Application of QMRA to MAR operations for safe agricultural water reuses in coastal areas

Costantino Masciopinto a, *, Michele Vurro a, Nicola Lorusso a, Domenico Santoro b, c, Charles N. Haas b

a Consiglio Nazionale delle Ricerche, Istituto di Ricerca Sulle Acque, Via F. De Blasio 5, 70132, Bari, Italy
b Architectural and Environmental Engineering, Drexel University, Drexel, 3141 Chestnut Street, 251 Curtis Hall, Philadelphia, PA, 19104, USA
c USP Technologies, 3020 Gore Rd, London, ON N5V 4T7, Canada

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A B S T R A C T
A pathogenic Escherichia coli (E.coli) O157:H7 and O26:H11 dose-response model was set up for a quantitative microbial risk assessment (QMRA) of the waterborne diseases associated with managed aquifer recharge (MAR) practices in semiarid regions. The MAR facility at Forcatella (Southern Italy) was selected for the QMRA application. The target counts of pathogens incidentally exposed to hosts by eating contaminated raw crops or while bathing at beaches of the coastal area were determined by applying the Monte Carlo Markov Chain (MCMC) Bayesian method to the water sampling results. The MCMC provided the most probable pathogen count reaching the target and allowed for the minimization of the number of water samplings, and hence, reducing the associated costs. The sampling stations along the coast were positioned based on the results of a groundwater flow and pathogen transport model, which highlighted the preferential flow pathways of the transported E. coli in the fractured coastal aquifer. QMRA indicated tolerable (<10⁻⁶ DALY) health risks for bathing at beaches and irrigation with wastewater, with 0.4 infectious diseases per year (11.4% probability of occurrence) associated with the reuse of reclaimed water via soil irrigation even though exceeding the E. coli regulation limit of 10 CFU/100 mL by five times. The results show negligible health risk and insignificant impacts on the coastal water quality due to pathogenic E. coli in the wastewater used for MAR. However, droughts and reclaimed water quality can be considered the main issues of MAR practices in semiarid regions suggesting additional reclaimed water treatments and further stress-tests via QMRAs by considering more persistent pathogens than E. coli.

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1. Introduction

Currently, managed aquifer recharge (MAR) is used as an attractive water source recycling technology useful for both agriculture and industry productions and, importantly, also drinking water (Asano et al., 2007; Dillon et al., 2008). Moreover, in coastal aquifers, MAR practices are also used to control the impact of seawater encroachments on groundwater quality. Climate change affects coastal areas leading to an increase in the sea level rise and more frequent droughts with subsequent water scarcity. MAR applied to the bedrock (limestone) coastal aquifers of the Salento Peninsula (Masciopinto et al., 2011) provides an efficient dynamic barrier controlling seawater intrusion to the groundwater and recovering freshwater storage suitable for irrigation and drinking supplies in touristic coastal areas in semiarid regions.

Accordingly, sustainable and effective MAR schemes have to maintain a good microbial/chemical quality of the recycled water to protect human health from the potential presence of dissolved toxic compounds and suspended enteric pathogens (Gibson and Schwab, 2011). Quantitative microbial risk assessment (QMRA) (Howard et al., 2006) provides a “quantitative” estimate of the degree of safety the population exposed to the contamination derived from MAR practices has (Toze et al., 2010), as opposed to “qualitative” microbial risk assessment (Ayuso-Gabella et al., 2011). Although viruses and protozoa can better survive in the environment than bacteria, suggesting the potential higher risk of viruses...
and protozoa to public health, recent data collected by National and European surveillance agencies show an alarming increase of diseases from the ingestion of food contaminated by pathogenic *Escherichia coli* (*E. coli*). In particular, the presence of *E. coli* in reused irrigation water (Lapen, 2019; Kouamé et al., 2017) has generally been considered as the main indicator of fecal water contamination in the last decade because of its significant impact on public health. Indeed, the Italian regulation (Lgs D. 185, 2003) for water reuse in irrigation (EU Commission, 2018) suggests an at least 80% of monitored water samples where only one sample at an *E. coli* count to 10 CFU/100 mL is allowed, while *Salmonella* spp. must be absent. The European Union (EU) proposal for a regulation of the minimum requirements for reclaimed water of Class A used in irrigation (EU Commission, 2018) suggests an *E. coli* limit ≤10 CFU/100 mL in at least 90% of monitored water samples along with microbial limits for legionella ≤100 CFU/100 mL and intestinal nematodes (Helminth eggs) ≤1. The EU limits are related to rawly consumed roots and crops of which the edible part is directly in contact with reclaimed water.

During the last 20 years, specific *E. coli* serotypes have led to cases of severe diseases, with increasing incidence in Europe. During 2018, the European Centre for Disease Prevention and Control (ECDC, 2020) recorded approximately 8161 cases of *Shiga*-toxin producing *E. coli* (STEC) infection, also referred to as *E. coli* Vero Toxin (VTEC) in the EU, with 73 of these cases recorded in Italy. These pathogenic *E. coli* strains are associated with various symptoms ranging from mild gastrointestinal illness to severe disorders such as Hemolytic Uremic Syndrome (HUS), which can lead to acute renal failure and, consequently, death, particularly in children.

In this study, we aim to quantify the number of diseases caused by the pathogenic – *E. coli* O157:H7 and O26:H11 – ingested by hosts via raw crops, i.e., vegetables and fruits where the edible part is directly in contact with effluent water from MAR facilities. In fact, quantitative health risk assessments associated with waterborne infections at MAR sites due to *E. coli* O157:H7 and O26:H11 have not been comprehensively investigated in the literature, in contrast to foodborne infections (Pang et al., 2017; Franz et al., 2010). Moreover, we estimate the quantitative health risk owing to the ingestion/inhalation of seawater contaminated by pathogenic *E. coli* in seasonal wastewater discharges that are frequent in coastal areas at bathing locations (Eregno et al., 2016; Ashbolt et al., 2010). Microbiological data collected during sampling and the results of stochastic forecasts were used to assess the quantitative health risk at a MAR site on a coastal area, such as the Forcatella facility in the Puglia region (southern Italy). In this region, because of the water scarcity exacerbated by global warming, the Puglia government has authorized the installation of 32 MAR plants since 2016. These plants produce 128 ML/day (Portoghese et al., 2019) of reclaimed water for irrigation and managed groundwater recharge following the tertiary treatment of effluents derived from municipal wastewater treatment plants.

### 2. Materials and methods

#### 2.1. The MAR facility

MAR sites in coastal areas are usually in the vicinity of touristic beaches, such as in the case of the Forcatella MAR facility. The reclaimed water (Table 1) from the MAR facility is mainly used for the irrigation of agricultural land, with a remaining flow-rate to control the seawater intrusion and groundwater over-exploitation through the MAR. Table 1 shows the *E. coli* modal (10 CFU/100 mL), median (5 CFU/100 mL), and 95th percentile (16 CFU/100 mL) from the statistical (Gamma) distribution of concentrations in 114 routine samples processed by the water utility between 2014 and 2018. The *C. perfringens* count was instead estimated from three water samples collected under critical operating conditions of the wastewater treatment plant.

