Estimating microbial risk in treated wastewater for reuse: a case study in Lund, Sweden

Shuang Liu and Kenneth M. Persson

ABSTRACT

The potential microbial risk from using treated wastewater is a burning issue to be studied. In Sweden, only a small part of treated wastewater is reused directly, although water reuse could be beneficial. Disinfection is virtually never practised and no protective guidelines for water reuse are found in Sweden. Based on a 1 year monitoring programme of water quality, this paper estimates the microbial risk of *Escherichia coli* and rotavirus in treated wastewater for different applications of irrigation, landscape, industry, unban non-potable water. A Quantitative Microbial Risk Assessment model is used and the samples were collected from the pond system of Källby wastewater treatment plant in Lund, Sweden. The results are used to evaluate if the treated wastewater after tertiary treatment process combined with pond system can be reused for different applications from a microbial point of view. The risk assessment results show that the studied water is only suitable for agriculture irrigation, while additional treatment or disinfection are needed for other applications since the potential risks are higher than the value that can be accepted. The protective guidelines are discussed based on the process and results of risk assessment and suggestions for establishing a structure of guidelines in Sweden are presented.

Key words | *E. coli*, guidelines, Quantitative Microbial Risk Assessment, reuse, rotavirus, treated wastewater

Shuang Liu (corresponding author)
Kenneth M. Persson
Division of Water Resources Engineering,
Lund University,
Box 118,
SE-221 00 Lund,
Sweden
E-mail: *zhaocailius@126.com*

INTRODUCTION

As the global water crisis is increasing, direct water reuse, which is recognised as a cost-effective way both for the easing of water shortage and the protection of water environment, is carried out in more and more countries at different levels. Of all challenges for further development, the safety of reuse is subjected to the most attention and study. Although the concentration of pollutants significantly lowers after treatment, many pollutants, especially the pathogenic microorganisms (e.g. bacteria, virus and parasites), which are the main source of risk to humans by using treated wastewater, can still be detected. Although much effort is put into the area, such as regulations, guidelines and standards being constantly developed and improved, the potential health risk from using treated wastewater, especially from microbial aspects, is still a burning issue to be studied and is considered seriously when treated wastewater is applied.

Risk assessment is a process to describe and quantify the probability of the adverse outcomes or unwelcome events. For water reuse, risk assessment is a systematic process that includes quantitative description of the probability of the occurrence and the extent, timing and character of the adverse effects to human and environment caused by exposure to chemicals and pathogenic microorganisms in reclaimed water through various exposure routes. It is considered as the most appropriate approach to protect the public from harm and to provide an important scientific basis of risk management by the National Academy of Sciences (USA) and the National Research Council (USA), and is widely applied in the studies of safe reuse of treated wastewater. For example, Aiello *et al.* (2013) assessed the

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health risk for irrigation of tomato crops by drip and subdrip irrigation with treated municipal wastewater in Sicily; Cutolo *et al.* (2012) assessed the parasitological risks of treated wastewater reuse from a stabilization pond in the city of Piracicaba (Brazil); Wang *et al.* (2013) carried out a health risk assessment of irrigation workers and the public to trihalomethanes from reclaimed water in landscape irrigation in Tianjin (China). To control the health risk and improve the safety of reuse, the microbial risk assessment work is recommended by WHO (2008) to be a complementary measure to guidelines which are based on epidemiological studies and use indicator parameters for general situation, for improving the safety of reuse treated wastewater.

In virtually all of Sweden, there is no incentive for directly using treated wastewater because abundant freshwater resources almost can meet all needs. Nevertheless, direct reuse of treated water is needed and has been carried out in some practice. There have been some projects to use the treated effluents directly in Sweden, mostly for irrigation and industry. For example, in the south eastern region of Sweden, which sometimes experienced dry and hot summers, expressed as low precipitation and high evaporation, for example in 2008, 2010 and 2013, there is an interest in reusing the tertiary treated effluents of wastewater treatment plant (WWTP) for irrigation. In south east Sweden, over 40 reuse projects are implemented. They consist mainly of treated effluent storage up to 9 months in large reservoirs before being used for irrigation. In some cases the effluent is blended with surface water. Treated wastewater can provide a stable alternative water source and reduce the loss of harvest caused by drought in dry summer and contributes to easing the water shortage and preserves surface and groundwater for other uses. Also, the use of treated wastewater for irrigation is an ecological solution to reduce the discharging of treated wastewater to the receiving water and can minimise infrastructure costs and save the fertilizer by recycled nutrients in treated wastewater for farming, and thus be profitable both for water utility and users. In Sweden, industry is the main part of water consumption (64% of total water withdrawals). Water recovery is an important way to reduce water consumption and started early in Sweden. Industrial wastewater, after proper treatment, is used for cooling, district heating and so on. The reclaimed water not only reduces fresh water consumption, but also saves energy. Besides, there are also some demonstrations in eco-villages, such as Toarp, which reuse treated wastewater for non-potable application, such as irrigation for agriculture and garden. Water reuse makes the water in eco-villages form a closed loop, which means virtually zero pollution discharge to the outside water body. Besides, the use of treated wastewater can be developed for sanitation or environmental protection purposes in response to increasingly stringent environmental regulations, such as is consistent with the EU policy of water reuse. Nevertheless, so far only a small part of treated water has been reused directly, mostly for agricultural irrigation and industry; also, there are no relevant guidelines founded and few studies for health risks aimed at water reuse cases in Sweden.

