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 Techonologies, 3020 Gore Rd, London, ON N5V 4T7, Canada

ARTICLE INFO

ABSTRACT

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*.

© 2020 Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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 limits the *E. coli* count to 10 CFU/100 mL in at least 80% of monitored water samples where only one sample at an *E. coli* count of 100 CFU/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 flowrate to control the seawater intrusion and groundwater overexploitation 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 m^3. 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 m^2 (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

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: D. Lgs. 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⁻)			0.01**	< 0.4±0.01	**< 0.4±0.01**
Specific conductivity (mS/cm)	3.0	2.5	2.5	**3.5±0.4**	**3.5±0.4**
Chloride mg/L (as Cl⁻)	250	250		**334±4**	**389±5**
Fluoride mg/L (as F⁻)	1.5	1.5	1.5	0.61±.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			0	1,000±190	0
E. coli: mode,median,95% percentile (mean)	10 in 80% (*and* 10 in 90% of samples)		0	10, 5, 16 (7.6)	0
Enterococci			0	0	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).
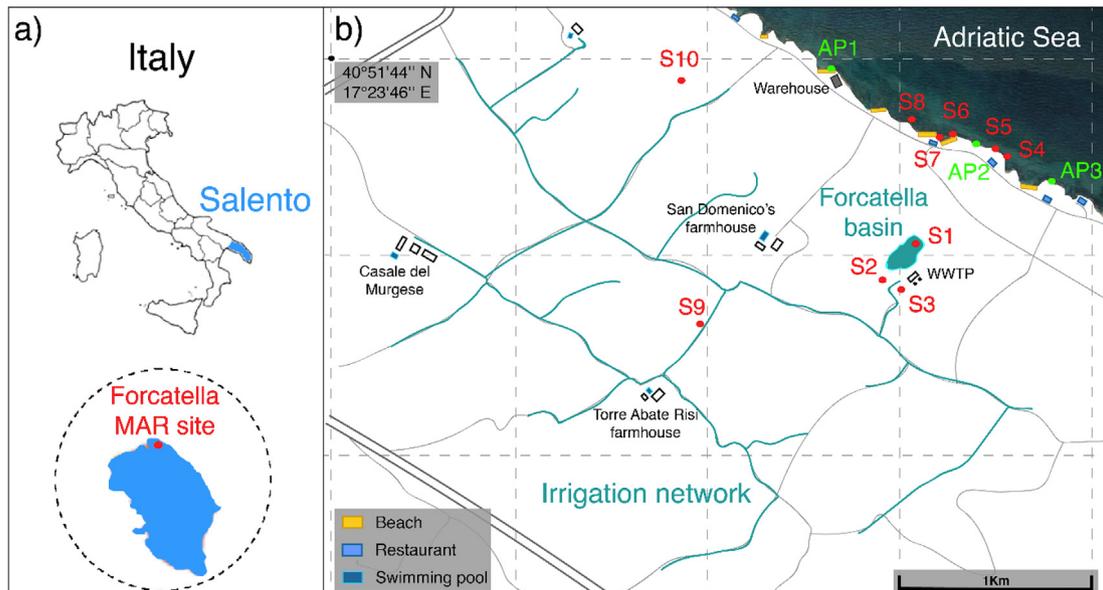

Fig. 1. Overview of the sampling stations: a) map of the study area; b) positions of the sampling stations of IRSA (from S1 to S10) and ARPA (from AP1 to AP3), and irrigation pipelines (blue lines).

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
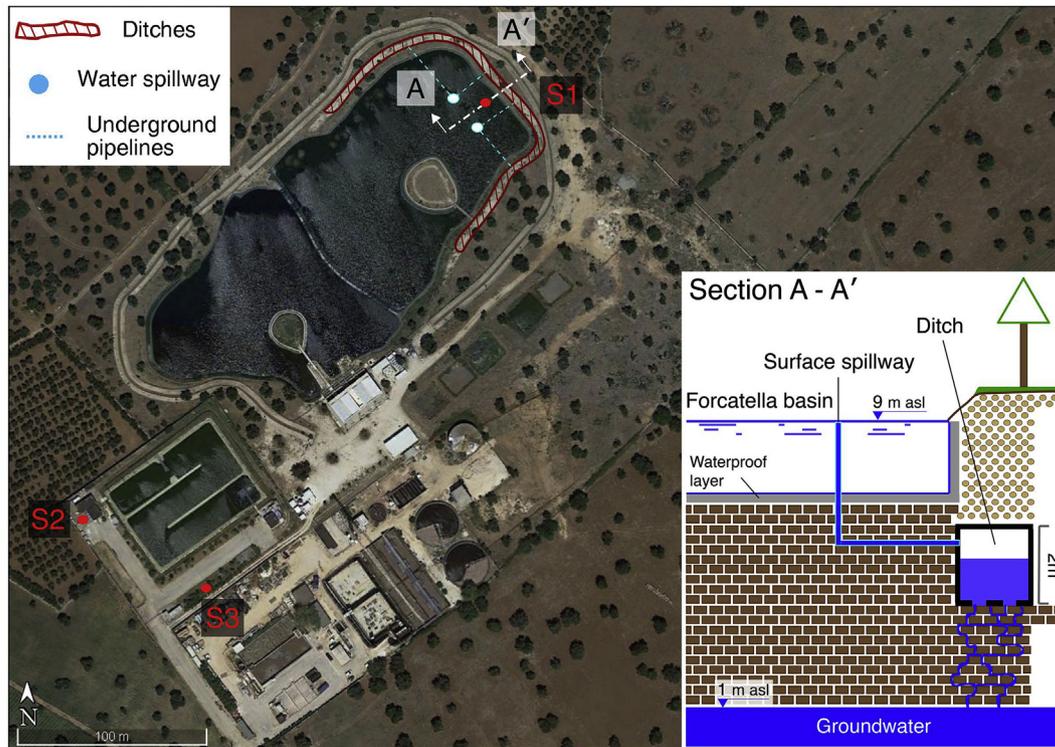
Fig. 2. Forcatella MAR plant in the coastal area (southern Italy).