The water samplings at the Forcatella MAR site of the present work were carried out during summer, as the chemical and microbial analysis of municipal wastewater indicates that this season was the most critical for the quality of water inflow to the MAR plant. Specifically, during summer 2019, the *Istituto di Ricerca Sulle Acque* (IRSA) monitored reclaimed water in five sampling stations to test the quality of the water supplied for irrigation (Fig. 1). Fig. 1 also shows farmhouses and restaurants that may occasionally discharge untreated wastewater into the sea. In particular, we sampled the reclaimed water from the Forcatella artificial basin between 15th and July 22, 2019 at station S1, close to the two spillways of the ditches for the managed recharge of groundwater, and at two further irrigation valve stations (S2 and S9; see Fig. 1b). Groundwater was sampled in two monitoring wells (S3 and S10). We also sampled bathing water at the other five sampling stations (S4 to S8) along the beaches to control possible MAR impacts on the seawater quality via groundwater sea outflows. The locations of the sampling stations along the coast were determined through mathematical model simulations, using specific in-house software (Masciopinto et al., 2008). More details on the groundwater model results showing that the sampling points intersect the plume, especially in limestone fractured rock aquifers, are described in Section 2.2. Fig. 1 also shows sea sampling locations monitored during 2019 by the Regional Agency of Environmental Protection (ARPA; http://www.arpa.puglia.it/). The tertiary wastewater treatments of the MAR plants in Puglia consist of clarification/flocculation/sedimentation and disinfection mainly with UV and sodium hypochlorite. The Forcatella facility also hosts a “side-stream” pilot plant where three advanced wastewater treatment schemes for indirect potable reuse are currently being tested. These water treatments will achieve high-quality standards of recycled water (Piras et al., 2020) for drinking purposes.

In this study, we considered only the mainstream scenario as being implemented at the full-scale in a MAR site, i.e., without the “side-stream” treatments. Under the considered scenario, the reclaimed water is directed to agricultural reuse via a pressurized aqueduct while overflows are collected into the downgradient water storage basin, where the water depth (2 m, on average) is constantly controlled by piezometers. The reclaimed water is mainly used for irrigation, with a mean flowrate of 7.34 ML/day using a network of pipelines (>30 km) that supplies an irrigation area of 1000 ha around the MAR site. The main crop production of Fasano, as well as the agricultural region, consists of salads, tomatoes, cucumbers, carrots, olives, and fruits, e.g. cherries, strawberries, grapes, almonds, and plums. The Forcatella MAR facility covers an area of 6 ha, with a total water storage capacity of 50,000 m³. Here, water flows via two spillways into two underground ditches positioned along the end-border of the basin, with a length of 250 m and a cross-section of 2 × 2 m² (see Fig. 2). The annual average recharge rate at the Forcatella site was estimated to be 1–2.6 ML/day. The bottom of the ditches is about 3 m above the surface head of the groundwater where the water flows under low pressure into the fractured limestone to reach the Adriatic Sea in the N-E direction (see Fig. 1). Via ditches, the seepage flow of reclaimed water through the karstic limestone reaches the water table within a few hours due to the high permeability of the vertical fractures (Masciopinto and Caputo, 2011).

#### 2.2. Model simulations of pathogen pathways

Mathematical models treating pathogens as colloids in groundwater, i.e. as suspended particles (bio-colloid or biotic
particulate matter fraction) rather than dissolved compounds, have been shown to be valuable tools for predicting pathogen counts in aquifers (Masciopinto et al., 2011). This makes them suitable to assess potential long-term health risks associated with the occurrence of enteric viruses and protozoa in fractured bedrock aquifers.

The mathematical model applied to the MAR facility in this work

**Fig. 1.** Overview of the sampling stations: a) map of the study area; b) positions of the sampling stations of IRSA (from S1 to S10) and ARPA (from AP1 to AP3), and irrigation pipelines (blue lines).

**Table 1**

Reclaimed water quality (average values) at the MAR site in a coastal area on routine samples during 2018. Values in red and green are those exceeding the regulation limits for drinking and irrigation water, respectively.

| PARAMETER | Regulation limits | Reclaimed water quality from MAR |
|-----------|------------------|---------------------------------|
|           | Irrigation: 185/2003 (and EU 2018) | Ground-water protection: EU Directive 2006/118 | Drinking water: EU Directive 98/83 | Irrigation and managed groundwater recharge | Drinking water* |
| Bromide (mg/L as Br) | 3.0 | 2.5 | 2.5 | 3.5±0.4 | 3.5±0.4 |
| Specific conductivity (mS/cm) | 0.01** | < 0.4±0.01 | < 0.4±0.01 |
| Chloride mg/L (as Cl) | 250 | 250 | 334±4 | 389±5 |
| Fluoride mg/L (as F) | 1.5 | 1.5 | 1.5 | 0.61±0.05 | 0.63±0.05 |
| Nitrates mg/L (as NO3) | 50 | 50 | 9±2.7 | 26±6.5 |
| Sulphate mg/L (as SO4) | 500 | 250 | 250 | 70±4.5 | 75±5 |
| Biochemical Oxygen Demand (as mg/L O2) | 20 (and) | 10 | | |
| Chemical Oxygen (COD) Demand (as mg/L O2) | 100 | 5.0 | 21.4±5.2 | 9.4±2.3 |
| Cyanide μg/L (as CN) | 50 | 50 | 50 | 29.9±1.2 | < 5±0.2 |
| Nitrites mg/L (as NO2) | 0.5 | 0.5 | 0.08±0.02 | 0.03±0.01 |

**Microbial fecal indicators (CFU/100 mL)**

|                  | Total Coliforms | 1,000±190 | 0 |
|------------------|----------------|-----------|---|
| Enterococci      | 0              | 0, 5, 16  | 0 |
| C. perfringens   | 0              | 63±7      | 0 |

*Advanced (pilot plant) oxidation (ozone) pre-process (AOP) and Bio-carbon (i.e., biological activated carbon) filtration for drinking reclaimed water supply (Piras et al., 2020).

**Provided as Bromate.

Assuming COD/total organic carbon (TOC) ratio of about 2.3 in wastewater effluents from municipal treatment plants (Masciopinto et al., 2007).
was accurately investigated and validated using tracer and groundwater monitoring tests (Masciopinto et al., 2008). The simulation results at the Forcatella site have visualized the preferential pathways in fractures that are followed by pathogens (Fig. 3) using the particle tracking computation method (Masciopinto et al., 2019). These pathogen trajectories into horizontal fractures are highlighted in Fig. 3.

The plume of infiltrated E. coli in the groundwater was visualized by applying an E. coli probability distribution to outflowing flow in fractures, conditioned by higher water velocities at each fractures intersection. This approach allowed us to exactly locate the positions of the sampling points along the coast to check for the possible seawater contamination by polluted groundwater outflows via MAR. Hydrogeological and geochemical data (i.e., water molarity and ionic strength, pH, temperature, and charge/potential of surfaces of pathogen collectors; Masciopinto and Visino, 2017) for the simulations were required to estimate groundwater flow velocities and the pathogen travel time in the fractured aquifer.

