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QMRA of *Ralstonia solanacearum* in potato cultivation: Risks associated with irrigation water recycled through managed aquifer recharge

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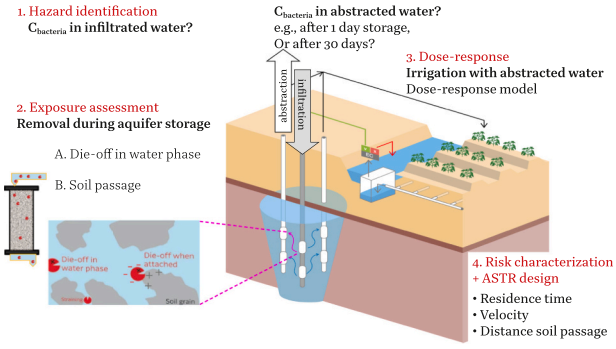
HIGHLIGHTS

- First QMRA focusing on plant health and risks of water reuse in agriculture.
- Aquifer storage provides irrigation water and removes bacterial plant pathogens.
- ASTR combines bacterial removal by water die-off and by attachment to aquifer sand.
- A one meter soil passage predicts sufficient bacterial removal by attachment.
- QMRA helps to support decision-making processes for water resource management.

GRAPHICAL ABSTRACT



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ABSTRACT

Agricultural aquifer storage recovery and transfer (ASTR) stores excess fresh water for later reuse in irrigation. Moreover, water quality improves because chemical pollutants and pathogens will be removed by degradation and attachment to the aquifer material. The source water may contain the bacterial plant pathogen *Ralstonia solanacearum* which causes plant infections and high yield losses. We used quantitative microbial risk assessment (QMRA) to investigate the removal of *R. solanacearum* during ASTR to predict infection risks of potato plants after irrigation with the recovered water. Laboratory experiments analyzed the ASTR treatment by investigating the bacterial die-off in the water phase and the removal by attachment to the aquifer sediment. Die-off in the water phase depends on the residence time and ranged between 1.3 and 2.7 $\log_{10}$ after 10 or 60 days water storage, respectively. A subpopulation of the bacteria persisted for a prolonged time at low concentrations which may pose a risk if the water is recovered too early. However, the natural aquifer sand filtration proofed to be highly effective in removing *R. solanacearum* by attachment which depends on the distance between injection and abstraction well. The high removal by attachment alone (18 $\log_{10}$ after 1 m) would reduce bacterial

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concentrations to negligible numbers. Upscaling to longer soil passages is discussed in the paper. Infection risks of potato plants were calculated using a dose-response model and ASTR treatment resulted in negligible infection risks of a single plant, but also when simulating the irrigation of a 5 ha potato field. This is the first QMRA that analyzed an agricultural ASTR and the fate of a plant pathogen focusing on plant health. QMRA is a useful (water) management tool to evaluate the treatment steps of water reclamation technologies with the aim to provide safe irrigation water and reduce risks disseminating plant diseases.

1. Introduction

Freshwater is a critical resource for irrigated agriculture to obtain sufficient crop yields and securing food security of the growing world population. Nevertheless, freshwater scarcity increases as a result of climate change and ongoing groundwater exploitation (FAO, 2022). Additionally, surface water may contain plant pathogens and is therefore not suitable for irrigation as pathogens present in irrigation water pose a threat to global crop production and food security causing high economical losses (Hong and Moorman, 2005). This study focuses on the plant pathogenic bacterium *Ralstonia solanacearum* which has been found in surface waters and plant disease outbreaks have been linked to contaminated irrigation water (Janse, 1996). The pathogen originated from South America and was probably introduced in Europe through latently infected seed material. The cold-adapted strain of *R. solanacearum* (phylotype II, race 3 biovar 2) threatens potato and tomato production in Europe and is found in surface waters where it sheds in plants growing along waterways. Irrigation with contaminated (surface) water has been recognized as primary source of infection on a local scale as described in the pest categorization of the *Ralstonia solanacearum* species complex (RSSC) performed by the EFSA Panel on Plant Health (EFSA et al., 2019). After severe disease outbreaks in Europe in the 1990's, irrigation of (seed) potatoes has been prohibited which successfully reduced disease incidences and less positive findings of *R. solanacearum* in surface water (Directive, 2006; Janse, 2012).

Managed aquifer recharge (MAR) is a nature based solution to provide a fresh water reservoir in times of need while simultaneously improving water quality (Pyne, 1995). Aquifer storage and recovery (ASR) is a specific type of MAR where water is intentionally infiltrated during wet periods via injection wells and recovered from the subsurface using the same well (Dillon, 2005). Source waters of different origins and water qualities (e.g., wastewater, urban stormwater, rain water) can be infiltrated and improvement of the water quality will strongly depend on the aquifer material, its geochemical composition, and microbial community (Bekele et al., 2018). In an ASR, pathogens or chemical pollutants will die-off or degrade to a certain extent after a given storage time. However, it is difficult to exactly predict the travel distance of the recharged water as it depends on the infiltration volume and the porosity of the different aquifer sand layers. In contrast, an aquifer, storage, transfer and recovery (ASTR) system uses a spatially separated abstraction well. This forces the water to flow from infiltration towards the abstraction well through the porous aquifer medium before it is recovered. This offers additional pathogen removal during the soil passage in the subsurface while the known distance between both wells adds a computable filtration step (Dillon et al., 2009). Pathogen removal occurs at the soil-water interphase and is governed by chemical, biological, and physical mechanisms (Ginn et al., 2002). Moreover, favorable conditions for attachment are related to the water's ionic strength, dissolved oxygen, soil and rock chemical mineral compositions, water temperature, pH, and salinity (Bradford et al., 2013; Johnson et al., 1996).

The treatment efficiency of AS(*T*)R and risks related to the reuse of water from different sources can be analyzed using quantitative microbial risk assessment (QMRA). QMRA in the context of MAR has already been used to quantify human health risks when MAR treated water is used for the irrigation of recreational areas, or the production of raw consumed vegetables (Ayuso-Gabella et al., 2011; Masciopinto et al., 2020; Page et al., 2015). These studies highlight the potential of MAR to enhance microbiological water quality and the potential of QMRA to reveal weak points of a MAR treatment scheme. For example, Masciopinto et al. (2020) showed that MAR recharge with treated wastewater resulted in low health risks when using the recovered water. However, the authors also found increased pathogen concentrations in reclaimed water after extended drought when the dry and touristic season coincide in the coastal study area. Then, higher amounts of wastewater were produced and dilution by rainwater was lacking. This resulted in increased pathogens loads in the reclaimed water. The related human health risks may not exceed a certain target concentration. For example, the acceptable concentration of *Campylobacter* in drinking water is 10^{-4} cells/L (WHO, 2011). Overall, studies focused on human health risks but neglected the risks of plant pathogens potentially being present in source waters intended for MAR recharge. Therefore, currently no target concentration ('safe' concentration) for plant pathogens in irrigation water exists. Only the plant pathogen Pepper mild mottle virus (PMMoV) has been investigated in risk assessments where it served as an indicator for human viruses in wastewater treatment (Symonds et al., 2018). Verbyla et al. (2016) included PMMoV as surrogate to study its removal during riverbank filtration and applied QMRA to analyze human health risks related to the consumption of raw lettuce that had been irrigated with MAR treated water. To our knowledge, no study used QMRA to evaluate AS(*T*)R treatment for the production of irrigation water with the focus on plant health.

In this research, we used QMRA to analyze an agricultural ASTR system for the irrigation of potato plants with the aim to assess changes in microbiological water quality and quantify the infection risks of potato plants after irrigation with ASTR treated tile drainage water. The treatment refers to the natural processes in the subsurface leading to pathogen removal where the setup and operation of the ASTR system will influence its treatment efficiency. Therefore, the aim of our study is to determine the critical parameters during ASTR operation that will enhance pathogen removal and to propose an operation scheme to reclaim water for irrigation without risking plant infections. The selected distance between injection and abstraction well may play a significant role as a greater distance will increase the natural filtration processes. Furthermore, bacterial die-off in the water phase and attachment to soil grains depend on velocity and residence time which will impact the fate of pathogens. The calculated plant health risks of using ASTR-treated water for irrigation have to be compared with the current situation in which all safety measurements are followed (e.g. prohibition of surface water irrigation). At present, infections of potato plants are still found incidentally indicating that transmission pathways other than irrigation with surface water play a role in the epidemiology of the pathogen (Janse, 2012). Moreover, the risk of drought related yield losses due to insufficient irrigation water has to be balanced with the risk for disease outbreaks after using ASTR treated irrigation water (Breukers et al., 2008).

The studied ASTR site uses tile drainage water (TDW) collected after excess rain events from the agricultural field. Although low pathogen concentrations are expected in the TDW, it may mix with *R. solanacearum* contaminated surface water. We hypothesize that the concentration of pathogens and other (agro)pollutants like fertilizers will get reduced during aquifer storage due to die-off over time and removal by sorption. However, it is currently not allowed to use the recovered water from an ASTR for the irrigation of seed potatoes. The

installation of water reclamation for agriculture is also hampered as there exist no target concentrations for plant pathogens in reclaimed water as for human pathogens, e.g., 10^{-4} cells/L of *Campylobacter* in drinking water (WHO, 2011). These reference values are missing to analyze the efficiency of a water treatment system. A recent policy used QMRA to establish minimum requirements for water reuse in irrigation regarding human health risks but neglected plant pathogens (Alcalde-Sanz and Gawlik, 2017; Commission, 2020). Therefore, the QMRA of agricultural MAR with focus on plant pathogens can serve as a tool in legislative decision processes to promote the implementation of MAR, and in specific ASTR, to secure agricultural production by providing safe irrigation water.

2. Material and methods

2.1. Agricultural MAR – site description

The QMRA was applied to a pilot ASTR system (Fig. 1) situated in an agricultural area in a polder in the North-Western part of the Netherlands (coordinates: 52.8883, 4.8221). The ASTR system stores water during wet periods in the underlying confined aquifer using wells from where it can be recovered in dry periods for irrigation. After rain events, tile drainage water (TDW) is collected from a 10 ha of agricultural land and injected via a vertical well in a sandy anoxic aquifer (11.5–33.0 m below surface level (b.s.l.)) of late Holocene and Pleistocene origin, below a confining Holocene clay/peat layer. The rain water reaches the tile drains (located about 0.7 m b.s.l.) as it percolates through the top soil from where chemical and biological agro(pollutants) may be released and carried along. The tile drains end up in a collection drain, from which TDW is discharged to a storage tank (ca. 1 m^3) when the phreatic groundwater level rises. As the water level exceeds a threshold, a pump within the storage tank is activated. First, disc filters (pore size: 40 μm) treat the pumped TDW to remove suspended solids to avoid clogging of the screens of the infiltration wells. However, these filters have no effect on pathogen removal due to their large pore size. The native aquifer is anoxic, brackish and has a constant temperature of about 10 °C. A freshwater storage is created through the infiltration of the oxic, fresh TDW. As consequence, the infiltrated water will undergo different biochemical reactions. For example, oxygen was reduced within two days and nitrate within 4–7 days using push-pull tests to assess aquifer reactivity (Kruisdijk and van Breukelen, 2021). Changes in water quality will depend on the aquifer composition, its hydrogeochemistry and the composition of the microbiota (Bekele et al., 2018). Moreover, changes will depend on the residence time of the water within the aquifer (time between infiltration and abstraction event) and the soil passage length which is determined by the distance between infiltration and abstraction well of an ASTR system. The outcomes of the QMRA will help in the design of an ASTR to determine the required soil passage to improve water quality sufficiently. The TDW may get contaminated with plant pathogens when contaminated surface water overflows adjacent fields after heavy rain events and enters the drainage system. Additionally, farmers may use level controlled drainage allowing surface water to enter the drainage system to increase water levels in the agricultural field. Pathogens and any other chemicals entering the tile drainage water need to be removed during the recharge process.

