

Energy forest irrigated with wastewater: a comparative microbial risk assessment

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ABSTRACT

In this study, risks for human infection associated with irrigation of municipal wastewater on short rotation willow coppice (*Salix*) were evaluated in three countries. The aim was also to determine the reduction of indicator organisms and pathogens in the treatment plants. Two of the field sites were chosen for further evaluation by QMRA (quantitative microbial risk assessment) applied to three scenarios: accidental ingestions of wastewater, exposure to aerosols and ingestion of groundwater. The risks of infection for bacteria (*Salmonella*), virus (rotavirus) and protozoa (*Giardia*, *Cryptosporidium*) were characterised as probability of infections per exposure and number of infections per year.

The highest risk for infection was associated with exposure to rotavirus in Culmore (Northern Ireland), by either accidental ingestion of wastewater or ingestion of groundwater ($P_{\text{inf}} 8 \times 10^{-1}$). For Kvidinge (Sweden) the risk for virus infection by ingestion of wastewater were in the same range ($P_{\text{inf}} 7 \times 10^{-1}$). The risk for *Giardia* infection differed between the two sites due to differences in concentration of this pathogen in the wastewater. The groundwater was found to have suffered faecal contamination due to the wastewater irrigation. Use of partially treated wastewater for irrigation of energy crops could be a sustainable option if site-specific recommendations are developed.

Key words | indicator organisms, irrigation, microbial risk assessment, pathogens, short rotation willow coppice, wastewater

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ABBREVIATIONS

DALY	disability adjusted life years
DAPI	4',6-diamidino-2-phenylindole
IMS	immuno-magnetic separation
PDF	probability density functions
PE	potential evaporation
pe	person equivalents
P_{inf}	median risk for infection per exposure
P_{yearly}	annual risk for infection
QMRA	quantitative microbial risk assessment

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INTRODUCTION

Irrigation with wastewater on agricultural crops has attracted an increasing interest in both industrial and developing countries during recent decades. The driving factors behind the increased reuse are an expanding water scarcity in many areas together with an increasing population, as well as recognition of wastewater as a resource (WHO 2006). At least 10% of the world's population was thought to consume foods produced by irrigation with wastewater in the late 1990s (Smit & Nasr 1992). According to Bontoux (1998), wastewater reclamation and reuse

worldwide is estimated to represent a potential extra water resource of approximately 15% of the existing water use and locally it represents a higher percentage. One of the largest wastewater irrigation systems in the world is located in central Mexico, with nearly 90,000 hectares irrigated with wastewater produced from Mexico City (Cifuentes 1998; Cifuentes *et al.* 2000).

Wastewater irrigation has been performed on various crops (Amahmid *et al.* 1999; Tsadilas & Vakalis 2003), as well as on public areas such as golf courses and parks (Foug *et al.* 1995; Bahri *et al.* 2001). One alternative application that has stimulated increased interest during the past decade is wastewater irrigation of energy crops used for production of biofuels (Perttu & Kowalik 1997). This application has a dual purpose. It purifies the wastewater in so-called vegetation filters and at the same time produces renewable energy (Larsson 2003). Using wastewater as a fertiliser has potential for saving finite resources in terms of plant nutrients, and results in lower use of chemicals and energy compared with traditional wastewater treatment (Larsson 2003).

A comparison of wastewater treatment in different regions in Europe, performed by the European Environment Agency (EEA) in 1997, showed that at that time, the percentage of the population connected to secondary or tertiary wastewater treatment was 14% and 10%, respectively for Greece, 62% and 18% for the United Kingdom and 6% and 87% for Sweden (EEA 1997).

Since, in addition to water, nutrients play an essential role in wastewater irrigation, the level of treatment is less important from this perspective. However, it plays a fundamental role for further assessments of the hygienic risks related to pathogen exposure. Raw municipal wastewater contains faecal microorganisms (both pathogens and non-pathogens); the numbers and types of pathogen vary with time and are dependent on the prevalence of infections in the population connected to the treatment plant. The removal of organisms depends on the treatment steps used; primary treatment such as primary sedimentation is considered to give a low removal: 0–1 log of viruses, bacteria and protozoans and 0– < 1 log of helminths (Feachem *et al.* 1983; Yates & Gerba 1998; WHO 2006). When secondary biological treatment is used (e.g. activated sludge, trickling filters), another 0–2 log units of viruses and

bacteria, 0–2 log of protozoan (oo)cysts and 1–2 log of helminths are removed (Feachem *et al.* 1983; Rose *et al.* 1996; Yates & Gerba 1998; WHO 2006). Additional removal occurs if the wastewater is further treated with, for example, chemical flocculation, sedimentation or filtration, giving an additional 0–1 log removal of bacteria and 1–3 log removal of viruses and protozoans (WHO 2006).

The level of wastewater treatment required before its use on various crops is not clearly regulated within Europe. As a result, the legal status for reuse of wastewater is not uniform and varies between countries. France, for example, has national recommendations, whereas Spain has various regional recommendations, and some countries (e.g. United Kingdom, Belgium, the Netherlands) do not have any specific legislation (Bontoux 1998). In Australia and some states within the USA, where wastewater reuse is commonly practised, criteria have been established, for example, regarding acceptable levels of faecal indicators and defined levels of pre-treatment needed (NRMCC & EPHCA 2005). In the USA, for unrestricted irrigation (including crops that are likely to be eaten uncooked), no detectable faecal coliforms are allowed in a 100-ml sample, and for irrigation of, for example, fodder crops, a guideline limit of ≤ 200 faecal coliform bacteria per 100 ml applies (Blumenthal *et al.* 2000). The actual standards vary between the states; California has the strictest standards for irrigation of food crops, requiring <2.2 total coliforms per 100 ml and <23 total coliforms per 100 ml for irrigation of pasture and landscape areas (US EPA & US AID 1992; Blumenthal *et al.* 2000).

The World Health Organization (WHO) has also published recommendations for the safe reuse of wastewater, including quantitative values on indicator organisms. As for the United States Environmental Protection Agency (US EPA), a higher quality is required if the wastewater is to be applied to edible crops, compared with so-called *restricted irrigation* including other types of crop such as energy crops (WHO 1989, 2006). The 2006 WHO guidelines differ from the 1989 guidelines by, for example, giving a new recommendation for faecal coliforms of $\leq 10^5$ FC 100 ml⁻¹ when used for restricted irrigation, compared with no recommended standard values for this parameter previously (Carr *et al.* 2004; WHO 2006).

Recommended guidelines for wastewater reuse in Greece have been suggested (Andreadakis *et al.* 2003),

with 200 faecal coliforms per 100 ml (median value) for restricted irrigation (e.g. forests, fodder, pastures, trees) and 5 faecal coliforms per 100 ml in 80% of the samples for unrestricted irrigation. In Sweden, local authorities give permission for wastewater irrigation on a site-specific basis.

When wastewater is used for irrigation, humans and animals can be exposed either by direct contact or through contaminated crops or soil in the irrigated area. Depending on the application method used, aerosols could be created and transported to the surrounding area. This applies especially if sprinkler irrigation is used. The groundwater in the irrigated area may also be contaminated depending on the irrigation load, type of soil, etc.

The aim of this study was to investigate the occurrence of indicator organisms and pathogens in municipal wastewater, to determine reduction of organisms (varying levels of treatment in treatment plants as well as a pond system), to measure the outgoing concentration of pathogens and to assess the risk of using this treated wastewater for irrigation in willow plantations for bio-fuel production.

In this study, municipal wastewater was used for irrigation of short rotation willow coppice (*Salix*) in three countries: Greece (GR), Northern Ireland (NI) and Sweden (SE). The level of treatment of the wastewater before application varied between the sites from mechanical treatment (primary) to biochemical (secondary) treatment. As described above, this affects the concentration of microorganisms in the irrigation water, and the subsequent risks upon exposure to the irrigation water applied. For the study a number of organisms, both indicator organisms and pathogens, were selected and analysed in the wastewater and groundwater. The selected organisms represent bacteria (pathogens *Salmonella* and *Campylobacter* and indicators *Escherichia coli*, intestinal enterococci and clostridia), viruses (represented by the coliphages) and protozoa (*Giardia* and *Cryptosporidium*). All of the pathogenic organisms are known to cause waterborne disease and are present in the populations in the three countries in this study.