The flow simulations yielded a map of groundwater contour heads and vectors of the water velocities during water filtration through the fractures under both natural (red contour lines) and recharge (blue contour lines) flow conditions (see Fig. 3). The managed recharge increased the groundwater surface-head (and groundwater discharge) for up to 1000 m upstream of the ditches. The model simulation provided an average travel time of 18–20 ± 3.2 d (i.e., a mean water velocity of 32 ± 5.8 m/d) for the infiltrated reclaimed water before outflowing into the sea, after 600 m of filtration in the fractured aquifer. Moreover, the E. coli removal rate by the soil aquifer treatment (SAT), i.e., by physical retention or straining (Gerba and Goyal, 1985), inactivation and attachment was set to 0.33 d⁻¹. This value was estimated using data derived from a previous study (Masciopinto et al., 2011; La Mantia et al., 2008) carried out at Nardò (Salento peninsula, Italy). These data were considered in the present work to estimate E. coli persistence in the groundwater of the Forcatella MAR site because the hydrogeology of the two sites is quite similar, e.g., both feature a fractured limestone aquifer. Thus, we report the E. coli counts that were monitored by IRSA in two Nardò wells 320 m and 500 m away from the injection location, in Fig. 4. The travel times of 2 and 6 days for E. coli in the injected reclaimed water reaching the monitoring wells were determined in this work using the mathematical model mentioned above. Fig. 4 shows that almost a complete removal of E. coli can be achieved after 10–15 days of filtration throughout the karstic fractured aquifer, even considering MAR operative conditions resulting in E. coli concentrations of 300 MPN/100 mL in the wastewater. This is because after 18–20 ± 3.2 days of wastewater filtration in the groundwater, SAT ensures that only a very low count (<0.2 MPN/100 mL) of pathogenic E. coli from the MAR facility site can affect the seawater and QMRA from STEC/VTEC infections is not required. The same cannot happen when seasonal untreated wastewaters are released from restaurants and farmhouses directly into the sea. QMRA due to pathogenic E. coli ingestion/inhalation was carried for wastewater reuse in irrigation and untreated wastewater discharged into the sea.

2.3. Sampling and sample processing

At each sampling point, we collected one sterile bottle (1.0 L) of water to analyze its chemical constituents and three 300 mL samples for microbiological tests. During each sampling, temperature (T), pH, and water electrical specific conductance (EC) were measured on-site. The bottles containing the collected samples were transported to the IRSA laboratory using a mini portable refrigerator (at 4 °C) and analyzed within 24 h. Standard Methods (APHA-AWWA-WEF, 2005) were applied to determine water chemical oxygen demand (COD), ammonia (NH₄⁺), nitrates (NO₃⁻), and total nitrogen (TN) in the sampled water.
2.4. Microbial analyses

The count of Total Coliforms (TCs) and *E. coli* (Chik et al., 2018) was determined using the Colilert®-18 Test (IDEXX Laboratories Inc., www.idexx.co.uk), according to the manufacturer’s protocol. The number of the positive tray wells both for TCs and *E. coli*, was converted into the most probable number (MPN) per 100 mL using the conversion factor provided by the IDEXX Laboratories Inc.

However, it was not possible to detect TCs in bathing seawater samples using the Colilert®-18 rapid method. This was because there were false-positives due to the enzymatic interference activities of algae, plants, and non-coliform bacteria with the β-d-galactosidase enzyme which belongs to Coliforms and *E. coli* (Eckner et al., 1998). The Enterococcus number (including *E. faecium* and *E. faecalis*) was evaluated by the Enterolert®-E approach (IDEXX Laboratories Inc., www.idexx.co.uk) according to the manufacturer’s protocol. This method required the same Colilert®-18 steps, except for the incubation process that was carried out at 41 °C for 24 h. The positive samples were directly visualized with a blue-white fluorescence after the exposure to a 6-W 365 nm UV light (Fluorescence Analysis Workstation — CM — 10A model/ Spectroline®, NY, USA) and the Enterococcus number indicated as MPN per 100 mL as was the case in the last Colilert®-18 step. The total viable bacteria count was evaluated utilizing the plate count method, with a yeast extract agar selective medium, following standard methods (APHA et al., 2005). Only Petri dishes containing less than 300 colonies were considered to estimate the colony-forming units (CFU) per mL of sample.

2.5. QMRA method

2.5.1. Exposure assessment

A Monte Carlo based exposure dose is usually applied in the QMRA of water supplies (Schijven et al., 2011; Whitaker et al., 2005) rather than a deterministic (point-value) dose assumption, which is also widely used. A Monte Carlo Markov Chain (MCMC) analysis,
based on the Gamma prior distribution (Schmidt et al., 2013, 2019) was applied in this study to determine the 5%, 50 and 95% percentiles of the target counts of E. coli in both contaminated reclaimed water of vegetables and polluted seawater at the Forcatella beaches. Specifically, we considered the host’s ingestion (or consumption) of raw vegetables, such as salads, tomatoes, cucumbers, and fruits (e.g. cherries and strawberries), that were contaminated by reclaimed wastewater coming from the MAR plant, as the primary root of the infection.

The ingestion/inhalation of contaminated seawater during swimming (or bathing) was instead considered separately, as the secondary root of infections caused by untreated wastewater discharges along the coast. Therefore, the estimation of the probability of infection was split into two distinct predictions, related to the quality of the reclaimed water for irrigation supplies and the seawater pollution by local wastewater discharges. The MCMC analysis was performed on the microbial sampling results using the OpenBUGS software (see Appendix) to define the most probable pathogenic E. coli concentrations at the sampled stations for both sampling groups of irrigation (S1, S2, S3, S9, and S10) and bathing (S4, S5, S6, S7, S8, AP1, AP2, and AP3). To get the pathogenic E. coli count in the target, we multiplied the sample concentrations and the volume of the contaminated water ingested by the host eating raw crops by 20 mL per day (Ayuso-Gabella et al., 2011). Moreover, we considered a volume of 50 mL of contaminated seawater that was inhaled and ingested per bathing or swimming by the host. This volume estimation (McBride et al., 2013) depends on several factors, such as the duration of bathing, age, and the ability of the host during swimming (Haas et al., 2014). Data collected using a questionnaire, involving 19,000 persons of various ages in the Netherlands (Schets et al., 2011), highlighted that men swallowed a water volume of 27–34 mL per swimming event, women 18–23 mL, and children 31–51 mL. Hence, we estimated an accidental inoculation of 50 mL of polluted water per bathing event and two bathing events per day to arrive at 100 mL per day since older children (>4 years) and adolescents (<15 years old) were considered for disease predictions in this work.

2.5.2. Dose-response model of the infection probability

The HUS typically arises because of severe enteric STEC/VTEC infection, mainly (in 70–80% of cases) caused by pathogenic E. coli strains that produce powerful toxins, the VTEC or STEC. These infections mainly affect immune-depressed people like children (and sometimes also the elderly) that have been exposed to E. coli via ingestion of foods contaminated by feces, such as unpasteurized cheese or raw vegetables containing wastewater or to the ingestion of polluted seawater during swimming or bathing.

