4.8-5.9

In the studies, the indicator organisms were supplemented with four selected pathogens: *Salmonella*, *Campylobacter*, *Giardia* and *Cryptosporidium.* These four pathogens are of special interest when recycling municipal waste due to their potential to cause diseases in both humans and animals, *i.e.* zoonoses. These pathogens were analysed in raw and treated wastewater in order to evaluate the frequency of positive samples and to determine the load reaching the irrigated sites. The mentioned pathogens were also analysed in faecal stools from animals living in the fields in order to evaluate any potential increase in the frequency of positive samples caused by wastewater irrigation. The sampling procedure and analyses are described in detail in the respective papers and are summarised in Table 5.

Table 5. *Summary of organisms analysed and methods used in the respective papers*

Indicator organisms	Substrate	Incubation time, temp.	Verification	Paper	Reference
Total coliforms	mEndoagar LES (Difco)	24±4h and 44±4h, 35±0.5°C	Oxidase test	II, IV, V	(ISO, 2000b)
Faecal coliforms (FC) and *E.coli* (EC)	mFC agar (Difco)	24±4h, 44±0.5°C	LTLSB	II-V	(ISO, 2000b)
Enterococci	mEnterococcus agar (Difco)	44±4h, 35±0.5°C	Esculine test	II-IV	(ISO, 2000a)
Clostridia spp	Perfringens agar (Difco)	20±4h and 44±4h, 37±1°C		II, IV, V	(ISO, 1986)
Bacteriophages			Host strain		
Somatic coliphage	Nutrient agar, Nutrient broth	18±2 h, 37±1°C	E.coli ATCC 13706	II, IV, V	(Adams, 1959) (ISO, 2000c)
Salmonella typhimurium phage type 28 B	Nutrient agar, Nutrient broth	18±2 h, 37±1°C	*S. typhimurium* type 5	I, III	(Lilleengen, 1948; ISO, 2000c)
Coliphage φx 174	Nutrient agar, Nutrient broth		*E. coli* WG5	III	(ISO, 2000c)
Pathogens					
Salmonella spp	Buffered peptone water (Difco), Rappaport-Vassiliadis medium (Oxoid) Brilliant green agar (Oxoid)	16-20h, 36±2 ºC 18-24h, 41.5±0.5ºC 18-24h, +36±2 ºC	Oxidase Mucab	II, V	(ISO, 1995)

Table 5. *cntd*

Pathogens	Substrate	Incubation time, temp.	Verification	Paper	Reference
Campylobacter spp	Campylobacter blood-free selective enrichment broth, Selective supplement (Oxoid), Blood free selective substrate (Oxoid)	1 h, +37ºC overnight, +37ºC 18 h, +42ºC	Gram staining	II, V	(NMKL, 1990)
Campylobacter (organ)	Preston Campylobacter-Selective Enrichment Broth, Preston Campylobacter-Selective agar	24h, +42°C 48h, +42°C		II	(Hansson *et al.*, 2004; Hansson *et al.*, 2005)
E.coli (verotoxigenic O-group 157)	Peptone water			II	(Wahlström *et al.*, 2003)
Parasites	Concentration		Purification	Paper	Reference
Giardia spp (water)	Centrifugation		IMS	II, V	(USEPA, 2001)
Giardia spp(organs and faeces)				II	(Anon, 1986; Thienpont *et al.*, 1986)
Cryptosporidium spp (water)	Centrifugation		IMS	II, V	(USEPA, 2001)
Cryptosporidium spp (organs and faeces)				II	(Henriksen & Pohlenz, 1981)

Results and discussion

Occurrence of indicator organisms and pathogens in wastewater

The occurrence of indicator organisms and pathogens in wastewater was analysed in five treatment plants and three wetland or pond systems and the results are presented in Papers II-V. The concentrations of organisms in the raw wastewater entering the treatment plants were in similar ranges between the sites (Table 6) and corresponded with literature data (Table 4; Stenström, 1986; Horan, 2003). An exception was the site in Roma, with 1 to 2 $\log_{10}$ lower concentrations of indicator organisms than the other sites, as reported for the inlet to the first oxidation pond. Raw sewage from the households entered the oxidation pond without any pre-treatment, but was diluted by the already treated wastewater, and the concentrations varied depending on the amount of raw wastewater being discharged into the oxidation pond and the time of sampling in relation to discharge of raw wastewater.

The concentrations of indicator organisms in the inlets of the two wetlands studied (Paper IV) are presented for comparison. The inlet water in the wastewater wetland was mechanically and chemically pre-treated before discharge to the wetland, resulting in 1-3 $\log_{10}$ lower concentration of organisms compared with the raw wastewater at the other sites. The incoming concentrations agree with an earlier reported study (Wittgren, Stenström & Sundblad, 1996). Low concentrations of indicator organisms were found in the inlet of the stormwater wetland. This may be due to a lower impact from animals in the surface run-off water from roads, industrial areas, *etc*. The occurrences of some indicator organisms may also represent their potential to grow in soil or decaying vegetation. Both the total coliforms and the *Clostridia* can be naturally present in the environment (Stenström, 1996). The wetland samples represent single samples for comparison only without any intention to discuss variations that could occur.

Table 6. Average c*oncentration of indicator organisms in untreated wastewater entering the treatment plants (± standard deviation). Values representing the inlet to the oxidation pond (Roma) and inlet to the wastewater (Oxelösund) and stormwater wetland (Flemingsberg) are given for comparison. Concentration expressed as* log_{10} *cfu or pfu 100* ml^{-1}

Site	Total coliforms	*E. coli*	Intestinal enterococci	*Clostridia*	Coliphages
Sweden					
Roma (n=2)[a]	5.1-5.7	4.6-5.4	4.1-5.1	4.0	4.1-4.6
Bromölla (n=6)	7.3±0.3	6.8±0.3	5.8±0.1	5.2±0.3	5.2±0.8
Kvidinge (n=7)	7.3±0.1	6.5±0.3	6.0±0.4	4.7±0.1	5.3±0.9
Kågeröd (n=6)	7.4±0.4	6.4±0.6	6.1±0.5	4.6±0.4	5.2±0.6
Oxelösund (n=2)[a]	4.0	2.6-3.3	3.4-3.7	3.2-3.4	3.8-4.9
Flemingsberg (n=1)	2.9	2.0	<1	1.8	1.0
International					
Larissa, Greece (n=2)[a]	7.5-8.2	6.6-7.1	5.9-6.5	5.1-5.5	6.3-6.8
Culmore, Northern Ireland (n=4)	7.7±0.4	7.0±0.3	5.8±0.1	5.5±0.5	6.0±0.7

[a]=presented as individual values

The treatment of the wastewater ranged from mechanical to biological-chemical in the conventional treatment plants, in some cases followed by additional treatment in wetland/pond systems. The degree of treatment was clearly reflected in the reduction of organisms and thus in the concentration of organisms in the irrigation water (Tables 7 and 8). The reduction in indicator organisms was assumed to reflect reduction of pathogenic organism groups potentially present in wastewater. The lowest removal, approximately 1 log_{10} for the indicator organisms, was found in the mechanically treated wastewater in Culmore. For the treatment plants in Kågeröd, Bromölla, Kvidinge and Larissa, the reduction in vegetative bacteria was 2-3 log_{10}, while the sporeforming bacteria (*C. perfringens)* and coliphages were reduced by 1-2 $log_{10.}$ This reduction pattern agrees with earlier findings (Ottoson, 2005).

The efficiency of organism reduction in the Roma pond system varied between the two sampling occasions, mainly for the coliform parameters but also for the others (Table 7). Although sampling was limited, the reduction in organisms in the oxidation pond and storage pond exceeded that in the different treatment plants. The reduction of organisms in pond systems and wetlands is dependent on *e.g.* retention time, sedimentation and UV-light (Davies-Colley, Donnison & Speed, 1997). Pond systems for treatment of wastewater are common in many countries

(Belmont *et al.*, 2004; Ntengwe, 2005), mainly in tropical regions with higher ambient temperature and sunlight intensity than in Sweden. The retention time in the wetland is short compared with what is normally applied in warmer climate, while the storage time in the pond system in Roma is much longer. This is also reflected by a low to medium removal in the wetland and a high reduction performance in the ponds. Since the low ambient temperature in Sweden favour microbial survival longer retention times or a well-defined monitoring programme may be necessary to ensure the quality.