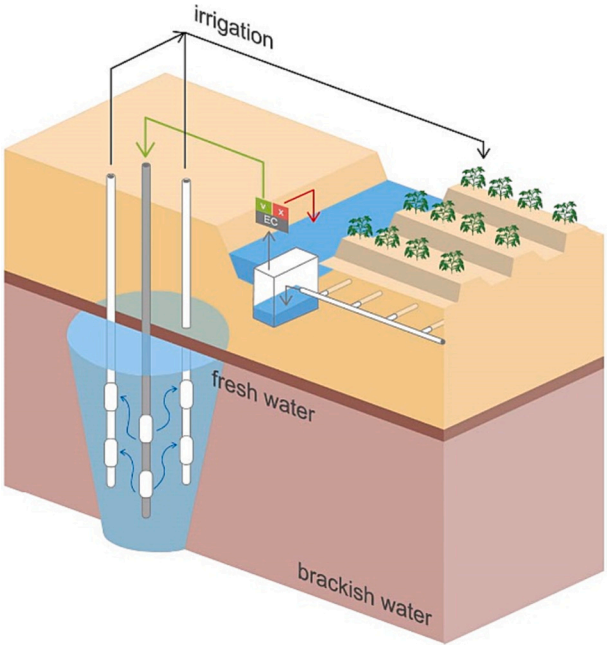

Fig. 1. Schematic representation of an agricultural field connected to a managed aquifer recharge site. The site is designed as an aquifer, storage, transfer, and recovery (ASTR) system. Excess rain water reaches the tile drainage system buried at about 70 cm depth. The collection drain terminates into a concrete reservoir where the electrical conductivity (EC) and turbidity of the tile drainage water is measured. If the EC or turbidity is below a set threshold value, the water is infiltrated via the injection well (depicted in gray). From there, the water travels through the sandy aquifer to the abstraction wells (depicted in white) and can be used for irrigation.

In the studied ASTR system, the soil passage is 7 m which cannot be changed anymore after drilling of the infiltration and abstraction wells. In contrast, the residence time is variable and can be controlled by the farmer. The water flow velocities within the aquifer are variable and depend on the pumping rate. For example, the studied system has two infiltration wells with a maximum pump rate of 10 $m^3\ h^{-1}$ per well which may result in flow velocities of up to 5.4 m day^{-1}, considering only radial horizontal water flow in the most permeable layer of the aquifer. Moreover, the four abstraction wells are installed with a pump rate of 20 $m^3\ h^{-1}$ per well. Although the pumping activities will result in different flow velocities, the background groundwater flow is about 0.01 m day^{-1} if no infiltration or abstraction is taking place.

2.2. Risk assessment

QMRA calculates risks probabilities associated to specific scenarios and comprises four steps: (i) hazard identification, (ii) exposure assessment, (iii) dose-response analysis and (iv) risk characterization as elaborated in the sections below (Haas et al., 2014). At first, the biological hazard causing harm for the crop health is identified. Then, the hazard's concentration in the source water and its removal during ASTR by different treatments are assessed to determine the exposure concentration. The dose-response analysis determines the infection risk of a potato plant given a certain exposure concentration. Finally, the results of hazard identification, exposure assessment and dose-response analysis are combined to formulate the risk characterization and analyze different scenarios including their variability and uncertainties.

Data analysis was performed using R (v.4.1.2, R Core Team (2022)) and the packages *gsl* (Hankin, 2021), *truncnorm* (Mersmann et al., 2018), and *fitdistrplus* (Delignette-Muller and Dutang, 2015). Graphics were prepared with the package *ggplot2* (Wickham, 2016). A random sample distribution was drawn using Monte Carlo sampling ($n = 10'000$) from all input parameters, using the parameter's mean value and standard deviation. Creating such a large random sample size allows accounting for uncertainty and variability of all parameters which are used in the risk model. The distributions of the results are presented in box-whisker-plots where the 75% percentile represents the conservative and the 25% the optimistic estimate. It was assumed that the parameters follow a normal distribution, if not stated otherwise (Table 1).

2.3. Hazard identification

This study focused on *Ralstonia solanacearum* as the biological agent which is a hazard in agricultural production. *R. solanacearum,* together with *R. pseudosolanacearum* and *R. syzigii,* comprise the *R. solanacearum* species complex (RSSC) (Fegan and Prior, 2005). The three individual species can cause bacterial wilt in >200 plant species worldwide ranging from tomato to ornamental flowers (Hayward, 1991; Tjou-Tam-Sin et al., 2016). Here, we focus on *R. solanacearum* (phylotype II) which causes brown rot in potato in temperate climates. *R. solanacearum* originated from South America and was introduced into the EU through international seed trading which is the main route of pathogen distribution (EFSA et al., 2019). The pathogen complex has a quarantine status and is regulated in the European Union (Directive, 2000). As consequence of severe disease outbreaks in Europe in the 1990's, irrigation of (seed) potatoes has been prohibited. Additionally, irrigation of starch and consumption potatoes is prohibited in areas with brown rot contaminated surface water (Directive, 2006). Member states of the EU are required to conduct yearly surveys to restrict the further spread of the pathogen with the aim to eradicate the disease. In the surveys, surface waters near potato producing areas and potato seed lots are investigated for the presence of *R. solanacearum* (Directive, 1998). Hosts like *Solanum dulcamara* (bittersweet nightshade) often grow along waterways where they can get infected with *R. solanacearum*. The pathogen multiplies within the host without showing disease symptoms and gets released into the water when the environmental conditions are favorable (EFSA et al., 2019). Furthermore, the pathogen can survive in topsoil for up to 200 days and its persistence may be prolonged if plant debris are present (Messiha et al., 2009; Tomlinson et al., 2011). In greenhouse experiments, the effect of different concentrations of *R. solanacearum* on two potato cultivars has been determined and a dose-response model was developed (Eisfeld et al., 2022a). It will be used in the QMRA to determine the infection risk of potato plants by *R. solanacearum* and is described in one of the sections below.

2.4. Exposure assessment

2.4.1. Source water quality

TDW collected after rain events is the source water used for storage in the ASTR system. However, the concentration of *R. solanacearum* in TDW has not been analyzed in this study. Tomlinson et al. (2009) surveyed *R. solanacearum* in the Nile delta of Egypt in canal waters along potato growing fields and found concentrations of 0.1–0.2 CFU mL^{-1} in canal waters. The authors also analyzed the drainage water in designated 'Pest-Free-areas' where the bacteria were not detectable. Consequently, the bacterial concentration in TDW is expected to be lower than in surface water or zero but there exists the risk that surface water containing *R. solanacearum* contaminates the drainage water. This may

Table 1
Input parameters for the quantitative microbial risk assessment (QMRA) to calculate the infection risk of potato plants by *Ralstonia solanacearum* after irrigation with managed aquifer recharge (MAR) treated water.

Model parameter	Unit	Value; standard deviation	Reference
Source water concentration			
C_s	CFU mL^{-1}	LOGN(0.08; 1.25)	van Duivenbode, 2020, NAK dataset from years 2018–2020 (during summer months)
Recovery efficiency			
R	–	N(0.91; 0.024)	Pradhanang et al. (2000)
Removal by die-off in water phase (Eq. (1))			
Weibull + tail model			Eisfeld et al. (2021); cf. Fig. 3(R5)/Table 3)
a	day^{-1}	N(0.05; 0.002)	
b	–	N(6.7; 2.0), truncated at (0; Inf)	
C_0	CFU mL^{-1}	N(15900; 1)	C_0 relates to the experimental inoculation concentration used in the batch experiments
C_{res}	CFU mL^{-1}	N(33; 1.2)	
Removal by irreversible attachment (Eq. (3))			
Attachment to quartz sand			Eisfeld et al. (2022b)
k_{att}	min^{-1}	N(0.007; 0.009), truncated at (0; Inf)	
α_L	cm	N(0.038; 0.008)	
$\alpha_{L\text{-}2}$ (100 cm soil passage)	cm	N(0.17; 0.037)	Upscaled longitudinal dispersivity
$\alpha_{L\text{-}3}$ (200 cm soil passage)		N(0.33; 0.072)	
Attachment to aquifer sand			
k_{att}	min^{-1}	N(0.121; 0.018), truncated at (0; Inf)	
α_L	cm	N(0.45; 0.30)	
$\alpha_{L\text{-}2}$ (100 cm soil passage)	cm	N(2.0; 1.32)	Upscaled longitudinal dispersivity
$\alpha_{L\text{-}3}$ (200 cm soil passage)		N(3.9; 2.6)	
Velocity			
$v_{average}$	cm min^{-1}	0.246	Average velocity during infiltration or abstraction occur (3.5 m day^{-1})
Volume of irrigation			
176	mm	per growing season	Acacia Water (2019b)
35.2	L	total volume per plant per growing season	
Dose-response model (Eq. (6))			
α	–	bp(0.17; 0.08)	Eisfeld et al. (2022a)
β	–	bp(3.6 $\times 10^5$; 9.7 $\times 10^5$)	
ASTR operation			
a) soil passage			
x_1	cm	23	Column length from Eisfeld et al. (2022b)
x_2	cm	70	Required soil filtration distance needs to be known during ASTR design stage; the pilot ASTR site has a 7 m soil passage
x_3	cm	100	
b) residence time			
t_1 - t_2 - t_3	days	10–30–60	Residence time can be set by the farmer

LOGN = lognormal distribution; N = normal distribution, UNIF = uniform distribution; bp = Beta-Poisson distribution; α_L = longitudinal dispersivity; k_{att} = irreversible attachment parameter.

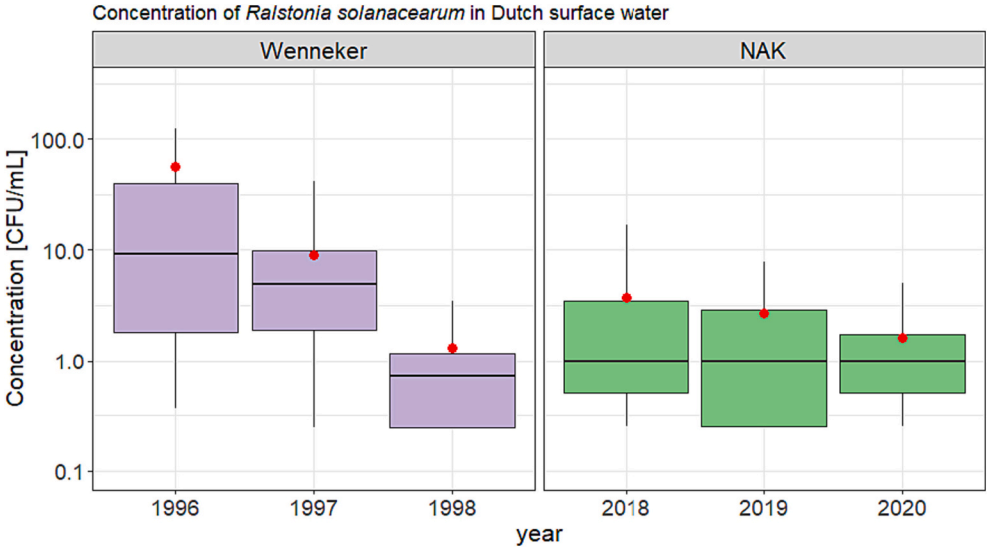

Fig. 2. Concentration of *Ralstonia solanacearum* in Dutch surface water shown as box-whisker plots with the 5–95% confidence interval. The bottom and top of the box represent the first and third quartiles (25th and 75th percentile values) and the red dot indicates the mean value. Data were obtained from yearly surveys reported by Wenneker et al. (1999) (left panel, $n = 104$) and the 'Nederlandse Algemene Keuringsdienst (NAK)' (van Duivenbode, 2020) (right panel, $n = 590$). The 'Wenneker' data was obtained during periodical sampling from 1996 to 1998 during winter and summer months. The 'NAK' data was obtained during sampling events in the summer months of June to August.

occur when agricultural fields are flooded during storm events by overflowing ditch water (Janse, 1996). Additionally, farmers use surface water in controlled drainage to raise water levels below the agricultural field (subirrigation) which is beneficial for plants during certain development stages (de Wit et al., 2022). However, if the surface water contains plant pathogens this increases the risks of plant infections. Instead, ASTR treated water can be used for such controlled drainage and reduce the risk of plant infections. Lastly, an ASTR system may use exclusively surface water for aquifer storage which poses a higher risk that plant pathogens enter the aquifer which have to be removed during storage.

