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Assessment of *E. coli* Attenuation during Infiltration of Treated Wastewater: A Pathway to Future Managed Aquifer Recharge

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Abstract: Treated wastewater (TWW) infiltration into non-potable aquifers has been used for decades in Western Australia for disposal and reuse. These wastewater treatment plants (WWTPs) are mostly pond systems, infiltrating secondary TWW with some activated sludge. There is no disinfection of TWW pre-infiltration. This study gave an opportunity to study the fate of *Escherichia coli* (*E. coli)* in aquifers, using compliance monitoring data (2006–2016) and is relevant if water reuse is to be implemented at these sites in the future. Microbiological water quality data (*E. coli*) were evaluated using an advanced statistical method able to incorporate the highly censored data at full scale operational infiltration sites. Subsurface *E. coli* removal from TWW was observed at all 17 infiltration sites investigated. Most sites (14) had less than six detections of *E. coli* in groundwater (58–100% non-detects; 7–117 samples/bore), thus the statistical method could not be applied. The observations could be used to infer between 1 to >3 $\log_{10}$ removal for *E. coli*. The remaining three sites had sufficient detections for probabilistic modelling analysis, the median removal efficiency for *E. coli* was quantified as 96% to greater than 99%, confirming at least 1 $\log_{10}$ removal with potential for several $\log_{10}$ removal. Reductions could not be explained through dilution with the native groundwater alone as there was a high proportion of TWW in observation bores. The observed reductions are likely the result of bacteria retention and inactivation in the aquifer. The magnitude of microbiological water quality improvement highlights the sustainable and reliable use of the aquifer to improve water quality to levels appropriate for low- and medium-risk non-potable uses without using engineered disinfection methods.

Keywords: *E. coli*; managed aquifer recharge; infiltration ponds; treated wastewater

1. Introduction

Infiltration ponds have been in use for decades in Western Australia for treated wastewater (TWW) disposal. This study was undertaken to determine whether current waste disposal operations can transition to Managed Aquifer Recharge (MAR) through evidence that the quality of water recovered is fit for its intended uses and the contaminant attenuation processes are sustainable. This paper focuses only on *E. coli* data collected as part of compliance monitoring with most sites having ten or more years data on groundwater quality, suggesting that impacted groundwater can be assessed for its ability to meet water quality requirements. The wastewater treatment plant associated with the infiltration ponds is usually a multi-pond treatment system producing secondary treated wastewater, though there are also a small number of activated sludge treatment plants. Both treatment systems exclude disinfection pre-infiltration.

While there is a wide spectrum of organic and inorganic contaminants in the TWW, pathogenic microorganisms such as bacteria and viruses pose the greatest risk to human health [1]. *Escherichia coli* (*E. coli*) and enterococcus are the two main microbial indicators that have been used to assess the microbiological quality of TWW in these infiltration systems.

Historically, microbial water quality parameters have been collected at these schemes for many years and, in many instances, decades of water quality data are available. Yet, despite the ongoing operation and analysis of infiltration scheme performance, much of the data has not undergone rigorous analysis to evaluate the treatment efficiency and long-term sustainability of non-potable aquifers to treat wastewater to meet environmental or water reuse objectives in the future. This is largely due to the highly censored nature of the data (i.e., less than detectable limit of quantification), making it difficult to analyse using conventional summary statistics. Currently, the benefit of pathogen removal in aquifers is not adequately appreciated, due to a limited understanding of the removal of pathogens in aquifers and the inability to readily validate removal.

However, the mechanisms that influence microbial transport and removal in aquifers have been reported in the literature. The mechanisms include filtration through the pond *schmutzdecke*, attachment to and detachment from the sediments, straining, and inactivation in the aqueous and solid phases [2]. Changes in the flow rate, temperature, aqueous (pH, ionic strength and major ion composition, redox state) and solid phase chemistry are amongst many factors known to influence the removal and transport of microorganisms in groundwater [3]. Several field studies have reported the potential removal of pathogens [4–6] during Managed Aquifer Recharge (MAR). High pathogen removals (>3 $\log_{10}$) have been reported for MAR systems using TWW [7–10], with microbiological indicators rarely detected in groundwater beneath the infiltration basins. However, many of these studies have not properly accounted for the censored data sets. Similarly, reviews of contaminant removal during MAR (including pathogens and faecal indicators such as *E. coli*) have been previously reported [11–16]. In most of these studies, *E. coli* removals are predominantly reported as occurring during filtration through the soil media—though die-off continues in the groundwater.

The fate and removal of bacteria and the factors influencing their presence in groundwater are important issues to be considered when infiltrating TWW. The main objective of this study was to assess the long-term efficacy of infiltration at the field scale for removing *E. coli*, and to demonstrate that MAR would offer considerable protection for public health and groundwater quality.

2. Materials and Methods

2.1. Scheme Descriptions

A total of 17 full-scale infiltration schemes were considered for analysis (Figure 1) and are summarised in Table 1. Wastewater was treated by ponds in 14 WWTPs, with annual average wastewater discharge between 4×10^3 m^3/year and 770×10^3 m^3/year. Activated sludge was used at four WWTPs in the Perth–Peel metropolitan region, typically with higher annual average wastewater discharge, between 560×10^3 m^3/year and 3500×10^3 m^3/year. The infiltration basins were managed differently across the 17 study sites. Where multiple infiltrations basins were in operation (66% of sites), rotation occurred at the operator's discretion based on the infiltration rates achieved. Basins tend to remain active for long intervals (months) due to the high infiltration capacity of the sandy soils. Observation bore placement in relation to WWTPs varied considerably, from adjacent to the infiltration area (6 m) to over 200 m away. Data used for the analysis were limited to TWW and groundwater samples, collected as part of compliance monitoring for the 17 WWTPs studied and supplied 'as is' from Water Corporation.