Table 7. *Removal of indicator organisms in wastewater treatment plants, expressed as average log_{10} reduction (± standard deviation). Removal in the pond system in Roma and the two wetlands are given for comparison*

Site	Treatment	Total coliforms	*E. coli*	Intestinal enterococci	*Clostridia*	Coli-phages
Sweden						
Roma (n=2)	Biological + storage pond	1-4.1	>2.6->5.4	>3.1->4.1	2.3-3	2.6->3.6
Bromölla (n=6)	Bio-chemical	2.3±0.4	2.4±0.5	2.5±0.5	2.0±0.4	0.8±0.4
Kvidinge (n=7)	Biological	1.7±0.4	2.0±0.8	1.8±0.7	1.2±0.4	1.5±0.3
Kågeröd (n=6)	Biological	2.6±0.7	2.9±0.7	2.5±1.0	0.8±0.2	1.6±0.4
Oxelösund (n=2)	Constructed wetland	0.9-1.1	>1.6-2.8	2.9-3.7	1.4-1.9	>0.2-1.7
Flemings-berg (n=1)	Stormwater wetland	0.9	1.9	-[a]	0.9	-[a]
Inter-national						
Larissa, (n=2)	Mechanical	1.8-1.9	2.7-3.3	2.9-3.2	1.1-1.5	2.1-2.6
Culmore, (n=4)	Mechanical	0.6±0.7	0.9±1.3	0.8±0.8	1.2±0.5	0.9±1.45

[a] Concentrations both at the inlet and the outlet near or below detection level (*i.e.* <2 log_{10} cfu or pfu 100 ml^{-1}), and removal efficiency could not be calculated

The oxidation and storage pond systems, as applied in Roma, substantially reduced the microbial load, thus producing irrigation water with high microbial quality and low risk of infection. *E. coli* and intestinal enterococci were below the applied analytical detection limit, <1 to <100 cfu 100 ml^{-1}. These low values indicate a substantial barrier effect and reduced risks for bacterial, viral and parasitic etiological agents. The number of samples taken of the water in Roma was limited in this study. However, other studies from the same site (Anonymous, 1996) showed somewhat higher concentrations of *E. coli* (2-3 log_{10} 100 ml^{-1}) in the storage ponds. The parasites are efficiently reduced in a pond or wetland, *e.g.* by sedimentation, as has been shown by Ellis, Rodrigues & Gomez (1993) and

Maynard, Ouki & Williams (1999). For this type of treatment, the nutrient content is also reduced through particle sedimentation and denitrification. The benefits for plants supplied with such pre-treated wastewater are therefore mainly related to their water requirements. Thus, the technique is especially useful in areas with low precipitation during the growing season.

At the other end of the scale we find the sites with mechanically treated wastewater at Culmore and Rosinedal (Vindeln, northern Sweden; Carlander, unpublished data) with high concentration of organisms in the irrigation water, resulting in high loads of organisms to the irrigated fields. The low removal of indicator organisms in the treatment plant corresponds to an enhanced risk for pathogen exposure in the irrigation water. In Culmore, the concentrations of vegetative bacteria were 5-6 $\log_{10}$ higher than in the water treated in the oxidation pond and stored in Roma. Collected data regarding occurrence and reduction of organisms in the wastewater are further used in the risk assessment part and in Paper V.

The total storage time for the wastewater treated in Roma can be up to 6 months, which is the governing factor for the high reduction of organisms, resulting in the high sanitary quality of the effluent water. The storage times for the two wetlands in this study (Oxelösund and Flemingsberg) are considerably shorter, with retention times of less than a week. The reduction in organisms in the water phase here was instead mainly dependent on the sedimentation of particles (Paper IV), also shown by *e.g.* Karim *et al*. (2004). In both wetlands, the major sedimentation of particles and associated organism reduction occurred in the first ponds, resulting in low concentration of organisms in the water phase in the latter part of the respective wetland. For the stormwater wetland, the major sedimentation occurred in a deep, open water area, which is more prone to sediment accumulation than shallower, open water areas. These differences in sedimentation are also discussed by Fennessy, Brueske & Mitsch (1994). In a stormwater wetland, the load of material supplied varies with time due to rainfall and seasonality. However, irrespective of the amount of inflowing particulate matter, a 95-97-% reduction occurred in this study (Paper IV).

Table 8. *Average concentration (± standard deviation) of selected indicator organisms in irrigation water, representing the outlet after wastewater treatment. Values are expressed as log_{10} cfu or pfu 100 ml^{-1}. Values for water treated in a pond system (Roma) or outlet concentrations from two wetlands (Oxelösund and Flemingsberg) are given for comparison*

Site	Treatment	Total coliforms	*E. coli*	Intestinal enterococci	*Clostridia*	Coliphages
Sweden						
Roma (n=2)	Biological + storage pond	1.7-4.2	-[a]	-[a]	1.0-1.7	1.5
Bromölla	Bio-chemical	5.0±0.4	4.3±0.4	3.3±0.5	3.2±0.3	4.4±0.9
Kvidinge	Biological	5.6±0.4	4.5±0.9	4.1±0.8	3.5±0.5	4.2±0.5
Kågeröd	Biological	4.8±0.6	3.6±0.6	3.6±0.8	3.8±0.3	3.6±0.4
Oxelösund (n=2)	Mech.+chem. +wetland	2.9-3.1	0.5-<1	<1	1.3-2.0	2.1-<4.7
Flemings-berg (n=1)	Storm water	2	0.1	[a]	0.9	[a]
Vindeln (n=2)[b]	Mech.	7.2-7.8	5.9-6.6	5.4-5.7	4.4-4.9	4.9-5.6
Inter-national						
Larissa (n=2)	Mech.	5.7-6.3	3.8-3.9	3.0-3.3	3.9-4.1	4.2
Culmore (n=4)	Mech.	7.2±0.6	6.1±1.3	4.9±0.8	4.3±0.6	5.1±1.2

[a] Below detection limit, *i.e.* <1-100 cfu 100 ml^{-1}.
[b] Carlander, unpubl. data

Pathogens in wastewater (Papers II, V)

In addition to indicator organisms, the occurrence of selected pathogens (*Campylobacter*, *Salmonella*, *Giardia* and *Cryptosporidium*) was analysed, and the results are presented in Papers II and V. The occurrence of pathogens in both the raw and the treated wastewater varied in frequency of positive samples, as exemplified with *Salmonella* and *Campylobacter,* and also in concentrations of organisms found at the different treatment plants, as exemplified with *Cryptosporidium* and *Giardia*.

The occurrence of *Campylobacter* in the wastewater, both raw and treated, was limited and the only positive sample was found in the first oxidation pond in Roma. In Sweden, as well as in several other countries *e.g.* USA (CDC, 2006a) *Campylobacter* is the most commonly identified bacterial agent associated with gastrointestinal infections. *Campylobacter* has a reported incidence for the year 2004 of 68.6 cases per 100000 persons in Sweden (SMI, 2005). In the literature, the reported concentration of *Campylobacter* in raw sewage varies from 1 to 5 log_{10} 100 ml $^{-1}$ (Stampi, Varoli & Luca, 1993; Curtis, 2003; Moreno *et al.*, 2003), and secondary treatment results in approximately a 2 log_{10} reduction (Stampi, Varoli & Luca, 1993). The reported concentrations in treated wastewater, possibly reaching an irrigated field, range from 0–210 organisms 100 ml^{-1} (Stampi, Varoli & Luca, 1993).

The absence of *Campylobacter* in the samples analysed could be explained by few *Campylobacter* infections occurring within the population connected to the sewage system at the time of sampling. For the Swedish field sites and compared to the potential incidence this is plausible. With 1400-10000 pe in the populations connected, the incidence of *Campylobacter* during the year would range between 1 to 7 infected people. However, this does not account for under-reporting. Some literature reports indicate that a viable but not culturable stage (Rollins & Colwell, 1986; Buswell *et al.*, 1998) could occur for *Campylobacter*, resulting in negative samples which may be a more likely explanation to the low findings, even though the positive analytical controls verified the analytical procedure.

Salmonella was detected in the raw wastewater in all the treatment plants where samples were analysed (Bromölla, Kvidinge, Kågeröd, Larissa, Culmore) in varying frequencies (see Table 3). In total, 35% of the samples from the inlet of treatment plants or the pond were positive for *Salmonella*, while for the treated wastewater used for irrigation, *Salmonella* occurred in 19% of the samples.

The occurrence and in some instances lack of occurrence of *Salmonella* reflect the prevalence of this bacteria in the human population connected to the sewage system. The incidence in Sweden for *Salmonella* was 40.5 cases per 100000 residents in 2004 (SMI, 2005), resulting in 0.5 to 4 estimated cases in the Swedish sites if calculated based on the populations connected. Comparing *Salmonella* and *Campylobacter*, and accounting for underreporting, this may further indicate that it is the analytical detection of *Campylobacter* that reflect the negative samples and not the prevalence in society. Animals, including wild animals and birds, may also be carriers of *Salmonella,* and contamination may occur from these. Since a semi-quantitative method was applied, the results are the frequencies of positive samples. It is therefore not possible to directly apply the results to estimate the reduction in *Salmonella*. However, based on findings by Feachem*, et al.* (1983) and Curtis (2003), it is realistic to assume that *Salmonella* closely follows the reduction characteristics of *E. coli*. They are both Gram negative bacteria included in *Enterobacteriaceae*, and have similar ranges of sensitivity against the impact of environmental pressure. Literature data indicate concentrations in raw wastewater of 90 to 11000 MPN (Most Probable Number) 100 ml^{-1} in Finland (Kiovunen, Siitonen & Heinonen-Tanski, 2003) with an incidence in the same range as for Sweden. Secondary treatment gave a removal of 94.8 to 99.9% of the measured *Salmonella* with remaining concentrations of <3 to 240 MPN 100 ml^{-1} in the effluent (Kiovunen, Siitonen & Heinonen-Tanski, 2003). Although the numbers of *Salmonella* not were measured in this study one out of five samples of the used irrigation water were positive for *Salmonella* indicating a potential risk for further transmission to humans or animals that come into contact with the effluent.