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 trough 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 (NH_4^+), nitrites (NO_2^-), nitrates (NO_3^-), and total nitrogen (TN) in the sampled water.
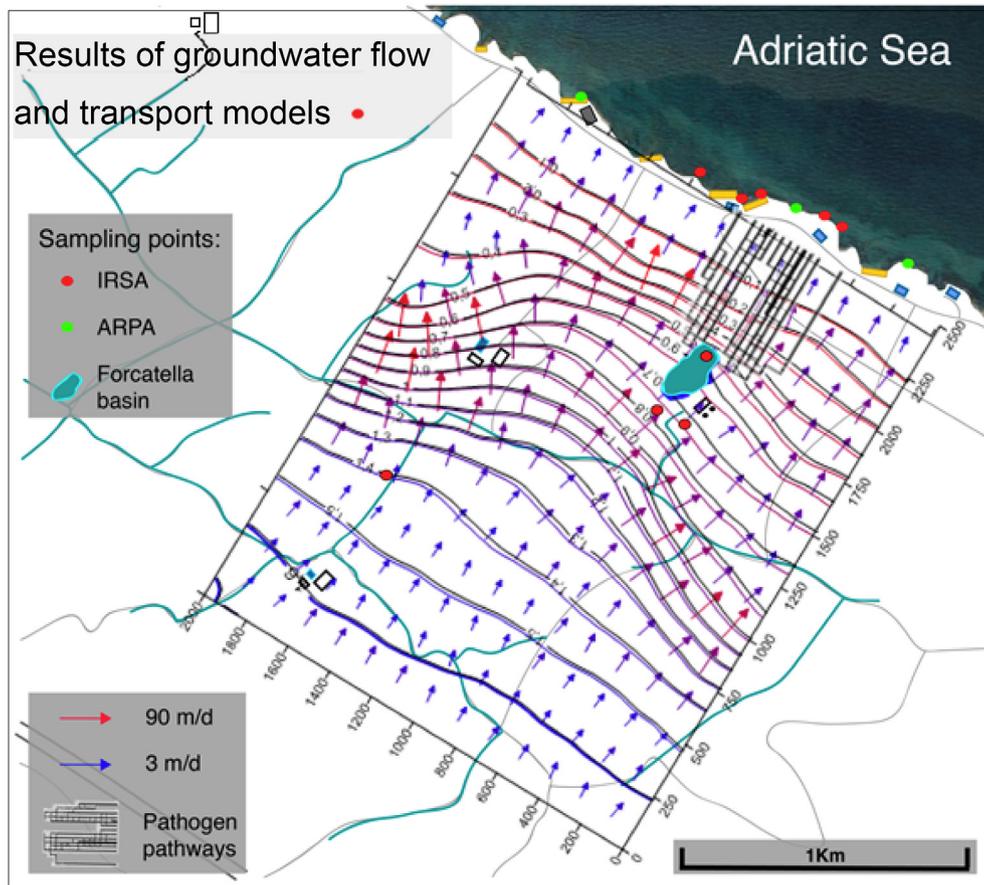
Fig. 3. Simulation results showing the flow velocity vectors and *E. coli* pathways (solid black lines) or plumes, during recharge operations at the Forcatella MAR plant. The results show piezometric surface heads under both *natural* (red contour lines) and *recharge* groundwater flow conditions (blue contour lines).
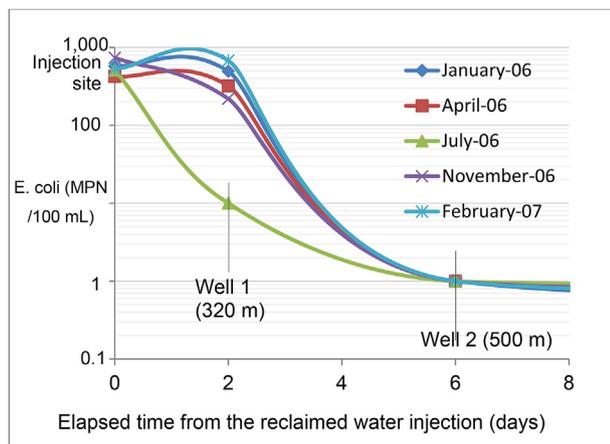

Fig. 4. Efficacy of soil aquifer treatment (SAT) on the *E. coli* removal from wastewater during filtration in fractured limestone aquifers.

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-ᴅ-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 (Fig. 5) 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

$$\text{No. of HUS} = 16.3 \cdot exp[0.04(year - 1988)] \qquad (1)$$

$$\text{No. of STEC / VTEC} = 3.1 \cdot exp[0.10(year - 1988)] \qquad (1a)$$

In this work, we applied the above equation to estimate the STEC/VTEC infections in the region during 2018, to have a practical verification of the QMRA results against recorded infections. In particular, 16 (Tota et al., 2013) of the 22 HUS notified cases during

the 2013 outbreak in Puglia during summer involved, children on the Adriatic Sea coast of Puglia, where high flowrates of secondary municipal effluents are discharged into the sea. This severe HUS outbreak led to 17 diseases in about 3 weeks (Germinario et al., 2016) affecting mainly children ranging from 11 months to 15 years (but no adults), and causing the death of a 13-months old child infected by close relatives via secondary morbidity due to the infection. Trace-back investigations were mainly focused on contaminated food from dairy products and raw vegetables.