Most cases of the European STEC/VTEC infections during 2018 were caused by the E. coli O157 serotype (~39.2%), while in Italy a different E. coli strain, O26:H11, emerged as the significant agent of STEC/VTEC infections (38/73 cases) in the same year.

Between 1988 and 2018 the Italian Istituto Superiore di Sanità (ISS, 2020) recorded a mean of 38.5 HUS cases per year in Italy, an incidence of 0.44 per 100,000 residents. The best-fit of the increasing trends of HUS ($R^2 = 0.7$) and STEC/VTEC ($R^2 = 0.8$) in Italy shown in Fig. 5, yields

$$P = \frac{1}{\Gamma(a + \beta)} \int_0^\infty \frac{r^{a-1} e^{-r} (1 - r)^{\beta - 1}}{\Gamma(a) \Gamma(\beta)} dr$$

(2)

where $r$ is the single-hit value of the beta probability of the infection of each virus ingested (i.e., host susceptibility), $\Gamma(r)$ is the gamma function, and $ID$ or $ID_{50}$ is the (dimensionless) count of the ingested/inhaled pathogen.

2.5.3. Infection/diseases prediction method

The static QMRA in this work was performed by multiplying the individual infection probability and the exposed population by accounting for the morbidity for an STEC/VTEC infection and host autoimmunity (Masciopinto et al., 2007). During the disease risk calculation in this study, an immunity of 15% of hosts younger than 15 years, was considered. Investigations carried out by Ludwig et al. (2002) on a control group of 327 persons in Germany showed that 27% had IgG antibodies against Shiga toxins –Stx2and/or–Stx1and that there was an age-related immunity increase starting from 11%, in children aged less than 4 years to 59% in the age group of 26–44 years.

Moreover, Tozzi et al. (2003) showed that evidence for an STEC infection in Italy was only found for 73.1% of the investigated patients < 15 years that were affected by gastrointestinal disorders. This means that despite the use of different diagnostic methods, for 26.9% of the HUS patients younger than 15 years there was no evidence for an STEC infection probably because some patients may have had an infection caused by a STEC strain belonging to a different E. coli serogroup not included in the screening panel. Thus, we considered the latter percentage as the maximum proportion of under-reported diseases due to STEC/VTEC infections of exposed hosts at the MAR site.
Primary and secondary morbidities due to the infection were fixed both at the rate of 10% (Masciopinto et al., 2019).

3. Results

3.1. Chemical and microbial quality of sampled water

Puglia is a semiarid region with an average rainfall of less than 600 mm during winter. Hence there are no natural water reservoirs but many ephemeral streams that convey surface runoff toward the sea or to the karst bedrock aquifer via sinkholes. The first water monitoring was performed during a “rainy day” preceding several days without rainfall or a “drought period”. At the sampling station S4, the high concentration of ammonium (5.70 mg/L as N-NH₄) highlights the local fecal contamination of the sea caused by a regular untreated wastewater discharge that we observed during sampling. The chemical constituents and microbial concentration in sampled water are reported in Tables 2 and 3, respectively. The S4 contamination was confirmed by the fecal indicator concentrations in Table 3. Thus, we took the untreated wastewater discharge

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Fig. 5. Number of notified HUS (blue circles) illnesses per year in Italy from 1988 to 2018 recorded by the Italian Istituto Superiore di Sanità (ISS, 2020) and STEC/VTEC by ECDC (brown circles), and best-fit trends in this work.

Fig. 6. Posterior Poisson density at selected target samplings for irrigation (upper) and bathing (below) groups; E. coli counts in X[3] and X[18] samples had the highest (i.e., the most frequent) posterior probability of 12% and 25%, respectively, whereas E. coli counts in X[1] and X[6] were closest to the mean value (5 and 8% of probability) of the Gamma prior probability distributions.
into consideration in the QMRA via the MCMC of the pathogenic E. coli count detected in the sampled water at S4, i.e., at the pipe outflow section of the wastewater into the sea. This sampling station, by affecting seawater quality, characterized QMRA of this work for bathing/swimming at the Forcatella beaches.

The sampled reclaimed water at S1, S2, and S9 (see Table 2) showed traces of ammonium with COD ranging from 41.1 to 55.1 mg/L as O₂. Tables 2 and 3 also show an impairment of reclaimed water collected in the Forcatella basin (S1) for MAR, especially during the drought period.

Bird and insect secretions (i.e., nutrient load), sunlight, plants, and algal growth had created eutrophic conditions (Greening and Janick, 2006) due to fecal pollution of the water basin used for managed recharge.

3.2. Exposure assessment results and target E. coli counts

The results of the MC simulations shown in Table 4 returned 5%, 50, and 95% percentiles of the Gamma prior probability distribution of the mean E. coli concentration of each considered sampling group. The maximum number of iterations performed in OpenBUGS ensured a fixed maximum Monte Carlo error of the results of less than 5.5%. Specifically, 330,000 and 35,000 iterations were required for the “irrigation” and “bathing” group predictions, respectively. The difference in the iteration number was due to the lower number (5) of samples available in the irrigation group compared with those in the bathing group (25). Following this, the parameters of the posterior Poisson probability distributions of the E. coli counts of each sample were obtained using the MCMC method. This method, starting from the Gamma distribution concentrations in Table 4, fitted the E. coli mean concentrations collected at each sampling station using the true posterior Poisson probability distribution.

The MCMC results are summarized in Table 5. OpenBUGS yielded the most probable E. coli count Poisson distribution, with percentiles of 5%, 50, and 95% that might be incidentally exposed to hosts.

Indeed, we considered, as target counts in QMRA, the E. coli counts of the predicted percentiles (Table 6) corresponding to the lowest measured concentration in 100 mL during the samplings, i.e., 14.8 (for irrigation) and 31.6 (for bathing) (see bold counts in Table 5) since they were the most probable values (12% and 25%, respectively; Fig. 6) with the lowest standard deviations. Further, for QMRA we also selected the MCMC percentiles corresponding to the maximum target E. coli count that might be ingested and/or inhaled.

These percentiles are provided in Table 5 via the measured E. coli concentrations of 42.58 and 31.01 MPN/100 mL during irrigation and bathing, respectively, which have a maximum posterior probability of 5% and 8%. The above concentrations were selected because they are closest to the mean (mu) values (see Table 4) of the Gamma distribution into the group of loading data, i.e., 67.51 (irrigation) and 30.65 CFU/100 mL (bathing), respectively.

Subsequently, based on the OpenBUGS results, we obtained the E. coli counts at the target of 9, 15 and 21 CFU/100 mL, respectively, due to the 5%, 50 and 95% percentiles of the most probable (12%) Poisson distribution of the E. coli count occurrence in contaminated reclaimed water of uncooked (raw) vegetables or fruits daily ingested by a single host.