As is further exemplified in Table 9, in some of the wastewater treatment plants the same strains of *Salmonella* were found in the treated and untreated water, while at other treatment plants different strains were found. All strains found may be prevalent in the human and animal populations. In fact, wastewater may be used to get an indication of which strains that are circulating in the society, although on the other hand (Sahlström *et al.*, 2004) shows that *Salmonell*a can become established in treatment plants and persist for prolonged times.

Despite low concentrations of indicator organisms, *Salmonella* and *Campylobacte*r occurred at the inlet to the oxidation pond in Roma on one occasion. Their presence could of course originate from the municipal wastewater but due to the open ponds, frequently visited by swimming birds, the occurrence of the pathogens could also originate from these birds. Wild birds act as reservoirs of enteropathogenic bacteria, *e.g*. *Salmonella* and *Campylobacter*, in nature, and potential transmission to humans has been discussed in the literature (Palmgren*, et al.*, 1997).

Table 9. *Occurrence of Salmonella spp in untreated and treated wastewater in six treatment plants, 1999-2001*

Site	Positive samples, strains in untreated wastewater	Positive samples, strains in treated wastewater
Culmore	1/4, *S. typhimurium* phagetype NT, *S. virchow*	1/4, *S. give*, *S. typhimurium* phagetype 12
Larissa	1/2, *S. bovismorbificans*	1/2, *S. bovismorbificans*
Bromölla	2/6, *S. thompson, S. fresno*	1/6, *S.indiana*
Kvidinge	2/6, *S. enteritidis* phagetype 4, S. *Java*	1/6, *S. enteritidis* phagetype 4
Kågeröd	2/6, *S. give, S. typhimurium, S. ferruch, S. thompson*	1/6, *S. give*
Roma	1/2	0/2
Total	**9/26**	**5/26**

Giardia spp. occurred in 88% of the samples of raw wastewater and in 52% of the samples of treated wastewater. The corresponding figures for *Cryptosporidium* were 75% for untreated wastewater and 48% for treated (Table 10). The concentrations of *Giardia* cysts in the raw wastewater varied greatly between the sites studied, from less than 2 cysts per litre for Roma to a maximum concentration of 15000 cysts per litre for Culmore. The difference should be linked to the prevalence of *Giardia* infections during the time for sampling. For 2004, a total of 1327 such cases were reported in Sweden, giving an incidence of 14.7 cases per 100000 head of population (SMI, 2005) indicating that at the Swedish sites, 1400 to 10000 person in size, during a year up to two persons could be infected. However, the frequencies of positive samples as well as the concentrations in the Swedish sites indicate a higher prevalence. According to (Hörman *et al.*, 2004), significant underreporting occurs in Sweden for *Giardia*, where for each recorded case 254 unregistered cases may occur annually.

The removal of cysts in the treatment plants ranged from as low as 7% (Culmore) up to 99.99% (Kvidinge), also resulting in a large concentration range in the irrigation water (Table 10). However, the concentrations were mostly below 20 cysts per litre. For Culmore, up to a maximum of 2800 cysts per litre was found.

For one of the Swedish sites, Kågeröd, the removal efficiency was rather low, and 5 out of 6 six samples from the irrigation water were positive for cysts (2 to 20 per litre). Although these concentrations may not seem high, a constant load of cysts must have reached the field. For the study sites in northern Sweden, (Vindeln) with forest irrigation of mechanically treated wastewater, *Giardia* cysts were spread in concentrations of 125 cysts per litre (Carlander, unpublished data).

Table 10. *Occurrence of Giardia (Cysts L^{-1}) and Cryptosporidium (Oocysts L^{-1}) and reduction (%) at five wastewater treatment plants and one pond system (Roma)*

		Culmore	Larissa	Bromölla	Kvidinge	Kågeröd	Roma
Giardia	In	24-15000	100-6326	5-580	<4-1760	3-185	N.D.
Positive samples	In	4/4	2/2	5/5	5/6	5/6	0/2
Giardia	Out	<0.7-2805	0.5-1.7	<0.5-5	<0.2-<4	2-20	N.D.
Positive samples	Out	2/4	2/2	1/5	2/6	5/6	0/2
Reduction		7->99.5	99.5-99.97	90-99.7	>83.3->99.99	27-92	
Crypto-sporidium	In	4-104	<2-4	<6-460	<4-360	3-45	N.D.
Positive samples	In	3/4	1/2	4/5	4/6	5/6	0/2
Crypto-sporidium	Out	<0.7-42	<0.5-8	<0.5-18	0.2-<4	0.3-8	N.D.
Positive samples	Out	2/4	1/2	2/5	3/6	3/6	0/2
Reduction		50-60	-	90->98.2	>90-99.9	33-96	

The concentrations of *Cryptosporidium* oocysts were lower, but *Cryptosporidium* occurred in similar frequencies as *Giardia* (Table 10). The concentrations of *Giardia* and *Cryptosporidium* in raw wastewater corresponded with concentrations found in other studies reported in the literature *e.g.* for Sweden (Ottoson, 2001; Ottoson *et al.*, 2006), England (Bukhari *et al.*, 1997), and Canada (Chauret, Springthorpe & Sattar, 1999).

The measured concentrations of *Giardia* and *Cryptosporidium* in the treated wastewater at some of the field sites were further used in the risk assessment (Paper V), and when discussing potential exposure scenarios.

Groundwater

Groundwater was sampled in the willow plantations irrigated with wastewater (Papers II and V). Total coliforms are normally of doubtful value as an indicator of faecal pollution. However, in groundwater, their presence indicates an external impact, which is the reason why this parameter was included in the study. Total coliforms could be detected in the groundwater at all field sites sampled (although in highly variable concentrations), indicating that the groundwater was affected by the wastewater irrigation (Table 11). The concentrations were sometimes high and in general exceeded the guideline values for drinking water by several orders of magnitude.

In addition to total coliforms, several of the other indicator organisms were detected in the groundwater samples, but the frequency of occurrence and the concentrations varied between the fields. The occurrence of indicator organisms in groundwater partly followed the degree of pre-treatment of the wastewater, with limited occurrence of organisms in Roma (oxidation pond + storage pond) and in Bromölla, which received biochemically treated wastewater.

Both in Kågeröd and Kvidinge and within some of the irrigation regimes in Culmore, several of the indicator organisms were present in the groundwater on a majority of the sampling occasions. In Kågeröd, total coliforms were present at all sampling sites in concentrations ranging from 2.9 to >5.3 $\log_{10}$ 100 ml^{-1} (average 4.3±0.6 $\log_{10}$). *E. coli*, intestinal enterococci and clostridia were detected in the groundwater at most of the sampling sites (average 2.6 to 2.9 $\log_{10}$ 100 ml^{-1}) as were the coliphages (viral indicator) in numbers of 1 to 3.4 $\log_{10}$ pfu 100 ml^{-1}. Similarly, total coliforms were present in the groundwater from Kvidinge in all samples, in average concentrations of 4.4±1.0 $\log_{10}$. *E. coli* bacteria were present in the majority of the samples, mostly below 3 $\log_{10}$, but occasionally up to 4.7 $\log_{10}$. The enterococci were normally also present in low numbers, except at one sampling in September 2001, when concentrations varied between 3 and 5.3 $\log_{10}$ at the four sampling sites. *Clostridia* were generally present, with concentrations ranging from 0.7 to >4.5 $\log_{10}$ 100 ml^{-1}. The concentration of coliphages was under detection level (*i.e.* <1 pfu per 100 ml) in the majority of the samples. If detected, the numbers were low.

In Culmore, Northern Ireland, total coliforms were also present at all groundwater sampling sites in varying concentrations, with the highest concentrations in the sites representing the regimes with sludge treatment and the highest wastewater load (*i.e.* 3 PE WW). The wastewater impact was confirmed by the finding of *E. coli*, intestinal enterococci and *Clostridia*, usually in concentrations below 3 $\log_{10}$ 100 ml^{-1}, but occasionally in higher concentrations in the high load irrigation regime (3 PE WW). Coliphages were present in approx. 40% of the samples, mostly in concentrations near the analytical detection level (*i.e.* <1 $\log_{10}$ 100 ml^{-1}), but on one occasion in concentrations of 3.6 $\log_{10}$ 100 ml^{-1} in the pure water treatment, and 3.9 $\log_{10}$ pfu 100 ml^{-1} in the 3 PE WW treatment. Coliphages were not detected in the plots irrigated with 1 PE WW, 2 PE WW or in the non-irrigated control plots.

Despite the large variation in abundance between field sites, the frequency of elevated concentrations together with the consistent positive findings of several or all of the analysed indicator organisms clearly indicate an impact on the groundwater due to the wastewater irrigation.

The impact on groundwater is dependent on the ability of water and organisms to percolate through the soil profile (Taylor *et al.*, 2004), and depends on the soil type, irrigation load and type of organisms (Corapcioglu & Haridas, 1984; Gannon *et al.*, 1990; Li, Loehle & Malon, 1996). The average concentrations of organisms in the irrigation water were compared with the average concentrations of organisms in the groundwater in order to arbitrarily calculate the removal efficiency in the respective soil profiles. The lowest removal was found in the soil in Kågeröd, with 0.5 $\log_{10}$ for total coliforms, 0.9 $\log_{10}$ for the rest of the vegetative bacteria and *Clostridia*, and 1.3 $\log_{10}$ for the coliphages (Table 11). In general, the removal of *Clostridia* was low, 0.6 $\log_{10}$ in Kvidinge to 2.3 $\log_{10}$ for the 1 PE WW treatment in Culmore. Coliphages were the organisms with the highest removal in the soil, with a 2.9 $\log_{10}$ -5 $\log_{10}$ removal in Culmore (3 PE WW and 1 and 2 PE WW, respectively), which was the highest reduction in organisms found in the study. The reduction in the vegetative bacteria was approximately 1-2 $\log_{10}$ lower.