In Europe, only 5% of diseases due to STEC/VTEC infections (411 on 8161 reported cases) were classified as HUS diseases during 2018, while this percentage was 15 times higher in Italy. Here, 77% (56 out of 73 cases) of the diseases due to STEC/VTEC infections were classified as HUS disorders, with the highest number of these occurring in southern Italy. The notified HUS cases in the Puglia region during 2018 (ISS, 2020) were 13 out of the total of 60 in Italy. A little child aged 16 months died in this region in 2017, as a result of being affected by HUS disorder.

In the QMRA of this study, the fraction of pathogenic serotypes − *E. coli* O157:H7 and O26:H11 − involved in STEC/VTEC infection, was assumed to be 17%, on average, of the total *E. coli* count, as suggested in the relevant literature (Soller et al., 2010; Truchado et al., 2018). Hence, the *E. coli* values derived from sample measurements and the MCMC were reduced to 17% to obtain id and id_m of the dose-infection probability model. The infection calculations were carried out by selecting the appropriate α and β coefficients of the individual β−Poisson probability of the infection model as a result of pathogenic *E. coli* ingestion by contaminated food or water (Teunis et al., 2004, 2008). In this dose-response infection model, we consider disaggregated pathogenic *E. coli* Poisson distributed in every single infectious dose exposed to a host, to have

$$P = 1 - \int_0^1 \left(\frac{\Gamma(\alpha + \beta)}{\Gamma(\alpha) \cdot \Gamma(\beta)} \cdot r^{\alpha-1} \cdot (1 - r)^{\beta-1} \right) \cdot e^{-\ r \cdot id} dr \qquad (2)$$

where r (−) is the single-hit value of the beta probability of the infection of each virus ingested (i.e., host susceptibility), $\Gamma()$ is the gamma function, and id or id_m 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 −*Stx2* and/or −*Stx1* and 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.
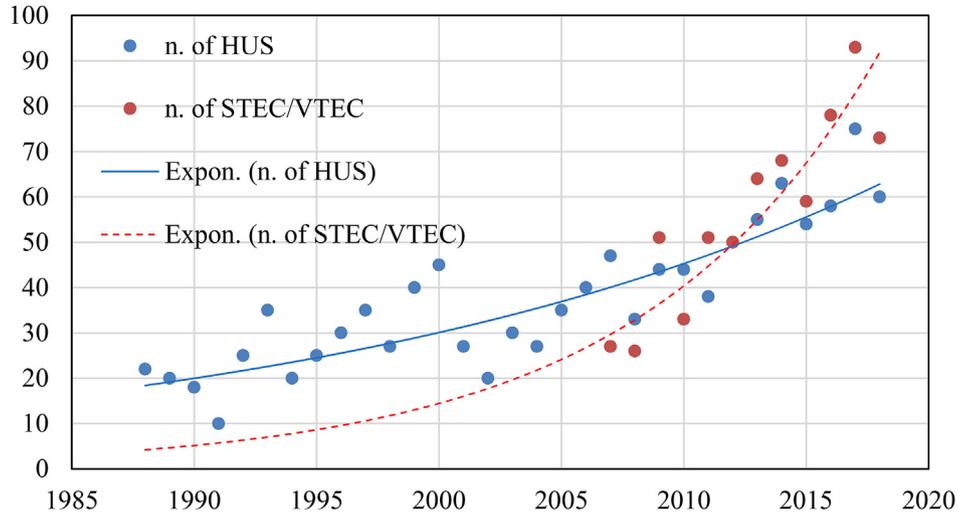
Fig. 5. Number of notified HUS (blue circles) illnesses per year in Italy from 1988 to 2018 recorded by the Italian *Istituto Superiore di Sanità* (ISS, 2020) and STEC/VTEC by ECDC (brown circles), and best-fit trends in this work.
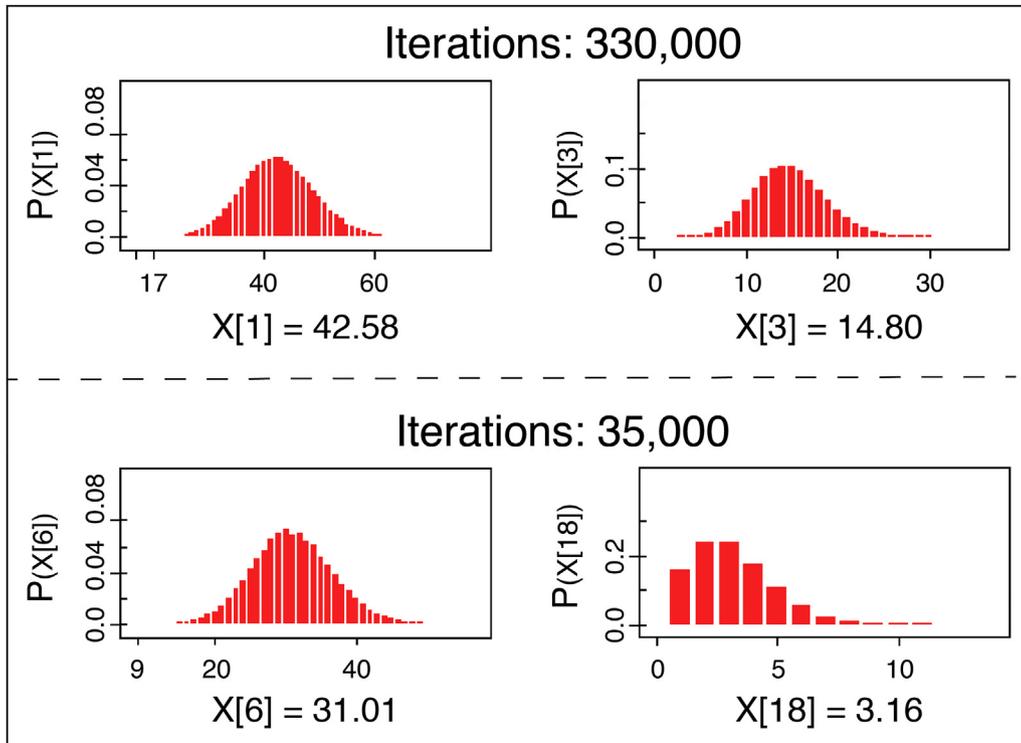
Fig. 6. Posterior Poisson density at selected target samplings for irrigation (upper) and bathing (below) groups; *E. coli* counts in X[3] and X[18] samples had the highest (i.e., the most frequent) posterior probability of 12% and 25%, respectively, whereas *E. coli* counts in X[1] and X[6] were closest to the mean value (5 and 8% of probability) of the Gamma prior probability distributions.