In this paper *Escherichia coli* and rotavirus were chosen as representatives for microbial risk assessment of direct reuse of treated wastewater for different non-potable applications, which were irrigation, landscape, industry water and urban non-potable water. The treated wastewater from Källby Wastewater Treatment Plant in Lund, Sweden, was studied and a Quantitative Microbial Risk Assessment model (QMRA) was applied for assessment in the work. The study is to evaluate if the treated wastewater after the tertiary conventional treatment process combined with the pond system, which is the typical wastewater treatment process in Sweden with lower energy consumption and lower cost than some advanced technologies, for example, a microfiltration-reverse osmosis system, could be safely reused for different application from a microbial point of view. The objective and scope of the study is to focus on providing a reference to water managers to formulate health-based protective guidelines and reuse policy according to the process and results of risk assessment.

METHODS

Water studied and sampling

The samples studied in the work were collected from the Källby WWTP located in the south of Lund, which is a city in the province of Skåne, southern Sweden. The WWTP treats an average of about 350 L/s (28,000 m^3/day) wastewater from 80,000 residents in Lund. The wastewater is treated by the tertiary treatment process, which is a typical process flow in Sweden including physical (screens, grit removal basins, primary and secondary clarifiers), biological (activated sludge in anoxic and aerobic conditions) and chemical treatment (ferric chloride is added to precipitate residual phosphorus), followed by chemical clarifiers and a sedimentation system using six ponds with a total average retention time of 2–3 days for final polishing. The total area is 87,000 m^2 and areas for each pond are 8,700, 35,000, 8,400, 8,600, 13,000, and 13,300 m^2, respectively. The depth of the ponds is 0.8 m and the protection height is 0.3 m. The monitoring data show that the pond system plays a certain role on the reduction of physical, chemical and microbial pollutants, such as suspended and dissolved solid, metals, organic matter, nitrogen, *E. coli* and fecal coliforms. After passing through six ponds by gravity, the effluent is discharged into the receiving water-stream Höjeå. This area is frequently used for recreation by the citizens of Lund, who enjoy walking or running on pathways along the ponds. The samples were normally collected biweekly from January to December 2012, but were occasionally obstructed by the severe weather, such as snow and rain. Twenty samples were collected in total for each sampling point. The grab samples were collected at around 0.5 m depth of the outlet of ponds 1–6 (Points 2–7) and the outlet of WWTP (treated wastewater before flowing into the pond system as Point 1). The location for sampling is shown in Figure 1.

Figure 1 | The locations of the sampling points.

Selection of microorganisms

E. coli and rotavirus were selected for risk assessment in the paper. Most strains of *E. coli* cause no harm when they are in the normal intestinal flora of humans and animals. However, in other parts of the body, *E. coli* can cause serious disease, such as urinary tract infections, bacteraemia and meningitis (WHO 2008). In addition, a limited number of enteropathogenic strains such as enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and enteroinvasive *E. coli* (EIEC) can cause acute diarrhoea (WHO 2008). The testing of pathogenic *E. coli,* which is more complicated and costly than total *E. coli,* is not practical for daily monitoring at a wastewater treatment plant. Therefore, total *E. coli* was chosen as an indicator for the assessment of a general microbial risk. This is further in accordance with that which important guidelines for water reuse suggest, such as the US Environmental Protection Agency (US EPA), which recommends it as the best indicator of health risk from water contact. Its presence indicates the potential for the co-existence of pathogenic organisms. Since dose-response models have not been developed for total *E. coli,* the best-fit dose-response parameters for the ingestion of non-enterohaemorrhagic strains (except O111) of *E. coli* defined by Haas *et al.* (1999) are used in QMRA, which was adopted by An *et al.* (2002) and used in this paper. The actual risk of pathogenicity is expanded for this replacement, but it could be safer for users if any protective guidelines or measures are formulated according to assessment results of expanded risk.

Rotavirus is a representative of the 'enteric viruses' group which can survive long enough to pose health risk (longer survival period in water than most intestinal bacteria) and are very important and commonly used in microbial risk assessment since they can cause most waterborne infections in developed countries and are highly infective and high health significance for human (Hamilton *et al.* 2006; WHO 2006; Muñoz *et al.* 2010). In WHO (2006), the study for the safe use of wastewater used combination of standard QMRA techniques and 10,000-trial Monte Carlo stimulation to estimate infections risk of virus including rotavirus, which means it is one of the important pathogens for reuse. Rotavirus could cause gastroenteritis and are highly infectious (Hamilton *et al.* 2006; Muñoz *et al.* 2010). It is the most important single cause of infant death in the world and is a common infective, especially in developed countries with an average of 6% of the population being susceptible (WHO 2008). Unfortunately, Sweden does not have a vaccination programme yet, although it is being considered. In addition, rotavirus has a higher resistance to disinfection than *E. coli*. Thus, *E. coli* is not a reliable indicator of its presence/absence and infectious risk (WHO 2008). WHO (2006) points that bacteriophages are indicator organisms for rotavirus rather than *E. coli* although both *E. coli* and rotavirus are excreta organisms. Besides, the inhalation of airborne rotavirus or aerosols containing the viruses, which is the route of exposure in the scenarios in this study, would appear to play an important role in exposure and spread of rotavirus (WHO 2008).

The detection of viruses is a rather time consuming, complex and expensive procedure due to pathogen variability, especially when large volumes of water must be tested. For the preliminary study as presented in this paper, it is better to find a straightforward way to obtain the data since the study focuses on the risk evaluation process and the development of protection guidelines based on QMRA rather than detection method and procedure. As there is a lack of data about enteric viruses, the concentration of rotavirus in this paper is estimated based on the concentration of fecal coliforms (FC), using a rotavirus:fecal coliform average ratio of $1{:}10^5$ (Oragui *et al.* 1987; WHO 2006). This relationship has been suggested in several studies, see for instance Muñoz *et al.* (2010).