The limited treatment in the treatment plant at Culmore, with high loads of organisms to the field, had a similar faecal impact on the groundwater as in Kvidinge and Kågeröd, although these sites had further pre-treatment of the irrigation water. The consistent contamination of groundwater in Kågeröd could be explained by a low removal of organisms in the soil profile (0.5-1.3 $\log_{10}$), possibly due partly to the high groundwater level (0.5 to 1.5 m below soil surface). Both soil type and pore size affect the potential transport of organisms in the soil (Brockman & Murray, 1997; Schäfer *et al.*, 1998), where earlier studies indicate a higher retention of organisms in clayey soils compared with sandy soils. However, in Paper I (further discussed below), a very rapid transport of organisms occurred in a clay soil compared with a markedly delayed transport with very high reduction of the studied organisms in a sandy soil. The soil type in Kågeröd consisted of silty loam, compared with sandy loam in Culmore. Besides soil type and groundwater level, the irrigation regime (*i.e.* the irrigation intensity) can also affect the transport of organisms through the soil. In Kågeröd and Bromölla, irrigation was conducted using tubes placed on the ground with one emitter covering approx. 100 m^2, and thus the irrigation load was spatially highly variable as compared with the sites at Roma, Kvidinge and Culmore, where sprinklers used distributed the wastewater fairly evenly. The high spatial variability in wastewater application in Kågeröd and Bromölla was reflected in highly variable plant growth, especially in Bromölla, as shown by biomass assessments conducted by Aronsson et al. (2002).

If organisms are transported down to groundwater level, the survival of these organisms is prolonged due to favourable conditions with low temperature, absence of UV-light, *etc.* (Keswick & Gerba, 1980). Lateral transport of the organisms in the groundwater can occur, with rapid transport over long distances under certain hydrogeological conditions (*e.g.* large gradient and highly permeable soils (Taylor *et al.*, 2004).

The transport and retention of organism in the soil profile varies with size, where viral transport is of major concern due to the small size of virus particles (Jin &

Flury, 2002). The parasitic protozoa *Giardia* and *Cryptosporidium* represent a group of organism with large size, much more likely to be reduced to a higher degree than bacteria and viruses. However, even *Giardia* cysts and *Cryptosporidium* oocysts were detected in the groundwater in low levels on one occasion in Bromölla 0.4 cyst and 0.8 oocysts per litre and at one occasion in Kvidinge (0.8 (oo)cysts per litre). Applying the risk assessment approach presented by WHO in the Guidelines for Drinking Water (WHO, 2004), this would mean that a further reduction of about 3 $\log_{10}$ of the concentration in groundwater needs to be achieved to meet the suggested health target level of 10^{-6} DALY (Disability Adjusted Life Years).

Table 11. *Concentration of indicator organisms in sampled groundwater fields from five field sites. Concentration presented as $\log_{10}$ cfu or pfu 100 ml^{-1}. Numbers within brackets represents reduction in the soil profile (within the 1 PE WW irrigated plots at Culmore and Roma) presented as $\log_{10}$ reduction.*

	Sampling occasions	Total coliforms	*E. coli*	Enterococci	*Clostridia*	Coliphages
Culmore	5	2-5.9 (3.6)	<1-4.9 (4.2)	<1-4 (2.3)	<1-4.1 (2.3)	<1-3.9 (5)
Bromölla	7	0.7-5.5 (1.7)	N.D.[a] (3.3)	<1-3.9 (>2.3)	<1-3.3 (1.1)	N.D.[a] (>3.4)
Kvidinge	7	<1-6.3 (1.2)	<1-4.7 (2.0)	<1-5.3 (1.1)	0.7->4.5 (0.6)	0.7-1 (3.3)
Kågeröd	6	2.9->5.3 (0.5)	<1-3.7 (0.9)	1-3.8 (0.9)	1.3-4.2 (0.9)	<1-3.4 (1.3)
Roma	1	2.4-3.4 (0.3)	<1-2.2	≤1	<1 (0.3)	<1 (0.5)

[a]N.D. = Not Detected. The detection limit in the majority of the samples was <1 $\log_{10}$, but in some <3$\log_{10}$. cfu or pfu 100 ml^{-1}

A limitation of the study in southern Sweden (Papers II and V) was that the groundwater quality was not measured in control plots (*i.e.* without wastewater irrigation). For the sites in Culmore and Roma, control plots were established with no irrigation or with irrigation with pure water. The frequency of occurrence of organisms in the groundwater samples taken from shallow groundwater was lower in the control plots than within the plots irrigated with wastewater, but several of the organisms were still found (especially at Culmore). This could be due to contamination by groundwater laterally transported from adjacent wastewater irrigated plots. This explanation is to some extent supported by elevated concentrations of chloride (from wastewater) occasionally found in the groundwater samples from control plots during periods of intensive wastewater irrigation (P. Aronsson, pers. comm.).

As a supplement to the studies of occurrence of faecal indicator organisms in groundwater in wastewater irrigated willow coppice, a tracer study with bacteriophages in artificial wastewater applied to willow cropped lysimeters (with a volume of 1200 or 68 litres) was conducted (Paper I).

Bacteriophages as tracers of the transport and retention of human viruses in soil have previously been used in several studies (Bales *et al.*, 1989; Jin *et al.*, 1997; McKay *et al.*, 1993). Using bacteriophages has several advantages. They mimic the behaviour of potentially pathogenic viruses and have a relatively good persistence in water and soil. They also allow a quantitative assessment of the barrier potential, *e.g.* filtration in the soil, due to the possibility to detect phages even after a very high degree of dilution of up to 10^{-12}. This is due to the large numbers that can be propagated and applied in a study, and to the fact that they can be easily detected and quantified. They are also harmless to humans, animals and the environment (Havelaar *et al.*, 1991).

The results from the study (presented in Paper I) indicated a large difference in transport of bacteriophages in the two soil types tested (sand and clay), with rapid transport in the clay soil. Bacteriophages were detected in the drainage water after only 2-24 hours ($8*10^{-4}$ to $7.6*10^{-3}$ pore volumes) compared to the large sand lysimeters with breakthrough in the drainage water after 126-246 days (0.45-0.88 pore volumes) (Table 12). The rapid transport of the indicator virus in the clay soil could only be explained by a considerable macropore flow of water through the soil matrix. This is in accordance with the findings in other studies of virus transport in well-structured soils (McKay *et al.*, 1993; McKay, Gillham & Cherry, 1993; McMurry, Sinton, Finlay & Scott, 1997; Coyne & Perfect, 1998). McMurry, Coyne & Perfect (1998) found a significant correlation between the transport pattern of water and faecal coliforms (bacteria) in a soil prone to preferential flow. The macropores responsible for most of the water transport also accounted for most of the transport of the coliforms.

In addition to the transport time and breakthrough, the cumulative numbers of phages that were transported through the columns also differed between the soil types. The cumulative numbers relate to the barrier efficiency of the soil transport. Although organisms were transported to the groundwater, a very high retention of organisms occurred, with nearly 3 $\log_{10}$ to >5 $\log_{10}$ reduction in the clay lysimeters. In the large sand lysimeters, the reduction in organisms ranged from more than 5 $\log_{10}$ to >9 $\log_{10}$ reduction. This contradicts the results from several other studies, where clayey soils have been found to be more efficient in retaining viruses than sandy and organic soils (Keswick & Gerba, 1980; Sinton, Finlay & Scott, 1997).

A major difference between the field sites and the lysimeter study was that the lysimeters received ‘wastewater’ in a single pulse compared with the continuous irrigation of wastewater conducted in the field during the whole irrigation period.

The results from the lysimeter study support the results from the field, with higher faecal impact of the groundwater in the fields with clayey soils compared with the sites with more sandy soils. A new leaching study using the same set of lysimeters but with raw wastewater added daily was started in 2005. Preliminary results from this study agree well with those presented in Paper I (Aronsson *et al.*, unpublished results).