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 $N-NH_4^+$) 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

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 O_2. 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 Open-BUGS 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 3.16 (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 × 20 mL corresponding 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 × 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, 10 and 15 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, **d_m**, that might be incidentally exposed to hosts via irrigation and bathing.

3.3. MAR infections

To determine the infectious dose *id* and *id_m* required for QMRA, the *d* and *d_m* 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 (mS/cm)	COD[a] (mg/L O_2)	NH_4^+ (mg/L N–NH_4)	NO_2– (mg/L N–NO_2)	NO_3– (mg/L N–NO_3)	TN (mg/L N)
July 15, 2019 (rainy day)								
S1	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
July 22, 2019 (drought period)								
S1	26.0	7.13	3.38	44.9	**7.65**	0.465	0.485	8.6
S2	–	7.24	3.39	55.1	**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	–	7.90	>20.00	NA	5.7	NA	NA	>5.7
S5	–	7.83	>20.00	NA	0.26	NA	NA	>0.26
S6	–	7.70	>20.00	NA	0.15	NA	NA	>0.15
S10	20.5	6.80	15.12	33.2	0.18	0.016	1.632	1.83

**NA stands for not analyzed due interference by high water salinity.
[a] The COD, here, represents the concentration of dissolved organic compounds in the filtered sampled water.

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: *IRSA 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
22/07	**S1**	>24,199	209.8	>2419	3390	3320
	S2	>24,199	118.3	>2419	6580	5200
	S3	>24,199	3.1	150	1740	640
Bathing: *IRSA samplings*						
15/07	**S6**	ND	10	0	46	92
	S7	"	0	0	14	21
	S8	"	0	10	5	25
22/07	**S4**	"	5172	145	400	11,760
	S5	"	228	10	50	<10
	S6	"	31	0	5	10
	S10	ND[a]	211	63	2300	440
	Intruded well					
Bathing: *ARPA samplings*						
	AP1	**AP2**	**AP3**			
19/04	8	14	6			Enterococci (CFU/100 mL)
	1	3	3			E. coli (CFU/100 mL)
17/05	0	0	0			Enterococci
	4	3	11			E. coli
14/06	0	4	5			Enterococci
	0	73	33			E. coli
12/07	0	0	0			Enterococci
	0	0	0			E. coli
13/08	0	3	5			Enterococci
	0	0	0			E. coli
06/09	6	18	17			Enterococci
	0	1	0			E. coli

[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.

The predicted individual infection probability given by Eq. (2) is shown in Table 7. The probability of infection was obtained selecting $\alpha = 0.44$ and $\beta = 49$ which are close to the α (0.38) and β (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 β–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) since 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 $4.98 \cdot 10^{-7}$ DALY in 2018 using the conversion factor of $54.7 \cdot 10^{-3}$ 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 4
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[a]	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	0.000	0.00	0.00	0.00	

[a] 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).

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., 0.4×32) diseases per year, with an individual probability of infection of $1.41 \cdot 10^{-3}$. 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 MCMC statistics obtained by OpenBUGS: mean and standard deviation, of the posterior (Poisson) probability distribution for each sampling; Monte Carlo errors; and corresponding percentiles, i.e., 5%, 50 and 95% of the *E. coli* count with a Poisson distribution at the sampling points. Selected *E. coli* concentrations, representing the 5 and 50% percentiles for the infection probability estimate, are circled.