The specific factors evaluated were related to differences between the systems (i.e. between the field sites in the various countries) from a risk perspective. This included climate, treatment levels, presence of pathogens, irrigation methods, distance to houses, etc. The potential exposure scenarios and transmission routes for pathogens that could

occur during irrigation were explored and possible barriers to reduce the risks for disease transmission were identified and discussed.

MATERIALS AND METHODS

Description of field sites

Six field areas irrigated with wastewater on short rotation willow coppice (*Salix*) formed part of the study, with four areas in Sweden (Bromölla, Kvidinge, Kågeröd, Roma), one in Greece (Larissa) and one in Northern Ireland (Culmore). The areas used for wastewater irrigation varied from 2 to 11 ha at the different sites and were planted with willow, approximately 12,000–14,000 cuttings per ha, in double rows. Willow plantations are usually harvested in winter-time every 3–5 years, after which the plants resprout. The irrigation water was distributed either through perforated tubes placed on the ground (drip irrigation) or through low-emitting sprinklers. The irrigation wastewater had different levels of pre-treatment, from just mechanical to biological/chemical. At Roma, Gotland, the wastewater was biologically treated in oxidation ponds and then stored in a final storage reservoir with a capacity of approximately 6 months' water production. The irrigation load represented the yearly mean evapotranspiration (1 PE) or multiples thereof at different subplots with a size of approximately 400 m² each (Roma, Culmore and Larissa). Pure water was used as a control at some sites (Roma, Culmore and Larissa). Comparisons were also made with sludge (Culmore; 100 tonnes ha⁻¹ applied at the start of the experiment) and human urine fertilisation (Roma; Larsson 2003). The different characteristics for each of the treatment plants and irrigated fields are summarised in Table 1.

The sites represent different climatic conditions. A typical Mediterranean climate prevails in Larissa, Greece, with hot dry summers and rainy winters (yearly average temperature 15.5°C and 415 mm of precipitation). Culmore, Northern Ireland, has a typical western European maritime climate with mild, wet winters and relatively cool summers (yearly average temperature 8.7°C and 943 mm of precipitation, evenly distributed during the year). Roma, Sweden, has a North European local-maritime climate with

Table 1 | Characteristics of the irrigation area and irrigation regimes at the six field sites: Culmore, Larissa, Bromölla, Kvidinge, Kågeröd and Roma (pe = person equivalents, PE = potential evaporation)

	Culmore, NI	Larissa, GR	Bromölla, SE	Kvidinge, SE	Kågeröd, SE	Roma, SE
Treatment (before irrigation)	Mechanical	Mechanical	Biological/Chemical	Biological	Biological	Oxidation ponds + storage pond
Size/daily load	114,000 pe (60,000 m ³)	250,000 pe (40,000 m ³)	10,000 pe	1,400 pe (400 m ³)	1,500 pe	1,400 pe
Soil type	Sandy loam	Loam/clay-loam/sandy-clay loam	Clayey sand till	Silty clay	Silt loam	Loam/clay-loam/sandy-clay-loam
Topography	Partly slope	Flat area	Flat area	Partly hilly	Flat area	Flat area
Irrigated area (ha)	5	2	11	10	10	5
Irrigation system	Low level sprinklers, ~30 cm	Drip irrigation	Surface irrigation	Low level sprinklers, ~25 cm	Surface irrigation	Drip irrigation
Irrigation period	April to October	April to October	March to November	May to October	May to October	April to October
Annual irrigation load (mm)	1 PE (308–581 mm), 2 PE/3 PE	1 PE (718–1,072 mm), 2 PE/3 PE	502–936	370–462	377–757	1 PE (352–533 mm), 2 PE/3 PE
Annual precipitation (mm)	943	413	560	724	760	514

relatively low, evenly distributed precipitation and a high number of sunshine hours (annual average temperature and precipitation 7.1°C and 514 mm, respectively). The three other Swedish field sites, Bromölla, Kvidinge and Kågeröd, situated in southern Sweden, have similar conditions to Roma with mean annual temperatures of 7.2°C and with annual precipitation of 560 mm, 724 mm and 760 mm, respectively. Groundwater samples taken at these sites represent the entire fields with more or less even irrigation loads.

Sampling

Raw and treated wastewater from each wastewater treatment plant was sampled on the same occasion (matched samples). Groundwater samples were collected within the irrigated willow plantations using permanent groundwater pipes (except Larissa, where the groundwater level was too low for sampling). Samples were taken during the vegetation periods at Kågeröd (1999–2000), at Bromölla and Kvidinge (1999–2001) and at Roma, Culmore and Larissa (2000–2001).

Samples taken from the Swedish sites were kept cold and transported to the Swedish Institute for Infectious Disease Control (SMI, Solna) and analysed within 24–36 hours after sampling. The samples from Greece and Northern Ireland were air-freighted to Sweden, resulting in a somewhat delayed start of analysis, at most 48–60 h after sampling. The groundwater samples from 1999 at Bromölla, Kvidinge and Kågeröd were taken from a minimum of four different pipes per field site, and were pooled together and analysed as one composite sample for each field and sampling occasion. For the following sampling years, each of the groundwater samples was analysed separately.

Microbial analysis

The water samples were diluted in 10-fold dilution series in a phosphate buffer. The bacteria were quantified using spread-plate techniques on selective agars in triplicate: mEnterococcus agar (Difco; 44 h, +35°C) for intestinal enterococci and mFC agar (Difco; 24 h, +44°C) for faecal coliforms and *E. coli*. Spores of anaerobic bacteria

(*Clostridium perfringens*, hereafter referred to as clostridia) were analysed using a standard method (International Organization for Standardization (ISO) 1986), preheating samples at 70°C for 20 minutes and applying the pour plate method. Coliphages were analysed with the double-agar layer method described in ISO (2000), with *E. coli* ATCC 13706 as the host strain. *Salmonella* were analysed (fluid samples 1–250 ml) by a semi-quantitative method (ISO 1995) with enrichment in buffered peptone water (Difco; 16 h, +36°C), and were subsequently transferred to Rappaport-Vassiliadis medium (Oxoid; 18 h, +41.5°C) and grown on brilliant green agar plates (Oxoid; 18 h, +36°C). *Campylobacter* were analysed semi-quantitatively with micro-aerophilic incubation in *Campylobacter* blood-free selective enrichment broth (1 h, +37°C), with overnight incubation with selective supplement added (Oxoid SR155E; 18 h, +37°C) and with further cultivation on blood-free selective substrate plates (Oxoid; 18 h, +37°C).

For analysis of *Giardia* and *Cryptosporidium* in wastewater and groundwater, the samples were concentrated by centrifugation (volume varied from 50 ml for raw wastewater up to 3 l for groundwater, dependent on amount of particulate matter). The samples were further analysed in accordance with US EPA (2001) by an immuno-magnetic separation (IMS) technique with selective removal of the cysts and oocysts from other particulates in the sample. The recovered parasites were stained with fluorescent antibodies and counted by microscope. The viability (DAPI positive) of *Giardia* and *Cryptosporidium* as described by US EPA (2001) was analysed in a limited numbers of samples. For the samples analysed in 1999, a flotation technique was used for concentration of the samples (US EPA 1996).

Risk assessment

A risk assessment based on a combination of qualitative and quantitative assessments was performed for the field sites described above. The concept of risk analysis previously described by Haas *et al.* (1999) was partly followed. Hazard analysis and exposure assessments were conducted for all sites on a broad scale. Specific exposures that were judged to be of highest importance were then quantitatively evaluated by further estimating the exposure to individuals

affected by the system. This was done by visits to the irrigated fields, questionnaires to the staff at the treatment plants and measured concentrations of organisms from the sampling occasions. Doses of selected pathogens were calculated and utilised in a dose-response assessment in order to quantify risks for infection. The stepwise procedure for the quantitative microbial risk assessment (QMRA) is further described below.

Hazard identification

The hazard identification is based on the range of pathogenic microorganisms that may occur in municipal wastewater originating from faeces and that can cause gastrointestinal infections. These infections mainly cause symptoms such as diarrhoea, abdominal cramps, vomiting, headache and fever, but more severe consequences may also occur. As model organisms in the QMRA, *Salmonella*, *Giardia*, *Cryptosporidium* and rotavirus were selected. Thus, at least one representative from the major prevailing groups of pathogens was included. The choice was further based on health significance and availability of data needed for the quantification.