The use of the ratio could simplify the detection, but the statement of the ratio is only supported by little evidence. A very weak correlation between rotavirus and fecal coliforms was found in some studies (Grassi *et al.* 2010; He *et al.* 2012), which means the reliability of quantitative relationship should be questioned by the little supported evidence. For that reason, more detection testing is needed for rotavirus data collection in further studies. In practical projects, for avoiding complicated and expensive testing, the model could be developed and applied for estimating the density of target pathogens according to other parameters that are easier to monitor or obtain.

Testing methods for testing and data input

The samples were sent to the laboratory of VASYD Company, Malmö, Sweden, to test *E. coli* and fecal coliforms. The method of IDEXX Colilert quanti-Tray®, which is the standard method of ISO (ISO 9308-2:2012) and approved by the US EPA, was used for analysing FC and *E. coli* which are incubated at 37 °C and 44 °C for 48 hours, respectively. The concentration data of FC and *E. coli* (EC) are shown in Table 1 and Figure 2(a) and (b). From the effluent of WWTP to pond 6, the fecal coliforms and *E. coli* are reduced to 0.3–1.2 log and 0.5–3.3 log, respectively. The concentration of fecal coliforms and *E. coli* in ponds 2–6 are lower and the removal efficiency of *E. coli* is a little higher in the summer time (May–August) than other seasons, probably due to the UV effect from much stronger sunshine and physicochemical conditions in that period (Roberto *et al.* 2011).

The extreme values (including outliers) of density in different periods are used for input in QMRA to calculate the risk range including the potential maximum risk for different scenarios. Whether an outlier should be included or excluded from a data analysis is both dependent on the reason why it is an outlier (e.g. provenance of the data, how they were collected and analysed) and the objective of analysis. In this study, most outliers of FC and EC were concentrated in February, while others appeared in the samples at the beginning of March. That means the unusual values are representative to some extent and maybe not outliers in the data of longer-period (several years) sampling. They were probably caused by weather conditions in that period, such as extremely low temperatures, snow and freezing on the surface, and the physicochemical conditions influenced by the weather. As there is a short data collection period (only 1 year) and limited data, it cannot conclude that the outliers are occasional samples or errors. More data collection for a longer period, for example 2–3 years, of sampling should be needed for evaluation of outliers. In the paper the objective is to estimate the maximum risk that may occur. All of the data including outliers which actually occur should be considered for the worst case.

Assessment method – QMRA

The QMRA, which is a useful tool for quantifying the microbial risk, was applied in the work. The model has

Table 1 | The concentration range of fecal coliform and *E. coli* in samples from January to December 2012

Unit:st/100 mL	**Point 1**	**Point 2**	**Point 3**	**Point 4**	**Point 5**	**Point 6**	**Point 7**
Fecal coliforms	2.40×10^4–$>2.42\times10^5$	2.25×10^3–$>2.42\times10^5$	9.68×10^3–1.73×10^5	7.15×10^3–1.3×10^5	6.11×10^3–7.27×10^4	4.11×10^3–5.48×10^4	2.8×10^3–5.17×10^4
E. coli	7.55×10^3–1.20×10^5	4.50×10^2–2.42×10^5	1.06×10^3–6.13×10^4	3.60×10^2–5.79×10^4	2.60×10^2–2.76×10^4	80–2.14×10^4	30–1.96×10^4

been widely studied and used in many conditions of estimating the microbial risk from using treated wastewater. For example, the QMRA model has been used as methodology for the assessment of implications of the evidence on the health risks from wastewater use on international guidelines. Some studies applied QMRA to assess the microbial risk for agricultural irrigation with treated wastewater (Hamilton *et al.* 2006; An *et al.* 2007; Muñoz *et al.* 2010). The QMRA model mainly includes three steps: exposure assessment, dose-response analysis and risk characterisation.

Exposure assessment

In the paper, QMRA is applied for four exposure scenarios of direct wastewater reuse for non-potable applications as follows: scenario 1 – agricultural irrigation; scenario 2 – industrial cooling water; scenario 3 – landscape water for recreational impoundment; scenario 4 – urban non-potable water for the irrigation of green space.

Scenario 1: The potato is used as an example crop for assessment since it is the most common crop with the largest irrigated area in Sweden and is irrigated during July and August when the shortage of water sometimes occurs. Thus, it is assumed that the farmers and children are exposed for 60 days during the irrigation period. The irrigation technology in the south of Sweden is mainly spray irrigation. For the potato, which is always eaten prepared (normally cooked), the risk of exposure mainly comes from aerosol inhalation rather than food intake. Children who are vulnerable and more easily infected by pathogens, especially rotavirus, and may be near, for example, playing in the irrigation area, are also considered in the work. Thus, the exposure risks, both for farmers and neighbouring children, through the route of inhalation for the reclaimed water irrigation is assessed in scenario 1. The exposure dose is calculated according to Equation (1) developed by US EPA (1997).

$$\lambda_1 = C_{\mathrm{air}}.\,v.t \tag{1}$$

where λ_1 is the exposure dose of respiratory pathway (st/d); C_{air} is the pathogen concentration in air (st/m^3); v is the respiration rate (m^3/d or m^3/h), and selected according to US EPA (1997), yard work scenario for farmer and