	Count #ID	Mean	SD	MC error	Percentiles			No. of iterations
					5.0%	50.0%	95.0%	
Irrigation Group	**X[1]**	42. 58 →	6.525	0.01231	**32.0**	**42.0**	**54.0**	330,000
	X[2]	27.91	5.279	0.009384	20.0	28.0	37.0	
	X[3]	14.8 →	3.849	0.006742	**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]	$7.143 \cdot 10^{-4}$	0.02672	$1.392 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[3]	$6.857 \cdot 10^{-4}$	0.02618	$1.309 \cdot 10^{-4}$	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]	211.0	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]	$8.571 \cdot 10^{-4}$	0.02926	$1.548 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[10]	0.001229	0.03503	$1.924 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[11]	0.001029	0.03205	$1.79 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[12]	0.001057	0.0325	$1.612 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[13]	6.027	2.441	0.01381	2.0	6.0	10.0	
	X[14]	14.02	3.744	0.02114	8.0	14.0	20.0	
	X[15]	$7.143 \cdot 10^{-4}$	0.02672	$1.393 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[16]	4.067	1.941	0.0107	1.0	4.0	8.0	
	X[17]	0.001086	0.03379	$1.725 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[18]	3.159 →	1.628	0.009153	**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	0.01258	2.0	6.0	10.0	
	X[21]	0.001114	0.03336	$1.786 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[22]	5.038	2.199	0.01125	2.0	5.0	9.0	
	X[23]	$9.714 \cdot 10^{-4}$	0.03115	$1.713 \cdot 10^{-4}$	0.0	0.0	0.0	
	X[24]	5.025	2.213	0.01168	2.0	5.0	9.0	
	X[25]	16.97	4.117	0.02064	11.0	17.0	24.0	

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.

Irrigation[a]			
	5%	**50%**	**95%**
d(12%)	0.67(0.37)[b]	1.11(0.74)	1.57(1.11)
d_m *(5%)*	2.38	3.12	4.01
Bathing			
d (25%)	1.0	3.0	6.0
d_m *(8%)*	22.0	31.0	41.0

[a] Estimated by considering 72 h of the natural *E. coli* inactivation rate of 0.33 d^{-1}.
[b] 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.

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 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_{exp}. The latter infection probability accounts for the duration of the exposure of a host.

	Bathing		
	5%	**50%**	**95%**
Id (25%)	0.2	0.5	1
P(id)	$2.2 \cdot 10^{-3}$	$4.9 \cdot 10^{-3}$	$9.0 \cdot 10^{-3}$
Id_m (8%)	3.7	5.3	7.0
P(id_m)	$3.2 \cdot 10^{-2}$	$4.4 \cdot 10^{-2}$	$5.7 \cdot 10^{-2}$
P_{exp} (id)	$1.53 \cdot 10^{-2}$	$3.38 \cdot 10^{-2}$	$6.13 \cdot 10^{-2}$
Diseases	**0.04**	**0.08**	**0.015**
P_{exp} (id_m)	$2.04 \cdot 10^{-1}$	$2.70 \cdot 10^{-1}$	$3.37 \cdot 10^{-1}$
Diseases	**0.5**	**0.6**	**0.8**
	Irrigation		
Id(12%)	0.11 (0.06)	0.19 (0.13)	0.27 (0.19)
P(id)	$1.41(0.97) \cdot 10^{-3}$	$2.12(1.59) \cdot 10^{-3}$	$2.83(2.12) \cdot 10^{-3}$
Id_m (5%)	0.40	0.53	0.68
P(id_m)	$0.40 \cdot 10^{-2}$	$0.51 \cdot 10^{-2}$	$0.64 \cdot 10^{-2}$
P_{exp}(id)	$1.75 \cdot 10^{-1}$	$2.18 \cdot 10^{-1}$	$2.65 \cdot 10^{-1}$
Diseases	**0.4 (0.3)***	**0.7 (0.4)**	**0.9 (0.5)**
P_{exp}(id_m)	$2.02 \cdot 10^{-1}$	$3.20 \cdot 10^{-1}$	$4.09 \cdot 10^{-1}$
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%	
17.8° 13 (notified)	**27.01°°**	**12.8 (9.6) 22.4 (12.8) 28.8 (19.2)**	

*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 ($0.44 \cdot 10^{-5}$ 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).

probability of 23.8% (5% percentile) in 2018, with an uncertainty of 1.6%.

The disease burden at the beaches with polluted seawater provided in 2018 a DALY of $2.0 \cdot 10^{-7}$, due to the incidental ingestion/inhalation of pathogenic *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 *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 *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 d^{-1}) of natural inactivation allows rapid decay of *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 *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 C. perfringens* and somatic coliphages, respectively, which are more persistent than *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 *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; Nevecherya et al., 2005) and that neither somatic coliphages nor *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 *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 $4.98 \cdot 10^{-7}$ with a probability of 11.4% of the cumulative risk of disease occurrence during MAR operations. The DALY was instead $2.0 \cdot 10^{-7}$ (23.8% of occurrence) during bathing at Forcatella's beaches due to the ingestion/inhalation of pathogenic *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 *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 *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.

Appendix

The MCMC Bayesian analysis was performed using the Open-BUGS open-source software (v. 3.2.3, rev 1012, http://www.openbugs.net/) using the source code:

```
model {
        # Prior on logmu 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 (j in 1:n) {
                theta[j] <- C[I[j]]*V[j]
                X[j] ~ dpois(theta[j])
        }
}
```

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.

References

APHA-AWWA-WEF, 2005. Standard Methods for the Examination of Water and Wastewater, twenty-first ed. American Public Health Association, Washington.

Asano, T., Burton, F.L., Leverenz, H.L., Tsuchihashi, R., Tchobanoglous, G., 2007. Water Reuse— Issues, Technologies and Applications, first ed. McGraw-Hill, New York.