Exposure assessment

Humans may be exposed by different routes during irrigation of willow coppice with municipal wastewater. The exposures to hazards were identified by systematic on-site surveys including the treatment plants and irrigated field areas. The staff at each site answered questions orally and completed questionnaires in writing. The visual inspections and written information formed a baseline for identifying potential exposure to hazards at each site. The densities of pathogens in wastewater and other materials (groundwater, leaves, faecal stools) that people could be exposed to were calculated by combinations of results from the microbial analyses of occurrence and reduction in pathogens and indicators, and on reported incidences of disease, further described in Results. Volumes ingested were estimated based on the information obtained from interviews and questionnaires, supplemented with previously published information. The frequency of exposure and the number of exposed people were also based on site-specific data, as well as on assumptions where data were lacking.

Dose-response assessment

The dose-response relationships used can all be found in Haas *et al.* (1999). Beta-Poisson dose-response models were used for *Salmonella* and rotavirus. For *Cryptosporidium* and *Giardia*, exponential models were used.

Risk characterisation

The exposure models were developed in Excel 2000 spreadsheets and run using @RISK 3.5.2. The input variables defined as PDF (probability density functions) were sampled using a Latin Hypercube simulation in @RISK with 10,000 iterations per simulation. The risks of infection were characterised as probability of infection per exposure and number of infections per year, taking frequency of exposure and number of individuals exposed into consideration.

RESULTS

Occurrence and reduction of indicator organisms in wastewater

The microbial quality of the water used for irrigation varied between the different field sites as a result of the wastewater treatment applied. The highest concentrations were found at Culmore, with a mean *E. coli* concentration of around $6 \log_{10}$ cfu 100 ml^{-1} and $5 \log_{10}$ cfu 100 ml^{-1} for enterococci and coliphages (Figure 1), while for Larissa, Bromölla, Kvidinge and Kågeröd the levels averaged between 3 and $4 \log_{10}$ cfu 100 ml^{-1} .

In the irrigation water at Roma, the levels of *E. coli* and intestinal enterococci were below the detection limits, <0.1 to $<2 \log_{10}$ cfu 100 ml^{-1} . Coliphages were detected on one occasion in low numbers ($1.5 \log_{10}$ 100 ml^{-1}). The clostridia concentrations in the irrigation water at Roma were 1.0 to $1.7 \log_{10}$ cfu 100 ml^{-1} , while at the other sites they occurred in the range 3.2 to $4.2 \log_{10}$ cfu 100 ml^{-1} .

The reductions within the treatment plants are presented in Figure 2. The reductions were calculated by using the mean inlet concentration and mean outlet concentration (including SDs) for each plant. The plant at Culmore, with only mechanical treatment, had high incoming concentrations and a low reduction, resulting in

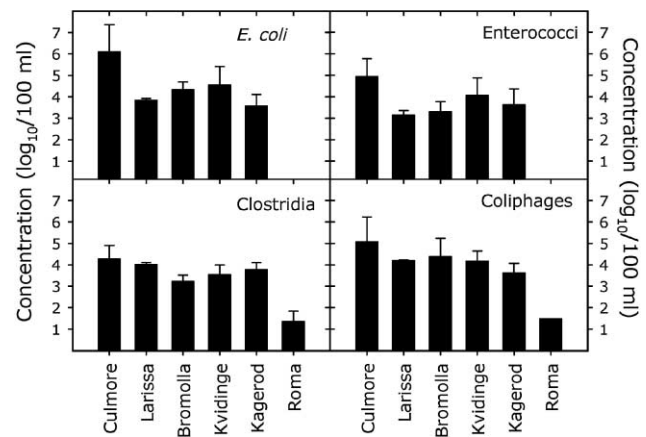


Figure 1 | Mean concentrations and standard deviations for the indicator organisms *E. coli*, enterococci, clostridia and coliphages in the irrigation water at six different treatment plants.

the highest numbers of microorganisms in the irrigation water. The pond system at Roma gave the highest reduction, although the incoming concentrations were low compared with the other treatment plants. For the organisms where no colony-forming unit or plaque-forming units were detected in the irrigation water, the reductions were based on, and are presented as, the detection level.

Occurrence and reduction of pathogens in wastewater

The occurrence of the four selected pathogens was analysed in the untreated and treated wastewater. For the untreated wastewater ($n = 26$, total number of samples), *Campylobacter* were detected only once in the water in the

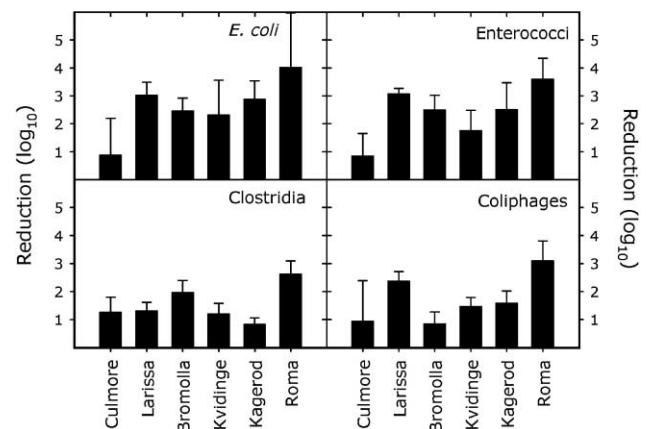


Figure 2 | Mean reduction and standard deviation for the organisms *E. coli*, enterococci, clostridia and coliphages in six different treatment plants.

first two ponds at Roma and not at all in the treated wastewater from any of the other sites ($n = 26$ for all six field sites). *Salmonella* were found in both the untreated and the treated wastewater from all sites: in total 35% of the untreated wastewater samples and 19% of the treated. At Larissa, *Salmonella bovis*morbificans, at Kvidinge, *Salmonella enteritidis* phage type 4 and at Kågeröd, *Salmonella give*, occurred both in the incoming and outgoing wastewater. The other positive samples did not show any consistent pattern and represented, for example, *Salmonella typhimurium* phage type NT, *Salmonella virchow*, *Salmonella thompson*, *Salmonella fresno* and *Salmonella indiana*.

Both *Giardia* and *Cryptosporidium* were also found at all sites, except for *Cryptosporidium* at Roma. *Giardia* cysts were found in 84% of the raw wastewater samples, and oocysts from *Cryptosporidium* in 68%. In the irrigation water, *Giardia* were detected in 48% of the samples, while *Cryptosporidium* were found in 44%. The concentrations of *Giardia* cysts in the raw wastewater varied greatly, both between the sampled sites but also within samples from one site (Table 2). Culmore had the highest incoming concentration (maximum of 15,000 cysts l^{-1}) followed by Larissa (maximum of 6,300 cysts l^{-1}). The reduction within the Culmore treatment plant varied between 7% and >99.5%, with concentrations in the irrigation water of between <0.7 and 2,800 cysts l^{-1} . For the other treatment plants the

concentration in the irrigation water was rather low, normally less than 5 cysts l^{-1} , even when the incoming concentration on the same sampling occasion was high. Kågeröd had a maximum in the outgoing water of 20 cysts l^{-1} .

The incoming numbers of *Cryptosporidium* oocysts varied from <2 to 460 cysts l^{-1} , with the highest incoming concentrations at Bromölla and Kvidinge (Table 2). The reduction in these treatment plants was 1–3 \log_{10} , resulting in low numbers in the irrigation water with a maximum concentration of 18 oocysts l^{-1} . The corresponding reduction in the mechanically treated water at Culmore was 50–60%, resulting in irrigation water levels of <0.7 to 42 oocysts l^{-1} . The viability (DAPI positive) of *Giardia* and *Cryptosporidium* was analysed in some of the samples and varied considerably. Since not all samples were checked for viability, the concentrations presented are the total numbers found.

Microbial load to irrigated fields

The concentrations of organisms in the irrigation water varied between the treatment plants and the pre-treatment used for the wastewater. The average concentrations (recovery not accounted for, detection limit used for less than values) in the treated wastewater together with the difference in amount of water applied resulted in varying loads of organisms to the irrigated fields (Table 3). The willow

Table 2 | 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
<i>Giardia</i>	In	24–15,000	100–6,300	5–580	<4–1,760	3–185	ND
Positive samples	In	4/4	2/2	5/5	5/6	5/6	0/2
<i>Giardia</i>	Out	<0.7–2,800	0.5–1.7	<0.5–5	<0.2– <4	2–20	ND
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	
<i>Cryptosporidium</i>	In	4–104	<2–4	<6–460	<4–360	3–45	ND
Positive samples	In	3/4	1/2	4/5	4/6	5/6	0/2
<i>Cryptosporidium</i>	Out	<0.7–42	<0.5–8	<0.5–18	0.2– <4	0.3–8	ND
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	

^{*}Detection limit dependent on the amount of suspended material in sample. This means that for negative samples the detection limit in theory can be higher than the lowest possible findings. For example, for Kågeröd the negative sample had a detection limit of <6 cysts l^{-1} while the lowest positive finding was 3 cysts l^{-1} . ND = not detected. Detection limit at inlet for Roma <2 (oo)cysts l^{-1} , while at outlet <0.2– <0.3(oo)cysts l^{-1} .