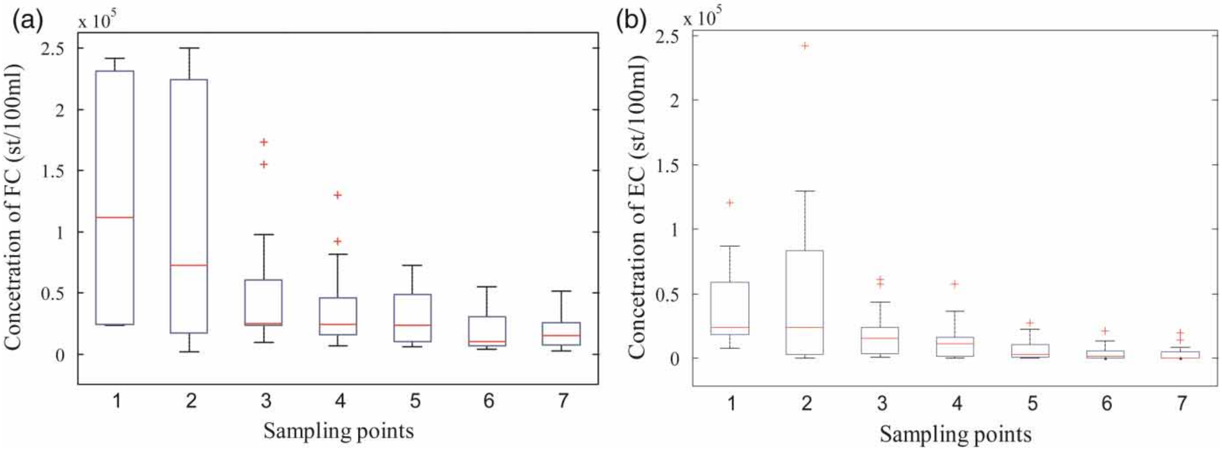

Figure 2 | (a) Boxplot of fecal coliform concentration. (b) Boxplot of *E. coli* concentration.

play scenario for child, respectively; t is the exposure time per day. C_{air} is calculated according to Equations (2) and (3) developed by Camann (1980). The parameter in the equations is selected according to local conditions.

$$C_{\mathrm{air}} = Q.\, D.\, R + B \tag{2}$$

where Q is the emission source intensity (st/s); D is the microbial aerosol diffusion coefficient (s/m^3), calculated by the Gaussian dispersion models and its value is a complex analytical function of atmospheric stability, downwind distance, wind speed and aerosol plume height (Camann 1980), and the spray height is assumed to be 1 m, while the evaluation point is assumed to be 3 m away from the spray point in the downwind direction; R is the attenuation coefficient of pathogenic microorganisms, and 1 is used since no die-off of pathogens is assumed for the worst possible case; B is the concentration of pathogenic microorganisms in the background (st/m^3), which generally could be ignored (≈0).

$$Q = C_w.\, q.\, A \tag{3}$$

where q is the spray intensity (m^3/s), estimated by the irrigation area and water needed; A is the atomization efficiency factor, and 0.01 is used for the worst case; C_w is the pathogen concentration in water (st/m^3), and the data from July to August are used for assessment. In addition, the wind speed is assumed to be 6 m/s for the average according to the statistics data, and the heights of an adult and a child are assumed to be 1.8 and 1.2 m, respectively.

Scenario 2: Cooling water is assessed for industrial application since it is the most common way of water recycling and consumes the most water in industry water use. The exposure way is mainly breathing in aerosols from cooling tower drift, which is recycled water containing all the minerals, chemicals and bacteria that are in the tower. The exposure dose is calculated with the same methods for recycled water atomization used for scenario 1. The quantity of drift is calculated according to Equation (4) (Kunz 2010):

$$Q_d = c.Q_r \tag{4}$$

where Q_d is the rate of water loss per second via drift (m^3/s); c is the drift percent, which typically is 0.1–0.3% of the circulation rate (Kunz 2010); 0.3% is used here for the worst condition; Q_r is the circulation rate (m^3/s) and 10 m^3/s is used here for the condition of the power plant with an installed capacity of 150 MW. The height of the cooling tower is assumed to be 3 m for mechanical draft and 100 m for a natural draft cooling tower. The exposure times are assumed to be 8 hours per day, 5 days a week for 52 weeks per year (260 days per year) for workers. The respiration rate is selected according to US EPA (1997), and the maximum for the walking scenario for adults is used. Other assumptions are the same as scenario 1.

Scenario 3: Recreational impoundment, which is one type of landscape water, is used for assessment, as this needs large amounts of water and is easy for individuals, especially the children, to have access to the reclaimed water and exposure to the pathogens. The route of exposure is mainly body (such as hand) contamination and the resultant transfer to the mouth or open wounds. A person is assumed to intake 100 mL of treated wastewater in a day (Haas 1983) and have access to the recreational impoundment for once a week over a 5 month period, which means 20 times per year, considering the climate in the south of Sweden. The data from May to September are used for assessment. No die-off of pathogens is assumed for the worse possible case.

Scenario 4: The irrigation of golf courses is selected for assessment in the applications of urban non-potable water since it needs large amounts of water. The irrigation of golf courses with an area of 500,000 m^2 needs about 2,700 m^3 of water per day. The exposure ways are mainly inhalation for occupational groups due to spray irrigation and direct contact for the golfers. The exposure dose of inhalation is calculated with the same method as scenario 1, and 1 mL per day is assumed for direct contact for the public (Asano *et al.* 1992). It is assumed that the occupational groups are exposed 8 hours per day for 2 days a week and once a week for the public over the period March–September considering the climate in the south of Sweden. In addition, the spray height is assumed to be 3 m, and the evaluation point is assumed to be 3 m away from the spray point in the downwind direction. Other assumptions are the same as scenario 1.