Similarly, we estimated 1.0, 3.0 and 6.0 CFU/100 mL as corresponding to the 5%, 50 and 95% percentiles of the most probable (25%) occurrence of Poisson distributed E. coli counts in contaminated seawater ingested (and inhaled) daily by a single host during swimming or bathing. Moreover, to account for the natural pathogenic E. coli inactivation, all irrigation doses were reduced according to an inactivation time of 72 h from the harvesting to the host ingestion of the contaminated food. An E. coli survival time between 3 and 6 days was experimentally estimated by Vergine et al. (2015) on lawn grass (Paspalum vaginatum), while it was higher, e.g., 10–13 days, on topsoil. The inactivation rate on vegetables and fruits was fixed here at 0.33 d⁻¹, i.e., less than the minimum natural rate of 0.6 d⁻¹ due to the UVB sunlight exposure proposed in the literature (Maraccini et al., 2016). This yielded E. coli counts, d, of 0.67, 1.11, and 1.57 reported in Table 6. Table 6 also shows (in parentheses) the apparent counts of 0.37, 0.74 and 1.11 given by the MCMC (i.e., 5, 15 and 21 instead of 9, 15, and 21) and the successive inactivation during 72 h, for the hypothetical scenario where E. coli concentrations from the irrigation water of S2 and S9 are fixed at the regulation limit of 10 CFU/100 mL. All the above calculations were repeated (see Table 6) to determine the maximum count of E. coli doses, dₘ, that might be incidentally exposed to hosts via irrigation and bathing.

### 3.3. MAR infections

To determine the infectious dose id and dₘ required for QMRA, the d and dₘ values in Table 6 had to be reduced to the pathogenic fraction of E. coli strains to cause STEC/VTEC infections and HUS diseases, as explained in Section 2.5.2.

### Table 2

**Chemical constituents of sampled water.**

| ID# | Sampling | T (°C) | pH | EC (μS/cm) | COD (mg/L O₂) | NH₄ (mg/L N–NH₄) | NO₂ (mg/L N–NO₂) | NO₃ (mg/L N–NO₃) | TN (mg/L N) |
|-----|----------|-------|----|-----------|--------------|----------------|-----------------|-----------------|-------------|
| S1  | July 15, 2019 (rainy day) | 24.3  | 7.05 | 1.37      | 41.1         | 4.15           | 0.67            | 0.465           | 5.29        |
| S2  |          | 26.0  | 7.70 | 3.44      | 52.8         | 7.48           | 1.34            | 0.667           | 9.49        |
| S6  |          | 26.4  | 7.71 | >20.00    | NA           | 0.17           | NA              | NA              | >0.17       |
| S7  |          | 26.2  | 7.43 | >20.00    | NA           | 0.11           | NA              | NA              | >0.11       |
| S8  |          | 21.0  | 7.30 | 19.70     | NA           | 0.06           | NA              | NA              | >0.06       |
| S9  |          | 26.1  | 7.52 | 3.40      | 50.5         | 6.92           | >3.5            | 0.635           | >11.06      |
| S1  | July 22, 2019 (drought period) | 26.0  | 7.13 | 3.38      | 44.9         | 7.65           | 0.465           | 0.485           | 8.6         |
| S2  |          | 26.1  | 7.24 | 3.39      | 53.1         | 7.96           | 0.304           | 0.277           | 8.54        |
| S3  |          | 18.9  | 7.47 | 0.13      | 15.1         | 0.53           | 0.035           | 2.65            | 3.22        |
| S4  |          | 18.9  | 7.90 | >20.00    | NA           | 5.7            | NA              | NA              | >5.7        |
| S5  |          | 7.38  | 7.83 | >20.00    | NA           | 0.26           | NA              | NA              | >0.26       |
| S6  |          | 7.70  | 7.70 | >20.00    | NA           | 0.15           | NA              | NA              | >0.15       |
| S10 |          | 20.5  | 6.80 | 15.12     | 33.2         | 0.18           | 0.016           | 3.632           | 1.83        |

**NA stands for not analyzed due interference by high water salinity.**

* The COD, here, represents the concentration of dissolved organic compounds in the filtered sampled water.
The predicted individual infection probability given by Eq. (2) is shown in Table 7. The probability of infection was obtained selecting $a = 0.44$ and $b = 49$ which are close to the $a$ (0.38) and $b$ (37.9) values determined by Teunis et al. (2008) for the 1995 outbreak in Oregon, USA, caused by *E. coli* O157 ingestion at concentrations from 3 to 93 CFU/g that were isolated from the two leftover pieces of beef jerky (200 g) that were assumed to have been eaten by the host.

The $\beta$–Poisson probabilities of the infections shown in Table 7 due to *E. coli* O157:H7/O26:H11 inoculation by a host, were determined by solving Eq. (2) in MathCad (PTC prime 4.0; www.ptc.com).

### 3.4. Risk quantification

The QMRA results, i.e. the number of diseases that might affect the population living near the MAR site, are shown in Table 7. The irrigation risk was estimated by considering the exposure period of 48 days per year (i.e., about 1 day per week) of the Fasano inhabitants (39,683) between 4 and 15 years (3,492) (ISTAT, 2020) as we assumed that older hosts affected by the infection were not hospitalized and thus, the cases remained unreported. Further, due to parental control, we also assumed that young children aged 4 and less did not have the opportunity to eat raw roots and vegetables contaminated by reclaimed water. The older children (>4 years) and adolescents (<15 years old) comprised 12.7% of the total residents in Fasano in 2018 (ISTAT, 2020). Seven days were considered as the exposure period for 10,000 tourists in 2–3 km of coastline in the vicinity to the MAR plant who went swimming or bathing at beaches with seawater contaminated by pathogenic *E. coli* during summer 2018. Moreover, only 15% (1500) of the tourists were considered to be exposed to the seawater contamination considering those aged less than 15 years.

Calculations of the expected diseases that occurred during 2018 at the MAR site (see Table 7) were performed using Microsoft Excel.

Table 7 also includes Puglia HUS and STEC/VTEC infections during 2018. Despite notifications of illnesses due to STEC/VTEC infections not being mandatory in Puglia and only 13 cases of HUS being notified in 2018 in this region, we estimated 27 cases of STEC/VTEC regional infections (≈1.51 × 17.8) (see Table 7) using the average National ratio of 1.51 STEC/HUS (≈5.3/3.5) infections per year reported in the ECDC database, from 2007 to 2018. This estimation increased the number of the HUS cases in Puglia from 13 to 17.8 in 2018 based on the Italian average incidence of 0.44 HUS diseases per 100,000 residents recorded by the National ISS (2020) between 1988 and 2018 (see Fig. 5), with an increasing rate of about 3.5 HUS diseases per year.

The QMRA results provided the most probable cumulative risk of 0.4 diseases due to STEC/VTEC infections of the exposed population at the MAR site in 2018, with the probability of 11.4% (5% percentile) and uncertainty of 3.8%, on average (see Table 5). The individual (i.e., per-capita) disability-adjusted life year (DALY) corresponding to STEC/VTEC disease burden was 4.98 · 10⁻² DALY in 2018 using the conversion factor of 54.7 · 10⁻³ of DALY per disease published by the World Health Organization (WHO) (Havelaar and Melse, 2003, p. 32) for the symptomatic diseases due to STEC O157 infections in the Netherlands. The resulting burden of diseases provided a DALY per year below the tolerable 10⁻⁶ upper limit of the reference level of risk set out in the WHO Guidelines for drinking water (WHO, 2011, p. 132) in high-income countries. This latter value, however, should not be considered a sharp threshold to classify the supplied water quality due to the uncertainty involved in risk assessment.

