Evaluation of pathogen risks using QMRA to explore wastewater reuse options: A case study from New Delhi in India

Rajashree Hajare, Pawan Labhasetwar and Pranav Nagarnaik

ABSTRACT

Selecting appropriate reuse for treated wastewater is a challenge. The current investigation outlines the utilization of quantitative microbial risk assessment (QMRA) to assist Effluent Treatment Plant (ETP) management to determine the best-possible reuse of treated wastewater from 11 ETPs in Delhi. Four representative pathogens: pathogenic *Escherichia coli* spp., *Salmonella* spp., *Cryptosporidium* spp. and *Giardia* spp. were selected to characterize microbial water quality. Reuse options selected based on the survey and interaction with ETP managers include crop irrigation, garden irrigation, toilet flush and industrial applications. The probability of infection was characterized for two exposure groups: workers and children. Water quality monitoring indicates the occurrence of pathogenic *E. coli* spp. (100%), *Salmonella* spp. (63%), *Cryptosporidium* spp. (81%) and *Giardia* spp. (45%) in the treated wastewater. QMRA reveals the annual median-probability of infection above acceptable limits for pathogenic *E. coli* spp., *Cryptosporidium* spp. and *Salmonella* spp. The probabilities of *Giardia*-associated infections were low. Adults showed a 1.24 times higher probability of infection compared to children. Sensitivity analysis indicated pathogen concentration as the most critical factor. The study highlights that the existing plans for chlorination-based treatment technology may prove insufficient in reducing the risk for selected reuse options; but, alternate on-site control measures and up-grading water reuse protocol may be effective.

Key words | *Cryptosporidium* spp, *Giardia* spp, health risks, pathogens, quantitative microbial risk assessment (QMRA), wastewater reuse

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HIGHLIGHTS

- The occurrence of bacteria and protozoan pathogens in treated wastewater.
- Most preferred reuse options: crop irrigation, garden irrigation, toilet flush and industrial reuse.
- Pathogenic *E. coli* spp., *Salmonella* spp. and *Cryptosporidium* spp. show a high probability of infection.
- Probabilities of *Giardia*-associated infections low for all reuse.

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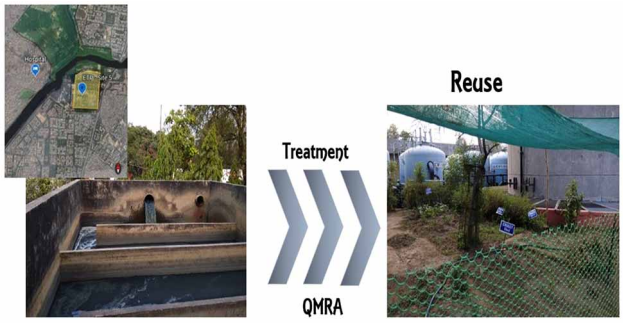
GRAPHICAL ABSTRACT



INTRODUCTION

Wastewater reuse is a reasonable approach to augment diminishing water supply and achieve sustainable development goals, specifically in the water-stressed areas (WHO/UNICEF 2015). Even in areas with surplus water, wastewater reuse reduces the release of treated effluent to freshwater bodies, avoiding potential contamination. The inadequately treated wastewater may contain pathogenic microorganisms, like diarrheagenic *Escherichia coli* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Aeromonas* spp., *Cryptosporidium* spp., *Giardia* spp., *Entamoeba hystolitica,* rotavirus, norovirus, adenovirus and helminths (*Ascaris),* which when exposed can cause gastroenteritis, typhoid, cholera and dermal infection (Hamilton *et al.* 2006; Seidu *et al.* 2008; Silverman *et al.* 2013; Becerra-Castro *et al.* 2015; Antwi-Agyei *et al.* 2016; Dickin *et al.* 2016; Vantarakis *et al.* 2016; Kozajda & Jeżak 2020).

The World Health Organization (WHO) recommends monitoring indicator microorganisms *(Coliforms, E. coli, Faecal streptococci, Clostridium perfringens, Enterococci*) in wastewater before discharge. It specifies a guideline value of 1,000 colony-forming units (CFU) per 100 mL for water reuse for irrigation (World Health Organization 2006). The majority of the countries' regulations specify monitoring only indicator microorganisms in the treated water before discharge or reuse. The information on the diversity and the abundance of pathogenic bacteria in raw and treated wastewater from Effluent Treatment Plants (ETPs) is limited and nearly non-existent for protozoa, viz. *Cryptosporidium and Giardia*. The prevalent pathogens, *E. coli O157:H7, S. faecalis, Salmonella* spp. *(S. typhi, S. typhimurium), Shigella* spp., *K. pneumonia, E. aerogenes*, and *Y. pestis*, are reported in treated wastewater. An extensive literature is available on the health concerns associated with these waterborne pathogens (Al-Gheethi *et al.* 2013).

ETPs' performance depends on hydraulic retention time, influent characteristics, and treatment design (Sidrach-Cardona & Bécares 2013; Cui *et al.* 2016). It appears that a maximum microbial reduction of 1.5–3 log is consistently achievable (Jamwal & Mittal 2010). The major concern is not the removal efficiency of microorganisms but their potential to regrow and multiply in the environment when favourable conditions prevail (Nguyen *et al.* 2017). Moreover, wastewater reuse can expose people to these infective microorganisms and adversely affect users' health (Hamilton *et al.* 2006; Jamwal & Mittal 2010; Dickin *et al.* 2016; Petterson & Ashbolt 2016).