Table 3 | Yearly load of organisms (number m^{-2}) that reach the irrigated field calculated as the average concentration of organisms per litre times the irrigation load (minimum and maximum)

Field site	Irrigation load (mm)	<i>E. coli</i>	Coliphages	<i>Giardia</i>	<i>Cryptosporidium</i>
Culmore, NI	Min 308	1.2×10^{10}	1.7×10^9	2.9×10^5	4×10^3
	Max 1,742	6.9×10^{10}	9.8×10^{10}	1.6×10^6	2.3×10^4
Larissa, GR	Min 718	5.1×10^7	1.1×10^8	7.9×10^2	3.2×10^3
	Max 2,750	2×10^8	4.3×10^8	3×10^3	1.2×10^4
Bromölla, SE	Min 502	1.4×10^8	4.3×10^8	8.5×10^2	2.2×10^3
	Max 936	2.6×10^8	8×10^8	1.6×10^3	4×10^3
Kvidinge, SE	Min 370	4.8×10^8	9×10^7	4.6×10^2	4.6×10^2
	Max 462	6×10^8	1.1×10^8	5.8×10^2	5.8×10^2
Kågeröd, SE	Min 377	2.3×10^7	2.7×10^7	4.1×10^3	1.9×10^3
	Max 757	4.5×10^7	5.4×10^7	8.3×10^3	3.8×10^3
Roma, SE	Min 352	$<1.8 \times 10^5$	1.1×10^5	$<5 \times 10^2$	$<5 \times 10^2$
	Max 1,599	$<8 \times 10^5$	4.8×10^5	$<2.2 \times 10^3$	$<2.2 \times 10^3$

field at Culmore received the highest microbial load: 6 times more (*Cryptosporidium*) to approximately 200 times (*Giardia*) more than the site with the second highest load. Roma and Kvidinge received the lowest amounts of organisms; for *Cryptosporidium* 1/50 of that at Culmore, while for viruses (based on the virus indicator coliphages) the load was almost $5 \log_{10}$ lower at Roma compared with Culmore.

Occurrence of indicator organisms and pathogens in groundwater

The groundwater was sampled and analysed for the occurrence of indicator organisms and pathogens. Total coliforms could be detected in the groundwater at all sampled field sites but with large variations in concentration (Table 4).

At Bromölla, total coliforms were present in the groundwater on four out of seven sampling occasions, in concentrations of $0.7\text{--}5.5 \log_{10}$ cfu 100 ml^{-1} (mean and standard deviation (SD) of positive samples: 3.3 ± 1.3). On one occasion, enterococci were detected at a concentration of $3.9 \log_{10}$ cfu 100 ml^{-1} , whereas the concentrations in the remaining samples were below the detection limit (i.e. $<1 \log_{10}$ cfu 100 ml^{-1}). Clostridia were detected in approximately half of the samples analysed at concentrations of $1\text{--}3.3 \log_{10}$ cfu 100 ml^{-1} . *Salmonella* were detected in the groundwater on one occasion.

At Kvidinge, total coliforms were present in the groundwater in all samples, in concentrations averaging $4.0 \pm 1.0 \log_{10}$ (maximum $6.3 \log_{10}$ cfu 100 ml^{-1}). *E. coli* were generally found but mostly below $3 \log_{10}$ (maximum $4.7 \log_{10}$ 100 ml^{-1}). Enterococci were also mostly present in low numbers in all samples, near or below the detection level except in September 2001, when concentrations from 3 to $5.3 \log_{10}$ cfu 100 ml^{-1} were found at the four sampling sites. Clostridia were also generally present in concentrations ranging from 0.7 to $>4.5 \log_{10}$ 100 ml^{-1} . The coliphages were mainly close to or below the detection level ($1 \log_{10}$ pfu 100 ml^{-1}).

At Kågeröd, total coliforms were present at all sites, in concentrations averaging $4.3 \pm 0.6 \log_{10}$ cfu 100 ml^{-1} . *E. coli*, intestinal enterococci and clostridia were also detected in the groundwater in the majority of the samples, in numbers averaging $2.9 \pm 0.7 \log_{10}$ cfu 100 ml^{-1} , as well as coliphages in concentrations between 1 and $3.4 \log_{10}$ pfu 100 ml^{-1} .

At Culmore, total coliforms were present at all groundwater sampling sites in varying amounts, with the highest numbers in the sludge-supplied plots ($4.9 \pm 0.6 \log_{10}$ cfu 100 ml^{-1}) and where the highest (i.e. 3 PE, three times the estimated evaporation) wastewater load was applied ($5.0 \pm 0.5 \log_{10}$ cfu 100 ml^{-1}). *E. coli*, intestinal enterococci and clostridia were present at the majority of sampling sites but mostly in numbers below $3 \log_{10}$ cfu 100 ml^{-1} but occasionally in higher concentrations, up to $4.9 \log_{10}$ cfu 100 ml^{-1} .

Table 4 | Concentration of indicator organisms in sampled groundwater fields from five field sites. Concentration presented as \log_{10} cfu or pfu 100 ml^{-1} . Number within brackets represents reduction in the soil profile (within the 1 PE wastewater irrigated plots at Culmore and Roma) presented as \log_{10} reduction. Numbers calculated by subtracting the average (\log_{10}) concentrations in the groundwater from the average (\log_{10}) in the irrigation water (recovery not accounted for, detection limit used for less than values)

	Sampling occasions	Total coliforms	<i>E. coli</i>	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)	ND (3.3)	<1–3.9 (>2.3)	<1–3.3 (1.1)	ND (>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)

ND = 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} .

for *E. coli*, 4.0 for intestinal enterococci and 3.6 for clostridia, all in plots with the 3 PE irrigation regime. Coliphages were present in approximately 40% of the total groundwater samples from Culmore, the positive samples originating from the sludge application, the high load treatment (3 PE) and the pure water treatment. Most of the positive samples had concentrations near the detection limit (i.e. $< 1\log_{10}$) but on individual occasions higher concentrations: $3.6\log_{10}$ pfu 100 ml^{-1} in the pure water irrigation regime and $3.9\log_{10}$ pfu 100 ml^{-1} in the 3 PE wastewater irrigation regime. No coliphages were detected in the plots irrigated with 1 PE or 2 PE wastewater or in the non-irrigated control plots.

The average concentrations of organisms in the irrigation water were compared with the average concentrations of organisms in the groundwater (1 PE WW) in order to calculate the removal efficiency in the respective soil profiles (Table 4). The lowest removals were found in the soil at Kågeröd, with $0.5\log_{10}$ for total coliforms to $0.9\log_{10}$ for the rest of the vegetative bacteria and clostridia, and $1.3\log_{10}$ for the coliphages. In general, the removal of clostridia was low, $0.6\log_{10}$ at Kvidinge to $2.3\log_{10}$ for the 1 PE wastewater irrigation treatment at Culmore. Coliphages were the organisms with highest removal in the soil, $2.9\log_{10}$ at Culmore (3 PE WW) to $5\log_{10}$ (1 and 2 PE wastewater at Culmore), Kågeröd excluded. The highest reduction in organisms occurred at Culmore with up to

$5\log_{10}$ reduction in the coliphages, and approximately $2.3\text{--}4.2\log_{10}$ in the vegetative bacteria. For Roma, the concentration of organisms in the irrigation water and the groundwater was in the same range. The removal values for the organisms were further used in the microbial risk assessment (see below).

Giardia cysts and *Cryptosporidium* oocysts were detected in the groundwater in low concentrations, 0.4 and 0.8 per litre respectively, on one occasion at Bromölla and on one occasion, 0.8 (oo)cysts per litre, at Kvidinge.