As a conservative scenario, we assume that concentrations of *R. solanacearum* in drainage water are equal to surface water. *R. solanacearum* has been detected in surface waters in the Netherlands at maximum concentrations of 10^2–10^3 CFU mL^{-1} in the summer months (Wenneker et al., 1999). Fig. 2 shows the distributions of *R. solanacearum* concentrations in surface water during six different years. There is a strong seasonal fluctuation influenced by water temperature (Caruso et al., 2005; Wenneker et al., 1999). During the winter months in the Netherlands, when water temperatures are below 10 °C, the pathogen was detected at very low concentrations (0.5 CFU mL^{-1}) or even below the detection limit (Wenneker et al., 1999). The seasonal variability of the bacteria in surface water is an important factor in temperate climates as the farmer can choose to infiltrate only during the winter months when lower pathogen concentrations are present and where most precipitation surplus can be expected. This may be different in other climate zones where temperature remain stable during the year as well as concentrations of *R. solanacearum* in surface waters.

The analysis of the distributions is done using *fitdistrplus* in R (Delignette-Muller and Dutang, 2015). The left panel ('Wenneker') is a dataset ($n = 104$) retrieved from the graphs of Wenneker et al. (1999) and bacterial concentrations were recorded during summer and winter months in 1996–1998. During the 1990's, higher concentrations of *R. solanacearum* were observed as a result of several brown rot outbreaks in Dutch potato cultivation. In September 1996, highest concentrations of max. 10^3 CFU mL^{-1} were observed at a water temperature of 25 °C. Strict hygiene measures, seed testing and a ban on the use of surface water for irrigation resulted in lower disease incidences. Consequently, the concentrations of *R. solanacearum* found in surface waters also decreased which are represented by the more recent dataset ($n = 590$) from 2018 to 2020, obtained from the 'Nederlandse Algemene Keuringsdienst (NAK)' (van Duivenbode, 2020). There, the maximum concentration of *R. solanacearum* was 65 CFU mL^{-1}. Sampling was performed according to the scheme for detection and identification of *R. solanacearum* in water samples described in the EU Directive (2006). The recent surveys are only performed twice per year during the warmer summer months, for example in 2020 from 3 to 19 of June and 4–14 of August. As input data for the risk assessment, the surface water concentrations of *R. solanacearum* in the years 2018–2020 (Fig. 2, 'NAK' data) were used which follow a log-normal distribution (Table 1). The concentrations in Dutch surface water are comparable with surveys of other countries. In the UK, high concentrations of max. 600 CFU mL^{-1} were found during July and August 1994 downstream of infected *Solanum dulcamara* plants while the bacterium remained undetectable from November until June (Elphinstone and Matthews-Berry, 2017). More recent river sampling in the England and Wales only detected the bacterium at a maximum concentration of 68 CFU mL^{-1} at 15 °C. Twenty-six rivers were tested at 54 locations in begin September 2014 and 2015 and only one river tested positive at both testing locations during both years (APHA, 2015). Moreover, Caruso et al. (2005) reported low concentrations (10–80 CFU mL^{-1}) of *R. solanacearum* in Spanish rivers and a dependency on water temperature.

2.4.2. Die-off in the water phase

The first natural treatment during aquifer recharge relies on the bacterial die-off in the water phase. It depends on the residence time which is the storage period between injection and abstraction events, and can be determined by the farmer. For the QMRA, three residence times of 10, 30 and 60 days were compared. Subsurface travel time of 60 days combined with specific setback distances is considered safe in drinking water production in the Netherlands (CBW, 1980). A short residence time of 10 days will remove less pathogens but represents a greater flexibility for the farmer using recovered water for different purposes. However, infiltration will mostly occur during the wet winter months while water recovery is needed during the drier cropping periods. Therefore, residence times of one or several months might be more realistic.

A non-linear Weibull + tail model described the bacterial die-off in natural oxic TDW and anoxic aquifer water from a MAR site (Eisfeld et al., 2021). The removal is described by $z_{die-off}$:

$$z_{die-off} = \frac{C}{C_0} = e^{-(at)^b} - \frac{C_{res}}{C_0}\, e^{-(at)^b} + \frac{C_{res}}{C_0} \quad (1)$$

where C_0 [M L^{-3}] is the inoculation concentration which has been used in the batch experiments (about 10^4 CFU mL^{-1}). C is the bacterial concentration after time t [T]. a [T^{-1}] and b [−] are the model parameter estimates which influence the curve shape. C_{res} [M L^{-3}]

represents a more persisting residual concentration which depends on the inoculation concentration for which is accounted using the ratio of C_{res}/C_0. The improvement in water quality of the infiltrated water after a given residence time is calculated as:

$$C_t = C_s * z_{die-off} \quad (2)$$

where C_s [M L^{-3}] is the bacterial concentration in the source water which will decrease to C_t [M L^{-3}] by water die-off after a given residence time t. The maximum removal which can be predicted is about 3-$\log_{10}$ due to the experimental conditions which observed the bacterial die-off from a inoculation concentration of 10^4 CFU mL^{-1} to about 10^1 CFU mL^{-1}.

The die-off was monitored under oxic and anoxic conditions as the redox conditions will change during ASTR. The infiltrated TDW is oxic but due to biological and chemical interactions with the aquifer material (e.g., organic carbon), oxygen will be depleted within 1–2 days (Kruisdijk et al., 2022a). For the risk assessment, the experimental parameters of the die-off of *R. solanacearum* in anoxic aquifer water at 10 °C are used (Table 1). There, the die-off was longest and bacteria were no more detectable after 55 days. The die-off was described with the Weibull + tail model. The die-off curve under these conditions was characterized by an initial shoulder phase where the bacterial concentration remained stable followed by a linear decline, and a third phase in which the residual concentration remained at a low level of a few cells per mL until the concentration dropped below the detection limit of 3 CFU mL^{-1}. The residual concentration will depend on the inoculation concentration for which is accounted using the ratio of C_{res}/C_0. This persistent bacterial populations poses a risk to cause plant infections if the residence time was too short. However, after 60 days the bacteria were no more detectable in experimental conditions where the bacteria may have either died-off completely or entered the viable but non-culturable (VBNC) state (Elphinstone et al., 1998). It is questionable if VBNC cells in the recovered water from an ASTR system will still pose a hazard in irrigation as they need to restore their viability and remain virulent (Kong et al., 2014).

2.4.3. Attachment to aquifer sediment

The second and simultaneous natural aquifer treatment process is pathogen removal by attachment to the sand grains, die-off after attachment and physical straining (size-exclusion). The removal was analyzed using soil column experiments using clean quartz sand or natural aquifer sand from the pilot site which was obtained during drilling operations (Eisfeld et al., 2022b). A Hydrus-1D model using an extended advection-dispersion equation accounting for bacterial attachment and detachment was used to fit the bacterial column breakthrough curves. When analyzing the bacterial transport, it cannot be distinguished between the different removal processes which were described above. In contrast, the removal processes are represented by the kinetic attachment parameter estimate k_{att}. As detachment was orders of magnitude lower than attachment (Eisfeld et al., 2022b), the detachment parameter can be neglected. Yet, detachment of pathogens should be considered if strong changes in fluid velocity or ionic strength of the recharge water are expected which may result in remobilization of environmental colloids (Pazmino et al., 2014). In this study, removal by irreversible attachment ($z_{attachment}$) to the sediment grains is assumed to be the main mechanism during bacterial transport in the subsurface (Schijven et al., 2000):

$$z_{attachment} = \frac{C}{C_0} = e^{x\frac{1-\sqrt{1+4\alpha_L\frac{k_{att}}{\nu}}}{2\alpha_L}} \quad (3)$$

C/C_0 describes the bacterial removal and x [L] represents the soil passage length determined by the distance between injection and abstraction wells in an ASTR system. α_L is the longitudinal dispersivity [L], ν the average interstitial water velocity [L T^{-1}] and k_{att} the attachment parameter estimate [T^{-1}]. All input parameters are shown in Table 1. Monte Carlo samples of the attachment parameter were sampled from a truncated normal distribution with the lower limit at zero to avoid the sampling of negative attachment values. Similar to the water die-off, the concentration C_x after the soil passage is the reduction of the source concertation C_s by $z_{attachment}$ which will depend on the soil filtration length. Note, that the water will flow out radially from the infiltration well and when abstracted, the water will have travelled different distances. However, the minimum travel distance will remain the distance between infiltration and abstraction well. Moreover, the aquifer sediment composition will influence the transport velocities and the bacterial removal.

$$C_x = C_s * z_{attachment} \quad (4)$$

In the column experiments, removal was much higher in the natural aquifer sand than in the quartz sand due to a more heterogeneous surface structure and grain size distribution of the aquifer sand which offer more favorable attachment sites. For example, positively charged metal oxides on the grain surface of natural sands will increase bacterial attachment (Johnson et al., 1996). Moreover, the column experiments with the bacterial plant pathogen *Pectobacterium carotovorum* have shown that fine aquifer material (d_{50} = 192 μm) resulted in much higher (31–40 $\log_{10}$) removal per meter than in medium aquifer material (d_{50} = 305 μm; 19 $\log_{10}$ m^{-1}). The aquifer material has been selected as it is a good representation of the average medium grain sizes found in the aquifer of the pilot site (Eisfeld et al., 2022b).

The attachment is also influenced by the average interstitial water velocity which is set to the point estimate of 0.246 cm min^{-1} (3.5 m day^{-1}). Note, that the column length in the experimental setup was 23 cm while the distance in the pilot ASTR system is about 700 cm. For the QMRA, the infection risks will also be calculated for 70 and 100 cm to study the effects of a longer soil passage on the bacterial removal. A soil passage of 100 cm was chosen as this would be the minimum distance which is technically feasible. Longer soil passages could not be simulated due to high removals in aquifer sand which caused too low infection risks. This will be further elaborated in the results and discussion. Although a longer soil passage is desirable to achieve high pathogen removal, the recovery efficiency of freshwater from a brackish aquifer will decrease as more mixing between the saline groundwater and infiltrated freshwater will occur (Maliva et al., 2006). Moreover, the longitudinal dispersivity will increase with the soil passage length by 1–10% and needs to be scaled accordingly (Gelhar, 1986). Therefore, the ratio between tested upscaled filtration length (e.g., 100 cm) and the column length (23 cm) was used to increase the value of the longitudinal dispersivity which in the example would result in 100/23 = 4.4.