Table 12. *Summary of results from phage experiments 1997 and 1998 (Paper I)*

Lysimeter No.	Date of phage application	No. of phages applied (pfu)	Breakthrough time and *(discharged pore volumes)*	Time at peak conc.	Peak conc. (pfu ml^{-1})	Accum. phage transport (pfu)	Accum. phage transport (%)
Large clay							
1c	8 Oct-97	$5.2*10^{10}$	<12 hr (*$3.79\ 10^{-3}$*)	125 d	7.7	$4.36*10^{5}$	$8.4*10^{-4}$
2c	8 Oct-97	$5.2*10^{10}$	<1 d (*$7.58\ 10^{-3}$*)	1 d	$1.2*10^{3}$	$1.41*10^{7}$	$2.7*10^{-2}$
3c	21 Apr-98	$7.8*10^{11}$	<1 d (*$7.32\ 10^{-3}$*)	3 d	$9.8*10^{3}$	$2.79*10^{8}$	$3.6*10^{-2}$
4c	21 Apr-98	$7.8*10^{11}$	<2 hr (*$7.98\ 10^{-4}$*)	4-6 hrs	$5.8*10^{5}$	$2.22*10^{9}$	$2.8*10^{-1}$
Large sand							
5s	8 Oct-97	$5.2*10^{10}$	159 d (*0.52)*	159 d	0.1	$1.91*10^{3}$	$3.7*10^{-6}$
6s	8 Oct-97	$5.2*10^{10}$	126 d (*0.45)*	128 d	7.7	$2.10*10^{5}$	$4.0*10^{-4}$
7s	21 Apr-98	$7.8*10^{11}$	246 d (*0.88)*	251 d	0.7	$6.41*10^{2}$	$8.2*10^{-8}$
8s	21 Apr-98	$7.8*10^{11}$	nd.	-	-	0	0
Small sand							
A	8 Oct-97	$1.1*10^{10}$	21 d (*1.65)*	180 d	5.1	$3.80*10^{5}$	$3.5*10^{-3}$
B	8 Oct-97	$1.1*10^{10}$	27 d (*2.35)*	180 d	3.6	$1.51*10^{5}$	$1.4*10^{-3}$
C	21 Apr-98	$3.9*10^{10}$	2 d (*0.21)*	2 d	$3.2*10^{3}$	$2.62*10^{7}$	$6.7*10^{-2}$
D	21 Apr-98	$3.9*10^{10}$	2 d (*0.21)*	4 d	$2.8*10^{2}$	$4.75*10^{6}$	$1.2*10^{-2}$
E	21 Apr-98	$3.9*10^{10}$	<1 d (*0.11)*	4 d	$2.9*10^{3}$	$7.14*10^{7}$	$1.8*10^{-1}$
F	21 Apr-98	$3.9*10^{10}$	2 d (*0.21)*	4 d	$9.0*10^{2}$	$1.44*10^{7}$	$3.7*10^{-2}$

Occurrence in the irrigated environment

The two sections above describe the treatment and the quality of the irrigation water used. Due to the type of irrigation (*i.e.* sprinklers, drip pipes or perforated tubes on the ground) the wastewater with its content of microorganisms was either deposited both on the foliage and on the ground (sprinklers) or just on the ground (pipes and tubes). Animals within the willow coppice stands can potentially either additionally deliver pathogens to the area by droppings, or pick up pathogens from the irrigated foliage/ground and transmit it to other areas.

A brief analysis was therefore performed by examining faecal droppings and organs from a limited number of animals and birds (Paper II). Fresh faecal droppings were analysed from hare, rabbits, fox, pheasant, roe deer, fallow deer, mink and badger (n=8 before irrigation, n=25 during irrigation). Although limited, these examinations did not reveal any pathogenic organisms relevant for zoonotic transmission in any of the samples. Organs were collected from rodents, hare, roe deer and pheasant (n=12). No pathogenic bacteria were found. For parasitic protozoa and worms, the occurrence and numbers of parasites corresponded with the normal parasitic occurrence in the animals, without any direct relationship to the special environment. Additional stool samples (n=10) from birds were collected from the irrigated field in Roma (unpublished results), but here too, no pathogenic bacteria or protozoa were found, indicating no increased infections due to the special environment. The results are so far limited regarding potential uptake of pathogens causing infections in animals living in wastewater irrigated areas, and the methods used for collecting faecal stools in the environment had limitations regarding the possibilities to identify the species producing the stools. In addition, although probably not in this study, the origin of potential positive pathogen findings (*e.g.* as a result of infection in the animals or through stools being contaminated by the irrigation water) may be difficult to assess without elaborate investigations. A possible future approach would be to keep sample populations of animals or birds, fenced or in cages, in the irrigated area, with successive sampling to investigate whether infections occur.

The foliage in two of the irrigated areas was sampled on two occasions; July (day temperature 20°C, sunlight) and October (day temperature <10°C, partly cloudy). One field site with sprinkler irrigation (Kvidinge) and one with surface irrigation (Kågeröd) were included. Each sampling plot, 10 x 10 m^2 (n=5), had a low-emitting sprinkler (Kvidinge) or a perforated tube (Kågeröd) in the centre of the plot. The samples: 1) 0-1m from the sprinkler and up to a height of 1 m, 2) 0-1m from sprinkler and 1-2 m from the ground 3) 1-4 m from sprinkler and 0-1 m from the ground, 4) 1-4 m from sprinkler and 1-2 m from the ground, were assumed to cover the range of exposure of wastewater.

The organisms detected on the leaves were total coliforms and *Clostridium perfringens* in concentrations varying between 10^2-10^5 cfu g^{-1} fresh weight (fw) for total coliforms and 5-8*10^2 cfu g^{-1} fw for the sporeforming bacteria, with similar results for both sites, independent of the type of irrigation method. Both these groups of organism are naturally found in the environment and do not necessarily indicate faecal pollution. For the *E. coli*, intestinal enterococci and coliphages, most of the analysed samples were below the methodological detection limit (*i.e.* $<10^2$ g^{-1}) applied. The results did not indicate any differences between the two different irrigation types regarding occurrence of indicator organisms in the foliage originating from the wastewater. In addition, no variation in quantities between the July and October sampling could be found. It can thus be concluded that the low-emitting sprinklers did not affect the concentrations of organisms in the foliage, and there was no apparent seasonal variation of the presence of the dispersed organisms.

Survival in the environment

The risk for transmission of pathogenic organisms from the environment to humans or animals is linked with the survival of the organisms in the environment. When the wastewater with its potential content of pathogens is spread in the environment, either to a wetland or to forest or agricultural land, the further survival depends on the ambient environmental conditions. Conditions that positively affect the survival of the organism are in general high humidity, low temperature, low or no exposure to sunlight and neutral or slightly alkaline pH, (WHO, 2006). During large parts of the year, the prevailing climatic conditions in Sweden and Northern Ireland would contribute to a favourable environment for many of the organisms. In addition, the type of crop (sticky surface, sheltering leaves) and soil content, clay soils and soils with high organic content favour pathogen survival (WHO, 2006). For organisms applied to wetland area, the survival is linked to several of the above-mentioned factors such as temperature and sunlight (Davies-Colley, Donnison & Speed, 1997). Survival studies on selected organisms in this work were performed both in sediment from wetlands (Paper IV) and in vegetation (Paper III).

Sediment

For the wetland sediments the die-off of total coliforms, *E.coli*, intestinal enterococci, *Clostridium* and coliphages was studied during 50 days in the laboratory, and the results in terms of the logarithmic reduction (T_{90}- values) are shown in Table 13. The bacterial indicators were reduced more rapidly than the coliphages and the anaerobic, sporeforming bacteria. The variation in die-off of coliphages between the sediment from the two sites was due to a much lower quantity of indigenous phages at the stormwater site.

Table 13. *T_{90}-values in days for faecal indicator organisms in the sediment in laboratory survival study*

T_{90} values	Total coliforms	*E.coli*	Intestinal enterococci	*Clostridium perfringens*	Coliphages
Oxelösund	16	27	27	252	370
Flemingsberg	17	24	53	396	51

Feachem *et al.* (1983) found a total survival time in fresh water and sewage at 20-30°C for *e.g.* faecal coliforms of < 60 days, but normally less than 30 days, and for enteroviruses less than 120 days but normally less than 50 days. The sediment environment is favourable for most of the organisms. The results from the survival study exceeded these indicated survival times for the organisms tested. An increased survival in sediment for several organisms compared with the water phase was also shown by Karim *et al.* (2004), with lower die-off of faecal coliforms, *Salmonella typhimurium* and coliphages in the presence of sediment compared with water. Although the survival was prolonged in the sediment, both total coliforms and coliphages had a higher die-off in the study by Karim *et al.* (2004) than in the present study, with 1 $\log_{10}$ reduction of total coliforms after 16-17 days and 51-370 days for coliphages. For the same organisms, the corresponding time for 1 $\log_{10}$ reduction was approximately 7 and 10 days, respectively. For *Giardia* and *Cryptosporidium* the survival conditions were the opposite, with a longer survival in the water phase compared with the sediment (Karim *et al.*, 2004).

The survival study was performed at room temperature (+20°C). The ambient temperature in a wetland during large parts of the year would be considerable lower, which would further prolong the survival. The construction of wetlands with optimisation of reduction in particulate material and minimisation of potential resuspension of sediment should be governing factors for minimising the potential health impact.

Vegetation

When partly treated wastewater or sludge is applied to forest areas, the potential risk is linked to the exposure of humans or animals to contaminated vegetation. The survival time for the pathogens applied in wastewater or sludge is important for the further risk for transmission, and as stated earlier, the climatic conditions to a large extent determine the survival of the organisms. In Paper III, the survival of selected indicator organisms and pathogens was studied in two different vegetation types, in light or darkness, and at two temperatures for the pathogens *Salmonella senftenberg* and *Campylobacter coli*, the bacteriophages *Salmonella typhimurium* 28B and φx174, and the indicator bacteria *Enterococcus faecalis* and *E. coli*. The survival time differed between the organisms, with *Salmonella senftenberg* detected in the vegetation throughout the study and still occurring after 35 days in concentrations between 10^5-10^8 cfu g^{-1} dw (semi quantitative method). Thus, this type of *Salmonella* survived well in this environment, while *Campylobacter* were rapidly reduced and not detectable 3 hours after being added to the vegetation. The prolonged survival of *Salmonella* in high concentrations in the vegetation indicates a potential risk for transmission to both humans and animals if this organism is present in wastewater or sludge used as fertiliser in forest. The detection of *Salmonella* during the study indicates a good survival compared with reported literature data on survival on crops, since in *e.g*. Feachem *et al.* (1983) a survival time of less than 30 but usually less than 15 days was found for *Salmonella* at temperatures of 20-30°C.