Qualitative risk assessment

General exposure assessments

The raw wastewater is treated to various levels in the treatment plant, with potential exposure of workers who could accidentally ingest contaminated water or inhale aerosols created during the treatment (Table 5, a). After treatment, the wastewater is pumped through plastic tubes to the willow cropped field, and irrigation is conducted by use of either low-mounted sprinklers (Culmore, Kvidinge) or surface irrigation (perforated plastic tubes placed on the ground) (Bromölla, Kågeröd) or drip irrigation (Larissa, Roma). In the field with sprinkler irrigation, the workers could be exposed to and inhale aerosols created during irrigation (Table 5, b). Microorganisms, especially viruses, can be transported with aerosol droplets and spread by

Table 5 | Potential exposure scenarios for the field sites when irrigating willow coppice with pre-treated wastewater

Type of exposure	Treatment plants
a. Ingestion or inhalation of wastewater or aerosols at treatment plant–workers	All
b. Inhalation of aerosols by workers in the field	Kvidinge, Culmore
c. Inhalation of aerosols by people living in the area or passing on nearby roads	Kvidinge, Culmore
d. Ingestion of contaminated groundwater as drinking water	All
e. Surface runoff–contact with water	All
f. Children playing near storage ponds	Roma
g. Ingestion of contaminated vegetation, soil or water pools–humans	All
h. Ingestion of contaminated vegetation, soil or water pools–animals	All

the wind (Carducci *et al.* 1995, 1999; Brandi *et al.* 2000). Any aerosols created could also be transported by the wind out from the willow cropped area and reach public roads or houses in the neighbourhood (Table 5, c). Various distances over which aerosols can be transported are reported in the literature: for example, 40 to 100 m downwind of irrigated fields (Teltsch & Katsenelson 1978) to 730 m downwind (Shuval *et al.* 1989, *cit.* Schwartzbrod 1995).

Depending on factors such as soil type, water uptake by the crop, irrigation load and precipitation, the drip irrigation water may create ‘ponds’ of various sizes before it infiltrates the soil. Microorganisms in the wastewater are normally retained in the soil but could also be rapidly transported through the soil profile down to groundwater level (Carlander *et al.* 2000). The risk of exposure to contaminated groundwater is dependent on whether the groundwater is used for drinking water (ingestion) (Table 5, d) and whether wells are located in the area.

Surface ponding could occur during both types of irrigation, but the risk is higher with the tubes placed on the ground, with their high point loads of irrigation water. If the irrigation creates ponds in the field, the risk of people or animals coming into contact with the water increases. Depending on the topography of the field, the surplus water

could also be subject to surface runoff, potentially entering nearby streams or lakes (and thus potentially affecting bathing water) or reaching pasture areas (Table 5, e).

For the field site at Roma, the pond system (Table 5, f) can create an extra exposure situation compared with the other field sites. Humans, mainly children playing at the ponds, could accidentally ingest the treated wastewater. Humans, mainly workers in the irrigated field, could come into contact with contaminated foliage, soil or water pools (Table 5, g). In addition to humans, animals in the irrigated areas could also potentially be infected by pathogenic organisms present in the wastewater (Table 5, h).

Site-specific exposure assessment

The results from microbial analysis and the on-site survey provide information regarding potential exposure scenarios for transmission for the different field sites (Table 6). In general, the treatment plant and willow cropped field at Culmore had the highest number of potential exposure points. The reduction in organisms within the treatment plant was low, giving high concentrations of potential pathogens in the irrigation water. The irrigation was conducted using sprinkler irrigation, which could have created aerosols. The cropped field is partly sloping, which could increase the risk of surface runoff if the irrigation results in ponding of surplus water; thus there is an increased risk of direct contact for humans, and for any animals grazing in adjacent fields. All indicator organisms were found in the groundwater at Culmore. However, the irrigated area was fenced, which limited the access for humans and larger animals.

Several of the exposure points of concern at Culmore were also of importance at Kvidinge (Table 6), with a rather low reduction in the indicator organisms within the treatment plant (on average 1.2 to 2 log₁₀) compared with the other treatment plants with secondary treatment. The irrigation was conducted using sprinklers, possibly creating aerosols. The Kvidinge field is partly sloping, which could increase the risk of surface runoff if ponding of irrigation water occurs or if heavy rains occur during or after irrigation. As for Culmore, this would have increased the risk of direct contact for humans and animals.

Table 6 | Characterisation of exposure routes for the six study sites (i.o. = indicator organism)

	Culmore, NI	Larissa, GR	Bromölla, SE	Kvidinge, SE	Kågeröd, SE	Roma, SE
Reduction	Low, 0.6 log ₁₀ (tot coliph.) –1.3 log ₁₀ (<i>Clostr.</i>)	Low for <i>Clostr.</i> (1.3 log ₁₀), 2.4 log ₁₀ for coliph., 1.9–3.1 log ₁₀ for vegetative bacteria	Average 2–2.5 log ₁₀ except for coliphages 0.8 log ₁₀	From 1.2 log ₁₀ (<i>Clostr.</i>) to 2 log ₁₀ (<i>E. coli</i>)	Low, for <i>Clostr.</i> (0.8 log ₁₀), for vegetative bacteria 2.5–2.9 log ₁₀	High, (2.5–4.0 log ₁₀), low numbers in incoming water
Type of irrigation	Low level sprinklers	Drip irrigation	Surface irrigation	Low level sprinklers	Surface irrigation	Drip irrigation
Distance to houses or roads	30 m	200 m	Farm ~100 m	Farm ~500 m	Farm ~500 m	800–1,000 m
Surface runoff	Part sloping	Flat area	Flat area	Partly hilly	Flat area	Flat area
Depth to groundwater	3.2 ± 0.2 m	At start: 1.5–3.6 m, increased to 8 m	1.5–5.0 m	1.0–4.5 m	0.5–1.5 m	From 0.5 to 3.14 m at start
Ground-water contamination	All i.o. present	–	Total coliforms present	All i.o. except coliphages	All i.o. present	Low levels of total coliforms
Birds/ animals in the area	Cattle graze adjacent fields. Rabbits within site. Birds in the surrounding area	Yes, mammals, birds and reptiles	Yes	Yes, cattle graze adjacent fields sometimes	Yes	Yes, pheasant, pigeons
Fenced area	Yes	Yes	No	No	No	No

All indicator organisms except coliphages were found in the groundwater.

At Larissa and Roma, drip pipes were used for application of the wastewater, limiting the potential exposure to workers in the field. At Larissa, a relatively good reduction in organisms was found in the wastewater treatment plant, despite the fact that the irrigation water was subject to mechanical pre-treatment only. At Roma, the hygienic quality of the irrigation water was good. It was not possible to sample the groundwater at Larissa. At Roma, the concentration of organisms in groundwater was low or below the detection limit.

Bromölla and Kågeröd had relatively good reductions in organisms, but with low reduction of clostridia at Kågeröd, and of coliphages at Bromölla. At both sites, irrigation was conducted using tubes placed on the ground with point emitters giving rather high point irrigation loads, increasing the risk of transport of microorganisms to groundwater. The risk of wastewater ponding also increased the risk of surface runoff, although the ground is relatively flat at both sites, limiting this possible exposure. At Bromölla, only total coliforms were found in the groundwater, whereas at Kågeröd all indicator organisms were found in the shallow groundwater.

Quantitative microbial risk assessment (QMRA)

Based on the results from the qualitative risk assessment above, two field sites were found to be of particular interest and were chosen for further evaluations. The treatment plant at Culmore in Northern Ireland generally had a low reduction in microorganisms, resulting in potentially high concentrations of various pathogens in the irrigation water. Culmore also had the highest microbial load. Kvidinge in Sweden had low reductions in organisms compared with the other field sites in Sweden. These two sites both practised sprinkler irrigation, and microorganisms were detected in the groundwater.

Accidental ingestion of wastewater was chosen as a 'general' risk associated with the systems. Exposure to untreated, or partly treated, wastewater within the treatment plants was not considered; that is, the system boundaries were set to be the incoming pre-treated irrigation water. The treated wastewater could be ingested

by different exposure routes, but several of them could be considered similar, the difference being mainly related to dilution, as for recreational water, for example. The number of individuals exposed and the frequency of exposure will naturally also vary. We chose the scenario of workers in the field being exposed as the most likely scenario, resulting in exposure to 'concentrated' wastewater for a low number of individuals, but occurring several times per year (see below). Exposure to aerosols was considered of particular interest since the practice of using sprinkler irrigation is quite specific for the systems. Intake of groundwater in the area of irrigation could result in potentially high exposure, since drinking water is consumed every day and in large volumes. As described above, three different scenarios were evaluated in the QMRA: accidental ingestion of wastewater, ingestion due to inhalation of aerosols and ingestion of groundwater (Table 7).