2.4.4. Exposure dose

The exposure of a potato plant to *R. solanacearum* is given as the dose D [CFU]:

$$D = \frac{C_s}{R} * z_{die-off} * z_{attachment} * V_{irr} \quad (5)$$

The dose is derived by multiplying the Monte Carlo samples of the bacterial concentration in the source water C_s [CFU mL^{-1}] divided by the recovery rate *R*, with the removal by water die-off or attachment and the irrigation volume V_{irr} [L]. The recovery rate *R* relates to the effectiveness of the detection method to recover the bacteria from an environmental sample. Here, dilution plating on selective medium was used to recover the bacteria from soil or water described in Pradhanang et al. (2000). In this study, the recovery efficiency of *R. solanacearum* using semi-selective South Africa agar medium was 88–97% which was used in the QMRA to describe *R*. $z_{die-off}$ and $z_{attachment}$ (values between 0 and 1 [−]) refer to the natural treatments during ASTR. The irrigation volume and frequencies are strongly dependent on the prevailing climatic conditions and the water requirement of the potato plant during the cropping season. In potato production, irrigation is essential to ensure a high tuber yield and potatoes are specifically sensitive to water stress during

tuber initiation (Alva, 2008). Within the "Spaarwater" project, drip irrigation volumes were monitored during the growing seasons of three consecutive years (2016–2018) and ranged between 53 and 176 mm (Acacia Water, 2019a). The variation in irrigation volume reflects the differences in climatic conditions. Per irrigation event, about 3 mm of water was supplied via drip irrigation. These irrigation volumes are also given in traditional sprinkler irrigation. In a conservative scenario for the QMRA, the highest total irrigation volume of 176 mm is used as point estimate to calculate the dose. This volume was supplied during the dry season in 2018 and relates to the expected future drought events as a consequence of climate change.

2.5. Dose-response analysis

The exact beta-Poisson dose-response model was used to calculate the infectivity of *R. solanacearum* when contaminated irrigation water is applied by soil-soak inoculation to potato crops simulating drip-irrigation (Eisfeld et al., 2022a).

$$P_{inf}(D|\alpha,\beta) = 1 - {}_1F_1\ (\alpha, \alpha+\beta; -D) \tag{6}$$

${}_1F_1$ is the confluent hypergeometric function and α and β are the infectivity parameters which are Monte Carlo sample pairs (joint distribution), reflecting uncertainty and variability of infectivity (Table 1). D is the exposure dose, the number of pathogens, with D = c*V where c is the pathogen concentration in a certain volume V. In the greenhouse experiments, two potato cultivars (Kondor and HB) were analyzed (Eisfeld et al., 2022a). Cultivar Kondor was less resistant to *R. solanacearum* and more infected and symptomatic plants have been observed. Therefore, the dose-response parameters of the experiment with cv Kondor are used in the QMRA which also reflects a more conservative approach. The infection risk is calculated for one potato plant. It is assumed that 5 potato plants grow on 1 m^2 (Beukema and Zaag, 1990) which will receive at total irrigation of 176 mm which results in a volume of about 35 L per plant during a whole cropping season.

In a second step, the probability of having symptomatic plants (illness) within the group of infected plants can be calculated with the hazard model of illness dose response where r and η are the illness parameters. The results of the parameters are described in Eisfeld et al. (2022a).

$$P_{ill|inf}(cV) = 1 - \left(1 + \frac{cV}{\eta}\right)^{-r} \tag{7}$$

In the dose-response model, there is a differentiation between risk of infection and risk of illness. Infected plants may not show disease symptoms (latent infections), whereas infection is conditional for illness which relates to visually symptomatic plants. However, *R. solanacearum* is a quarantine organism for which currently a zero tolerance applies including latent infections. Therefore, the risk assessment is only executed calculating the risk of infection but not illness due to the zero tolerance policy. An infected plant without symptoms cannot by recognized during field inspection but the bacteria can move within the plant and infect the progeny tubers which may be detected during seed testing. For the seed testing, a random sample of 200 tubers is selected per 25 tons of harvested potato tubers (Directive, 1998). It is assumed that each tuber originates from a different plant.

2.6. Sensitivity analysis

The sensitivity analysis can help to identify the input parameters which mostly influence the infection risk and therefore, the most critical

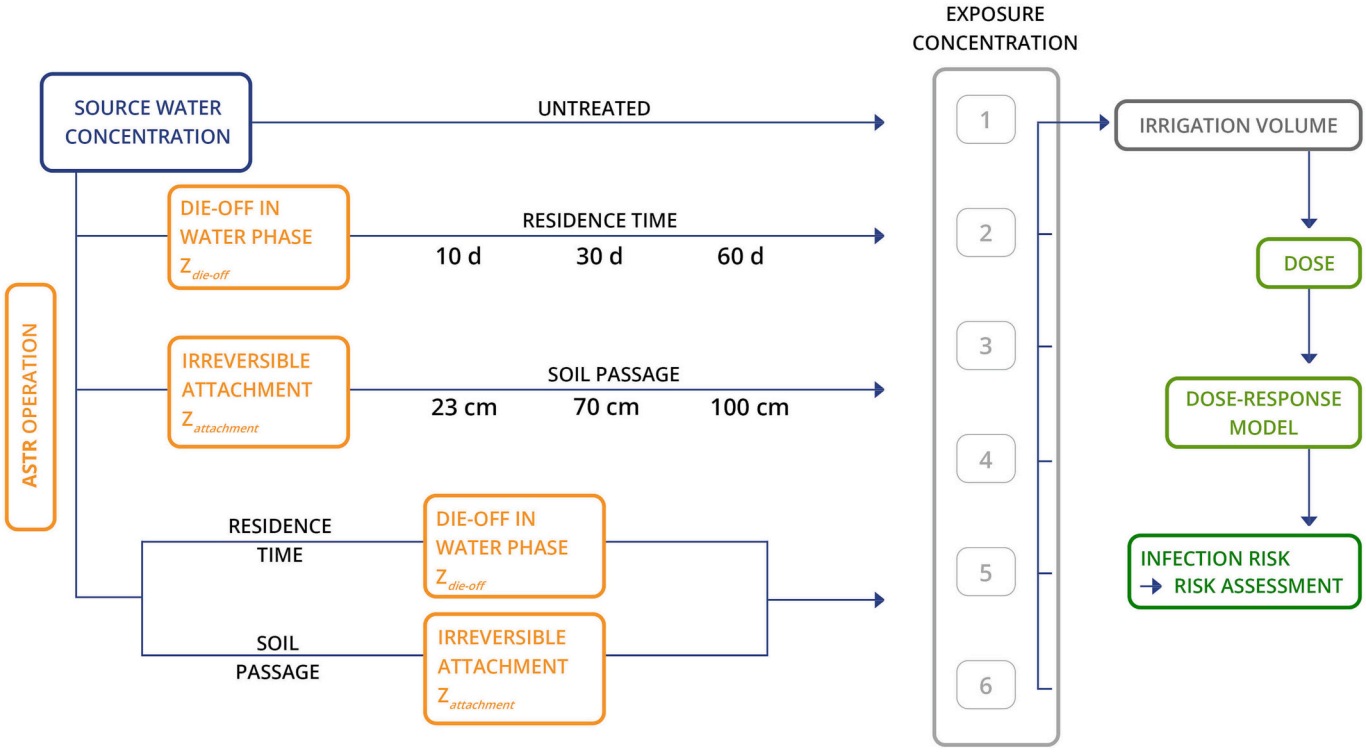

Fig. 3. Scheme for the steps of quantitative microbial risk assessment of an agricultural managed aquifer recharge system using aquifer storage transfer and recover technology (ASTR). In scenario 1, the exposure of potato plants after using untreated and *Ralstonia solanacearum* contaminated source water is predicted. Then, different ASTR configurations are compared: In scenario 2, the source water concentration is reduced by the die-off in the water phase which depends on the residence time (10, 30 or 60 days). Scenario 3 and 5 consider the natural treatment only by attachment to quartz sand or aquifer sediment, respectively, which depend on the soil passage length (23, 70, 100 cm). Scenario 4 and 6 evaluate the exposure concentration after the combined treatment by water die-off and attachment to either quartz or aquifer sediment. From the different treatment scenarios an exposure concentration of *R. solanacearum* is calculated. Using a dose-response model, the infection risk of a potato plant after irrigation with ASTR treated water is estimated.

treatment step within ASTR. Of each parameter used to calculate the dose D (Eq. (5)), the variance is calculated and divided by the variance of the respective infection risk (Eq. (6)):

$$Sensitivity = \frac{variance(log(parameter))}{variance(log(infection\ risk))} \quad (8)$$

Note, the parameters have an equal effect on the final dose through their linear relationship. In the sensitivity analysis, the variances of the parameters impacting the variation in infection risk are compared. Therefore, different ASTR configurations and the impact of residence time and soil passage length can be analyzed. Comparison of the different scenarios will give an insight about which treatment step mostly contributes to the variance of the infection risk.

2.7. Risk characterization

Fig. 3 shows the steps of the QMRA with an overview of different scenarios and possible ASTR configurations like the required soil passage length and the optimal residence time. The baseline scenario 1 refers the infection risk when potato plants are irrigated with untreated surface water using the 'NAK' dataset which detected *R. solanacearum* during yearly surveys in the summer (Fig. 2). Scenario 2 only considers bacterial die-off in the water phase as treatment of the source water which depends on the residence time. In comparison, scenario 3 only considers bacterial removal by soil attachment to quartz sand and scenario 5 removal by attachment to aquifer sediment, which both depend on the soil passage length. The comparison of the different scenarios will display which treatment will have the greatest impact on pathogen removal similar to the sensitivity analysis. In scenarios 4 and 6, the combined removal by water die-off and by attachment to quartz or aquifer sand is estimated, respectively. Hence, the comparison of the scenarios also allows to investigate different MAR configurations and compare ASR with ASTR. In ASR, water is pumped into the subsurface with the same well used for infiltration and abstraction. The water will travel away from the infiltration well when the freshwater storage expands where pathogen attachment and removal will occur. However, the exact soil passage length cannot be predicted. Consequently, an ASR system can only rely on die-off in the water phase for a predictable risk estimation. In contrast, an ASTR system uses an infiltration well and a spatially separated abstraction well which guarantees a minimum travel length of the water through the subsurface. From all scenarios and different AS(T)R operations, the exposure dose after using AS(T)R treated irrigation water is calculated to obtain the infection risk per plant. The AS(T)R operation will be simulated using different residence times of 10, 30 or 60 days. Moreover, soil passages of 23, 70 and 100 cm will be simulated.

3. Results

3.1. Removal by die-off in the water phase and attachment

Table 2 lists the estimated $\log_{10}$ removals by water die-off as a result of different residence times (10, 30 or 60 d). A simulated residence time from 10 to 30 days almost doubled the removal while a further increase to 60 days achieved about the same removal as for 30 days. As stated earlier, the removal is based on lab experiments which observed the die-off from about 10^4 CFU mL^{-1} to about 10^1 CFU mL^{-1}. Therefore, 2.7-$\log_{10}$ removal after 60 days residence time is the theoretical maximum although higher removals may be achieved with a longer storage time. The distributions of the $\log_{10}$ removals at 10 and 30 days showed a wide range (e.g., 0.7–1.9 $\log_{10}$ at 10 days) depicting their minimum, maximum and most likely values. They resulted from the Monte Carlo sampling ($n = 10'000$) which included uncertainty and variability in the parameter estimates.