### Table 3

Microbial quality of sampled water.

| Sampling date (2019) | Sampling ID | TCs (MPN/100 mL) | *E. coli* (MPN/100 mL) | Enterococci (MPN/100 mL) | TB 22° (CFU/1 mL) | TB 37° (CFU/1 mL) |
|---------------------|-------------|------------------|------------------------|--------------------------|------------------|------------------|
| **Irrigation:** IRA samplings | | | | | | |
| 15/07 | S1 | 12,997 | 42.6 | 72.8 | 996 | 2448 |
| | S2 | >24,199 | 27.9 | >2419 | 3632 | 5568 |
| | S9 | >24,199 | 14.8 | >2419 | 5520 | 5216 |
| | S2 | >24,199 | 118.3 | >2419 | 6580 | 5200 |
| | S3 | >24,199 | 3.1 | 150 | 1740 | 640 |
| **Bathing:** IRA samplings | | | | | | |
| 15/07 | S6 | ND | 10 | 0 | 46 | 92 |
| | S7 | * | 0 | 0 | 14 | 21 |
| | S8 | * | 0 | 10 | 5 | 25 |
| 22/07 | S4 | ND | 5172 | 145 | 400 | 11,760 |
| | S5 | ND | 228 | 10 | 50 | <10 |
| | S6 | ND | 31 | 0 | 5 | 10 |
| | S10 | ND | 211 | 63 | 2300 | 440 |
| **Bathing:** ARPA samplings | | | | | | |
| 19/04 | AP1 | 8 | 14 | 6 | Enterococci (CFU/100 mL) | E. coli (CFU/100 mL) |
| | AP2 | 1 | 3 | 3 | Enterococci |
| | AP3 | 4 | 3 | 11 | Enterococci |
| 17/05 | AP1 | 0 | 0 | 0 | Enterococci |
| | AP2 | 0 | 0 | 0 | Enterococci |
| 14/06 | AP1 | 0 | 73 | 33 | Enterococci |
| | AP2 | 0 | 0 | 0 | Enterococci |
| 12/07 | AP1 | 0 | 0 | 0 | Enterococci |
| | AP2 | 0 | 0 | 0 | Enterococci |
| 13/08 | AP1 | 0 | 3 | 5 | Enterococci |
| | AP2 | 0 | 0 | 0 | Enterococci |
| 06/09 | AP1 | 0 | 18 | 17 | Enterococci |
| | AP2 | 0 | 1 | 0 | Enterococci |

*a* ND stands for “not detected” value due interference (false positive) by a high salinity causing not identifiable fecal coliforms with a simultaneous occurrence of non-coliform bacteria in the same water sample.
4. Discussion

The MCMC Bayesian analysis provided the probability distribution of the target concentrations by minimizing the required number (and related costs) of field water samplings since a small data set is the main weakness in QMRA. The probability was then used for the prediction of STEC/VTEC infections and diseases at the Forcatella MAR site shown in Table 7. Uniformly extending the Forcatella reclaimed water health risk to all 32 MAR sites of the Puglia region, we arrived at a cumulative risk probability of 11.4% to have 12.8 (i.e., 0.4 $/C_2$) diseases per year, with an individual probability of infection of 1.41$\times 10^{-3}$. This is a reasonable number when we assume the same quality of reclaimed water supplied for irrigation by all 32 regional MAR plants along with the managed

Table 5
Summary of the Monte Carlo statistics for each group of sampling stations: mean (mu), standard deviation (sigma), error, and 5%, 50% and 95% percentiles of the prior Gamma probability distribution.

| Data set          | cfc/100 mL Percentiles | Iterations |
|-------------------|------------------------|------------|
|                   | Mean (mu)   | SD (sigma) | MC error | 5%      | 50%     | 95%     |               |
| Irrigation Group  | lambda$^*$  | 64.30      | 30.37    | 0.052   | 26.35   | 58.4    | 120.3   | 330,000 |
|                   | mu         | 67.51      | 17.52    | 0.092   | 37.66   | 68.12   | 95.08   |          |
|                   | rho        | 1.29       | 0.69     | 0.001   | 0.435   | 1.155   | 2.609   |          |
|                   | sigma      | 63.58      | 17.00    | 0.042   | 37.56   | 62.38   | 93.23   |          |
|                   | tau        | 0.02       | 0.01     | 0.000   | 0.008   | 0.017   | 0.038   |          |
| Bathing Group     | lambda     | 296.6      | 36.87    | 0.179   | 241.1   | 293.9   | 361.1   | 35,000  |
|                   | mu         | 30.65      | 4.01     | 0.033   | 24.14   | 30.63   | 37.22   |          |
|                   | rho        | 0.11       | 0.02     | 0.000   | 0.070   | 0.104   | 0.149   |          |
|                   | sigma      | 94.7       | 4.98     | 0.053   | 85.23   | 96.07   | 99.71   |          |
| tau               | 0.00       | 0.00       | 0.00     | 0.00    | 0.00    | 0.00    |          |          |

$^*$ Mean (mu) and standard deviation (sigma) are the parameters of the distribution of the measurements, whereas lambda and tau are two transformation variables of mu and sigma (see Appendix).

Table 4
Summary of MCMC statistics obtained by OpenBUGS: mean (mu), standard deviation (sigma), error, and 5%, 50% and 95% percentiles of the posterior (Poisson) probability distribution for each sampling point. Selected E. coli concentrations, representing the 5 and 50% percentiles for the infection probability estimate, are circled.