Dose-response modelling

The Beta–Poisson model is used for the dose-response analysis of *E. coli* and rotavirus and the equations (Haas *et al.* 1993) are the following:

$$P_I(\lambda) = 1 - \left(1 + \lambda/N_{50}(2^{1/\alpha} - 1)\right)^{-\alpha} \qquad (5)$$

where $P_I(\lambda)$ is the daily probability of infection from viruses; N_{50} is the median infectious dose (mass); α is the slope parameter; λ is the exposure dose per person per day (mass).

The annual probability of infection $P_I(A)$ can be calculated by Equation (6):

$$P_I(A) = 1 - (1 - P_I(\lambda))^N \qquad (6)$$

where N is the number of exposure events per year (day). Equation (5) is described by two parameters: N_{50} and α. According to Haas *et al.* (1993) and Haas *et al.* (1999), N_{50} is 6.17 and α is 0.2531 for rotavirus, while N_{50} is 8.60×10^7 and α is 0.1778 for *E. coli*, respectively. The model was calculated straight forwardly using Excel 2007.

Risk characterisation

The annual acceptable microbial probability of infection of 10^{-4} is applied for risk characterisation, which is suggested by the US EPA. If the $P_I(A)$ is below the benchmark of 10^{-4}, it means that in that scenario the water is acceptable for reuse purposes.

According to Haas *et al.* (1993), the range of 10^{-4}–10^{-6}, which is used by the US EPA as a target reference risk for carcinogens in drinking water, was considered a reasonable level of risk for communicable disease transmission, and annual risk values of above 10^{-4} were considered high for infection. The value of 10^{-4} (1 per 100,000 people infected per year) developed by the US EPA was used in this preliminary study as the benchmark of annual acceptable risk used to examine whether or not the studied water is acceptable for different applications. The lower benchmark means the more stringent treatment. For Sweden, where the direct reuse of treated wastewater is at the initial phase, it probably technically and economically reduces the interest of reuse treated wastewater if heavy and very strict treatment requirements are introduced at the beginning. It is perhaps better to approach the development in steps and start with the basic requirement. Besides, 10^{-4} is the most commonly applied benchmark in risk assessment (Regli *et al.* 1991) and is adopted in many studies of assessment of microbial risk from using treated wastewater, such as Muñoz *et al.* (2010) and An *et al.* (2007).

However, some researchers proposed that it may be argued that the acceptable risk of infection from a particular disease should be dependent upon the duration and severity of the symptoms rather than using the same benchmark. The reference level of acceptable risk could be expressed in Disability Adjusted Life Years (DALYs) which is calculated as the sum of the Years of Life Lost due to premature mortality in the population and the Years Lost due to Disability for people living with health conditions or their consequences

(WHO 2013). WHO (2006) suggested a level of $\leq 10^{-6}$ DALY per person per year (pppy) as a tolerable additional disease burden for wastewater use in agriculture. The tolerable risk of infection could be translated from the tolerable additional annual burden of disease by Equation (7) due to the pathogen of concern (WHO 2006). The expression as DALYs could be adopted for further study to make the benchmarks more targeted for pathogens due to the different characteristics rather than too general for all pathogens

$$\text{Tolerable disease risk pppy} = \frac{\text{Tolerable DALYs pppy}}{\text{DALYs per case of disease}} \tag{7}$$

Additionally, the need to establish some measure of acceptable or tolerable risk for wastewater reuse has been widely acknowledged. Relevant authorities in Sweden should set up their own benchmark according to local situations which vary greatly in different countries. There are several approaches such as a predefined probability approach, a 'currently tolerated' approach, a disease burden approach, an economic approach and the public acceptance of risk approach, and it is suggested by WHO that all of them should be relied on by public health practitioners when establishing acceptable risk.

RESULTS AND DISCUSSION

Discussion on the results of QMRA

The annual probability of infection calculated by dose–response modelling for the different scenarios is shown in Table 2. It should be known that the ponds have rich wildlife, particularly birds, such as ducks and swans, and fish. Fecal material from the animals may in some cases randomly cause an increase in indicator organism content. From the results of the dose-response model, the availability of reuse regarding the water can be discussed. It can be seen that the annual probability of infection of *E. coli* and rotavirus for farmers in scenario 1 is lower than 10^{-4} in ponds 2, 3, 5 and 6, which means that this part of the water is safe for farmers to use for irrigation from the microbial point of view. However, it is not safe enough for children since the probability of infection of rotavirus is over 10^{-4}. In addition, the annual probabilities of infection of rotavirus in other scenarios are all over 10^{-4}, which means the water cannot be accepted for safe reuse although most of the probability of *E. coli* is low enough. The difference between the risk of *E. coli* and rotavirus is mainly the median infectious dose.

Normally in the same scenario, the potential risk gradually decreases as water passes different sampling points. Nevertheless, some abnormal results do exist. The risk of rotavirus of point 5 in scenario 1 is unusually larger than in points 3 and 4, which is probably caused by deviation due to limited sampling. Averaging multiple sampling could reduce the deviation.

Discussion on the uncertainty of QMRA

There are two fundamental approaches to construct a QMRA model: deterministic, of which the inputs are represented as point estimates, and stochastic, of which the inputs are probability density functions and describe uncertainty both in the model inputs and outputs. Deterministic modelling is promulgated as a practical approach in several major national and international guidelines, such as the US, WHO and Australian guidelines since it has the pragmatic advantage of simplicity in analysis and results and is more readily embraced by water resource managers. The scope of the study is to provide the reference views to water managers regarding if the treated wastewater could be reused for different applications and the development of protection guidelines according to the results of QMRA. The characteristic of deterministic modelling that is simplicity and more easily understood by managers could meet the requirements of the study. Thus, the deterministic modelling, rather than stochastic modelling, is used in the study.