For the indicator organisms, no single factor (vegetation type, light or temperature) governed the reduction, but the persistence of these organisms was generally best supported in the moss vegetation at +3 °C in the dark (Tables 14 and 15). This agrees with the assumption, based on earlier investigations, that these factors may favour the survival of the organisms.

Table 14. *Survival rates of the indicator bacteria E. faecalis and E. coli from linear regression analysis. T_{90}-values are given with 95% confidence intervals for significant regressions*

			E. faecalis			*E. coli*		
Vegetation	Light dose (MJ m^2d^{-1})	Temp. (°C)	k-value	p-value	T_{90} (days)	k-value	p-value	T_{90} (days)
Lichen	1.2	+7	-0.087	0.001	12 (11-13)	-0.154	0.020	6 (5-8)
Lichen	0.0	+27	-0.086	<0.001	12 (11-12)	-0.181	0.272	- [b]
Lichen	1.5	+27	-0.024	0.067	- [b]	-0.235	0.255	- [b]
Moss	1.5	+27	-0.016[a]	0.777	- [b]	-0.063	0.054	16 (12-23)
Moss	0.0	+3	-0.039[a]	<0.001	25 (24-27)	-0.120	<0.001	8 (8-9)

[a]Initial sample included in the regression analysis
[b]Not significant

The lowest die-off rate during the study was found for the bacteriophage *Salmonella typhimurium* 28 B, followed by the phage φx174, with T_{90} values in the moss, darkness and low temperatures of 31 and 27 days, respectively.

Table 15. *Survival rates of the bacteriophages S. typhimurium 28 B and φx174 from linear regression analysis. T_{90}-values are shown with 95% confidence intervals for significant regressions*

			S. typhimurium 28 B			φx174		
Vegetation	Light dose (MJ m^2d^{-1})	Temp. (°C)	k-value	p-value	T_{90} (days)	k-value	p-value	T_{90} (days)
Lichen	1.2	+7	-0.071	<0.001	14 (13-15)	-0.068	0.070	-b
Lichen	0.0	+27	-0.072	0.016	14 (12-17)	-0.051	0.655	- b
Lichen	1.5	+27	-0.109	0.027	9 (7-12)	-0.050a	0.363	- b
Moss	1.5	+27	-0.108	<0.001	9 (9-10)	-0.055	0.017	18 (10-112)
Moss	0.0	+3	-0.033	0.004	31 (27-35)	-0.037	0.005	27 (23-31)

[a]Undetectable levels on day 8 and 21 were set at the detection level and included in the regression analysis.
[b]Not significant.

The high persistence of *Salmonella* in the study indicates a potential risk for transmission when contaminated wastewater is applied to forest vegetation. The need to control the occurrence of *Salmonella* is further emphasised by the risk for regrowth, which may occur even from low levels of *Salmonella*, partly undetectable in the material. Gibbs *et al.* (1997) reported this in stabilised wastewater sludge. In the same investigation, a regrowth was also found for faecal coliforms, where the concentrations were reported to increase to higher levels than those in the beginning of the trials. In our study, a regrowth of *E. coli* was indicated during the last two weeks at the high temperature in moss. The regrowth of *Salmonella* and faecal coliforms is generally supported by favourable nutrient conditions, and Sidhu *et al.* (1999) reported similar growth rates of *Salmonella serovars* and *E. coli* strains in composted biosolids.

A field study on the survival or organisms was also conducted. Vegetation was sampled in Vindeln, northern Sweden, for analyses of occurrence of indicator organisms and pathogens in the irrigated forest area (Carlander, unpublished data). Two irrigated areas receiving mechanically treated wastewater were sampled, as well as a non-irrigated control plot. The occurrence of total coliforms in the vegetation was similar in the irrigated areas and in the control plot, with concentrations of around 4 $\log_{10}$ cfu g^{-1} fw in the vegetation before irrigation started (Fig. 3). *E. coli*, intestinal enterocci and coliphages were also analysed in the vegetation during the study. Before irrigation started, concentrations were below the detection limit, *i.e.* <2 $\log_{10}$ (Table 16). Mechanically treated wastewater was applied (see Table 8, Section 4.1), and the occurrence and concentrations of the indicator organisms analysed increased in the vegetation with 1-2 $\log_{10}$ for total coliforms, *E. coli*, intestinal enterococci and coliphages. Two weeks after the irrigation had ceased, it was still possible to detect all indicators in the vegetation, although in 1 $\log_{10}$ lower concentrations compared with during irrigation. Four weeks after irrigation had ceased, it was still possible to detect all indicator organisms except intestinal enterococci in the material, indicating a high survival in the environment for several of the organisms. This supports the results from the laboratory study.

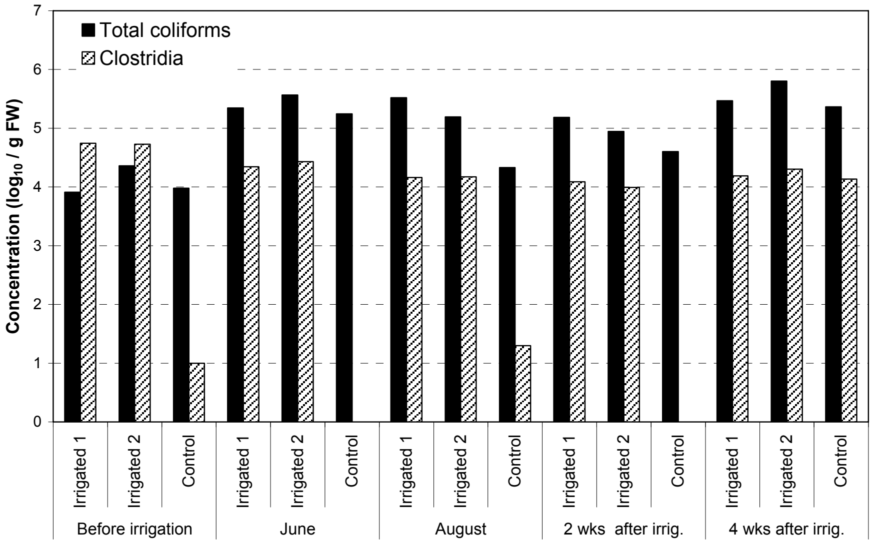

Fig. 3. Occurrence of total coliforms and *Clostridia* in vegetation sampled in a wastewater irrigated forest area in Vindeln, northern Sweden. Two irrigated sites (1 and 2) and one non-irrigated control site were sampled.

Campylobacter and *Salmonella* were also analysed in the vegetation. *Campylobacter* were not detected in the vegetation on any of the sampling occasions, while *Salmonella* were detected in the wastewater-irrigated vegetation on one occasion (August). In the samples taken after irrigation had ceased, no *Salmonella* were detected, nor were *Salmonella* detected in the vegetation sampled in the control site.

Water from the suction cups (*i.e.* tension lysimeters) installed in the irrigated area for sampling of soil water was analysed for the occurrence of indicator organisms on two occasions. In the majority of the samples analysed, the levels of organisms were below detection level, indicating that the retention of the organisms in the vegetation and soil was high. However, the method for extracting soil water by the use of suction cups has not been validated as regards the effect on microorganisms.

Table 16. *Concentration of indicator organisms in vegetation samples from a wastewater irrigates forest at Vindeln, northern Sweden, presented as log_{10} cfu or pfu per gram (fresh weight)*

Occasion	Sample	*E. coli*	Intestinal Enterococci	Coliphages
Before irrig.	Irrigated 1-2	1.7-<2	<2-2	<1.7
	Control	<2	<2	<1.7
June	Irrigated 1-2	3.6-3.8	3.5-3.7	2.8-3.7
	Control	<2	<2	<1
August	Irrigated 1-2	3.9-4.1	3.5-3.7	2.7
	Control	2.9	<2	<1
2 wks after irrig.	Irrigated 1-2	1.5-1.7	2.4-2.5	2.1-2.5
	Control	<2	<2	<1
4 wks after irrig.	Irrigated 1-2	2.2-2.5	<2	1.6-2.3
	Control	2.2	<2	1.4

Risk assessment

Irrigation with wastewater could, as stated previously, constitute a risk for transfer of infections to humans or animals if they are exposed to pathogens in the wastewater. In Paper V, baseline information was collected regarding differences between the fields and treatment plants, concentrations and reductions in organisms in the wastewater, occurrence of organisms in groundwater and foliage, as well as occurrence of pathogens in animals living in the irrigated area. This information, together with potential exposure scenarios most likely to occur at the different field sites, formed the basis for the risk assessment (Paper V, Table 7). The concept of risk analysis previously described by (Haas, Rose & Gerba, 1999) was partly followed. Hazard analysis and exposure assessment were conducted for all sites on a broad scale. Specific exposures that were judged to be of significance were then quantitatively evaluated by further estimating the exposure to individuals affected by the system. Doses of selected pathogens were calculated and utilised in a dose-response assessment in order to quantify risks of infection.