| Count #ID | Mean (mu) | SD (sigma) | MC error | 5%     | 50%     | 95%     | No. of iterations |
|-----------|-----------|------------|----------|--------|---------|---------|------------------|
| Irrigation Group | X[1]      | 42.58      | 6.525    | 0.01231| 32.0    | 42.0    | 54.0             | 330,000 |
|           | X[2]      | 27.91      | 5.279    | 0.00938| 20.0    | 28.0    | 37.0             |          |
|           | X[3]      | 14.8       | 3.849    | 0.00674| 9.0     | 15.0    | 21.0             |          |
|           | X[4]      | 209.8      | 14.48    | 0.02398| 186.0   | 210.0   | 234.0            |          |
|           | X[5]      | 118.3      | 10.9     | 0.01969| 101.0   | 118.0   | 137.0            |          |
| Bathing Group | X[1]      | 10.0       | 3.161    | 0.01553| 5.0     | 10.0    | 15.0             | 35,000  |
|           | X[2]      | 7.143$\times 10^{-4}$ | 0.02672| 1.392$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[3]      | 6.857$\times 10^{-4}$ | 0.02618| 1.309$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[4]      | 5172.0     | 71.75    | 0.3686  | 5054.0  | 5172.0  | 5291.0           |          |
|           | X[5]      | 228.0      | 15.06    | 0.07905 | 203.0   | 228.0   | 253.0            |          |
|           | X[6]      | 31.01      | 5.599    | 0.02805 | 22.0    | 31.0    | 41.0             |          |
|           | X[7]      | 21.0       | 14.57    | 0.07753 | 187.0   | 211.0   | 235.0            |          |
|           | X[8]      | 7.998      | 2.834    | 0.01559 | 4.0     | 8.0     | 13.0             |          |
|           | X[9]      | 8.571$\times 10^{-4}$ | 0.02926| 1.548$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[10]     | 0.001229   | 0.03503  | 1.924$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[11]     | 0.0001029  | 0.03205  | 1.79$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[12]     | 0.0001067  | 0.0326   | 1.612$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[13]     | 6.027      | 2.441    | 0.01381 | 2.0     | 6.0     | 10.0             |          |
|           | X[14]     | 14.02      | 3.749    | 0.02114 | 8.0     | 14.0    | 20.0             |          |
|           | X[15]     | 7.143$\times 10^{-4}$ | 0.02672| 1.393$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[16]     | 4.067      | 1.941    | 0.0107  | 1.0     | 4.0     | 8.0              |          |
|           | X[17]     | 0.0001086  | 0.03379  | 1.725$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[18]     | 3.159      | 1.628    | 0.00915 | 1.0     | 3.0     | 6.0              |          |
|           | X[19]     | 17.98      | 4.257    | 0.02374 | 11.0    | 18.0    | 25.0             |          |
|           | X[20]     | 6.002      | 2.435    | 0.01259 | 2.0     | 6.0     | 10.0             |          |
|           | X[21]     | 0.001114   | 0.03336  | 1.786$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[22]     | 5.038      | 2.199    | 0.01125 | 2.0     | 5.0     | 9.0              |          |
|           | X[23]     | 9.714$\times 10^{-4}$ | 0.03115| 1.713$\times 10^{-4}$ | 0.0 | 0.0 | 0.0 |
|           | X[24]     | 5.026      | 2.213    | 0.01668 | 2.0     | 5.0     | 9.0              |          |
|           | X[25]     | 16.97      | 4.117    | 0.02064 | 11.0    | 17.0    | 24.0             |          |
recharge scheme and, groundwater flow. Uniform conditions in Puglia are quite realistic because almost all MAR plants are managed by the regional water utility (AQP S.p.A, www.aqp.it), and groundwater flows into the same karstic fractured limestone. To check the consistency of the results, we made a comparison between the estimated diseases and the recorded cases in Puglia in 2018 shown in Table 7. The comparison of regional diseases presented in this table shows that the total predicted, e.g., the most probable number of diseases (12.8 or 9.6, when the maximum E. coli concentration was at 10/100 mL) with an occurrence of 11.4% or an expected count of roughly 1.46 diseases per year, is plausible in comparison with the total of 27 STEC/VTEC recorded cases in 2018.

QMRA for polluted seawater during bathing provided a cumulative risk of 0.04 diseases due to STEC/VTEC infection with a

Table 6
Percentiles of 5%, 50%, and 95% of the Poisson distributed E. coli counts at the target that might be incidentally exposed to hosts and the related probability of occurrence.

|                | 5%          | 50%         | 95%          |
|----------------|-------------|-------------|--------------|
| Irrigation     |             |             |              |
| d (12%)        | 0.67(0.37)  | 1.11(0.74)  | 1.57(1.11)   |
| dm (5%)        | 2.38        | 3.12        | 4.01         |
| Bathing        |             |             |              |
| d (25%)        | 1.0         | 3.0         | 6.0          |
| dm (8%)        | 22.0        | 31.0        | 41.0         |

* Estimated by considering 72 h of the natural E. coli inactivation rate of 0.33 d
-1.

* Values in parentheses are obtained by forcing the E. coli count in S2 and S9 to 10 CFU/100 mL, i.e., the limit of D. Lgs 185/2003 regulation for water reuse in irrigation.

Table 7
Results of the cumulative risk of disease of the exposed population at the MAR sites to STEC/VTEC infections and occurrence probabilities. Median, 5% and 95% percentiles of infectious doses of pathogenic E. coli O157:H7 and O26:H11, and corresponding β-Poisson probabilities P and P<sub>exp</sub>. The latter infection probability accounts for the duration of the exposure of a host.

|                | 5%          | 50%         | 95%          |
|----------------|-------------|-------------|--------------|
| Bathing        |             |             |              |
| ld (25%)       | 0.2         | 0.5         | 1            |
| P(id)          | 2.2·10<sup>-3</sup> | 4.9·10<sup>-3</sup> | 9.0·10<sup>-3</sup> |
| ld<sub>5</sub> (8%) | 3.7         | 5.3         | 7.0          |
| P(id<sub>5</sub>) | 3.2·10<sup>-2</sup> | 4.4·10<sup>-2</sup> | 5.7·10<sup>-2</sup> |
| P<sub>exp</sub> (id) | 1.5·10<sup>-2</sup> | 3.3·10<sup>-2</sup> | 6.1·10<sup>-2</sup> |
| Diseases       | 0.04        | 0.08        | 0.015        |
| P<sub>exp</sub> (id<sub>5</sub>) | 2.0·10<sup>-1</sup> | 2.7·10<sup>-1</sup> | 3.3·10<sup>-1</sup> |
| Diseases       | 0.5         | 0.6         | 0.8          |

|                | 5%          | 50%         | 95%          |
|----------------|-------------|-------------|--------------|
| Irrigation     |             |             |              |
| Id (12%)       | 0.11 (0.06) | 0.19 (0.13) | 0.27 (0.19)  |
| P(id)          | 1.4(0.97)·10<sup>-3</sup> | 2.1(1.59)·10<sup>-3</sup> | 2.8(2.12)·10<sup>-3</sup> |
| ld<sub>5</sub> (5%) | 0.40        | 0.53        | 0.68         |
| P(id<sub>5</sub>) | 0.4·10<sup>-2</sup> | 0.5·10<sup>-2</sup> | 0.6·10<sup>-2</sup> |
| P<sub>exp</sub> (id) | 1.7·10<sup>-1</sup> | 2.1·10<sup>-1</sup> | 2.6·10<sup>-1</sup> |
| Diseases       | 0.4 (0.3)*  | 0.7 (0.4)   | 0.9 (0.5)    |
| P<sub>exp</sub> (id<sub>5</sub>) | 2.0·10<sup>-1</sup> | 3.2·10<sup>-1</sup> | 4.0·10<sup>-1</sup> |
| Diseases       | 1.0         | 1.2         | 1.5          |

|                |              |                  |
|----------------|--------------|------------------|
| Puglia HUS during 2018 | Puglia 2018 STEC/VTEC | Probable diseases from 32 MAR sites of the Puglia region |
| Probability of occurrence per percentile | 11.4% | 6.0% | 0.6% |

*In parentheses estimations of diseases are reported when the maximum count of 10 CFU/100 mL of E. coli is fixed for water irrigation supply.

*Estimated by the mean incidence (0.44·10<sup>-5</sup> per resident) of notified HUS in Italy between 1988 and 2018 (http://old.iss.it/seu/).