However, the deterministic modelling has the defect of failing to address the inherent uncertainty in the estimates of risk and probably overstating the true risk if the single values obtained from this method are considered as upper bound estimates of risk to a maximally exposed individual. The uncertainty should be analysed and modelled for further study to fully characterise the risk and evaluate the implications and limitations of the risk assessment, which is increasing in popularity amongst researchers in recent

Table 2 | Annual risk span of infection for *E. coli* and rotavirus from four exposure scenarios of reuse treated wastewater from different sampling points

		Scenario 1 – Irrigation for potato		**Scenario 2 – Cooling water in cooling towers**		**Scenario 3 – Recreational impoundment**	**Scenario 4 – Irrigation for golf courses**	
Risk of infection (pppy)		**Farmer**	**Child**	**Mechanical draft (3 m)**	**Natural draft cooling tower (100 m)**	**Person playing in the impoundment**	**Occupational group**	**Golfer**
Influent of pond system	*E. coli*	**$8.0*10^{-7}$–$3.0*10^{-6}$**	$3.1*10^{-5}$–$1.2*10^{-4}$	$3.6*10^{-2}$–$4.4*10^{-1}$	$1.3*10^{-5}$–$2.1*10^{-4}$	$1.6*10^{-2}$–$1.2*10^{-1}$	$5.6*10^{-5}$–$5.9*10^{-4}$	**$8.1*10^{-6}$–$8.7*10^{-5}$**
	Rotavirus	$8.1*10^{-5}$–$6.7*10^{-4}$	$3.1*10^{-3}$–$2.6*10^{-2}$	$9.8*10^{-1}$–$9.9*10^{-1}$	$2.5*10^{-3}$–$2.5*10^{-2}$	$8.9*10^{-1}$–$9.9*10^{-1}$	$9.7*10^{-3}$–$9.4*10^{-2}$	$2.8*10^{-2}$–$2.4*10^{-1}$
Outlet of pond 1	*E. coli*	**$8.2*10^{-7}$–$5.7*10^{-6}$**	$3.2*10^{-5}$–$2.2*10^{-4}$	$2.2*10^{-3}$–$6.8*10^{-1}$	$8.0*10^{-7}$–$4.3*10^{-4}$	$9.0*10^{-4}$–$1.7*10^{-1}$	$3.1*10^{-6}$–$6.7*10^{-4}$	**$4.5*10^{-7}$–$9.8*10^{-5}$**
	Rotavirus	$3.1*10^{-5}$–$6.7*10^{-4}$	$1.2*10^{-3}$–$2.6*10^{-2}$	$4.7*10^{-1}$–$9.9*10^{-1}$	$2.3*10^{-4}$–$2.5*10^{-2}$	$2.3*10^{-1}$–$9.9*10^{-1}$	$9.2*10^{-4}$–$9.3*10^{-2}$	$2.7*10^{-3}$–$2.4*10^{-1}$
Outlet of pond 2	*E. coli*	**$5.0*10^{-8}$–$1.8*10^{-7}$**	**$1.9*10^{-6}$–$7.1*10^{-6}$**	$5.1*10^{-3}$–$2.6*10^{-1}$	$1.9*10^{-6}$–$1.1*10^{-4}$	$2.1*10^{-3}$–$4.7*10^{-2}$	$7.3*10^{-6}$–$4.2*10^{-4}$	**$1.1*10^{-6}$–$6.1*10^{-5}$**
	Rotavirus	**$2.7*10^{-5}$–$6.6*10^{-5}$**	$1.1*10^{-3}$–$2.6*10^{-3}$	$9.3*10^{-1}$–$9.9*10^{-1}$	$1.0*10^{-3}$–$1.8*10^{-2}$	$6.4*10^{-1}$–$9.5*10^{-1}$	$3.9*10^{-3}$–$6.8*10^{-2}$	$1.1*10^{-2}$–$1.8*10^{-1}$
Outlet of pond 3	*E. coli*	**$1.7*10^{-8}$–$9.4*10^{-8}$**	**$6.6*10^{-7}$–$3.6*10^{-6}$**	$1.7*10^{-3}$–$2.4*10^{-1}$	**$6.4*10^{-7}$–$1.0*10^{-4}$**	$7.2*10^{-4}$–$3.4*10^{-2}$	$2.5*10^{-6}$–$4.0*10^{-4}$	**$3.6*10^{-7}$–$5.8*10^{-5}$**
	Rotavirus	**$3.1*10^{-5}$–$1.0*10^{-4}$**	$1.2*10^{-3}$–$4.0*10^{-3}$	$8.7*10^{-1}$–$9.9*10^{-1}$	$7.6*10^{-4}$–$1.4*10^{-2}$	$5.4*10^{-1}$–$9.6*10^{-1}$	$2.9*10^{-3}$–$5.1*10^{-2}$	$8.4*10^{-3}$–$1.4*10^{-1}$
Outlet of pond 4	*E. coli*	**$1.2*10^{-8}$–$7.7*10^{-8}$**	**$4.8*10^{-7}$–$3.0*10^{-6}$**	$1.3*10^{-3}$–$1.3*10^{-2}$	**$4.6*10^{-7}$–$4.9*10^{-5}$**	$5.2*10^{-4}$–$1.6*10^{-2}$	$1.8*10^{-6}$–$1.5*10^{-4}$	**$2.6*10^{-7}$–$2.2*10^{-5}$**
	Rotavirus	$1.7*10^{-5}$–$1.4*10^{-4}$	$6.6*10^{-4}$–$5.6*10^{-3}$	$8.2*10^{-1}$–$9.9*10^{-1}$	$6.5*10^{-4}$–$7.7*10^{-3}$	$4.9*10^{-1}$–$9.8*10^{-1}$	$2.5*10^{-3}$–$2.6*10^{-2}$	$7.2*10^{-3}$–$7.4*10^{-2}$
Outlet of pond 5	*E. coli*	**$3.8*10^{-9}$–$2.8*10^{-8}$**	**$1.5*10^{-7}$–$1.1*10^{-6}$**	$3.9*10^{-4}$–$9.9*10^{-2}$	**$1.4*10^{-7}$–$3.8*10^{-5}$**	$1.6*10^{-4}$–$4.9*10^{-3}$	**$5.5*10^{-7}$–$9.1*10^{-5}$**	**$8.0*10^{-8}$–$1.3*10^{-5}$**
	Rotavirus	**$1.8*10^{-5}$–$9.6*10^{-5}$**	$6.9*10^{-4}$–$3.7*10^{-3}$	$6.9*10^{-1}$–$9.9*10^{-1}$	$4.3*10^{-4}$–$5.8*10^{-3}$	$3.9*10^{-1}$–$9.5*10^{-1}$	$1.8*10^{-3}$–$1.7*10^{-2}$	$5.2*10^{-3}$–$4.7*10^{-2}$
Outlet of pond 6	*E. coli*	**$1.4*10^{-9}$–$2.0*10^{-8}$**	**$5.5*10^{-8}$–$7.7*10^{-7}$**	$1.5*10^{-4}$–$9.1*10^{-2}$	**$5.3*10^{-8}$–$3.5*10^{-5}$**	$6.0*10^{-5}$–$3.8*10^{-3}$	**$2.1*10^{-7}$–$8.2*10^{-5}$**	**$3.0*10^{-8}$–$1.2*10^{-5}$**
	Rotavirus	**$1.4*10^{-5}$–$7.3*10^{-5}$**	$5.5*10^{-4}$–$2.8*10^{-3}$	$5.5*10^{-1}$–$9.9*10^{-1}$	$3.0*10^{-4}$–$5.4*10^{-3}$	$2.8*10^{-1}$–$9.1*10^{-1}$	$1.1*10^{-3}$–$1.3*10^{-2}$	$3.3*10^{-3}$–$3.8*10^{-2}$