Ashbolt, N.J., Schoen, M.E., Soller, J.A., Roser, D.J., 2010. Predicting pathogen risks to aid beach management: the real value of quantitative microbial risk assessment (QMRA). Water Res. 44, 4692–4703. https://doi.org/10.1016/j.watres.2010.06.048.

Ayuso-Gabella, N., Page, D., Masciopinto, C., Aharoni, A., Salgot, M., Wintgens, T., 2011. Quantifying the effect of Managed Aquifer Recharge on the microbiological human health risks of irrigating crops with recycled water. Agric. Water Manag. https://doi.org/10.1016/j.agwat.2011.07.014.

Borchardt, M.A., Bradbury, K.R., Gotkowitz, M.B., Cherry, J.A., Parker, B.L., 2007. Human enteric viruses in groundwater from a confined bedrock aquifer. Environ. Sci. Technol. 41 (18), 6606–6612. https://doi.org/10.1021/es071110.

Chik, A.H.S., Schmidt, P.J., Emelko, M.B., 2018. Learning something from nothing: the critical importance of rethinking microbial non-detects. Front. Microbiol. 9, 2304. https://doi.org/10.3389/fmicb.2018.02304.

Dillon, P., Page, D., Vanderzalm, J., Pavelic, P., Toze, S., Bekele, E., et al., 2008. A critical evaluation of combined engineered and aquifer treatment systems in water recycling. Water Sci. Technol. 57 (5), 753–762.

ISTAT, 2020. Istituto Nazionale di Statistica, Rome (I); (Accessed March 12, 2020): http://demo.istat.it/strasa2019/index02.html.

Eckner, K.F., 1998. Comparison of membrane filtration and multiple-tube fermentation by the Colilert and Enterolert methods for detection of waterborne coliform bacteria, *Escherichia coli*, and Enterococci used in drinking and bathing water quality monitoring in Southern Sweden. Appl. Environ. Microbiol. 64 (8), 3079–3083. https://aem.asm.org/content/aem/64/8/3079.full.pdf.

Eregno, F.E., Tryland, I., Tjomsland, T., Myrmel, M., Robertsona, L., Heistad, 2016. Quantitative microbial risk assessment combined with hydrodynamic modelling to estimate the public health risk associated with bathing after rainfall events. Sci. Total Environ. 548–549, 270–279. https://doi.org/10.1016/j.scitotenv.2016.01.034.

EU Commission, 2018. Regulation of the European Parliament and of the Council on minimum requirements for water reuse, COM (2018) 337 final- Annexes. Brussels. Accessed on. https://ec.europa.eu/environment/water/pdf/water_reuse_regulation_annex.pdf. (Accessed 21 May 2020).

Franz, E., Tromp, S.O., Rijgersberg, H., van der Fels-Klerx, H.J., 2010. Quantitative microbial risk assessment for *Escherichia coli* O157:H7, *Salmonella* and Listeria monocytogenes in leafy green vegetables consumed at salad bars. J. Food Protect. 73 (2), 274–285.

Gerba, C.P., Betancourt, W.Q., 2017. Viral aggregation: impact on virus behavior in the environment. Environ. Sci. Technol. 51, 7318–7325. https://doi.org/10.1021/acs.est.6b05835.

Gerba, C.P., Goyal, S.M., 1985. Pathogen removal from wastewater during groundwater recharge. In: Chapter 9 in Artificial Recharge of Groundwater. Butterworth Publisher, London, UK, pp. 283–318.

Germinario, C., Caprioli, A., Giordano, M., Chironna, M., Gallone, M.S., Tafuri, S., Minelli, F., Maugliani, A., Michelacci, V., Santangelo, L., Mongelli, O., Montagna, C., Scavia, G., et al., 2016. Community-wide outbreak of hemolytic uremic syndrome associated with Shiga toxin 2-producing Escherichia coli O26:H11 in southern Italy, summer 2013. Euro Surveillance Report 21 (38), 1–9. https://doi.org/10.2807/1560-7917.ES.2016.21.38.303432.

Gibson, K.E., Schwab, K.J., 2011. Detection of bacterial indicators and human and bovine enteric viruses in surface water and groundwater sources potentially impacted by animal and human wastes in Lower Yakima Valley, Washington. Appl. Environ. Microbiol. 77, 355–362. https://doi.org/10.1128/AEM.01407-10.

Greening, H., Janicki, A., 2006. Toward reversal of eutrophic conditions in a subtropical estuary: water quality and seagrass response to nitrogen loading reductions in tampa bay, Florida, USA. Environ. Manag. 38 (2), 163–178. https://doi.org/10.1007/s00267-005-0079-4.

Haas, C.N., Rose, J.B., Gerba, C.P., 2014. Quantitative Microbial Risk Assessment, second ed. John Wiley & Sons, Inc., Hoboken, New Jersey. Published by.

Havelaar, A.H., Melse, J.M., 2003. Quantifying Public Health Risk in WHO Guidelines for Drinking-Water Quality – A Burden of Disease Approach. RIVM report 734301022/2003, p. 30. RIVM, P.O. Box 1, 3720 BA Bilthoven, The Netherland. https://www.who.int/water_sanitation_health/dwq/rivmrep.pdf.

Howard, G., Pedley, S., Tibatemwa, S., 2006. Quantitative microbial risk assessment to estimate health risks attributable to water supply: can the technique be applied in developing countries with limited data? J. Water Health (1), 49–64. https://doi.org/10.2166/wh.2005.058, 04.