Table 2 also lists the estimated $\log_{10}$ removals of *R. solanacearum* during the natural soil passage in the subsurface. In the column experiment, the removal within 23 cm was studied. In the QMRA, the filtration length was upscaled by modifying the soil passage length in the model to 70 or 100 cm which increased the removal. Considering clean quartz sand as aquifer material, the $\log_{10}$ removal were much lower (0.3-$\log_{10}$ removal at 23 cm, or 1.2 $\log_{10}$ removal at 100 cm soil filtration) than in the natural aquifer sand (4.2-$\log_{10}$ removal at 23 cm, or 18 $\log_{10}$ removal at 100 cm soil filtration).

When upscaling the soil passage length to 100 cm, the longitudinal dispersivity can be adapted linearly. In quartz sand, α_L scaled from 0.038 to 0.17 cm at 100 cm which did not reduce the mean $\log_{10}$ removal but influenced the 95% distribution of the results. In aquifer sediment, α_L scaled from 0.28 to 2.0 cm at 100 cm which reduced the mean bacterial attachment from 18 to 14-$\log_{10}$.

Table 2
$\log_{10}$ removals during aquifer storage transfer and recovery (ASTR).

-$\log_{10}$ removal by die-off in the water phase (scenario 2)						Infection risk			
Residence time [days]		mean	5%	50%	95%	mean	5%	50%	95%
10		1.3	0.7	1.3	1.9	1.4×10^{-2}	1.2×10^{-4}	2.8×10^{-3}	6.6×10^{-2}
30		2.5	2.0	2.7	2.7	1.6×10^{-3}	8.6×10^{-6}	1.6×10^{-4}	5.3×10^{-3}
60		2.7	2.6	2.7	2.7	1.0×10^{-3}	7.2×10^{-6}	1.3×10^{-4}	3.5×10^{-3}
-$\log_{10}$ removal by attachment to quartz sand (scenario 3)									
Soil passage [cm]	α_L [cm]	mean	5%	50%	95%	mean	5%	50%	95%
23	0.038	0.3	7.5×10^{-3}	0.1	1.1	5.8×10^{-2}	1.2×10^{-3}	2.9×10^{-2}	2.2×10^{-1}
70		0.9	2.3×10^{-2}	0.2	3.3	4.3×10^{-2}	1.7×10^{-5}	1.4×10^{-2}	1.9×10^{-1}
100		1.2	3.2×10^{-2}	0.3	4.7	3.9×10^{-2}	7.7×10^{-7}	1.0×10^{-2}	1.8×10^{-1}
		Upscaled longitudinal dispersivity							
100	0.17	1.2	3.2×10^{-2}	0.3	4.6	3.9×10^{-2}	9.1×10^{-7}	9.6×10^{-3}	1.9×10^{-1}
200	0.33	2.4	0.1	0.6	9.0	3.0×10^{-2}	4.3×10^{-11}	4.3×10^{-3}	1.6×10^{-1}
-$\log_{10}$ removal by attachment to aquifer sand (scenario 5)									
Soil passage [cm]	α_L [cm]	mean	5%	50%	95%	mean	5%	50%	95%
23	0.28	4.2	3.2	4.1	5.5	8.4×10^{-5}	8.1×10^{-8}	4.2×10^{-6}	2.1×10^{-4}
70		13	10	12	17	5.2×10^{-11}	$<1.8 \times 10^{-14}$	1.8×10^{-14}	2.0×10^{-11}
100		18	14	18	24	1.1×10^{-14}	$<8.9 \times 10^{-16}$[a]		8.9×10^{-16}
		Upscaled longitudinal dispersivity							
100	2.0	14	10	14	20	2.5×10^{-11}	$<2.5 \times 10^{-11}$[a]	1.4×10^{-3}	1.9×10^{-11}
200	3.9	24	16	22	37	1.2×10^{-15}	$<1.2 \times 10^{-15}$[a]		

[a] The high removal resulted in very low exposure concentrations and in very low infection risks that were so small that R program mostly showed zero values in the 10,000 Monte Carlo samples.

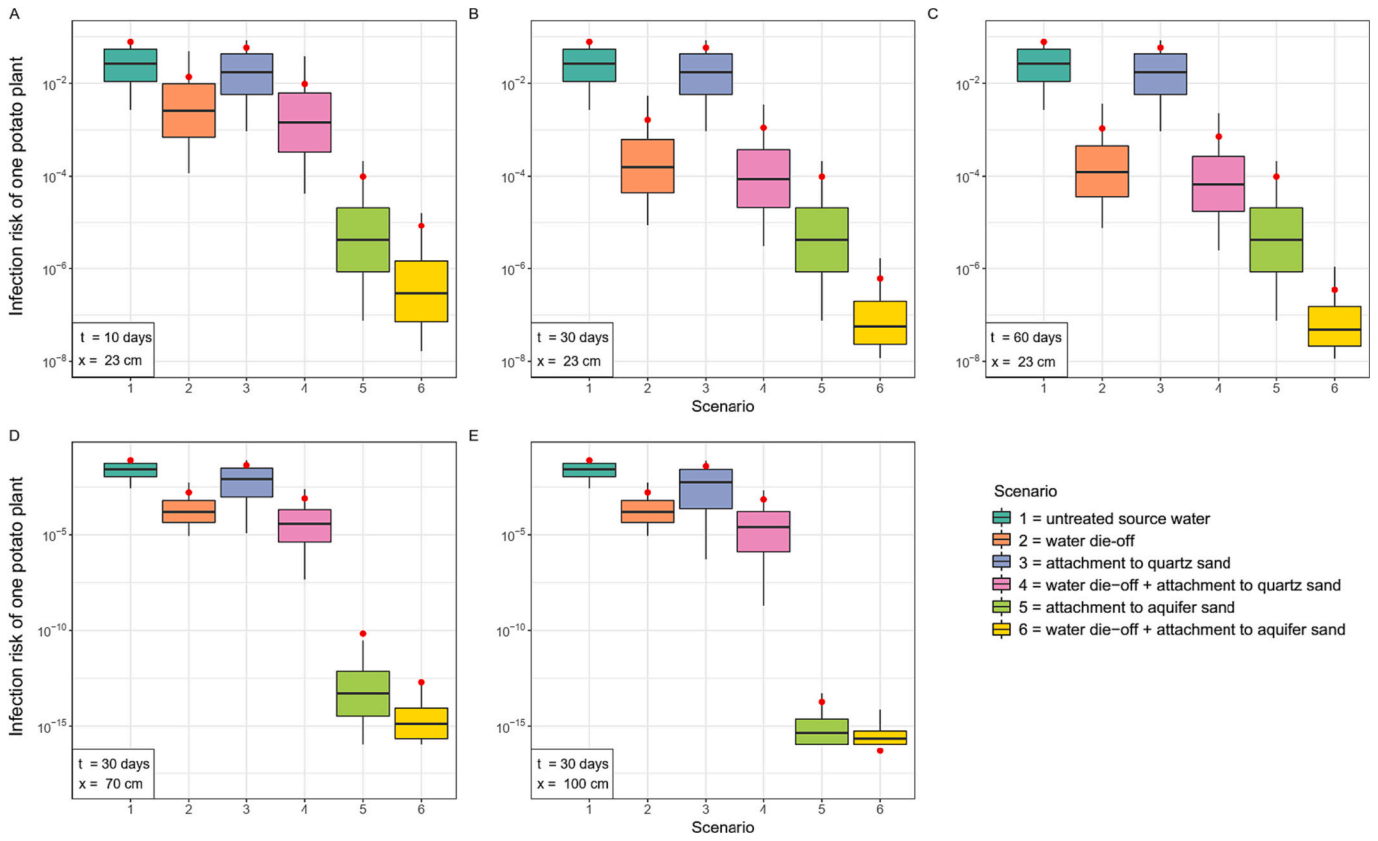

Fig. 4. Infection risks by *Ralstonia solanacearum* per one potato plant after irrigating with water treated through aquifer storage transfer and recovery – ASTR. Box-whisker plots describe the distribution of the data and its 5–95% confidence interval. The bottom and top of the box represent the first and third quartiles (25th and 75th percentile values) and the red dot indicates the mean value. Each graph shows the effect on the infection risk depending on the different treatments during ASTR (scenario 1–6). Scenario 1 in all graphs shows the infection risk if untreated source water is used in irrigation (no residence time or soil passage). The treatment depends further on the characteristics of the ASTR operation. A-C: increase in residence time, from 10 d (A), to 30 d (B) and 60 d (C). D + E: Increase in soil passage length from 70 cm (D) to 100 cm (E). The operational characteristics of the ASTR site are described in the left corner of each graph specifying the simulated residence time (t) and the soil passage length (x).

3.2. Infection risks

Table 2 also lists all related infection risks which were calculated with the depicted $\log_{10}$ removal rates. Note that filtration through aquifer sediment at a soil passage >70 cm resulted in high pathogen removals of at least 13-$\log_{10}$ and therefore, a very low exposure concentrations. Consequently, the calculated infection risks resulted in very small numbers near zero which could not be computed by the R program anymore and most of the 10′000 Monte Carlo sample distributions contained zeros. Therefore, it was not possible to upscale to 7 m to simulate the soil passage in the pilot ASTR site. The resulting infection risks were shown as zeros when simulating filtration through aquifer sediment at lengths of >1 m as the removal was predicted to be so high. Fig. 4 visualizes the infection risks of a single potato plant after irrigation with ASTR treated water that may have still contained *R. solanacearum*. Graphs A-E in Fig. 4 relate to different ASTR operations (residence time, soil passage length) that influenced the infection risk. In each graph, scenarios 1–6 relate to different treatment scenarios of the source water by die-off in the water phase, attachment to the sand or a combination of both. In graphs A-C, an increase in residence time (10, 30, 60 d) was simulated with a constant soil passage of 23 cm. Graphs D + E simulated an increase in soil filtration length (70, 100 cm) at a constant residence time of 30 days.

The infection risks of scenario 1 in Fig. 4A-E were all the same as the untreated source water (*R. solanacearum* contaminated surface water) was used for irrigation. An increase in residence time resulted in a higher $\log_{10}$ removal by water die-off (Table 2) and also reduced the infection risk as shown in scenario 2 (Fig. 4A-C). Scenario 3 simulated water flow through an aquifer consisting of clean quartz material. The removal by quartz filtration was very low and the infection risk remained higher than after removal by water die-off. Even a longer soil passage length of 100 cm through quartz sand (Fig. 4E, scenario 3) resulted in an infection risk of 4% per 1 potato plant, while a 30 day residence time reduced it to 0.2%. Scenario 4 shows the infection risks of a combined treatment by water die-off and quartz sand removal (30 d, 100 cm), which was lower (0.07%) than the individual treatments. In contrast, removal by attachment to the natural aquifer sand alone (scenario 5) reduced the infection risk by six order of magnitude in comparison with the untreated source water (Fig. 4A-C). Increasing the filtration length to 100 cm (Fig. 4E) further reduced the infection risk by magnitudes to about 10^{-14} per one potato plant. The lowest infection risk (3.3×10^{-17}) was achieved by scenario 6 in Fig. 4E, simulating a residence time of 30 days and a 100 cm natural aquifer sand soil passage. The mean values of the Monte Carlo samples are located between the 75% and 95% percentile with the exception of Fig. 4E, scenario 6. As stated earlier, the removal by die-off and filtration through aquifer sediment was so high that the resulting sample distribution contained many zero values which cannot be displayed on the $\log_{10}$ scale.