Of the model organisms, *Giardia* and *Cryptosporidium* were directly analysed in the pre-treated wastewater. Triangular distributions were created by using minimum, maximum and average (as most likely) concentrations. *Salmonella* was also analysed, but by a semi-quantitative method. To estimate the concentrations of *Salmonella* and rotavirus in incoming wastewater, surveillance data (Swedish Institute for Infectious Disease Control (SMI) 2005) and published incidence data from the UK (Wheeler *et al.* 1999) were used. This method of calculation gave very high estimated concentrations of rotaviruses in the irrigation water, and, as a comparison, literature values for concentrations in sewage were also used (Rao *et al.* 1987). The time of excretion, the density of pathogens in faeces during the infection (excretion numbers) and an estimated amount of faeces excreted per day (150 g) were also needed for the assessment, similar to the values used in Arnbjerg-Nielsen *et al.* (2004). The concentration in pre-treated wastewater (the irrigation water) was calculated by applying the reduction in indicators for the respective treatment plants. Groundwater concentrations were estimated in a similar way by calculating the reduction of various indicators and applying these values to the modelled pathogens, as further described in Table 7.

Both areas were assumed to be irrigated twice per week, for 7 months at Culmore and 6 months at Kvidinge. For the direct exposure to wastewater, one person (worker) at each

Table 7 | Description of calculations for estimating the concentrations of pathogens in treated wastewater (wastewater and aerosols) and groundwater, and assumptions made for the three scenarios evaluated in the QMRA (WWTP = wastewater treatment plant, ww = wastewater, gw = groundwater, red = reduction)

Exposure route	Culmore			Kvidinge		
	Wastewater	Aerosols	Groundwater	Wastewater	Aerosols	Groundwater
<i>Salmonella</i> calculated by	Community surveillance* red. in <i>E. coli</i> in the WWTP	Community surveillance* [†] red. in <i>E. coli</i> in the WWTP	<i>Salmonella</i> in ww; red. by comparing <i>E. coli</i> in treated ww and gw	Incidence in Skåne county [‡] ; red. in <i>E. coli</i> in the WWTP	Incidence in Skåne county [‡] ; red. in <i>E. coli</i> in the WWTP	<i>Salmonella</i> in ww; red. by comparing <i>E. coli</i> in treated ww and gw
Excretion time (days) [§]	30			30		
Excretion numbers (per gram faeces) [§]	1 × 10 ⁶			1 × 10 ⁶		
<i>Giardia</i> calculated by	Direct analysis	Direct analysis	<i>Giardia</i> in ww; red. calc. by comparing clostridia in ww and gw	Direct analysis	Direct analysis	<i>Giardia</i> in ww; red. calc. by comparing Clostridia in ww and gw
<i>Cryptosporidium</i> calculated by	Direct analysis	Direct analysis	<i>Cryptosporidium</i> in ww; red. calc. by comparing clostridia in ww and gw	Direct analysis	Direct analysis	<i>Cryptosporidium</i> in ww; red. calc. by comparing clostridia in ww and gw
Rotavirus calculated by	Community surveillance*, red. in coliphages in WWTP	Community surveillance*, red. in coliphages in WWTP	Rotavirus in ww; red. calculated by comparing coliphages in ww and gw	Community surveillance*, red. in coliphages in WWTP	Community surveillance*, red. in coliphages in WWTP	Rotavirus in ww; red. calculated by comparing coliphages [†] in ww and gw
Excretion time (days) [§]	13.5			13.5		
Excretion numbers (per gram faeces) [§]	1 × 10 ¹⁰			1 × 10 ¹⁰		
Volume ingested	1 ml	Exposure time: 1 h, sprinkler flow: 0.047 l s ⁻¹ , sprinkler height: 0.3 m, distance: 100 m	1 l	1 ml	Exposure time: 1 h, sprinkler flow: 0.044 l s ⁻¹ , sprinkler height: 0.25 m, distance: 500 m	1 l
Number of exposed people	1	50	5	1	10	5
Frequency of exposure (per year)	12	30	275	10	25	250

*Wheeler *et al.* (1999).[†]No coliphages were detected in the groundwater at Kvidinge.[‡](SMI 2005) and corrected for underreporting by community surveillance (Wheeler *et al.* 1999).[§]Estimated from PDFs used in Arnbjerg-Nielsen *et al.* (2004).

site was set to be exposed by accidentally ingesting 1 ml every fifth irrigation. This assumption resulted in an annual frequency of exposure of 12 for Culmore and 10 for Kvidinge (Table 7). The ingested volume has previously been used in risk estimates for inadvertent ingestion of reclaimed wastewater (Asano *et al.* 1992) and urine (Höglund *et al.* 2002).

Kvidinge is situated in the countryside, approximately 500 m away from the nearest farm. During the irrigation period, ten individuals living near or working in the field were assumed to be exposed every second irrigation event, resulting in a frequency of exposure of 25 times per year. For Culmore, the willow field is situated closer to houses as well as to a public road, and 50 people were assumed to be exposed to the aerosols potentially created every second irrigation event, resulting in an annual frequency of exposure of 30.

The volume and thus the dose ingested by exposure to aerosols is calculated using the equation for a Gaussian plume model (Matthias 1996) where X is the number of pathogens per m^3 at a specific location:

$$X(x, y, z, H) = \frac{Q}{(2\pi\sigma_y\sigma_z u)} \times \exp\left(-\frac{y^2}{2\sigma_y^2}\right) \times \left[\exp\left(-\frac{(z-H)^2}{2\sigma_z^2}\right) + \exp\left(-\frac{(z+H)^2}{2\sigma_z^2}\right) \right] \quad (1)$$

where Q = pathogen l^{-1} wastewater; x = distance (Table 7); $\sigma_y = ax^b$ ($a = 0.36$, $b = 0.86$); $\sigma_z = cx^d$ ($c = 0.22$, $d = 0.86$); $u = 5 \text{ m s}^{-1}$; $y = 0 \text{ m}$; $z = 1.7 \text{ m}$; H = height of sprayer (Table 7).

We considered a person inhaling 0.83 m^3 of air containing wastewater aerosols per hour (Dowd *et al.* 2000) at two different distances from the point of spraying (Table 7). At both sites, groundwater was assumed to be used for drinking water (1 litre per person and day) for one family (5 persons), during 275 (Culmore) or 250 days (Kvidinge) per year.

In Table 8, 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. Furthermore, the number of cases in the exposed population was calculated by multiplying the exposed population by the annual risk of infection:

$$P_{\text{yearly}} = 1 - (1 - P_{\text{inf}})^n \quad (2)$$

where P_{inf} is the risk per 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 sites; the highest risk was attributable to accidental ingestion of wastewater at Culmore ($P_{\text{inf}} = 2 \times 10^{-6}$), and groundwater as drinking water at Kvidinge ($P_{\text{inf}} = 2 \times 10^{-6}$). The lowest estimated risk of infection was by aerosols at Kvidinge, with a P_{inf} of 1×10^{-10} .

For the protozoans, *Giardia* posed the highest risk of infection, with a P_{inf} of 3×10^{-1} when groundwater was ingested at Culmore, and a P_{inf} of 2×10^{-2} for accidental ingestion of wastewater. The risks for these organisms were considerably lower for Kvidinge (Table 8).

Exposure to rotavirus also gave a high risk of infection when quantifying the risks using community surveillance data. The lowest risk of infection was related to exposure to aerosols at Kvidinge ($P_{\text{inf}} 3 \times 10^{-2}$). The highest risk was associated with wastewater ingestion at Culmore, with 8 out of 10 exposed persons potentially becoming infected. The same numbers were also estimated for ingestion of groundwater at Culmore. When the lower literature value for the concentration of rotavirus in wastewater was used, the risks for infection were considerably lower, especially for exposure to aerosols (i.e. 3 to $4 \log_{10}$). The risk of infection by direct contact with wastewater was reduced to $P_{\text{inf}} 2 \times 10^{-2}$ at Culmore and to $P_{\text{inf}} 4 \times 10^{-5}$ at Kvidinge. The corresponding changes in risks of infection were also seen for ingestion of groundwater. In summary, the result for the annual number of cases more clearly illustrates the differences in risk from various pathogens and transmission routes.