National Capital Region, Delhi of India, is roughly 1,485 km^2 in area with more than 10 million population, resulting in more than 9,000 people per square kilometre (Census 2011). The increased urbanization, migration and population rise impinge pressure on available water reserves. There is a rapid decline in aquifer levels in Delhi over the last 17 years (Jamwal & Mittal 2010). The city has 13 ETPs located in the industrial areas with a total treatment capacity of 221×10^3 cubic metres of wastewater per day (m^3/d). The ETPs treat organic matter in the wastewater from small-scale industries but do not have treatment units to remove microorganisms. Due to the increasing demand for wastewater treatment and spare design capacity with the ETPs, they also treat domestic sewage from nearby hospitals and households. Such scenarios are increasing across the world, mainly due to urbanization. At present, the data regarding the chemical characteristics of treated wastewater from

ETPs is readily available, but data of pathogenic microbial diversity and their abundance is limited.

The potential for reuse of the treated wastewater is maximum in such high-density urban centres. The selection of the reuse application of treated wastewater requires stakeholders' involvement, public perspectives and user acceptability (Jamwal & Mittal 2010). Even with all the stake-holders on board, the following two critical questions remain:

1. Is the treated wastewater quality suited for the intended reuse application without adverse impacts; and
2. Can this information be disseminated to all the stakeholders to empower them to make better-informed decisions on the choice of reuse applications?

Quantitative Microbial Risk Assessment (QMRA) is an established framework (Haas *et al.* 1999; World Health Organization 2006) for quantifying the probability of infection/illness, which is easy to understand and interpret for policy/decision-makers (Bichai & Smeets 2013). Like The Netherlands, few nations perceive QMRA as a better regulatory tool for water quality management than the conventional monitoring of faecal indicators (Ashbolt *et al.* 2010). Studies showcase the application of the QMRA framework to explore exposure pathways (Beaudequin *et al.* 2016), predict health hazards (Seidu *et al.* 2008; Mok & Hamilton 2014; Kouamé *et al.* 2017), identify critical contaminants in water (Ruecker *et al.* 2007; Silverman *et al.* 2013; Hassaballah *et al.* 2020), locate contamination zones (Jamal *et al.* 2020) and select interventions (Tyagi *et al.* 2006; Katukiza *et al.* 2014; Petterson 2016). For evaluation of reuse options, QMRA is used to estimate the risk of infection/illness while reusing treated wastewater for crop irrigation (Silverman *et al.* 2013; Becerra-Castro *et al.* 2015; Antwi-Agyei *et al.* 2016; Kouamé *et al.* 2017), toilet flushing (Chhipi-Shrestha *et al.* 2017; Busgang *et al.* 2018), recreational use (Ashbolt *et al.* 2010; Chhipi-Shrestha *et al.* 2017) and aquifer recharge (Toze *et al.* 2010). The estimation of risk of infection or illness due to waterborne pathogens is often under-reported due to the limited data on the concentrations of pathogenic bacteria and protozoa in the treated effluent, especially in the developing countries. The challenge is to identify correct exposure routes, analyse accurate microbial load, and define appropriate model constants to avoid over- or under-estimating the risk of wastewater reuse. These factors may have significant temporal and geographical variations (World Health Organization 2006; Seidu *et al.* 2008).

This study explores applying the QMRA-based framework to identify possible reuse of treated wastewater from 13 ETPs in the high-density urban area of the National Capital Region of India. The study aims to provide adequate information to the ETPs' management to identify the best possible non-potable reuse of treated wastewater and assess the existing control measures for designated safe reuse. The study also generates the essential data of microbial diversity and abundance of pathogenic protozoa (*Cryptosporidium* spp. and *Giardia* spp.) and bacteria (pathogenic *E. coli* spp., *Salmonella* spp.) in the ETPs' effluent.

METHODOLOGY

Sampling locations and sampling method

Out of 13 installed ETPs in the region, the functional ETPs (11) were selected for sampling and survey. The details are masked to protect their identity, and they are referred to as Site 1 through Site 11. All ETPs have the same process flow with a grid chamber and screens for physical separation followed by equalization tanks with agitators/mixers, baffled pre-chlorination tanks, flash mixers and tube settlers. The treated water passes through the dual media and activated carbon filters to remove sludge traces and colour. Only one ETP (Site 9) has a secondary/biological treatment system in addition to physicochemical treatment. A portion of the treated wastewater is released to nearby drains (under the control of local regulatory bodies) and partly reused for farm irrigation (currently, unintentional and unregulated reuse application).

The samples were collected from the treated effluent tank in the pre-monsoon season (month of June) during morning hours (8.30 am–11.30 am). At each sampling point, 1 L grab water samples for bacteria testing and 10 L grab water samples for protozoa testing (*Cryptosporidium* spp. and *Giardia* spp.) were collected in pre-sterilized, labelled, polypropylene bottles. Three samples were collected from different sampling points in the treated effluent tank of each ETPs. A 100 mL additional sample was collected in a glass beaker to perform on-site physicochemical testing for pH, turbidity and temperature (APHA/AWWA/WEF 2017). Samples were transported to the laboratory within 4 to 6 hours of collection by maintaining the temperature below 4°C until sample processing.