Infection risks were also calculated for variations in longitudinal dispersivity which was scaled linearly with the soil passage length. Results are shown in Fig. 5A+B. The ASTR configuration in Figs. 5A and 4A were the same and resulted in slightly higher infection risks when the dispersivity was scaled with the soil passage length. The scaling was also increased to a soil passage of 200 cm and in both cases, the infection

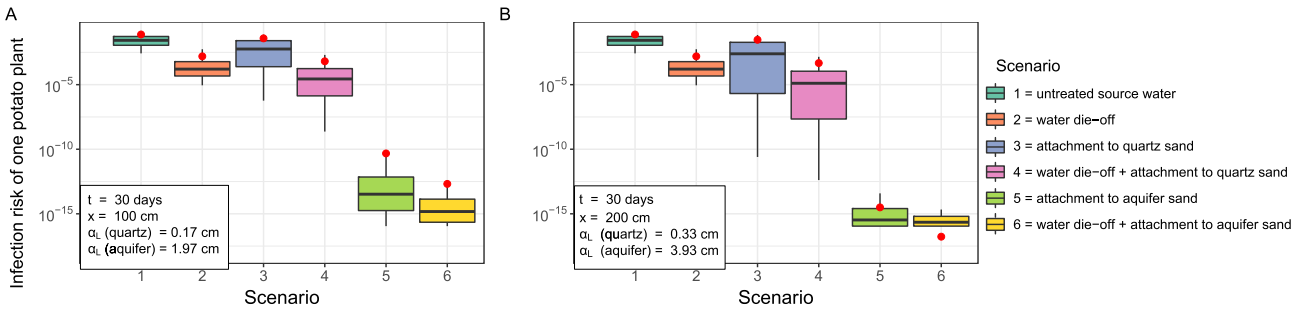

Fig. 5. Infection risks by *Ralstonia solanacearum* per one potato plant after irrigating with water treated through aquifer storage transfer and recovery – ASTR. Upscaling of longitudinal dispersivity at a soil passage length of 100 cm (A) or 200 cm (B). Box-whisker plots describe the distribution of the data and its 5–95% confidence interval. The bottom and top of the box represent the first and third quartiles (25th and 75th percentile values) and the red dot indicates the mean value. Each graph shows the effect on the infection risk depending on the different treatments during ASTR (scenario 1–6). Scenario 1 in all graphs shows the infection risk if untreated source water is used in irrigation (no residence time or soil passage). The treatment depends further on the characteristics of the ASTR operation which are described in the left corner of each graph specifying the simulated residence time (t), soil passage length (x) and longitudinal dispersivity (α_L).

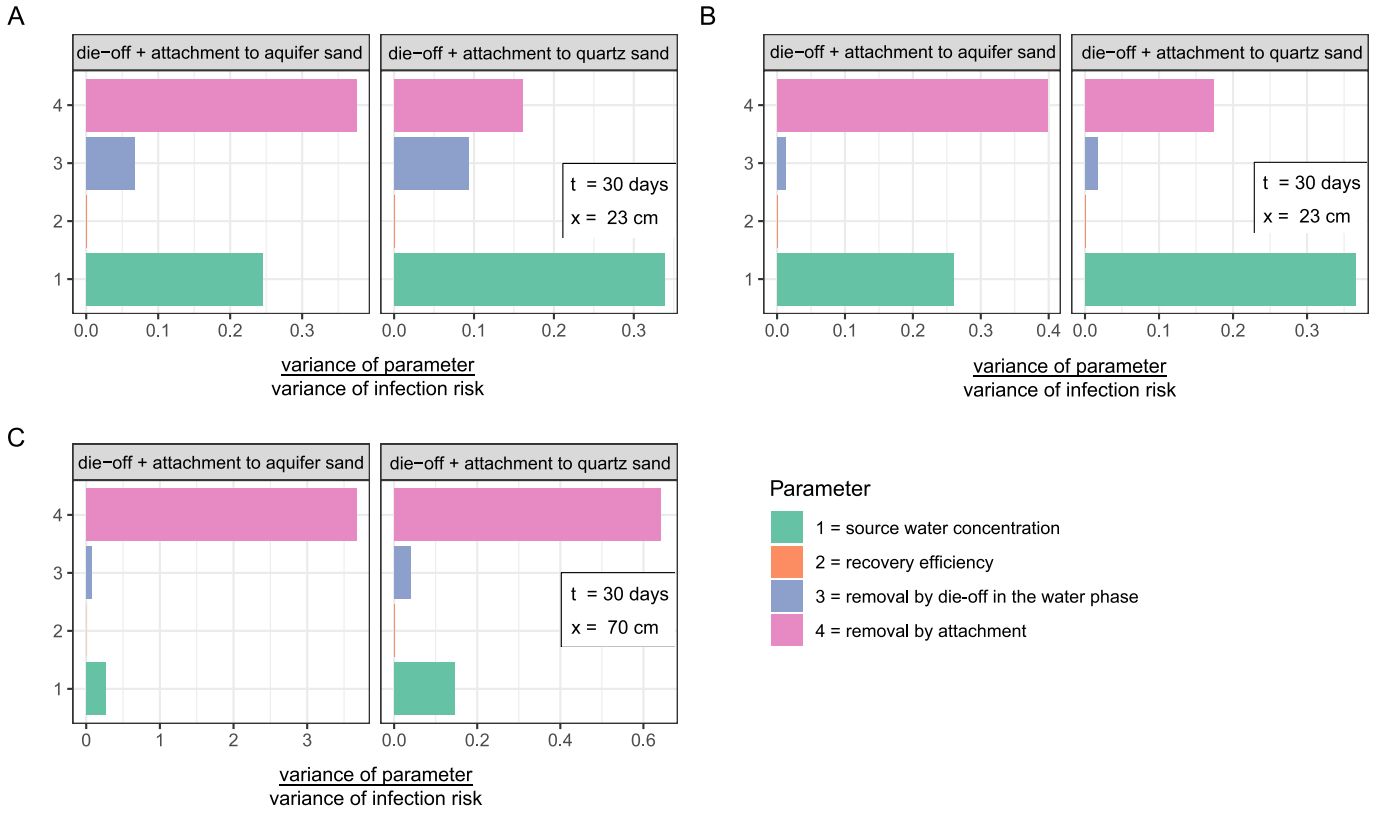

Fig. 6. Sensitivity analysis to analyze the impact of parameters on the infection risk. A-C: Analysis of different ASTR operations as described in the box on the right side of each graph. Left panel always analyses ASTR with pathogen removal by die-off in the water phase and attachment aquifer sand, right panel analyses ASTR with pathogen removal by die-off in the water phase and attachment quartz sand. Scenario 1–4 relate to the different input parameters that impact the final infection risk. The value of the ratios is irrelevant but allows to compare the different parameters with each other.

risks of one plant remained at very low values ($<10^{-10}$).

3.3. Sensitivity analysis

Fig. 6 shows the results of the sensitivity analysis and graphs A-C compared different ASTR operations, while 1–4 describe the different input parameters which were used to calculate the dose. The resulting value of the ratios of variance of the parameter to variance of the risk is irrelevant but allows to compare the different parameters with each other. The left panel of each group describes ASTR with bacterial removal by die-off in the water phase and filtration through aquifer sand while the right panel describes ASTR with removal by die-off in the water phase and filtration through quartz sand. Under all conditions, the recovery efficiency had a negligible impact on the infection risk. In all simulations with aquifer sand (Fig. 6A-C, left panels), the variance in removal by attachment to aquifer sand had the greatest impact on the infection risk. In contrast, the variance in removal by attachment to quartz sand had less impact on the infection risk (Fig. 6A+B, right panels). Here, the variation in source water concentration was the most critical parameter to influence the infection risk. However, when increasing the soil passage length to 70 cm (Fig. 6C, right panel), the variance of the removal parameter had the greatest impact on the infection risk. The sensitivity analysis showed that variation in source water concentration and removal by attachment will have the greatest

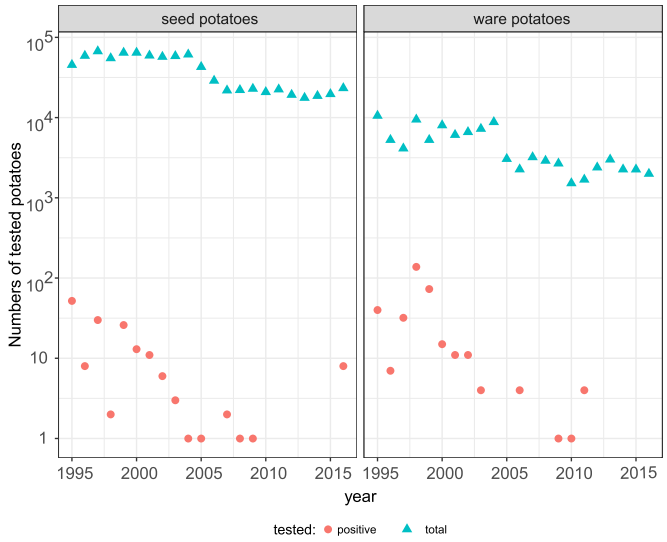

Fig. 7. *Ralstonia solanacearum* infected potato samples detected during yearly surveys (1995–2016) of the domestic potato production in the Netherlands (Commission, 2017). Per sample, 200 randomly selected tubers from a 25 ton potato tuber lot are tested for the presence of *R. solanacearum*. The total number of tested tubers is shown in blue triangles and the positive ones are shown in orange circles. Left panel shows the tested potatoes for seed production where all tuber lots have to be tested. Right panel shows tested ware potato lots, testing of lots is voluntarily.

impact on the infection risk.

4. Discussion

4.1. Baseline risk of infection

The calculated infection risks after using ASTR treated irrigation water are compared with the current risk of brown rot incidences in the Netherlands. To do so, the results of the mandatory seed testing were analyzed. There, the harvested tubers (200 tubers per 25 ton lot) intended as seed tubers are tested for the presence of *R. solanacearum*. Testing of ware potatoes is voluntary and only required if an infection is suspected (Commission, 2017). Fig. 7 shows the total number of tested tubers together with the numbers of positive (infected) tubers. In the mid 1990's, the number of positive tested tubers peaked but declined to zero or few cases until 2005 as a result of the implemented eradication scheme (Janse, 2012). From the ratio of positive tested seed tubers out of the total number of tested seed tubers we the defined the baseline risk of infection (1.9×10^{-4}) which exists although hygiene measures are followed (e.g., tested seed tubers, ban on surface water irrigation).

4.2. Upscaling to a 5 ha potato field

The presented infection risks (Fig. 4) were determined for the irrigation of a single potato plant with ASTR treated water. Using a more applicable scenario, the number of infected plants on a 5 ha field with 250 thousand potato plants was calculated using the infection risk of a single plant as input. 5 ha were chosen as this is the average parcel size for seed potato production in the Netherlands (CBS, 2022). From the infection risks calculated in this study (Fig. 4) the number of infected plants on a 5 ha field were estimated and results are shown in Table 3. For comparison, we also calculated the number of infected plants using the baseline risk.