DISCUSSION

The microbial risks relating to wastewater irrigation depend on the pathogen load in the raw wastewater and the reduction efficiency of the subsequent treatment steps. When information on pathogen loads is limited, the quantities of indicator organisms in the untreated wastewater function as a baseline for assessing the barrier efficiency and the subsequent risks of exposure. The relevance of the comparisons in this study was underlined

Table 8 | 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)

Exposure route		Culmore			Kvidinge		
		Waste-water	Aerosol	Ground-water	Waste-water	Aerosol	Ground-water
<i>Salmonella</i>	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}
<i>Giardia</i>	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
<i>Cryptosporidium</i>	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 [*]	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 [†]	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

*Community surveillance.

†literature value (Rao *et al.* 1987).

by the fairly consistent inlet concentrations of microorganisms found in five of the six treatment plants, that is, concentrations in the same range for the respective groups of organisms: *E. coli* $6.4\text{--}7 \log_{10} 100 \text{ ml}^{-1}$, intestinal enterococci $5.8\text{--}6.1 \log_{10} 100 \text{ ml}^{-1}$ and coliphages $5.2\text{--}6.8 \log_{10} 100 \text{ ml}^{-1}$. These values also correspond well with previously reported ranges of indicator organisms in raw wastewater (Stenström 1986; Curtis 2003). The only deviation was at the field site Roma, which had $1\text{--}2 \log_{10}$ lower counts. Raw wastewater at Roma is discharged into an oxidation pond for treatment and is diluted with partly treated wastewater, resulting in the lower concentrations.

The subsequent treatment and thus the reduction varied between the sites and resulted in broader concentration ranges of indicators in the outgoing irrigation water. The removal was site- and organism-specific: for example, the mechanical treatment at Culmore reduced the vegetative bacterial indicators by $0\text{--}1 \log_{10}$, whereas the corresponding value at Larissa, also representing the coliphages, was a reduction of $2\text{--}3 \log_{10}$. In both these plants, the spores of clostridia were reduced somewhat more than $1 \log_{10}$. Similar or lower reductions have previously been reported (Payment *et al.* 2001). However, the transport time of the samples to the laboratory may account for part of the variability for vegetative bacteria. Further treatment will most likely result in higher reduction, as shown in the biological or biological-chemical treatment plants with a

reduction of 1.7 to $2.9 \log_{10}$ for the vegetative bacteria. However, both the clostridia and the coliphages were reduced to a lesser extent, $0.8\text{--}2.0 \log_{10}$ and $0.8\text{--}1.6 \log_{10}$, respectively. This corresponds with findings by Ottoson (2005) of higher removal of vegetative bacteria than of spores and coliphages in treatment plants.

The extended storage for up to 6 months in oxidation ponds resulted in concentrations below the analytical detection limit for several of the organisms analysed in the irrigation water at Roma. In combination with the low incoming concentrations, further exposure through all transmission routes at Roma were below the P_{inf} level of 10^{-4} , and may thus be regarded as insignificant, even though low levels of total coliforms, clostridia and, on one occasion, coliphages were detected at the outlet.

The four pathogens analysed verified the general picture given by the microbial indicators. The actual concentrations of these and other pathogens vary with time because of the prevalence of infection in the population connected to the sewerage system. This in turn is reflected by the size of the populations connected to plants in this study, which ranged from 1,400 pe at Kvidinge and Roma up to 230,000 pe at Larissa, as well as the regional variations that the sites represent. The positive finding of *Salmonella*, detected in 35% of the raw wastewater and in 19% of the irrigation water, is mainly a reflection of their occurrence in the populations connected to the systems. The same serotypes

occurred in both the raw wastewater and the irrigation water at Larissa, Kvidinge and Kågeröd but other serotypes were also found on occasion, showing a variability in occurrence and in analytical precision in the qualitative methodology. *Campylobacter* were only detected on one single occasion (out of 26), this in the oxidation ponds at Roma. The absence of *Campylobacter* in the samples tested could of course be due to no or low numbers occurring in the population connected to the systems at the time of sampling, but comparing the incidence for *Salmonella* (40.5/100,000) and *Campylobacter* (68.6/100,000) in Sweden (SMI 2005) and the findings of *Salmonella*, *Campylobacter* should have been more frequently detected. In the literature a viable but not culturable stage of *Campylobacter* has been reported (Rollins & Colwell 1986; Buswell *et al.* 1998). The *Campylobacter* found in the pond at Roma probably did not originate from the wastewater, based on the low findings of the organisms and may instead have been introduced by birds using the open ponds as a habitat. *Campylobacter* has been found in almost all bird species that have been tested (Fricker 1999), and the finding illustrates the necessity to account for different sources of microbial pollution in an assessment of microbial risks related to wastewater systems.

The quantitative variability in pathogen occurrence and reduction is illustrated by the parasitic protozoa, with maximum concentrations of 15,000 *Giardia* cysts per litre of untreated wastewater at Culmore, while none was detected at Roma. Their removal varied but was not consistent with the type of pre-treatment. At Kågeröd, the secondary treatment only removed 0–1 log₁₀, while more than 4 log₁₀ reduction occurred at Kvidinge, also with secondary treatment. The primary mechanical treatment sometimes gave high but variable reduction, up to 2–3 log₁₀, but at other instances, as at Culmore, it resulted in no reduction at all within the treatment plant. Subsequently, the concentrations in the irrigation water varied from below detection level on several occasions up to a maximum of 2,800 cysts per litre in the mechanically treated wastewater at Culmore. The microbial quality and related risks associated with the irrigation water therefore varied between the study sites, with variations in the microbial loads to the irrigated fields. Culmore had the highest microbial load per surface area, which functioned as input

data for the risk assessments. In contrast, the low microbial loads at Roma excluded that site from further calculations of groundwater contamination and the risk of exposure for humans in the area. The limited number of samples analysed for protozoa may also explain part of the large variations.

The sampled groundwater was faecally polluted at several of the other field sites, most likely because of the wastewater irrigation. The occurrence of indicator organisms in groundwater partly reflected the degree of pre-treatment of the wastewater, with limited occurrence of organisms at Roma (oxidation pond + storage) and Bromölla (biological-chemical). Both at Kågeröd and Kvidinge, as well as within some of the irrigation regimes at Culmore, several of the indicator organisms were found in the groundwater on the majority of the sampling occasions, indicating a faecal impact of the groundwater due to the wastewater irrigation. Groundwater used as drinking water from private wells is not treated before use. Both at these sites and on several occasions at the less polluted ones (i.e. Roma, Bromölla), the concentrations exceeded the Swedish target value for drinking water from private wells, for total coliforms (<500 cfu 100 ml⁻¹) and for *E. coli* (10 cfu 100 ml⁻¹) (SOS FS 2003) by several orders of magnitude.

The limited treatment at Culmore, giving a high load of organisms to the field, had a similar faecal impact on the groundwater as at Kvidinge and Kågeröd, although the latter sites had further pre-treatment of the irrigation water. The consistent contamination of groundwater at Kågeröd could be explained by a low removal of organisms in the soil profile (0.5 to 1.3 log₁₀), possibly affected by the high groundwater level (0.5 to 1.5 m below surface). The lower groundwater level at Culmore, which had a higher retention of the organisms in the soil profile, reduced the faecal impact when the wastewater load was in the range of, or double, the estimated evapotranspiration (i.e. 1 PE and 2 PE). The coliphages were also reduced to a high extent. A higher degree of groundwater contamination was evident at Culmore when a more intense wastewater irrigation (3 PE) or sludge fertilisation was applied.

At Kågeröd, the irrigation was conducted using surface application, with the aim of supplying an area of approximately 100 m² from each outlet hole. This resulted in

localised high loads of water compared with the sprinkler irrigation applied at Culmore and Kvidinge, where each sprinkler irrigated 30–50 m² fairly evenly. The sites with the most pronounced groundwater impact also received the highest annual precipitation, which was considered when calculating the irrigation loads for each site.