Detection and enumeration of microorganisms

The microorganisms were selected based on literature search, guidelines from the pollution control board, relevance to the risk of infection during reuse, interactions with the ETPs in charge of water quality, and limitations in the sampling and analysis. The study included pathogenic *E. coli* spp., *Salmonella* spp., *Cryptosporidium* spp. and *Giardia* spp. as representatives of pathogenic bacteria and protozoa.

The *E. coli* spp. and *Salmonella* spp. were tested using standard membrane filtration techniques (Method 9260 B and 9260 F (APHA/AWWA/WEF 2017)). Samples of 10 mL and 1 mL were filtered through 0.45-micron nitrocellulose filter membranes using a magnetic filter assembly. For pathogenic *E. coli*, the membrane was placed over modified MacConkey Agar containing sorbitol and 4-methylumbelliferyl-β-D-glucuronide (MUG) (HiMedia Laboratories®, India) and incubated overnight for 24 hours at $37 \pm 1^{\circ}$C. The presence of colourless presumptive colonies with no fluorescence upon UV irradiation was regarded as positive. Positive colonies subsequently were subjected to confirmatory biochemical tests and streaked over Eosin Methylene Blue (EMB) agar plates for confirmation. *Salmonella* spp. were detected on Xylose Lysine Deoxycholate (XLD) agar; regarded positive on red/pink colonies' appearance with a black centre. The confirmation included biochemical tests using HiAssorted™ Biochemical Test kits (Himedia Laboratories, India).

For *Cryptosporidium* and *Giardia* enumeration, the United States Environmental Protection Agency (USEPA) standard protocol (Method 1623.1) was used. A 10 L sample was filtered through 0.1 micron hollow fibre ultra-filter membranes and centrifuged at 1,500× *g* to concentrate the sample. The immunomagnetic separation was performed to separate the (oo) cysts from the concentrated sample using antibody-coated magnetic beads. The final confirmation was done after visualization of the sample under a fluorescent microscope (OLYMPUS B3) using immuno-fluorescent EasyStain™.

During the sample collection and analysis, quality checks were performed to ensure confidence and accuracy in performance and data analysis. These checks included: field blank, transport blank, filtration blank and spiked samples; two per sampling day. All the sampling bottles for the blanks were filled with sterile distilled water in the laboratory before sampling. The field blank was opened and closed in the field for a similar duration required to collect one sample, while the transport blank remained unopened throughout the sampling duration and was opened before the analysis. Both the blanks were analysed and processed along with the water samples to assure no contamination during sample collection and transport.

Spiked samples included positive and negative controls. Pure *E. coli O157:H7* and *Salmonella* spp. culture were procured from National facilities for microbial culture repositories in India (NCIM, Pune, India). For *E. coli O157:H7* and *Salmonella* spp., a loopful of pure culture was added to the nutrient broth and incubated overnight at 37°C. Appropriate dilutions (as defined in Standard Methods 9260) of overnight cultures were done to obtain 100 CFU/mL in phosphate buffered saline (PBS). The positive spiked samples ensured a check on the analytical enumeration method and recovery. For negative controls, non-pathogenic *E. coli*, *Klebsiella and Shigella* spp. were used. Negative spiked samples ensured no false positives after a confirmatory biochemical test of the coloured colonies with similar characteristics. *Cryptosporidium* and *Giardia* (oo)cysts were procured from the distributors for ColorSeed™. For *Cryptosporidium* and *Giardia,* 100 (oo) cysts of each obtained from ColorSeed™ were spiked in 10 L sterile distilled water and recovery was recorded. Five replicates were used for calculating average recovery.

QMRA approach

The QMRA framework estimates the health risk of reusing treated wastewater for different non-potable applications. The pathogen concentrations in the water sample were used for distribution fitting. In the instances where the pathogens were not detected (or zero values), the value was replaced by the limit of detection (LoD) calculated based on the 1 CFU per quantity of sample analysed. For pathogenic *E. coli* spp. and *Salmonella* spp., the LoD was 1 CFU/100 mL (10 CFU/L), and for *Cryptosporidium* and *Giardia* it was 1 (oo)cysts/10 L (0.1 oocysts/L). The microbial concentration was fitted to several distributions to identify the best fit – e.g., such as normal, log-normal, inverse Gaussian, gamma, and Weibull, using XLSTAT®. Monte Carlo simulation was used to generate values for the identified distribution. The following key assumptions were considered for estimating the treated wastewater reuse health risk to the exposed group:

i. peer-reviewed dose relationship established for the sub-tropical countries;
ii. only direct accidental/incidental ingestion exposure route;
iii. no secondary transmissions.

Table 1 summarizes the details of the data of the parameters required for the exposure assessment. The

Table 1 | Input parameters for QMRA

Input Parameter	Units/model	Value	Scenario
Best fit equations			
Pathogenic *E. coli* spp.	Log-normal	–	Laboratory analyses (In this Study)
Salmonella	Log-normal	–	Laboratory analyses (In this Study)
Cryptosporidium	Log-normal	–	Laboratory analyses (In this Study)
Giardia	Log-normal	–	Laboratory analyses (In this Study)
Dose-Response Parameters			
Pathogenic *E. coli* spp.	Beta-Poisson	α – 0.373 N_{50} – 214.94	Teunis *et al.* (2008)
Salmonella	Beta-Poisson	α – 0.3126 N_{50} – 23600	Haas *et al.* (1999)
Cryptosporidium	Exponential	k – 0.09	Haas *et al.* (1999)
Giardia	Exponential	k – 0.0199	Rose *et al.* (1991)

frequency of exposure was estimated from the stakeholders' survey, including managers and workers at ETPs. The survey included a set of questions (refer to supplementary file) to capture stakeholders' views and intentions on reuse applications, accessibility to treated wastewater, targeted users, expected supply volumes, hours of exposure, frequency of supply, and awareness. The survey's purpose was explained to the respondent, and oral consent was obtained before the interaction. The possible treated wastewater reuse options were shortlisted from the survey, and the four most common reuse options were selected for the exposure scenario. The daily/annual exposure frequency for each exposure group – scenario combination was noted from the survey.