The baseline risk of infection for seed tubers was 1.9×10^{-4} which would predict 47 infected plants on a 5 ha potato field. Furthermore, it was estimated that 13% of a 5 ha field (6.2×10^{4} plants) would be infected after irrigation with untreated surface water. Similar high numbers can be expected if the MAR treatment would solely rely on water die-off. Consequently, an ASR system where water is stored no longer than 30 days will still result in too high infection risks. An increase in residence time to 60 days would still result in about 919 infected plants. Equally, ASTR treatment which uses quartz sand filtration (23–100 cm) would produce irrigation water of insufficient water quality as many infected plants are predicted ($> 10^{4}$ infected plants on 5 ha field). We even simulated a quartz sand soil passage of 700 cm length which still resulted in a high number of infected plants (2.4×10^{4}). However, it is not expected to find such a sand type in a natural setting as biological activity and weathering will change the surface structure. In our simulations, only the combination of attachment to quartz sand ($x = 700$ cm) with water die-off ($t = 30$ days) would reduce the number of infected plants to 96. Therefore, newly installed slow sand filters (SSF) filled with commercial 'clean' sand used for water treatment require aging of the sand layer to develop a better pathogen removal. Previous research has shown that SSF is an effective method to reduce plant pathogens by magnitudes of order. For example, a horizontal slow sand filter with a length of 27.6 m removed 99.5% (equal to a $\log_{10}$ reduction of 2.3) of *Fusarium* propagules (Prenafeta-Boldú et al., 2017). Moreover, Schijven et al. (2013) demonstrated up to 5-$\log_{10}$ removal of *E. coli* WR1 in a SSF-unit. SSF could also be considered as additional pretreatment for the infiltrated water and improve an existing ASR system. The active biological layer in a SSF also reduces chemical agropollutants such as pesticides (Majsztrik et al., 2017) which showed little sorption in aquifer injection experiments (Kruisdijk et al., 2022b).

In contrast, attachment to aquifer sand (23 cm) alone would predict about the same number of infected plants ($n = 56$) as the baseline risk ($n = 47$). A longer soil passage of 70 cm would already reduce the total of infected plants to a negligible number (4.7×10^{-6}). Finally, an ASTR

Table 3
Estimated number of infected potato plants after irrigating a 5 ha field (250 thousand potato plants) with untreated or ASTR treated surface water.

Baseline risk[a]			47					
	Scenario							
	1	Untreated source water	6.2×10^{4}					
ASTR operation		**residence time [days]**	**10**	**30**	**60**			
	2	water die-off	1.8×10^{4}	1.4×10^{3}	919			
		filtration length [cm]	**23**	**70**	**100**	**700**		
	3	attachment to quartz sand	5.4×10^{4}	4.8×10^{4}	4.5×10^{4}	2.4×10^{4}		
	5	attachment to aquifer sand	56	4.7×10^{-6}	6.3×10^{-10}	–[b]		
		residence time [days]	**10**	**30**	**60**	**30**	**30**	**30**
		filtration length [cm]	**23**	**23**	**23**	**70**	**100**	**700**
	4	water die-off + attachment to quartz sand	1.3×10^{4}	866	585	594	504	96
	6	water die-off + attachment to aquifer sand	4	0.18	0.12	8.1×10^{-6}	$<6.3 \times 10^{-10}$	–[b]

[a] Risk of brown rot infections while all hygiene measures are followed.
[b] Risk cannot be calculated by the model as the dose and risk are too low.

operation with 30 days residence time and a soil passage of 100 cm (aquifer sand attachment) would estimate almost zero ($<6.3 \times 10^{-10}$) infected plants when using ASTR treated irrigation water. Therefore, the ASTR treatment predicts lower number of brown rot infections than the current disease incidences where all hygiene measures area followed. In this context, the ASTR system provides irrigation water of sufficient quality as it would not increase the baseline risk.

4.3. Probability of detection

Seed tuber testing is an important regulations to avoid the transmission of latently infected seed material. In practice, 200 tubers are selected per 25 ton tuber lot and analyzed for the presence of *R. solanacearum* in the tuber material. Given a disease incidence in a tuber lot (P_i), the probability to detect (P_d) at least one infected tuber when sampling 200 randomly selected tubers is calculated as:

$$P_d = 1 - \prod_{i=1}^{n=200} (1 - P_i) \quad (9)$$

The sample number ($n = 200$) is based on detecting at least one infected tuber with a 95% probability, assuming a disease incidence of 1.5% within a tuber lot of infinite size (Janse and Wenneker, 2002). Here, we equated the disease incidence with the infection risks of a single potato plant (P_i) calculated in this study. The same ASTR configuration and scenarios were analyzed to calculate the probability of finding at least one infected tuber in a 200 tuber sample and results are shown in Fig. 8. For comparison, the baseline risk of brown rot incidences in the Netherlands has been used to calculate the probability of detection of at least one infected tuber ($P_d = 4.0 \times 10^{-6}$, shown as dashed purple line in Fig. 8). As discussed earlier, using ASTR treated water can result in higher disease incidences than the baseline risk and consequently, higher probabilities of detection. Therefore, this may be used as indication if an AS(*T*)R system results in a lower or higher calculated probability of detection in comparison to the current situation where the baseline risk exists. The use of untreated surface water had the highest risk of infection and resulted in 100% probability to detect an infected tuber ($P_d = 1$). The same result was observed when simulating attachment to quartz sand regardless the considered soil passage length (Fig. 8A-E). The resulting distributions have a very narrow range as high infection risks result in a high probability of detecting the disease. Moreover, the distributions show a smaller sample size ($n = 200$). Bacterial removal by die-off in the water phase (60 d) reduced the probability of detecting at least one infected tuber to about 7% (Fig. 8C). After removal by attachment to aquifer sand (23 cm), the probability to detect and infected tuber reduced by ten times lower to about 0.7% (Fig. 8A). The combined removal by water die-off and attachment to aquifer sand reduced the probability of detecting an infected tuber to a negligible value. However, only a soil passage of at least 70 cm resulted in lower probabilities of detection than the probability calculated with the baseline infection risk. In general, this demonstrated that the current testing scheme of 200 tubers has a low chance of detecting infected tubers given the few brown rot incidences that are still observed. A bigger sample size could increase the probability of detection but may not be reasonable regarding the cost-benefit of changing the current testing strategy (Breukers et al., 2008). To conclude, both, the upscaling of our infection risks to a 5 ha potato field and the probability to detect

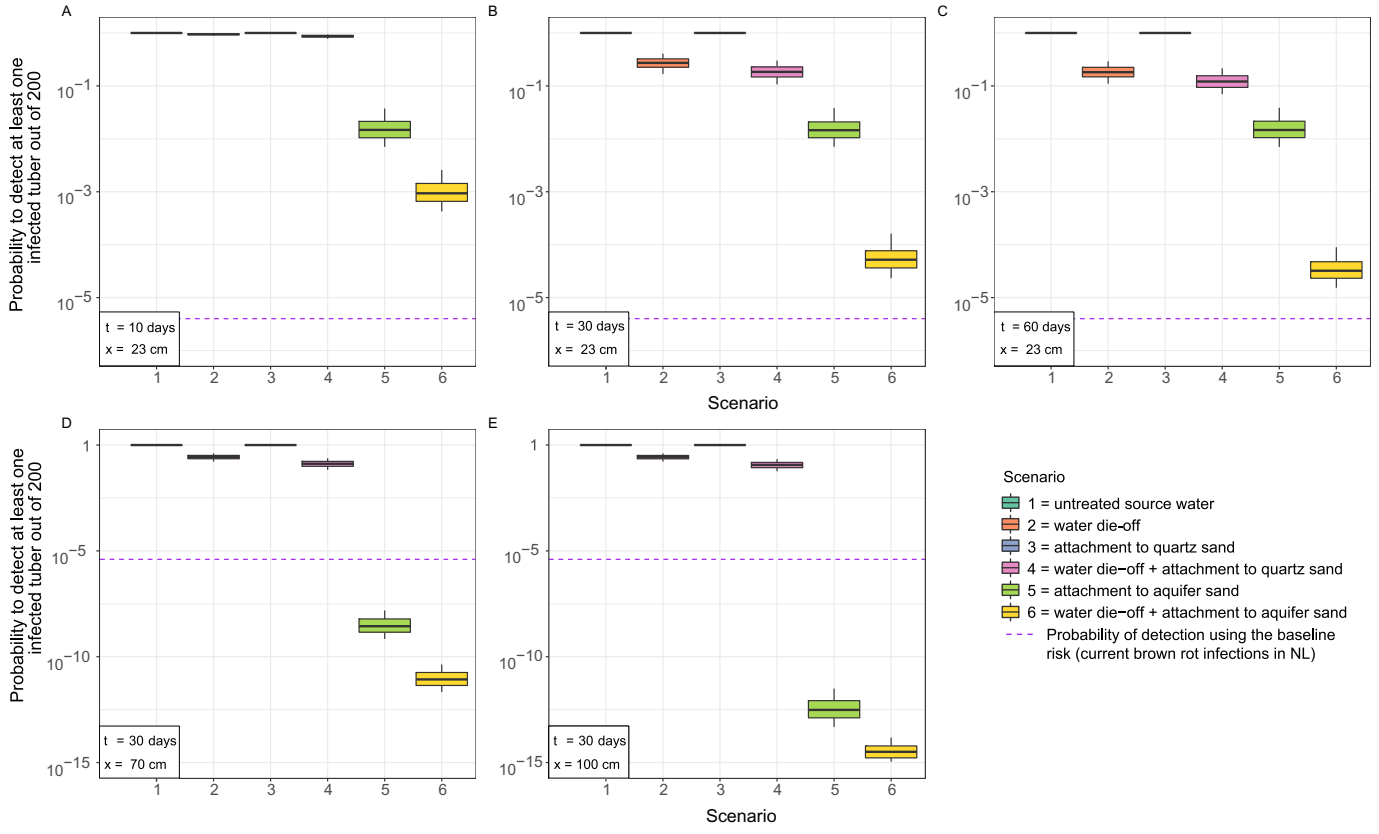

Fig. 8. Probability of detection of at least one infected tuber by *Ralstonia solanacearum* within a sample of 200 randomly selected tubers coming from a potato lot of infinite size and given an infection risk P_i. Box-whisker plots describe the distribution of the data and its 5–95% confidence interval. The bottom and top of the box represent the first and third quartiles (25th and 75th percentile values). Each graph shows the probability of detection depending on the different treatments during ASTR (scenario 1–6). The treatment depends further on the characteristics of the ASTR operation. A-C: increase in residence time from 10 d (A), to 30 d (B) and 60 d (C). D + E: Increase in soil passage length from 70 cm (D) to 100 cm (E). The operational characteristics of the ASTR system are shown in the left corner of each graph specifying the simulated residence time (t) and the soil passage length (x). The dashed purple lines shows the probability of detection calculated with the baseline risk resulting from the occurrences of brown rot infection in the Netherlands (NL).

at least one infected tuber, aimed to place the obtained results within a practical frame and compare the results with the current legislation. The current policies do not consider irrigation with ASTR treated water. Nevertheless, the results of the QMRA and the comparison with the existing risks demonstrated that irrigation with ASTR treated water did not increase infection risk.