Kouamé, P.K., Nguyen-Viet, H., Dongo, K., Zurbrügg, C., Biémi, J., Bonfoh, B., 2017. Microbiological risk infection assessment using QMRA in agriculture systems in Côte d'Ivoire, West Africa. Environ. Monit. Assess. 189–587. https://doi.org/10.1007/s10661-017-6279-6.

La Mantia, R., Masciopinto, C., Levantesi, C., Tandoi, V., 2008. Fate and transport of faecal contamination microbial indicators, pathogenic protozoa and Campylobacter in the artificially recharged fractured aquifer of Salento, Italy. Water Sci. Technol. 57 (6), 849–856. https://doi.org/10.2166/wst.2008.183.

Lapen, D., 2019. Using quantitative microbial risk assessment to explore waterborne pathogen infection risks associated with interventions of agricultural management practices. Abstracts/Int. J. Infect. Dis 79 (S1), 1–150. https://doi.org/10.1016/j.ijid.2018.11.187.

Lgs D. 185, 2003. In: *Italian*: Ministero dell'Ambiente e della Tutela del Territorio: Regolamento recante norme tecniche per il riutilizzo delle acque reflue in

attuazione dell'articolo 26, comma 2, del decreto legislativo 11 maggio 1999, n. 152, vol. 169. Gazzetta Ufficiale della Repubblica Italiana, pp. 10–13. https://www.gazzettaufficiale.it/eli/id/2003/07/23/003G0210/sg.

Ludwig, K., Sarkim, V., Bitzan, M., Karmali, M.A., Bobrowski, C., Ruder, H., Laufs, R., Sobottka, I., Petric, M., Karch, H., Müller-Wiefel, D.E., 2002. Shiga toxin-producing Escherichia coli infection and antibodies against Stx2 and Stx1 in household contacts of children with enteropathic hemolytic-uremic syndrome. J. Clin. Microbiol. 40 (5), 1773–1782. https://doi.org/10.1128/JCM.40.5.1773–1782.2002.

Maraccini, P.A., Catharine, M., Mattioli, M., Sassoubre, L.M., Cao, Y., Griffith, J.F., Ervin, J.S., van De Werfhorst, L.C., Boehm, A.B., 2016. Solar inactivation of enterococci and Escherichia coli in natural waters: effects of water absorbance and depth. Environ. Sci. Technol. 50, 5068–5076. https://doi.org/10.1021/acs.est.6b00505, 2016.

Masciopinto, C., Caputo, M.C., 2011. Modeling unsaturated–saturated flow and nickel transport in fractured rocks. Vadose Zone J. 10, 1045–1057. https://doi.org/10.2136/vzj2010.0087.

Masciopinto, C., Visino, F., 2017. Strong release of viruses in fracture flow in response to a perturbation in ionic strength: filtration/retention tests and modeling. Water Res. 126, 240–251. https://doi.org/10.1016/j.watres.2017.09.035, 2017.

Masciopinto, C., La Mantia, R., Carducci, A., Casini, B., Calvario, A., Jatta, E., 2007. Unsafe tap water in households supplied from groundwater in the Salento region of southern Italy. J. Water Health 5 (1), 129–148. https://doi.org/10.2166/wh.2006.054.

Masciopinto, C., La Mantia, R., Chrysikopoulos, C.V., 2008a. Fate and transport of pathogens in a fractured aquifer in the Salento area. Italy, Water Resour. Res. 44, W01404 https://doi.org/10.1029/2006WR005643.

Masciopinto, C., La Mantia, T., Tandoi, V., Levantesi, C., Divizia, M., Donia, D., Gabrieli, R., Petrinca, A., 2011. Analytical solution for the modeling of the natural time-dependent reduction of waterborne viruses injected into fractured aquifers. Environ. Sci. Technol. 45, 636–642. https://doi.org/10.1021/es102412z.

Masciopinto, C., De Giglio, O., Scrascia, M., Fortunato, F., La Rosa, G., Suffredini, E., Pazzani, C., Prato, R., Montagna, M.T., 2019. Human health risk assessment for the occurrence of enteric viruses in drinking water supplied from wells: role of flood runoff injections. Sci. Total Environ. 666, 559. https://doi.org/10.1016/j.scitotenv.2019.02.107, 2019.

Mc Bride, G.B., Stott, R., Miller, W., Bambic, D., Wuertz, S., 2013. Discharge-based QMRA for estimation of public health risks from exposure to storm waterborne pathogens in recreational waters in the United States. Water Res. 47, 5282–5297.

Mok, H.-F., Barker, S.F., Hamilton, A.J., 2004. A probabilistic quantitative microbial risk assessment model of norovirus disease burden from wastewater irrigation of vegetables in Shepparton, Australia. Water Res. 54, 347–362. https://doi.org/10.1016/j.watres.2014.01.060. Available at:

Nevecherya, I.K., Shestakov, V.M., Mazaev, V.T., Shlepnina, T.G., 2005. Survival rate of pathogenic bacteria and viruses. Groundwater Water Resources 32 (2), 209–214. Translated from Vodnye Resursy, 32(2), 2005, 232–237.

Ottoson, J., Hansena, A., Björleniusc, B., Norderd, H., Stenströma, T.A., 2006. Removal of viruses, parasitic protozoa and microbial indicators in conventional and membrane processes in a wastewater pilot plant. Water Res. 40, 1449–1457. https://doi.org/10.1016/j.watres.2006.01.039.

Pang, H., Lambertini, E., Buchanan, R.L., Schaffner, D.W., Pradhan, A.K., 2017. Quantitative microbial risk assessment for Escherichia coli O157:H7 in fresh-cut lettuce. J. Food Protect. 80 (2), 302–311. https://doi.org/10.4315/0362-028X.JFP-16-246.