The daily dose of the pathogen was calculated according to the following equation (Kouamé *et al.* 2017):

$$D = I_v * M \tag{1}$$

where I_v is the ingested volume during the incidence (L), M is the average reference pathogen dose (CFU/L), and D is the average daily pathogen dose (CFU/day).

Beta- Poisson (Mok *et al.* 2014) (Equation (2)) for pathogenic *E.coli* spp. and *Salmonella* spp. and exponential for *Cryptosporidium* spp. and *Giardia* spp (Haas & Eisenberg 2001) (Equation (2)) were used in the study. The dose-response models' infectivity parameters used for this study are listed in Table 1.

$$Beta\text{–}Poisson: \; = P_{inf,d} = 1 - \left[1 + \frac{D}{N_{50}}\left(\frac{1}{2^{\alpha}} - 1\right)\right]^{-\alpha} \tag{2}$$

$$Exponential\ model: P_{inf,d} = 1 - \exp(-k^{*}D) \tag{3}$$

In Equations (1) and (2) $P_{inf,d}$ is the daily probability of infection, D is the daily exposure pathogen dose, k and α are infectivity parameters, and N_{50} is a median infectious dose (Mok *et al.* 2014). The QMRA integrates exposure assessment and dose-response modelling information to estimate the probability of infection on the reuse of treated wastewater for each pathogen. The annual exposure frequency is used to calculate annual probability of infection (Equation (4)) using Monte Carlo simulations.

$$P_{inf,annual} = 1 - \left(1 - P_{inf,d}\right)^{n} \tag{4}$$

Here, $P_{inf,\ annual}$ is the annual risk of infection defined per person per year (pppy), and n is the total number of exposure events in a year. It should be noted that the risk annualization function assumes uniform daily risk throughout the year based on the one-time sampling. It is likely there may be a variation in the pathogen concentration over the year, especially between seasons, which may shift the annualized estimates.

Sensitivity analysis is performed to determine the uncertainty and variability factors that have the maximum impact on the estimates. The tornado analysis approach was adopted, which calculates the magnitude of the difference when the input parameter under consideration varies between the 5th and the 95th percentile value while keeping other variables at their median values. The larger the difference in the estimates, the more sensitive the input parameter.

Data management and analysis

All the data collation and analysis was performed using Microsoft Excel®, its add-ons and XLSTAT. An Excel spreadsheet

was used to collate data and perform Monte Carlo simulations for calculation of risk estimates. The mean and standard deviation of pathogens were determined by maximum likelihood estimation using monitoring data. For all the simulations and number generations, 10,000 iterations in the inbuilt random number generation function were used.

RESULTS AND DISCUSSION

Selection of potential intended reuse options

Figure 1 represents the preferred reuse option by the stakeholders and the basis for selection. All the ETP managers have professional education with more than 30 years in the wastewater treatment plants. Nearly 81% of ETP managers showed interest in reusing treated wastewater, while the rest preferred to discharge in drains. Nearly 36% of ETP managers mention using treated wastewater to irrigate plants and crops within the ETPs' premises. ETP managers (73%) were aware of the use of drain water to irrigate crops (like spinach, cucumber, tomatoes, okra, chilli) by farmers living next to the drain. The most common reuse applications are crop irrigation, garden irrigation, toilet flush, and industrial reuse from the survey. Water requirement, user acceptability and physical constraints govern their choice of reuse. These four options were selected for the exposure assessment in this study.

Quality of treated wastewater

Water analysis highlights the presence of pathogens in the treated wastewater (Table 3). Pathogenic *E. coli* spp. is detected in all (100%) samples with a mean of 177.5 CFU/mL. *Salmonella* spp. is detected at 63% of sampling points with a mean of 4.7 CFU/mL. The findings were similar to previous study reports analysing pathogens in treated municipal wastewater (Kadam *et al.* 2008; Tyagi *et al.* 2008; Al-Gheethi *et al.* 2013).

The *Cryptosporidium* was detected in 81% (mean concentration of 3.8 oocysts/L) and *Giardia* in 45% (mean concentration of 1.9 cysts/L) samples. The mean protozoa concentration count includes both infectious and non-infectious (oo)cysts. This study's findings are comparable to the reported literature; for instance, Gennaccaro *et al.* (2003) detected *Cryptosporidium* oocysts in 67% of the disinfected effluent samples. Dungeni & Momba (2010) reported 29% of samples positive for *Cryptosporidium* and 41% samples positive for *Giardia* at four wastewater treatment plants of South Africa. Also, the concentrations detected for protozoa in this study were in the range reported in the literature of 0 to 2,436 cysts/100 L and 0 to 1,745 oocysts/100 L for *Cryptosporidium* and *Giardia*, respectively (Gallas-Lindemann *et al.* 2013). Table 2 summarizes the mean and standard deviation of individual pathogen concentration for each ETP.