4.4. MAR to produce irrigation water

Currently, the use of surface water for the irrigation of seed potatoes is prohibited as disease outbreaks have been linked to contaminated irrigation water (Janse, 1996). The results of this QMRA support this regulation as even low levels of bacteria (max. 65 CFU mL^{-1}) may cause an infection if high irrigation volumes are applied. However, as ASTR is highly effective in removing bacterial pathogens the regulations on water reuse for irrigation should be adapted. From this QMRA, minimal allowable concentrations of the bacterial pathogens could be formulated by decision makers similar to drinking water guidelines (e.g., WHO (2011)). Furthermore, the QMRA allowed to compare infection risks when using an ASR or ASTR system. Considering only die-off in the water phase, an ASR system was insufficient to remove the concentrations of *R. solanacearum* found in surface water as the risk assessment still estimated high infection risks after irrigation. Only if the source water would contain a lower concentration (accidental contamination) or is known to be free of pathogens, treatment by water die-off can be regarded as sufficient. In this case, the regular monitoring of the source water for the pathogen of interest would be a prerequisite. Note, the recharge water within an ASR will still move through the porous medium of the aquifer when the freshwater lens is expanding in the subsurface. Then, recharged water with a longer residence time will have travelled further away from the infiltration well while freshly infiltrated water will have a short travel distance. In order to predict the water flow within an ASR, and thereby bacterial removal by attachment, requires precise knowledge about the hydrological and geological composition of the aquifer. This was also discussed by Page et al. (2015) who analyzed the aquifer treatment of either ASR or ASTR after stormwater infiltration and the related human health risks. The authors concluded that ASR may also be an option considering the application purpose e.g., when using the recovered water for irrigation in parks. Nevertheless, they argued that ASR may deliver less uniform treatment as residence time and travel distance of the recovered water are not certainly predictable which can result in fluctuating pathogen levels. In contrast, a MAR system designed as an ASTR system with a soil passage is highly effective in removing bacterial pathogens as shown in previous work (Pang, 2009; Schijven et al., 2000). In our study, a soil passage length of >70 cm in combination with water die-off due to a longer residence time resulted in negligible infection risks even when upscaling to a 5 ha potato field. This is in agreement with the multi-barrier approach established in aquifer recharge in drinking water production (Aertgeerts et al., 2003). It suggests that water treatments with multiple treatment steps are safer to produce water as each of the barriers reduces pathogen concentrations. Moreover, the barriers should act independently to compensate the failure of one of system (Haas and Trussell, 1998). Here, the soil passage of the ASTR depicted an additional treatment to the water die-off. Furthermore, the sensitivity analysis confirmed that removal by attachment to aquifer sand was the most critical parameter to impact the infection risk. To conclude, the QMRA illustrated that aquifer treatment of an ASTR system showed higher pathogen removal than an ASR system.

4.5. Limitations of QMRA for agricultural MAR

The QMRA was based on several assumptions to simplify and evaluate a complex set of sequential processes. However, the limitations due to simplifications were opposed by conservative assumptions. First, concentrations of *R. solanacearum* found in surface water during summer were used as conservative scenario instead of measurements of the TDW where the concentrations are expected to be zero or lower. Nevertheless, the results demonstrated successful removal of bacterial pathogens by ASTR treatment even if TDW mixes with contaminated surface water as it may happen during storm events and flooding, or if contaminated surface water is directly used for infiltration. Furthermore, the presence of *R. solanacearum* in surface water depends on the water temperature. In temperate climates, a seasonal fluctuation of the pathogen concentration in surface water is observed and bacteria are only present at very low concentrations during winter or remain undetectable (Wenneker et al., 1999). Consequently, infiltration of surface water depicts another safety measure to prevent contamination of the aquifer. Next, the aquifer treatment is based on laboratory studies where the pathogen removal could be studied under well controlled conditions (Eisfeld et al., 2022b; Eisfeld et al., 2021). Most QMRAs which analyzed aquifer treatment mostly measured pathogen concentration in the infiltrated and abstracted water to derive a total $\log_{10}$ removal rate that did not differentiate between removal by water die-off or attachment; a general removal rate is selected to consider both processes during aquifer treatment (e.g., Ayuso Gabella (2015); Masciopinto et al. (2020)). Our study allowed to analyze the individual results of die-off in the water phase or removal by attachment, and a combination of both processes. As this requires the experimental data for both processes of all pathogens of interest other studies collected existing removal rates from literature especially when different microorganisms (virus, bacteria, protozoa) needed to be considered in the QMRA. The experimental conditions of the literature data might not always simulate the aquifer treatment faultlessly. For example, most bacterial die-off in water phase is described by linear die-off models even though the die-off graph often follows a non-linear pattern. As for *R. solanacearum*, the bacterial population undergoes morphological changes during the die-off and different subpopulations may exhibit different resistances to the environmental conditions (Elsas et al., 2001). The die-off experiments with *R. solanacearum* showed that a persistent population exists that remains viable at a low concentration which may pose a risk in aquifer treatment if the water is recovered too early. Even fewer studies investigated the transport of bacterial (plant) pathogens in saturated porous media although our study demonstrated the great potential of aquifer recharge to remove *R. solanacearum*. Therefore, future studies should study the transport of pathogens in different media. However, the complexity of a natural system cannot be fully pictured in lab experiments although natural water and aquifer sand from the MAR study site have been used in the experiments.

Predicting pathogen removal in the field from lab column studies has to be done with caution as lab experiments can overestimate the removal (Oudega et al., 2021; Pang, 2009). Therefore, in this study, we also accounted for the effect of increasing dispersivity which scales linearly with the tested filtration length. From column experiments, dispersivity was determined by monitoring the transport of a non-reactive salt tracer solution through a 23 cm column. To account for the scaling effect, the column-derived longitudinal dispersivity was multiplied with the ratio of the soil passage length field/column. The resulting higher dispersivity values decreased the $\log_{10}$ removal rates in quartz and aquifer sand but calculated infection risks remained still very low. Although the change in dispersivity should not be neglected it only had a very minor effect considering a sandy aquifer of fine to coarse pore structure. Furthermore, pumping activity will influence the groundwater velocity within the aquifer which will affect the transport and attachment of the bacteria. According to colloid filtration theory, the attachment of pathogens will increase with velocity (Tufenkji and Elimelech, 2004). However, column and field studies have shown bacterial removal decreased when applying a higher flow rate which the authors explained by a shorter residence time and more reversible attachment (Hendry et al., 1999; Oudega et al., 2022). Additionally, bacterial breakthrough experiments in a sandy (Hijnen et al., 2005) and gravel aquifer (Oudega et al., 2022) showed that higher pumping rates did not always increase attachment

rates because attachment also depends on the chemical composition of the influent water or the geochemical composition of the aquifer matrix. These studies showed that a higher velocity will influence bacterial removal during aquifer treatment but also indicated that the overall effect on removal at higher velocities remained negligible. Lastly, attachment of bacteria to sand grains is a complex process by itself which may even depend on the bacterial species with regard to velocity changes (Hendry et al., 1999). Plus, differences in bacterial transport were also observed in our column study using the same porous media where removal varied depending on the species (Eisfeld et al., 2022b). In this study, detachment of bacterial pathogens during soil transport has been neglected as the results of the column study showed that detachment was magnitudes lower than attachment. However, if different water types with varying water quality and ionic strength (IS) are used for infiltration this may increase detachment of environmental colloids. For example, Masciopinto and Visino (2017) demonstrated enhanced virus detachment in laboratory studies with natural sediments caused by a reduction in IS. In our study, fluctuations in IS are not expected and unwanted as the recovered water is reused in crop irrigation where low salt concentrations are required. Therefore, the infiltrated and extracted water is monitored for its electrical conductivity (EC).

The dose-response model used in the QMRA analyzed the effect of contaminated irrigation water using a single inoculation event and experiments had to be done in a greenhouse due to the quarantine status of *R. solanacearum*. Therefore, the infection situation in the field may differ. In the field, plants may be irrigated several times with contaminated water using smaller volumes. Here, as conservative scenario, plants were inoculated during a single irrigation event. The inoculation solution contained a dose of bacterial pathogens that the plant would receive throughout a whole cropping season if the irrigation water was contaminated. Moreover, the irrigation method (drip, sprinkler) may influence the infection risks (Café-Filho et al., 2018; Dixon, 2015). The effects of irrigation frequency and method on disease incidences should be analyzed in future studies. The dose-response model was essential to determine the final infection risk. Two different potato cultivars could be tested in the dose-response experiments. Potato cultivar Kondor was more sensitive to irrigation with *R. solanacearum*. Hence, this dose-response model was selected for a more conservative risk estimation in comparison to the more resistant cultivar. Nevertheless, only one host-pathogen interaction could be studied as these experiments are expensive (especially when working with quarantine organisms) and laborious. In the water die-off and soil column experiments, two other plant pathogenic bacteria were analyzed (Soft Rot *Pectobacteriaceae*) but they could not be included in the QMRA as dose-response models are lacking which has to be addressed in future research.

Although the risk assessment contains several uncertainties due to simplification of certain processes, the overall effect of ASTR treatment on the improvement of water quality was clearly identifiable. The attachment to aquifer sand alone could minimize infection risks if ASTR treated water was used for irrigation. Moreover, conservative assumptions (e.g., *R. solanacearum* concentration in the source water) were used in the risk assessment. Plus, uncertainties of the input parameters were including during the Monte Carlo sampling. The resulting infection risks were presented as distribution where the 95% quantile should be used as conservative value in decision making processes. The results indicate that storage time is not needed as the soil passage causes high removal. However, a residence time and thereby removal by water die-off would give an additional safety to the farmer to guarantee pathogen removal. To conclude, the QMRA demonstrated that ASTR is a robust system to store excess tile drainage water in the subsurface and reuse it for potato irrigation. The QMRA predicted that the soil aquifer treatment removes bacterial plant pathogens in order to recover the water for irrigation without risking crop infections.

5. Conclusion

The QMRA presented in this research can help to answer questions regarding microbial water quality during MAR where critical treatment steps of the MAR operation were analyzed. MAR systems designed as ASR or ASTR system both store fresh water in the subsurface where bacterial pathogens can be removed before the water is reclaimed for irrigation. An ASR system will only depend on the residence time and bacterial die-off in the water phase which was slow resulting in low log-removal. Consequently, if the infiltrated water contains levels of *R. solanacearum* as found in surface waters it may require several months of storage time to reduce the bacterial concentrations sufficiently. On the contrary, an ASTR system has an additional soil passage of known length which adds a second treatment barrier where bacteria were removed effectively by attachment to the aquifer sand. The processes during ASTR are complex but QMRA helped to understand the individual treatment steps and their effect on the water quality of the recovered water used for irrigation (or other applications). As consequence, our results demonstrated that a residence time is not required because of the high log-removal by attachment during the soil passage alone (>1 m). However, an additional residence time makes the system's potential to improve water quality more robust. The remaining risks to infect potato plants after using ASTR treated irrigation water were very low or negligible depending on the residence time and soil passage length. This is the first QMRA that focused on plant health with the aim to prevent plant infections. As for drinking water production, QMRA can be used in decision making processes to evaluate water reclamation projects for agriculture. Then, the risk of a plant infection after using ASTR treated irrigation water should be compared with the risk of crop losses due to insufficient water quantity as consequence of droughts. To conclude, QMRA can serve as a valuable tool for risk managers to examine the suitability of MAR with the aim to provide safe irrigation water.

CRediT authorship contribution statement

Carina Eisfeld: Conceptualization, Methodology, Formal analysis, Writing – original draft. **Boris M. van Breukelen:** Funding acquisition, Project administration, Conceptualization, Writing – review & editing. **Gertjan Medema:** Funding acquisition, Conceptualization, Writing – review & editing. **Jan M. van der Wolf:** Funding acquisition, Conceptualization, Writing – review & editing. **Jouke Velstra:** Funding acquisition, Conceptualization. **Jack F. Schijven:** Funding acquisition, Supervision, Conceptualization, Methodology, Writing – review & editing.

Declaration of competing interest

Author JV was employed by company Acacia Water B.V. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

The supplementary data contains the R code which was used to perform the steps of the QMRA analysis. Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.166181.

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