For the quantitative microbial risk assessment (QMRA) two fields were selected, Culmore, Northern Ireland and Kvidinge, southern Sweden. For Culmore, mechanically treated wastewater was used for irrigation while for Kvidinge, biologically treated wastewater was used. The exposure routes judged to be of main importance were (1) direct exposure and accidental ingestion of wastewater, (2) exposure for aerosol (sprinkler irrigation at both sites), and (3) use of groundwater as drinking water.

As model organisms for the QMRA, *Salmonella*, *Giardia*, *Cryptosporidium* and rotavirus were selected. Thus, at least one representative from each of the major prevailing groups of pathogens was included. Further information regarding the estimates of concentrations of the pathogens in wastewater and groundwater is presented in Paper V. In Table 17, the results from the QMRA are presented as the median risk (50-percentile) for infection per exposure (P_{inf}) for each of the pathogens and exposure routes included. In addition, the annual risk of infection in the exposed population (P_{yearly}) was calculated by taking the yearly exposure into consideration:

$$P_{yearly}=1-(1-P_{inf})^n$$

where P_{inf} is the risk of exposure and *n* the number of exposures per year. P_{yearly} was then used for calculating the number of cases in the exposed population.

The risk of infection with *Salmonella* was low at both field sites, with the highest risk being attributable to accidental ingestion of wastewater in Culmore (P_{inf} $2*10^{-6}$) and using groundwater as drinking water in Kvidinge (P_{inf} $2*10^{-6}$). The lowest risk for *Salmonella* infection occurred through exposure to aerosols in Kvidinge, with a P_{inf} at $1*10^{-10}$. The risk of infection by *Salmonella* by accidental ingestion of treated wastewater or inhalation of aerosols is dependent on the numbers of organisms in the wastewater exposed for. Higher concentrations in the raw wastewater can prevail if an outbreak occurs in the connected population. Thus, the reduction in the treatment plant before irrigation is conducted in the field is of importance. In this study, the removal in the treatment plant was calculated using the removal values for *E.coli.* Compared with the other sites, the removal efficiency in the treatment plant was low for both Culmore and Kvidinge, with an average reduction of 0.9 and 2.0 log_{10} respectively. For Bromölla, Kågeröd and Larissa the removal of *E.coli* was 2.4 to 3.3 log_{10}, reducing the concentrations in wastewater for irrigation, and accordingly, also the risk of infection.

For the protozoans, *Giardia* posed the highest risk of infection with a P_{inf} of $3*10^{-1}$ if groundwater was ingested in Culmore, followed by the risk associated with ingestion of wastewater, with a P_{inf} of $2*10^{-2}$. The risks for these scenarios were considerably lower for Kvidinge (Table 17). There, the highest risks were associated with the use of groundwater for drinking, giving a P_{inf} at $8*10^{-3}$.The high risk for direct exposure in Culmore was due to the rather high average concentration in the irrigation water. The other field sites had lower concentrations of *Giardia* cysts in the irrigation water and the risk would be in the same range as in Kvidinge. For calculating the concentration in groundwater the removal of organisms in the soil was used, with removal of clostridia in Kvidinge being 0.6 log_{10} and in Culmore 1.8 log_{10}.

Exposure to rotaviruses gave a high risk of infection when quantifying the risks using community surveillance data. The lowest risks for infection were related to exposure to aerosols in Kvidinge (P_{inf} $3*10^{-2}$), whereas the highest were associated with groundwater ingestion in Culmore, with 8 out of 10 people exposed potentially getting infected. When the lower literature value for the concentration of rotaviruses in wastewater was used, the risks of infection were considerably lower, especially for exposure to aerosols (3 to 4 log_{10}). The risk of infection by direct contact with wastewater was reduced to P_{inf} of $2*10^{-2}$ in Culmore and to P_{inf}

$4*10^{-3}$ in Kvidinge. The corresponding changes in risk of infection were also seen for ingestion of groundwater.

Table 17. *Results from the QMRA for three exposure scenarios chosen in the irrigated field sites Culmore, Northern Ireland and Kvidinge, Sweden, expressed as median risk of infection per exposure (P_{inf}), and as the annual number of cases in the exposed population (cases)*

Field site		Culmore			Kvidinge		
Exposure route		Waste-water	Aerosol	Ground-water	Waste-water	Aerosol	Ground-water
Salmonella	P_{inf}	$2*10^{-6}$	$2*10^{-8}$	$5*10^{-7}$	$2*10^{-7}$	$1*10^{-10}$	$2*10^{-6}$
	cases	$2*10^{-5}$	$4*10^{-5}$	$7*10^{-4}$	$2*10^{-6}$	$3*10^{-8}$	$2*10^{-3}$
Giardia	P_{inf}	$2*10^{-2}$	$3*10^{-4}$	$3*10^{-1}$	$3*10^{-5}$	$2*10^{-8}$	$8*10^{-3}$
	cases	0.2	0.4	5	$3*10^{-4}$	$6*10^{-6}$	4.36
Crypto-sporidium	P_{inf}	$7*10^{-5}$	$8*10^{-7}$	$1*10^{-3}$	$7*10^{-6}$	$5*10^{-9}$	$2*10^{-3}$
	cases	$8*10^{-4}$	$1*10^{-3}$	1.27	$7*10^{-5}$	$1*10^{-6}$	1.7
Rotavirus[a]	P_{inf}	$8*10^{-1}$	$4*10^{-1}$	$8*10^{-1}$	$7*10^{-1}$	$3*10^{-2}$	$7*10^{-2}$
	cases	1	50	5	1	5.61	5
Rotavirus[b]	P_{inf}	$2*10^{-2}$	$2*10^{-4}$	$2*10^{-2}$	$4*10^{-3}$	$3*10^{-6}$	$2*10^{-3}$
	cases	0.88	0.26	4.96	$4*10^{-2}$	$7*10^{-4}$	2

[a] Community surveillance

[b] Literature value (Rao, Metcalf & Melnick, 1987)

In the simulations of the risk of infection, the highest risk was found for rotaviruses both on single exposure and as calculated on a yearly basis. The concentration of rotavirus in treated wastewater was high (3-4 $\log_{10}$ per L) based on estimates from community incidence and applying the measured reduction of coliphages. The number of virus particles excreted during an infection is higher than for bacterial pathogens, *e.g. Salmonella*, which partly reflects the high input concentration used in wastewater. Even higher excretion numbers (up to 10^{12}) and longer excretion times than used in the risk calculations (as a most likely value) have been reported for rotaviruses (Gerba *et al.*, 1996). On the other hand, excretion values represented the whole excretion period and knowledge is limited regarding variations during the time of infection and the excretion is potentially substantially lower during part of this time, resulting in lower excreted numbers. In conjunction with the community incidence calculations, literature values of rotaviruses in wastewater were also used. (Rao, Metcalf & Melnick, 1987) reported a rotavirus concentration of approximately 10^2 per L in wastewater. Using this value instead for calculation, the infections risks were still high but reduced, for aerosols in Culmore from $P_{inf} = 4*10^{-1}$ to $P_{inf} = 2*10^{-4}$. The somewhat lower risk of infection in Kvidinge compared to Culmore was partly due to the longer distance from the irrigation area to the potentially exposed population, *i.e.* 500 m, while for Culmore this distance was set to 100 m. In Culmore the nearest house or road was in fact only 30 m from the field, indicating that the actual risk of infection could be even higher than the calculated.

In addition to rotaviruses, an elevated risk for *Giardia* infection prevailed for the three routes of exposure in Culmore. The highest risks were associated with ingestion of groundwater, $P_{inf} = 3*10^{-1}$, as well as through direct exposure, $P_{inf} = 2*10^{-2}$. For Kvidinge the corresponding risks were substantially lower. The differences were due to a higher concentration of cysts in the raw wastewater and the lower reduction in the treatment plant in Culmore. The groundwater contamination was simulated using the reduction in *clostridia* spores in the soil. *Clostridia* spores have been suggested as a useful surrogate parameter for the removal efficiency of protozoan oocysts in drinking water treatment (Payment & Franco, 1993; Hijnen *et al.*, 2000). The simulated concentrations in groundwater of oocysts when using the concentration in treated wastewater of oocysts and removal of *Clostridia* spores agree well with measured levels, with the exception of *Giardia* cysts in Culmore, which could be seen as a 'worst case scenario'. The protozoa, due to their rather large size, should have a high retention in the soil (Thurston *et al.*, 2001). Although the concentrations in Kvidinge were substantially lower than in Culmore, a risk of infection occurred when using the groundwater for drinking.

General conclusion

Conventional treatment and handling of wastewater normally results in discharge of the treated wastewater to recipient waters. Potential exposure situations could occur if the discharged wastewater affects nearby bathing areas where people come into contact with the contaminated wastewater. Introducing alternative ways of wastewater treatment reduces this exposure, but also change the general exposure whereby other exposure routes come into focus. However, both in conventional systems and in the reuse systems described in this study, workers are exposed to the raw and partly treated wastewater in the treatment plant. Applying the wastewater to willow coppice, coniferous forest or wetland areas creates additional exposure opportunities for workers in the fields monitoring the irrigation and/or pond systems. However, the number of people exposed in this group is limited and since this is part of their normal work duties, they should be informed about the risk of infection through direct contact and ingestion of wastewater. Protective measures against accidental ingestion of wastewater are related to worker behaviour as well their use of protective clothing and protective face masks during work. Good hand hygiene should always apply.