The present investigation confirms the detection of pathogens in all the selected sampling locations. Variations in the abundance and diversity of pathogens are observed in this study, primarily due to variation in the raw wastewater quality (Bitton 2005; Al-Gheethi *et al.* 2013). No dedicated disinfection system is installed at any of the ETPs

Exposure characteristics

Table 3 summarizes the exposure assessment input parameters for each reuse option based on the literature and the survey conducted in this study. The potential exposure groups for the reuse options were identified from the

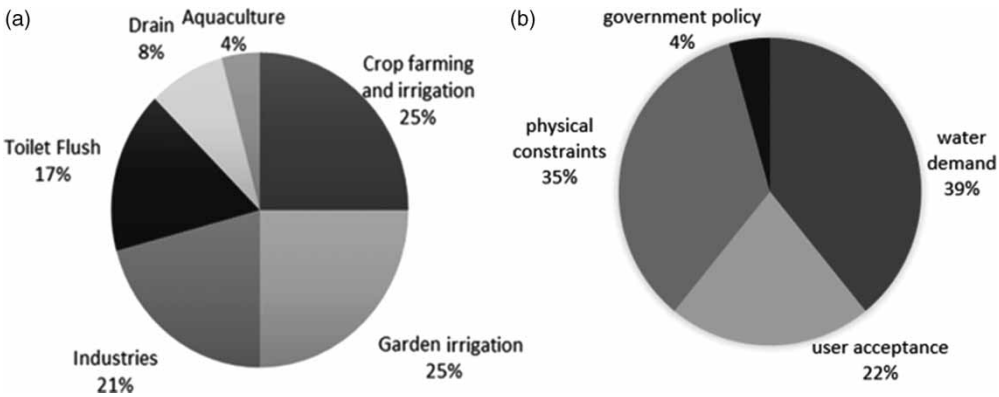


Figure 1 | Survey details describing (a) preference of reuse options and (b) basis for selection of reuse options.

Table 2 | Site-wise concentration of pathogenic *E. coli* spp., *Salmonella spp.*, *Cryptosporidium* spp. and *Giardia* spp. in treated wastewater

Parameters	*p. E.coli* spp.	*Salmonella* spp.	*Cryptosporidium* spp.	*Giardia* spp.
Units	CFU/mL	CFU/ml	Oocysts/L	Cysts/L
Site 1	79.7 ± 7.5	6.4 ± 1.9	1.8 ± 1.9	0
Site 2	183.0 ± 69.3	0	0.4 ± 3.4	7.7 ± 3.99
Site 3	296.0 ± 89.2	7.0 ± 3.5	0.03 ± 1.7	0
Site 4	271.0 ± 37.8	8.5 ± 4.7	3.0 ± 7.2	0
Site 5	200.0 ± 14.2	0 ± 2.5	6.0 ± 4.4	6.9 ± 4.2
Site 6	105.2 ± 13.8	0	11.0 ± 9.0	8.7 ± 3.8
Site 7	169.3 ± 63.3	0	0	0
Site 8	171.9 ± 13.7	0.34 ± 1.4	6.7 ± 10.5	0.7 ± 4.2
Site 9	158.0 ± 66.0	10.6 ± 3.3	0	0
Site 10	200.5 ± 8.5	6.7 ± 5.8	3.4 ± 9.5	0
Site 11	121.3 ± 55.2	12.2 ± 3.4	5.4 ± 4.1	0.04 ± 1.1

p., pathogenic.

survey. For irrigation, hosepipe is the most adopted practice without any protective gear (masks, gloves, shoes). Apart from the ETP workers, their children had unrestricted access to the fields and other irrigated areas, subjecting them to direct wastewater exposure. During the sampling visits, children were observed playing in the field alongside the adults. They were observed helping during irrigation at three locations. From the survey, 100% of workers at the ETPs believe treated wastewater is free from contaminants (pathogens and chemicals both), and any exposure is therefore safe. Exposure to children during industrial reuse or toilet flushing is not anticipated in the current scenario.

Predicted risk of treated wastewater reuse

The probability of infection is estimated separately for each pathogen and selected reuse options, incorporating existing control measures in the analysis. Table 4 summarizes the median estimates of the risk of each reuse option with 95% confidence intervals. The WHO (2006) maximum acceptable annual probability of infection of 1×10^{-4} per person per year (i.e., less than 0.01% infected per year) is considered the benchmark for this study. The highest median annual probability of infection between all the reuse applications was due to pathogenic *E. coli* spp. (1.51–1.68%) followed by *Cryptosporidium* spp. (0.08–0.09%), *Salmonella* spp. (0.010–0.013%), and *Giardia* spp. (0.0001– 0.0003%). The probability of infection from only *Giardia* spp. was below the acceptable value for all the reuse options.

The probability of infection is calculated based on only the ingestion exposure route. The risk associated with exposure to aerosol is included through the ingestion exposure route. The detected pathogens can directly enter the human body through the digestive tract via inhalation/ingestion of aerosols and direct water consumption (Busgang *et al.* 2018). Several disease outbreaks have been associated with exposure during the reuse of contaminated water (Hamilton *et al.* 2006; Petterson 2016). In most cases, the outbreaks are attributed to one pathogen, most commonly *E. coli O157:H7* or *Cryptosporidium parvum*; however, the risk of reuse may be from multiple pathogens (Jamwal & Mittal 2010).