*Estimated multiplying HUS (~17.8) by the Italian STEC-VTEC/HUS mean ratio (1.51) between 2007 and 2018 (ECDC).
The disease burden at the beaches with polluted seawater provided in 2018 a DALY of \(2.0 \times 10^{-7}\), due to the incidental ingestion/inhalation of pathogenic \textit{E. coli} during bathing or swimming close to the wastewater discharge (S4) along the coast. Likewise, this DALY is below the tolerable level provided by the WHO. Nevertheless, since the illnesses caused by STEC/VTEC infections may increase with the number of untreated wastewater discharges along with the volumes and degree of pollution of wastewater and wastes coming from offshore, a higher disease burden is expected in the anthropic coastal areas of the region.

The results of QMRA highlight that a small increase in the \textit{E. coli} count concerning the regulation limit in irrigation can produce a health risk in 2018 with a tolerable DALY at MAR sites. At the investigated MAR site, the increase in the \textit{E. coli} concentration in reused water for irrigation from 10 CFU/100 mL to \(14.8-24.8\) CFU/100 mL (in S9) and 118.5 CFU/100 mL (in S2), led to a negligible risk increase from 0.3 to 0.4 diseases caused by STEC/VTEC infections per year. This is because the rate \((>0.33\text{ d}^{-1})\) of natural inactivation allows rapid decay of \textit{E. coli} during 72 h, i.e., the minimum time between the last irrigation and the ingestion of contaminated raw crops by hosts. Hence, an increase in the \textit{E. coli} regulation limit of 10 could reduce the operating costs at MAR sites.

Finally, the sampling results of this work and the data provided by the water utility show that the quality of reclaimed water is affected by drought periods. In fact, reclaimed water from MAR had a lower quality after many days without rainfalls as compared with that sampled during the rainy day. Indeed, droughts are the main issues affecting the reclaimed water quality of MAR sites in coastal areas, especially where touristic presence abruptly increases the flowrate of wastewater inflow to the treatment plants. Supplementary reclaimed water disinfection trains (Pecson et al., 2017; Ottoson et al., 2006) and QMRA should then provide further insights during droughts at MAR sites in semiarid regions by controlling wastewater treatment requirements to avoid public health risks. This could be achieved by monitoring the possible occurrence of protozoa and enteric viruses in reclaimed water during droughts by means of pathogen indicators, such as spores of \textit{C. perfringens} and somatic coliphages, respectively, which are more persistent than \textit{E. coli} to the water chlorination treatments (Masciopinto et al., 2007). Since the laboratory methods to detect the above indicators are very inexpensive they should be mandatory in semiarid regions and included in current regulations for water reuse in Italy (D. Lgs 185/03) and Europe. These indicators would greatly reduce the possible risk of infection from \textit{protozoa} and/or enteric viruses under critical operating conditions of wastewater treatment plants. QMRA stress-tests could then determine, for instance, the most critical conditions requiring extra disinfection treatments. Conversely, supplementary QMRA stress-test results could define possible disease burdens caused by enteric virus ingestion as a result of wastewater irrigation (Mok et al., 2014) and groundwater pollution by managed recharge. In QMRA stress-tests the exposure assessment should consider that the upper limit of the survival time of viruses in fractures has been estimated to be 15 months (460 days) in groundwater (Borchardt et al., 2007; Nevezcherya et al., 2005) and that neither somatic coliphages nor \textit{C. perfringens} can emulate viruses or protozoa removal in wastewater. Indeed, Gerba and Betancourt (2017) showed that most of the enteric viruses in polluted water samples appear in aggregated forms to increase their survival or resistance to the water disinfection treatments.

Furthermore, during droughts the quality of reclaimed water should be improved at MAR sites of coastal areas by, for instance, eliminating potential seawater infiltrations into sewage collectors. Moreover, a dilution of reclaimed water collected into recharge basins using treated rainfall-runoff outflows (Schijven et al., 2011) may improve water quality for managed recharge by providing a more efficient action to control seawater intrusion in coastal aquifers due to the subsequent increase of the recharge flowrate. Additionally, an increase in freshwater depth in the recharge basins can reduce eutrophication.

5. Conclusions

The merits of the QMRA approach discussed in this paper, combined with the groundwater flow and transport modeling output, lie in the possibility to forecast microbial water concentrations, based on the probabilistic model-based analysis of the water quality obtained in field measurements. This approach is suitable to provide a comprehensive set of information. The groundwater flow and pathogen transport model allowed us to properly select the locations for seawater sampling by ensuring that pathogenic \textit{E. coli} cannot affect the quality of seawater at batches, whereas the Bayesian MCMC minimized the number (and related costs) of field water samples required to define the Poisson distributed target concentrations.

The risk assessment provided in 2018 a tolerable disease burden corresponding to a DALY of \(4.98 \times 10^{-7}\) with a probability of 11.4% of the cumulative risk of disease occurrence during MAR operations. The DALY was instead 2.0 \(\times 10^{-7}\) (23.8% of occurrence) during bathing at Forcatella’s beaches due to the ingestion/inhalation of pathogenic \textit{E. coli} in the vicinity of wastewater discharges to the sea.

The uncertainty of the MCMC results ranged from 3.8% for irrigation to 1.6% for bathing risk estimations and it was inversely correlated with the number of samplings.

The results of QMRA highlight that even if the \textit{E. coli} concentrations exceeded the regulation limit of 10 CFU/100 mL in irrigation water by 5.3 times, the health risk was yet tolerable, due to the rapid natural \textit{E. coli} inactivation during the elapsed time (>72 h) from the last irrigation before harvesting and the ingestion of raw vegetables and fruits. The potential increase in the regulation limit could reduce wastewater treatment costs.

However, droughts have been demonstrated to be the main problem affecting the water quality of the effluent from MAR sites in coastal areas of semiarid regions. Here, supplementary reclaimed water treatments for water disinfection and QMRA stress-tests of infections from the ingestion of protozoa and enteric viruses are recommended during the critical operating conditions of wastewater treatment plants to improve the efficacy of MAR practices and ensure tolerable public health risks.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

The MCMC Bayesian analysis was performed using the OpenBUGS open-source software (v. 3.2.3, rev 1012, http://www.openbugs.net/) using the source code:
model {
  # Prior on log(mu U(-1,2) and logsigma U(-1,2)
  logmu ~ dunif(-1,2)
  logsigma ~ dunif(-1,2)
  mu <- pow(10,logmu)
  sigma <- pow(10,logsigma)
  rho <- mu * mu / (sigma * sigma)
  lambda <- sigma * sigma / mu
  tau <- 1/lambda
  # Model for temporal concentration variability
  for (i in 1:M) {
    C[i] ~ dgamma(rho,tau)
  }
  # OpenBUGS uses reciprocal of scale parameter (tau=1/lambda)
}
# Model with Poisson-distributed counts
# for (i in 1:N) {
  theta[i] ~ dlnorm(V[i])
  X[i] ~ dpois(theta[i])
#}
}

The results yield the *E. coli* Poisson posterior probability, or the kernel density, at each sampling station based on Gamma’s prior probability, which was obtained by fitting the sampled concentrations given by Monte Carlo elaborations.

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