When sprinkler irrigation is used, creation of aerosols can occur. The number of people who are potentially exposed to aerosols varies due to the specific sites and the irrigation techniques employed. These persons should be identified and targeted in the risk management. Those most likely to be exposed are people living near the irrigated fields, and thus the distance from the irrigated area to the nearest house or public road affects the size of the exposed population, as well the risk of infection (Paper V). For the irrigated forest site investigated, no houses were situated in the neighbourhood. Instead, people using the forest for recreational activities would be the group that could be exposed to aerosols if sprinkler irrigation is used or to direct contact with contaminated ground or by eating contaminated berries and mushrooms. The risks can partly be counteracted by specific treatment requirements or by clear information posted at access points to the area in question.

Faecal contamination of the groundwater due to the wastewater irrigation was found in most of the irrigated areas. The further fate of the organisms and groundwater flow are thus central for the potential impact of groundwater wells in the vicinity. Exposure will occur if households in the area use the groundwater as drinking water. Communal wells are monitored regularly, but this is not the case for private water sources. In the quantitative risk assessment applied in Paper V, private wells in the area were assumed to exist, even if this was not the case in the fields studied. The number of people exposed will depend on the number and siting of private wells and the size of the households. When planning for new irrigation sites, the occurrence of drinking water wells in the vicinity should always be assessed. When in doubt regarding groundwater transportation, hydrogeological investigations should be included in the assessments.

Surface runoff can occur from the irrigated area to nearby localities, depending on the topographical conditions. The number of people or animals that could be exposed is site-specific. For example, the irrigated fields at Kvidinge and Culmore were partly hilly, with potential surface runoff to adjacent fields with grazing animals. In general, large numbers of people could be exposed if the surface runoff affects nearby lakes or rivers, where bathing or recreational activities occur. As for groundwater, the risks can partly be incorporated into the planning phase for a specific site, which should not be located in an area where an impact may occur. If this is not possible, restrictions may be imposed on nearby activities, more intensive pre-treatment may be employed or the impact may be judged in relation to irrigation technique. In this instance sprinkler irrigation may actually be better, due to a more even distribution over the irrigated area.

In all situations it is the exposure that is the focus. For the wetlands and pond systems, potential exposure to pathogenic organisms will occur if humans (or animals) are in direct contact with the wastewater or stormwater. Some treatment wetland areas have also been promoted as recreational sites, with an enhanced likelihood of direct contact with the water or sediment, which is also the main risk of exposure. The group most vulnerable for this is children playing at the waterfront. The risk can naturally be minimised by restricting access to the areas. This may not necessarily exclude people from the sites, but with proper planning can be done through the localisation of paths, excluding picnic sites from areas where the water front can be reached, zonation of the areas, with access to sites where proper treatment of the water has been verified. One should also ensure a sufficient residence time in the dams to minimise the risks in outlet area, and if these are used for recreational activities, institute a proper monitoring programme.

At all field sites, *i.e.* both the wetlands and the irrigated willow coppice and forest, animals could be exposed to pathogens occurring in the wastewater or sludge. The extent of this exposure should in general be assessed and, where appropriate, fencing could play a role for minimising this exposure situation to larger animals. However, this would not limit the exposure for birds and smaller animals, *e.g.* rodents.

In summary, the transmission of pathogenic organisms occurring in the wastewater or sludge and applied to willow coppice, forests or wetlands can affect humans or animals through:

- Direct contact with the wastewater at the treatment plant (workers), irrigated fields or wetland and accidental ingestion of minor amounts, but still enough to cause infections,
- Inhalation of aerosols containing pathogenic microorganisms if sprinkler irrigation is applied,
- Consumption of berries and mushrooms in the specific case when wastewater or sludge is applied in forest areas,
- Ingestion of contaminated groundwater. The risk was documented for all the irrigated field areas in this study, although the level of faecal contamination varied greatly between the studied fields.

The exposure and subsequent risks vary in time and due to transmission routes. Exposure can occur either occasionally or more frequently depending on field characteristics and the exposure situation. Irrigation with wastewater takes place daily or a couple of times per week during the vegetation period, both in the willow coppice and in the forest. The highest concentration of pathogenic organisms occurs during and soon after irrigation, on the vegetation and on the soil surface. After an irrigation event, the concentrations decline more or less slowly in the irrigated area, reducing the risk of subsequent exposure, while exposure to aerosols occurs during and right after irrigation. In the QMRA, irrigation was assumed to take place twice per week during the irrigation period and the exposed people living in the area were set to be exposed every other irrigation event. The risks for humans is minimised if the irrigation is conducted during night-time, but this will not limit the risk for animals living in the area.

The risks associated with surface runoff depend on irrigation load and rain events. Measures to reduce surface runoff need to incorporate both careful selection of sites for irrigation, and type and intensity of irrigation.

If the groundwater is contaminated in an area with public or private wells, the exposure and risks could be judged as more or less constant through the daily intake of drinking water. A contamination of groundwater could also last for long time due to the favourable conditions prevailing in the groundwater zone for most pathogenic organisms.

At wetlands supplied with wastewater, workers and occasional visitors are the two groups that could be exposed to pathogens. For both these groups the exposure is probably occasional and of limited importance. Instead, zoonotic transfer of pathogens through birds and mammals probably constitutes the main risk for transfer of infections.

As was exemplified in the risk assessment, all major organism groups able to cause infection in humans and excreted in faeces in the European population, *i.e.* bacteria, protozoa and viruses, will pose a risk, the magnitude of which varies in relation to the organism in question. This risk is reduced by pre-treatment of the wastewater, subsequently reducing the concentrations of the organisms before discharge or irrigation. A lower reduction during pre-treatment could for some of the transmission routes be compensated for by taking advantage of site-specific conditions, *e.g.* fencing, or irrigation method. The type and concentration of organisms reaching an irrigated field depend on the prevailing infections in society but also on the type of pre-treatment occurring. The quantitative risk assessment for two of the field sites indicated that the highest risks were associated with viral infections, here represented by rotavirus, followed by the parasitic protozoa, as exemplified by *Giardia*. The risk assessment tool, applied on scenarios and further refined by site-specific data, can thus function as a predictive management tool for future studies, as well as for validation of site-specific risk factors.

In addition to the general concluding remarks above, my studies have shown that:

- The reduction in organisms, indicator organisms as well as the pathogenic *Giardia* and *Cryptosporidium,* varied between the treatment plants, in general following the treatment level, but for some organisms it was more site-specific,
- The reduction was highest for vegetative bacteria, followed by sporeforming bacteria and coliphages, which need to be accounted for in future monitoring strategies,
- The varying treatment level resulted in large differences in microbial load applied to the fields,
- The treatment of wastewater in oxidation pond and storage pond gave a high microbial quality of the irrigation water with most indicator organisms below detection levels, resulting in limited risk for further transmission to humans,
- The faecal impacts due to wastewater irrigation were documented for groundwater. The level of contamination followed the pre-treatment of the wastewater with high concentrations and frequencies of positive samples at Culmore, Kvidinge and Kågeröd. The reduction in organisms in the soil profile varied between the field sites,
- The contamination of groundwater was verified in lysimeter studies assessed with tracer bacteriophages. Very rapid transport was found in clay lysimeters, explained by substantial macropore flow of water through the soil column. Considerably lower transport was found in sand lysimeters, with breakthrough after 0.45 to 0.88 pore volumes, indicating a convective/dispersive transport in the sand column,
- Although a rapid transport occurred in the clay soil, the retention of organisms was very high in both soil types tested,
- Prolonged survival of microorganisms occurred after application of wastewater to vegetation as well as in wetland sediments, determined in laboratory microcosm investigations and partly verified in the field. The

sediment constitutes a favourable environment for prolonged survival, which may result in subsequent risk events after resuspension with sudden high concentrations to the water-phase,
- Occasional sampling of the water phase does not indicate the actual risk of exposure for organisms occurring in the sediment,
- The risk assessment indicated an elevated risk for viral infections for all three exposure situations identified (accidental ingestion of wastewater, inhalation of aerosols and ingestion of groundwater), but this varied with input concentrations in the wastewater. Parasitic protozoa also showed an elevated risk.

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Abbreviations

cfu: Colony Forming Units
DALY: Disability Adjusted Life Years
DW: Dry Weight
EIEC: Enteroinvasive Escherichia coli
EPEC: Enteropathogenic Escherichia coli
ETEC: Enterotoxine producing Escherichia coli
EHEC: Enterohaemorrhagic Escherichia coli
EPA: Environmental Protection Agency
EU: European Union
FW: Fresh Weight
ha: Hectare
ISO: International Standardisation Organisation
$\log_{10:}$ logaritm
MPN: Most Probable Number
NMKL: Nordisk Metodikkomité for Naeringsmidler
µm: micrometre
N: Nitrogen
pe: person equivalents
PE: Potential Evaporation
pfu: Plaque Forming Units
SMI: Smittskyddsinstitutet / Swedish Institute of Infectious Disease Control
SRF: Short Rotation Forestry
SRWC: Short Rotation Willow Coppice
SVA: Statens Veterinärmedicinska Anstalt /National Veterinary Institute of Sweden
QMRA: Quantitative Microbial Risk Assessment
USEPA: United States Environmental Protection Agency
UV: Ultra Violet
yr: year
WHO: World Health Organization
WW: wastewater