The root sum squared of the annual median probability of infection of all pathogens for each reuse application is calculated to estimate the total risk. In the existing scenario, the least risk is associated with industrial reuse (0.17%) followed by toilet flushing (0.36%), crop irrigation (1.28%) and garden irrigation (1.69%). The root sum squared of the probability of infection may result in over-estimation compared with the field outbreak data. Still, it provides an estimate inclusive of the probability of infection from all the pathogens combined, which can be useful for comparing different reuse options. Thus, the root sum squared should be taken just for comparison and not as absolute risk.

The lower risk for industrial reuse, like floor and machine cleaning, is due to the low frequency of exposure and indirect water contact. Still, the risk of reuse for industrial applications is above the WHO guideline value due to high water consumption with lower frequency. On the contrary, the annual risk for reuse in the toilet is above the accepted value due to higher frequency but lower water consumption. The higher risk is observed in crop and garden irrigation due to the higher possibility of direct accidental ingestion. The risk variation within crop irrigation and garden irrigation is due to the number of days for exposure. For crop irrigation, the exposure time is limited to 3–4 months throughout the year, while exposure in the garden irrigation is daily.

When focusing on exposure groups across intended reuse options, adults show a 1.24 times higher median probability of infection than children. The highest risk is observed in workers during garden irrigation (1.63%) and in children during crop irrigation (1.19%). Among protozoa, the risk associated with *Cryptosporidium* spp. was marginally above the acceptable limits. Still, prolonged exposure is a concern, particularly for children with low immune responses, resulting in malnourishment, impaired development, and increased vulnerability to other diseases (Shoultz *et al.* 2016).

Table 3 | Exposure scenarios and the input parameters required for calculating reuse-wise exposure to pathogens from the literature and the survey

Reuse option	Exposed population	Exposure route	Scenario details	Input Parameters	Reference/ Remark
Crop Irrigation	Field Workers	Accidental ingestion while performing farm activities (e.g., watering the plants)	Irrigation is done with treated wastewater through a hose pipe manually without any protective gear at (8 out of 11 ETPs). Exposure through aerosol formation and droplet splashed while watering	V_i: 0.1–0.2 mL/Event Distribution: Uniform	Busgang *et al.* (2018)
			• Avg. Agricultural Land per ETP: 0.25 Acres (Google Earth) • Avg. Person working per irrigation event: (Range: 1–3, Mean: 2, n: 14) • No. of days which require irrigation (days): (Range: 30–45, mean: 39, n: 14)	f: 60–90 Event/yr Distribution: Uniform	The survey from this study
	Children	Accidental/incidental ingestion while playing in the fields	Playing or working (e.g., transfer of domestic animals) in the fields during irrigation without any protective gear. Ingestion through aerosol or accidentally while playing	V_i: 0.1– 1 mL/Event Distribution: Uniform	The survey from this study
			• Avg. Children working/playing per irrigation event: (Range: 2–4, mean: 3, n: 14) • Assumption: average working days in the field in 50% of the field workers • No. of days which require irrigation (days): (Range: 15–25, mean: 19, n: 14)	f: 30–45 Event/yr Distribution: Uniform	The survey from this study
Garden irrigation	Field Workers	Accidental ingestion while performing garden activities (e.g., watering the plants)	Irrigation is done with treated wastewater through a hose pipe manually without any protective gear (at 5 out of 11 ETPs). • Exposure through aerosol formation and droplet splashed while watering	V_i: 0.05–0.1 mL/Event Distribution: Uniform	Chhipi-Shrestha *et al.* (2017)
			• ETP had trees, small plants and shrubs around entire fencing • No. of people working per irrigation event: (Range: 1–2, Mean: 1.5, n: 6) • No. of days which require irrigation (days/Year): (Range: 120–180; Mean 76; n: 6)	f: 180–270 Event/yr Distribution: Uniform	The survey from this study
	Children	Accidental/incidental ingestion while playing in the garden during irrigation	Children are all the time present during irrigation.	V_i: 0.09–0.11 mL/ Event Distribution: Uniform	Chhipi-Shrestha *et al.* (2017)
			• Avg. children present per irrigation event: (Range: 1–2, n: 5) • No. of days present per week (1–2, n: 5)	f: 52–104 Event/yr Distribution: Uniform	The survey from this study

(*continued*)

Table 3 | continued

Reuse option	Exposed population	Exposure route	Scenario details	Input Parameters	Reference/ Remark
Toilet flush*	Workers (Adults)	Aerosol ingestion during flushing	• Exposure to aerosol during flushing containing pathogen	V_i: 0.009–0.011 mL/ Event Distribution: Uniform	Chhipi-Shrestha *et al.* (2017)
			• Avg. workers working per ETP: (Range: 8–12, mean: 10; n: 6) • Working hours per day: 8–10hrs, 6 days/ week. • Freq. of use of toilets: (Range: 2–4/day, mean: 3, n: 6)	f: 626–1,248 Event/yr Distribution: Uniform	The survey from this study
Industrial reuse*	Workers	Accidental ingestion of aerosols that may travel during washing/cleaning	Exposure while cleaning equipment or machines manually without the use of any masks and gloves	V_i: 0.09–0.11 mL/ Event Distribution: Uniform	Chhipi-Shrestha *et al.* (2017)
			• Avg. cleaning freq: (Range: once in 6 months or monthly; mean: 6; n: 5) • Cleaning hours per event: (Range: 0.25–2 hr; Volume: 10 L–100 L; mean 55; n:5)	f: 2–12 Event/yr Distribution: Uniform	The survey from this study

Here 'n' represents the number of respondents interviewed.
*Only occupational risk anticipated.