Surface irrigation was also performed at Bromölla, but with less groundwater contamination. Bromölla differed from Kågeröd by a higher degree of pre-treatment, lower precipitation and a lower groundwater level. Differences in the soil could be an additional explanation. Soil type has previously been shown to affect the transport of organisms, with a high retention of organisms in clay soils compared with sandy soils due to the smaller pore size, resulting in strong physical and electrostatic forces acting on the microorganisms. However, the difference can also be due to partial wetting and drying. In a lysimeter study (Carlander *et al.* 2000) with artificial wastewater applied to clayey and sandy soil cropped with willow, very slow transport and a high retention of the organisms applied (bacteriophages) were found in the sandy soil compared with a rapid transport in the clayey soil, indicating a substantial macropore flow in the clayey soil.

The topographical conditions differed between the sites, with partly hilly conditions in the fields at Culmore and Kvidinge, which could give horizontal transport (Keswick & Gerba 1980) of the potentially contaminated groundwater. At Kågeröd the area was flat, which would limit the risk of horizontal transport of the groundwater to larger areas but at the same time reduce the degree of dilution of the groundwater with less polluted groundwater laterally transported from the surroundings.

In the simulations of the risk of infection, the highest risk was found for rotaviruses both at single exposure and as calculated on a yearly basis (Table 8). The concentration of rotaviruses in treated wastewater was high (10³–10⁴ per litre) based on estimates from community incidence and applying the reduction of coliphages but corresponding with literature values. The number of virus particles excreted during an infection is higher than for bacterial pathogens such as *Salmonella*, which partly reflects the high numbers found in wastewater. Even higher excretion numbers (up to 10¹²) and longer excretion time 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 whether the actual excretion is substantially lower during part of the infection, resulting in lower accumulated number of excreted viruses. In parallel with the values based on community incidence, other calculations based on literature values of rotaviruses in wastewater were made. Rao *et al.* (1987) reported a rotavirus concentration of approximately 10² per litre in wastewater. Using this value instead, the infections risk would still be high but reduced: for aerosols at Culmore from $P_{\text{inf}} 4 \times 10^{-1}$ to $P_{\text{inf}} 2 \times 10^{-4}$. The somewhat lower risk of infection at Kvidinge compared with Culmore is due to the longer distance from the irrigation area to exposed people, 500 m compared with 100 m. At Culmore, the nearest house or road is only 30 m from the field, indicating that the actual risk of infection could be even higher than calculated.

In addition to rotaviruses, an elevated risk of *Giardia* infection prevailed for the three routes of exposure at Culmore. The highest risks were from ingestion of groundwater, $P_{\text{inf}} 3 \times 10^{-1}$, and from direct exposure, $P_{\text{inf}} 2 \times 10^{-2}$. For Kvidinge, the corresponding risk of infection was substantially lower. The differences were due to a higher concentration of cysts in the raw wastewater and a lower treatment removal at Culmore. The simulation for groundwater contamination was made using the reduction in clostridia spores in the soil profile. Clostridia spores have been suggested as a surrogate parameter for the removal efficiency of protozoan (oo)cysts in drinking water treatment (Payment & Franco 1993; Hijnen *et al.* 2000). The simulated concentrations of (oo)cysts in groundwater when using the concentration of (oo)cysts in treated wastewater and the removal of clostridia spores agree well with measured levels with the exception of *Giardia* cysts at Culmore, which could be seen as a worst case scenario. Owing to their rather large size, the protozoa *Giardia* should have a high but variable retention in the soil profile (Logan *et al.* 2001; Hijnen *et al.* 2005). Although the concentrations in groundwater at Kvidinge were substantially lower than at Culmore, a risk of infection when using the groundwater as drinking water occurred.

Viruses are generally identified as posing the highest risk of groundwater contamination as a result of their small

size and associated possibility of transport through the soil. The risk is also dependent on the type of pathogenic virus that is assessed, since the charge of the virus particles influences their possibility of transport. In this study the coliphages represented the removal of rotavirus. Other viruses, such as noroviruses or hepatitis A may be more prevalent than rotaviruses, but the lack of a suitable dose-response model currently limits the possibilities of using these in risk modelling. For all groups of organisms, survival can be prolonged when they reach the groundwater because of the low temperature and absence of impact from UV-light, for example (Yates *et al.* 1985).

The risk of infection for a worker directly exposed to the wastewater was calculated for Kvidinge and Culmore but is also applicable to the other sites. The risk is linked with the incidence of a certain pathogen in the population connected to the sewerage system, which varies with time, together with the reduction in organisms within the treatment plant. To minimise the risk of workers being exposed, information regarding good hygiene practice when working in the field is important, together with wearing personal protective equipment. In order to limit the access for unauthorised persons, the irrigated area could be fenced, which was the situation at Culmore and Larissa.

The risk of infection through aerosols is only applicable for the field areas irrigated with sprinklers (Culmore and Kvidinge). In order to limit the exposure, sprinkler irrigation can be conducted during the night, when few people are outside. This is also the way that irrigation is conducted in Culmore. In addition to the time of the day when irrigation is performed, 'barrier zones' without irrigation may be created in the fringe areas of the field, which would reduce the risk of aerosol transport from the area. This is also being practised at Culmore, where the willow coppice surrounding the experimental plots is irrigated using pipes.

Contamination of groundwater due to faecal impact was demonstrated in several of the irrigated areas and is potentially an important exposure route in all irrigated fields, although the major contamination was measured at Culmore, Kågeröd and Kvidinge. The calculated dose is based on the measured levels and does not take into consideration any additional horizontal transport of the water, which would further reduce the concentrations of organisms in the groundwater and thereby also the risk of

infection. Apparently, the risk of groundwater contamination must be addressed when designing new treatment facilities such as irrigation of willow coppice or other energy crops. For this, soil type and hydrological information is needed, for example, with respect to both vertical and lateral transport.

The topography is of importance, not only for the groundwater transport but also for surface runoff to nearby areas. In the study, Kvidinge and Culmore had hilly parts in the irrigated fields while the other sites were fairly flat, without obvious lateral hydrological gradients. The risk of infection with surface runoff is again dependent on the quality of the water. In surface runoff when wastewater has recently been applied to the field, the initial risk equals the calculated direct exposure. However, the wastewater is normally diluted by rain, which would reduce the concentration of organisms and risk of infection. Surface runoff should be considered in relation to the topography, as well as to the use of the receiving watercourse.

Contact with, or ingestion of, contaminated vegetation, soil or water are potential exposures for humans and animals. The humans exposed in these fields would probably be workers with direct contact with the wastewater. The calculated risk of direct exposure to wastewater was rather low and the risk of potential further ingestion of soil or vegetation contaminated with the wastewater should be further reduced because the wastewater is spread over a large surface and because of the passing of time from irrigation to potential contact with declining numbers of organisms. The environment for animals living in the irrigated area is unique as a result of the seasonal more or less constant exposure to wastewater and thereby potential pathogens. Larger animals would be excluded from contact at Larissa and Culmore owing to the fence, but rodents and birds might be exposed. In a report by Carlander & Stenström (2001), stools and organs from animals living in irrigated areas were analysed for the occurrence of pathogens in order to evaluate a potential increase in infections. The results were based on a limited number of samples, but did not indicate any increased incidence of infections.

By using a risk assessment approach, it would also be possible to calculate/estimate the necessary reduction of various pathogens in order to reach acceptable risk levels, as

defined by stakeholders. WHO has suggested an acceptable risk of 10^{-6} DALYs (disability adjusted life years) per year from exposure to drinking water as well as to wastewater or excreta. This level, building on the DALY concept, corresponds roughly to a risk level of approximately 10^{-4} (i.e. 1 additional infection per 10,000 persons), a limit that also has been suggested by US EPA for drinking water (Regli *et al.* 1991). This approach, together with site-specific conditions, may in the future determine the requirements for the treatment of wastewater that should be utilised for irrigation of energy crops on an individual site level.

CONCLUSIONS

The microbial quality of the irrigation water at the six field sites investigated varied greatly, in general reflecting the pre-treatment, but there were also site- and organism-specific differences. Treatment in oxidation ponds with storage gave high quality irrigation water with a limited risk of further transmission of organisms. The groundwater in the fields was found to have suffered faecal contamination due to the wastewater irrigation.

The risk assessment indicated a high risk of viral infections for all three exposure situations identified but the actual risk varied depending on the initial concentrations of pathogens in the wastewater applied. Risks from wastewater irrigation of willow coppice may be reduced by, for example, informing the public with signs and fencing irrigated areas. Site-specific recommendations ought to be developed by a combination of quantitative MRA and on-site surveys of the surroundings.

Reuse of partially treated wastewater for irrigation of energy crops could be a sustainable option if precautions are taken to minimise disease transmission.

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