Acceptable risks are in bold font.

years, such as Schmidt & Emelko (2011) and Donald *et al.* (2011). The uncertainty comes from three parts according to the United States Environmental Protection Agency (1992): (a) uncertainty of event background, e.g. incompleteness of analysis caused by description of event and missing information; (b) uncertainty of the parameters' selection; and (c) uncertainty of the models, e.g. model simplification and extrapolation. Specific to this study, the limited data of pathogen density and background information, amounts of assumptions, e.g. the parameters' selection involved in the exposure process discussed above and the dose-response model, e.g. N_{50} and α, extrapolated from data of animal experiments, are the main sources of uncertainty. Several methods for quantitative analysis of uncertainty have been developed, such as sensitivity analysis, Taylor Series Approximation (mathematical approximation), Monte Carlo Analysis (simulation approach) and Bayesian statistical modelling. Of these, Monte Carlo simulation, which is based on statistical sampling techniques to produce a stochastic approximation of the result and evaluate the uncertainty surrounding estimated values represented by credibility intervals, is the most widely used in quantitative analysis. For example, An *et al.* (2007) used the Monte Carlo model based on QMRA to estimate the microbial risk of *E. coli* in reclaimed wastewater irrigation on paddy fields; Tanaka *et al.* (1998) used Monte Carlo simulation to calculate the risk of infection resulting from food crop irrigation with secondary effluents; WHO used this approach in the latest version of wastewater reuse guidelines (WHO 2006). Monte Carlo simulation could be used for further study to evaluate uncertainty and inherent variability and obtain a more nuanced characterisation of risk by the resulting distribution of risk than the simpler point-estimation approach used in the paper.

Discussion on the development of protection guidelines based on QMRA

For protecting the users from using treated wastewater, some measures in terms of the microbial perspective should be formulated and applied. From the QMRA model point of view, the factors affect the risk of infection not only concerning the density of pathogens in the treated wastewater, but also in the ways of application, the length of exposure time, the distance from the source, the intensity of use, etc., which should also be included in the guidelines of safe reuse for different applications. For the water sources and scenarios in the paper, some suggestions for protection measures are discussed below.

The concentration of pathogens in treated wastewater is one of the main factors affecting the risk. From the assessment results it can be seen that the pathogens in the water are too high to be safely reused, except regarding irrigation in scenario 1 m if there is no disinfection process added. In that case the reduction of pathogens mainly relies on natural disinfection by UV from sunlight (Roberto *et al.* 2011), which is not stable and not efficient enough, especially for fecal coliforms, which is the indicator of pathogens in most of the water reuse guidelines. Thus, two methods from the technique perspective could be applied for the improvement of the reduction of pathogens. One way is the adding of the disinfection process. For this, UV which has a high effect in a short contact time on all kinds of pathogens, e.g. bacteria, viruses, spores and protozoa, is recommended previous to chlorination and ozone since UV does not produce harmful by-products and dissolved solids, and no chemical involved in the process, as well as it being ecological and environmentally friendly. Another way is by increasing the hydraulic retention time of ponds or storage time to extend the duration of sunshine. However, the change of water quality during the long-term storage should be considered and some measures should be taken for safe reuse. Based on the results of QMRA, agriculture is recommended to be a preferred application in Sweden since it needs amounts of water with lower quality than other applications and no additional treatment process is needed for meeting basic health security requirements from a microbial perspective.