Pecson, B.M., Triolo, S.C., Olivieri, S., Chen, E.C., Pisarenko, A.N., Yang, C.-C., Olivieri, A., Haas, C.N., Trussell, R.S., Trussell, R.R., 2017. Reliability of pathogen control in direct potable reuse: performance evaluation and QMRA of a full-scale 1 MGD advanced treatment train. Water Res. 122, 258–268. https://doi.org/10.1016/j.watres.2017.06.014.

Piras, F., Santoro, O., Pastore, T., Pio, I., De Dominicis, E., Gritti, E., Caricato, R., Lionetto, M.G., Mele, G., Santoro, D., 2020. Controlling micropollutants in tertiary municipal wastewater by O3/H2O2, granular biofiltration and UV254/H2O2 for potable reuse applications. Chemosphere 239. https://doi.org/10.1016/j.chemosphere.2019.124635. January 2020, 124635.

Portoghese, I., Demichele, F., Fidelibus, D., Vurro, M., 2019. Atlas of infiltration ponds for treated wastewater disposal in the Apulia region: suitable indicators of potential environmental threats and benefits. In: Poster, Int. Symposium on Managed Aquifer Recharge. ISMAR10, Madrid. May 2019.

Schets, F.M., Schijven, J.F., de Roda Husman, A.M., 2011. Exposure assessment for swimmers in bathing waters and swimming pools. Water Res. 45, 2392–2400. https://doi.org/10.1016/j.watres.2011.01.025.

Schijven, J.F., Teunis, P.P.M., Rutjes, S.A., Bouwknegt, M., Husman, A.M.D., 2011. QMRAspot: a tool for quantitative microbial risk assessment from surface water to potable water. Water Res. 45 (17), 5564–5576. https://doi.org/10.1016/j.watres.2011.08.024.

Schmidt, P.J., Pintar, K.D.M., Fazil, A.M., Topp, E., 2013. Harnessing the Theoretical Foundations of the Exponential and Beta-Poisson Dose-Response Models to Quantify Parameter Uncertainty Using Markov Chain Monte Carlo. https://doi.org/10.1111/risa.12006.

Schmidt, P.J., Emelko, M.B., Thompson, M.E., 2019. Recognizing structural non-identifiability: when Experiments do not provide information about important parameters and misleading models can still have great fit. Risk Anal. https://doi.org/10.1111/risa.13386, 0 (0).

Soller, J., Embrey, M., Tuhela, L., Ichida, A., Rosen, J., 2010. Risk-based evaluation of Escherichia coli monitoring data from undisinfected drinking water. J. Environ. Manag. 91, 2329–2335.

Teunis, P., Takumi, K., Shinagawa, K., 2004. Dose response for infection by Escherichia coli O157:H7 from outbreak data. Risk Anal. 24 (2), 401–407.

Teunis, P.F.M., Ogden, I.D., Strachan, N.J.C., 2008. Hierarchical dose response of E. coli O157:H7 from human outbreaks incorporating heterogeneity in exposure. Epidemiol. Infect. 136, 761–770. https://doi.org/10.1017/S0950268807008771.

Tota, M., 2013. Newspaper: in Italian: Puglia: 16 casi di Seu in 40 giorni, rischio epidemia. I medici: "Calma.". In: Il Fatto Quotidiano | 21 Agosto, 2013; (Accessed. https://www.ilfattoquotidiano.it/2013/08/21/puglia-13-casi-di-seu-in-40-giorni-esperti-situazione-sotto-controllo-ma-gente-ha/688326/. (Accessed 10 February 2020).

Toze, S., Bekele, E., Page, D., Sidhu, J., Shackleton, M., 2010. Use of static Quantitative Microbial Risk Assessment to determine pathogen risks in an unconfined carbonate aquifer used for Managed Aquifer Recharge. Water Res. 44 (4), 1038–1049. https://doi.org/10.1016/j.watres.2009.08.028.

Tozzi, A.E., Caprioli, A., Minelli, F., Gianviti, A., De Petris, L., Edefonti, A., Montini, G., Ferretti, A., De Palo, T., Gaido, M., Rizzoni, G., et al., 2003. Shiga toxin-producing Escherichia coli infections associated with hemolytic uremic syndrome, Italy, 1988–2000. Emerg. Infect. Dis. 9 (1), 106–108.

Truchado, P., Hernandez, N., Gil, M.I., Ivanek, R., Allende, A., 2018. Correlation between E. coli levels and the presence of foodborne pathogens in surface irrigation water: establishment of a sampling program. Water Res. 128, 226–233. https://doi.org/10.1016/j.watres.2017.10.041.

Vergine, P., Saliba, R., Salerno, C., Laera, G., Berardi, G., Pollice, A., 2015. Fate of the fecal indicator Escherichia coli in irrigation with partially treated wastewater. Water Res. 85, 66–73. https://doi.org/10.1016/j.watres.2015.08.001.

Whitaker, H., Best, N., Nieuwenhuijsen, M.J., Wakefield, J., Fawell, J., Elliott, P., 2005. Modelling exposure to disinfection by-products in drinking water for an epidemiological study of adverse birth outcomes. J. Expo. Anal. Environ. Epidemiol. 15, 138–14.

WHO, World Health Organization, 2011. Guidelines for Drinking-Water Quality - 4th Ed. NLM Classification: WA 675. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.