Sensitivity analysis indicates pathogen concentration in the treated wastewater as the most significant factor in all the reuse options (refer to supplementary information). In other words, implementing effective disinfection before any reuse would have the most significant impact on reducing the probability of infection (Busgang *et al.* 2018). Implementing an effective disinfection process depends on the log reduction targets for each microorganism. The log reduction targets are estimate values determined to reveal the pathogen dose concentration that reduces the annual probability of infection below the acceptable level. Table 5 lists the reuse-wise targeted log reduction values required to ensure safe reuse. For the current scenario, the disinfection treatment must ensure a log reduction of 2.24 $\log_{10}$ (95% CI 2.21–2.26) and 0.96 $\log_{10}$ (95% CI 0.93–0.98) for pathogenic *E. coli* spp. and *Cryptosporidium* spp., respectively, to ensure safe reuse of water for the intended purpose.

Chlorine-based disinfection and low-dose UV irradiation are effective disinfection processes against both bacteria (*E. coli* and *Salmonella)* with almost 4 logs of microbial load reduction (Chauret *et al.* 2001; Gagnon *et al.* 2005; Hijnen *et al.* 2006; Hassaballah *et al.* 2020). Chlorine tablets work as a good disinfectant only against bacterial, with a log reduction of 7 to 8 $\log_{10}$ per 100 mL (Rodda *et al.* 1993). Studies show that UV disinfection technology works better against all types of pathogens, including the most resistant *Cryptosporidium* and *Giardia* (Hijnen *et al.* 2006). Since wastewater quality is dynamic, disinfection dose and contact time requirements may need more site-specific considerations. The selection of disinfection treatment capable of reducing the highest pathogen risk estimate below the acceptable limit should ensure the infection risks from other pathogens to low levels (Rodda *et al.* 1993). The consensus in the literature is 2–6 $\log_{10}$ and 0–0.5 $\log_{10}$ removal of *E. coli* and *Cryptosporidium* spp., respectively, when chlorination is used as a disinfectant. Alternative treatment options for protozoa removal are using large-pore membrane filtration along with chlorination, ozonation or UV.

Limiting the exposure through on-site control measures may be considered an interim measure until an effective disinfection unit is installed. For reusing treated wastewater for irrigation, improving practices from hosepipe flood irrigation to sprinkler or drip irrigation can reduce exposure by reducing the quantity of accidental ingestion of treated wastewater. For toilet flush and industries reuse, reducing aerosolization through modified faucets, improving cleaning protocols and using protective gear (masks, gloves, coats)

Table 4 | Annual median probability of infection estimates with 95% confidence interval due to four pathogens for different non-potable reuse scenarios and different exposure groups

			p. *E.coli* spp.		*Salmonella* spp.		*Cryptosporidium* spp.		*Giardia* spp.	
Intended reuse	**Value**		**Workers**	**Children**	**Workers**	**Children**	**Workers**	**Children**	**Workers**	**Children**
Crop irrigation	Median		$\mathbf{1.24\times10^{-2}}$	$\mathbf{1.19\times10^{-2}}$	8.58×10^{-5}	4.55×10^{-5}	$\mathbf{3.89\times10^{-4}}$	$\mathbf{2.31\times10^{-4}}$	8.53×10^{-7}	5.22×10^{-7}
	CI 95%	LL	$\mathbf{1.12\times10^{-2}}$	$\mathbf{1.04\times10^{-2}}$	8.33×10^{-5}	4.02×10^{-5}	$\mathbf{3.57\times10^{-4}}$	$\mathbf{2.00\times10^{-4}}$	6.25×10^{-7}	4.63×10^{-7}
		UL	1.28×10^{-2}	2.23×10^{-2}	8.69×10^{-5}	5.12×10^{-5}	$\mathbf{4.08\times10^{-4}}$	$\mathbf{3.10\times10^{-4}}$	9.49×10^{-7}	5.80×10^{-7}
Garden Irrigation	Median		$\mathbf{1.63\times10^{-2}}$	$\mathbf{1.92\times10^{-3}}$	$\mathbf{1.17\times10^{-4}}$	8.69×10^{-5}	$\mathbf{5.32\times10^{-4}}$	$\mathbf{4.40\times10^{-4}}$	1.05×10^{-6}	8.53×10^{-7}
	CI 95%	LL	$\mathbf{1.51\times10^{-2}}$	$\mathbf{1.80\times10^{-3}}$	$\mathbf{1.06\times10^{-4}}$	8.10×10^{-5}	5.07×10^{-4}	$\mathbf{3.74\times10^{-4}}$	9.57×10^{-7}	8.31×10^{-7}
		UL	$\mathbf{1.68\times10^{-2}}$	$\mathbf{2.19\times10^{-3}}$	$\mathbf{1.36\times10^{-4}}$	8.92×10^{-5}	$\mathbf{5.74\times10^{-4}}$	$\mathbf{4.38\times10^{-4}}$	1.22×10^{-6}	8.75×10^{-7}
Toilet flush	Median		$\mathbf{2.66\times10^{-3}}$	–	1.97×10^{-5}	–	$\mathbf{9.02\times10^{-4}}$	–	1.72×10^{-6}	–
	CI 95%	LL	$\mathbf{2.62\times10^{-3}}$	–	–	-	–	-	–	-
		UL	$\mathbf{2.97\times10^{-3}}$	–	–	-	–	-	–	-
Industrial use	Median		$\mathbf{1.65\times10^{-3}}$	-	–	–	6.04×10^{-5}	–	1.11×10^{-7}	–
	CI 95%	LL	$\mathbf{1.56\times10^{-3}}$	–	–	-	–	-	–	-
		UL	$\mathbf{1.83\times10^{-3}}$	–	–	-	–	-	–	-