When the treated wastewater is applied for agricultural irrigation as described in scenario 1, there should be a regulation for prohibiting children close to the spray source since the annual probability of infection for children is higher than adults and children who have a weaker self-protection awareness. When infected by pathogens, children do not have such a strong resistance as adults and fall ill easier. In addition, the methods and intensity of irrigation are important factors. When using treated wastewater, the drip irrigation is recommended to reduce the atomization and spread of treated wastewater. Drip irrigation has the advantages of high water use efficiency and potential for significant reduction of crop contamination with an additional pathogen reduction of 2–4 log units (WHO 2006), which means the risk of infection will be reduced significantly. This has been convinced and documented by many studies, e.g. Drechsel *et al.* (2006), Drechsel *et al.* (2008) and Capra & Scicolone (2004). Although the cost of drip irrigation is always higher than spray, it should still be applied from a risk control and protection perspective, especially in Sweden which is an economic developed country. The drip system should be regulated in the Swedish reuse guideline as a mechanism to prevent the pollution risk. The large intensity of spray should be avoided; otherwise, the buffer zone should be set up for risk control (WHO 2006).

For irrigation, whether agricultural or urban green space (golf courses and park), by affecting the microbial aerosol diffusion coefficient, the distance from the spray source seriously affects the risk of infection for occupational groups. The person should not stay in a downwind direction near a spray source. The 'safe distance' during irrigation should be regulated in guidelines. Further, the wearing of suitable equipment such as masks is needed for occupation groups.

The treated wastewater should be marked obviously to the public when it is used for recreational water or the irrigation of urban green space, and the public should not be exposed to it immediately. The time for die-off of pathogens is needed, and how long is safe should be studied and regulated. Further, the public should avoid contacting reclaimed water, and washing after contact is necessary to avoid the ingestion of treated wastewater accidently.

For cooling water of industry application, the results show that the height of the cooling tower affects the probability of infection significantly. As the height increases, the risk of infection decreases although it still cannot be accepted. Thus, the treated wastewater is recommended to a natural draft cooling tower which always is much higher than a mechanical draft one. If the treated wastewater is applied for a lower cooling tower, the pathogens in water should be reduced greatly before use, and the factors such as 'safe distance', protection wears and residence time for workers in a risk area should be regulated.

Discussion on the limitation of the study

The study, however, has limitations of which the most important is there are some assumptions used for the calculation of the model, which could deviate from the actual conditions. The assumptions were defined mainly according to: (a) previous studies for similar situations and documents, e.g. intake volume per day in scenario 3 and the definition of parameters' value in aerosol experiments; (b) general local conditions, e.g. height of person, wind speed and the height of the cooling tower; (c) the worst case, e.g. the exposure time and no die-off of pathogens. The assumption inevitably causes the deviation of estimation of potential risk. For example, there were no aerosol experiments conducted to determine microbial aerosol diffusion coefficients in the study, which directly affect the density of pathogens in the air possible to be in contact with humans. The calculation method and the determining of parameter value from literature and documents were supposed to be suitable for local conditions. The aerosol diffusion model and relevant parameters should be defined based on the data of local situations, such as weather conditions and topography, which should be collected by monitoring experiments in the practical project for individual cases. The assumption for the worst case with low probability of occurring probably expanded estimates as well. For example, a worker is probably not exposed for the whole working time (8 h per day) when the treated wastewater is used for the cooling tower or irrigation. However, this assumption has to be used for obtaining the maximum potential risk because there was no reference which could be learned and no relevant data were collected. Also, the assumption of no die-off of pathogens was used since it is possible that the person is in contact and ingests the pathogens at the very beginning when no die-off happens.

The overestimation of potential risk will cause a too strict treatment process with higher costs than needed, which reduce cost-effective ways of reusing treated wastewater, although it is good for protecting users by excessively serious measures based on estimates. To improve the reliability of the results, significant investigation work should be carried out for data collection in future studies or practical cases. For example, questionnaires could be designed and applied for collecting the data of, for example, the frequency and timing of exposures, detailed information such as the distance from the spray source and water swallowed when swimming, and basic demographic characteristics such as age, gender, socioeconomic status and physical health. In addition, the adoption of assumption causes uncertainty of risk assessment, which should be considered in the assessment process to make the results more significant and reliable.

CONCLUSIONS

Treated wastewater is an important unconventional water source for both easing the water shortage and the protection of the water environment. The safe reuse, especially the microbial aspect, is an important issue being studied a great deal. In the study the results of the QMRA model show that the treated wastewater by tertiary treatment combined with the pond system is only available for agriculture irrigation, recommended as a preferred application, in the south of Sweden, while the risks for other applications are higher than the value that can be accepted if no further treatment or disinfection are added. The method is straightforward and easy to apply for different scenarios. It also gives a good first estimate of where any increased risk may occur for the different ways of water reuse practices. It gives basic advice for risk assessment regarding water reuse.

For safe reuse, the health-based protective guidelines using QMRA should be formulated, not only focusing on the pathogen limits but also considering the exposure conditions for different applications. The regulation of the ways of reducing the risk of exposure and infection are as important as the technical reduction of the pathogens concentration in treated wastewater.

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