The risk values shown in bold are above the WHO acceptable limit <1 in 10,000 probability of infection per persons in a year.
LL, lower limit; UL, upper limit.

reduces exposure to pathogenic microorganisms (WHO 2016). These interim measures will reduce the probability of infection for any reuse. Based on the sensitivity analysis, after the concentration of pathogens, reducing the annual frequency has the greatest impact on the probability of infection the most. In the current scenario, the marginal log reduction values (LRVs) are required for safe reuse of treated wastewater, e.g., *E. coli* spp. and *Cryptosporidium* spp. requires 1.22 to 2.24 and 0.59–0.96 log reduction value, respectively. In the absence of advanced disinfection technologies at the ETPs, achieving the desired LRV for any reuse with chlorination will be difficult. However, a combination of chlorination and reducing the annual frequency of exposure would reduce the probability of infection due to *E. coli* spp. and *Cryptosporidium* spp. below the acceptable limits. The selection of measures to achieve indicative LRVs requires technical expertise, understanding the limitations of existing treatment /controls, consideration of capital investments and user awareness. Overall, this study provides baseline information on the requirements of additional control measures for expected reuse applications, considering exposure from bacteria and protozoa.

Limitations of the study

The study had several significant limitations. First, the analysis is based on the single round of sampling to 11 ETPs and does not consider the seasonal variability. The variation is expected to be more pronounced during the monsoon season, impacting the annualized probability of infection. The estimated probabilities are conservative, as the samples were collected during the peak flow in the pre-monsoon season, where the concentrations are expected to be maximum. However, seasonal outbreaks cannot be ruled out (Ono *et al.* 2001; Castro-Hermida *et al.* 2008). Secondly, the detection methods utilized for monitoring reference pathogens did not consider the pathogenicity

Table 5 | Indicative target Log_{10} reductions for representative pathogens based on median risk estimates

	Log_{10} Reductions*			
Reuse options	***p. E. coli* spp. median (95% CI)**	***Salmonella* median (95% CI)**	***Cryptosporidium* median (95% CI)**	***Giardia* median (95% CI)**
Crop irrigation	2.11 (2.08–2.15)	–	0.59 (0.55–0.63)	–
Garden Irrigation	2.24 (2.21–2.26)	0.1 (0.02–0. 13)	0.73 (0.63–0.85)	–
Toilet Flush	1.43 (1.40–1.46)	–	0.96 (0.93–0.98)	–
Industrial use	1.22 (1.16–1.25)	–	–	–

Where N_0 = observed pathogen concentration in CFU/L.
N = predicted pathogen concentration in CFU/L for achieving the probability of infection 1×10^{-4} per person per year.
*$Log10\ Reduction = Log_{10}\ (N_0/N)$.

and assumed it to be 100%. In order to increase the certainty of pathogenicity, serological tests or other methods (e.g., polymerase chain reaction) should be considered in future investigations. The study did not consider viral pathogens like *rotavirus*, which contribute significantly to gastrointestinal infections on exposure (Sigei *et al.* 2015). Another limitation is the limited number of surveys conducted; hence, the exposure characteristics must be viewed cautiously. Lastly, the reuse options considered the ETP managers' willingness and not that of the (potential) users. The approach adopted in this case study is conservative as multiple pathogens, several sources of uncertainties, and conservative log reduction values from literature are considered in the QMRA assumption.

CONCLUSION

The current investigation focuses on applying a risk-based assessment approach to choose non-potable reuse application of treated wastewater. The study assessed occupational associated health risks to workers and children who would directly be exposed to treated wastewater. The study reports the presence of pathogenic protozoa and bacteria above acceptable levels in the treated wastewater. The calculated probability of infection for all selected reuse applications exceeds the acceptable level. The high probability of pathogenic *E. coli* spp. and *Cryptosporidium* spp. associated infection exists if water is reused without additional control measures. The study emphasizes the need for potent disinfection treatment and on-site preventive measures such as improved irrigation and wearing protective gear while using treated wastewater.

The study highlights that the intuitive approach of using chlorination could prove insufficient in reducing the probability of infection below the acceptable levels due to chlorine-resistant *Cryptosporidium*. The use of on-site control measures and disinfection may be more effective in reducing the risk for the intended application. This approach also highlights the need to establish a code of practice for safe wastewater reuse with a balance between treatment barrier process requirements and human health safety. Moreover, in-depth water quality examination is required to make the risk-assessment framework more rational and comprehensive before deciding on the appropriate reuse option and control measures.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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