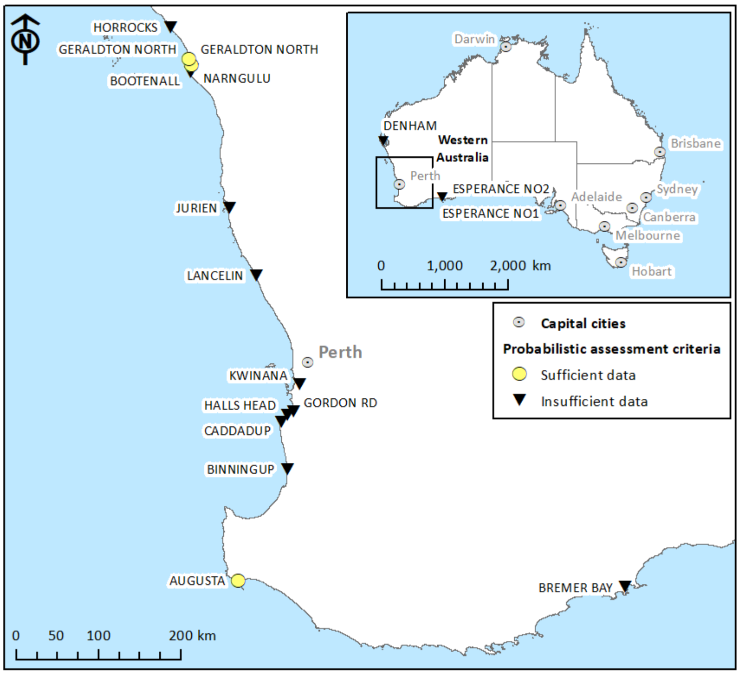


Figure 1. Water Corporation treated wastewater (TWW) infiltration sites.

Table 1. Treated wastewater (TWW) infiltration scheme summaries.

Site Name	Treatment Type	Effluent TWW Quality Data Range	Volume Discharge 2011–2016 Average (Range) (10^3 m^3/Year)	Minimum Distance to Observation Bore (m)
Denham	Pond; facultative and maturation	2005–2016 (*E. coli*) 2000–2016 (other data)	57 (43–72)	90
Horrocks	Pond; primary	2008–2016 (*E. coli*) 2002–2016 (other data)	4.4 (3.8–5.7)	45
Geraldton North	Pond; primary and secondary	2006–2016 (*E. coli*) 1996–2016 (other data)	228 (206–249)	6
Geraldton No. 2	Pond; primary, secondary and storage	2006–2016 (*E. coli*) 1995–2016 (other data)	715 (688–754)	35
Narngulu	Pond; primary and settling	2009–2016 (*E. coli*) 2009–2016 (other data)	773 (704–820)	70
Bootenall	Pond; primary and maturation	2008–2016 (*E. coli*) 1995–2016 (other data)	23 (19–27)	95
Jurien	Pond; primary and secondary	2006–2016 (*E. coli*) 1996–2016 (other data)	64 (50–77)	55
Lancelin	Pond; primary and secondary	2006–2016 (*E. coli*) 1996–2016 (other data)	60 (28–121)	50
Kwinana	Activated sludge (oxidation ditch)	2002–2016 (*E. coli*) 2000–2016 (other data)	1630 (1580–1710)	14
Gordon Road	Activated sludge (oxidation ditch)	2006–2016 (*E. coli*) 1996–2016 (other data)	3500 (3060–3800)	215
Halls Head	Activated sludge (oxidation ditch)	2006–2016 (*E. coli*) 1995–2016 (other data)	1120 (1010–1220)	40
Caddadup	Activated sludge (oxidation ditch)	2006–2016 (*E. coli*) 1997–2016 (other data)	562 (458–655)	25
Binningup	Pond; primary	2006–2016 (*E. coli*) 1995–2016 (other data)	21 (20–23)	95
Augusta	Pond; primary, secondary and tertiary	2006–2016 (*E. coli*) 1995–2016 (other data)	52 (50–56)	90
Bremer Bay	Pond; primary and secondary	2006–2016 (*E. coli*) 1997–2016 (other data)	17 (13–19)	40
Esperance No. 1	Pond; partially aerated and maturation	2006–2016 (*E. coli*) 1996–2016 (other data)	525 (424–562)	55
Esperance No. 2 *	Pond; partially aerated and maturation	2006–2016 (*E. coli*) 1996–2016 (other data)	239 (106–393)	30

* The Esperance No. 1 WWTP is the source of treated wastewater infiltrated at Esperance No. 2.

Only three sites—Geraldton North, Geraldton No. 2 and Augusta—had sufficient uncensored data to proceed with the probabilistic approach described below (Table 2). The remainder of the sites had insufficient groundwater samples above the detection limit for *E. coli* (<10 *n*/100 mL) to perform the statistical analysis.

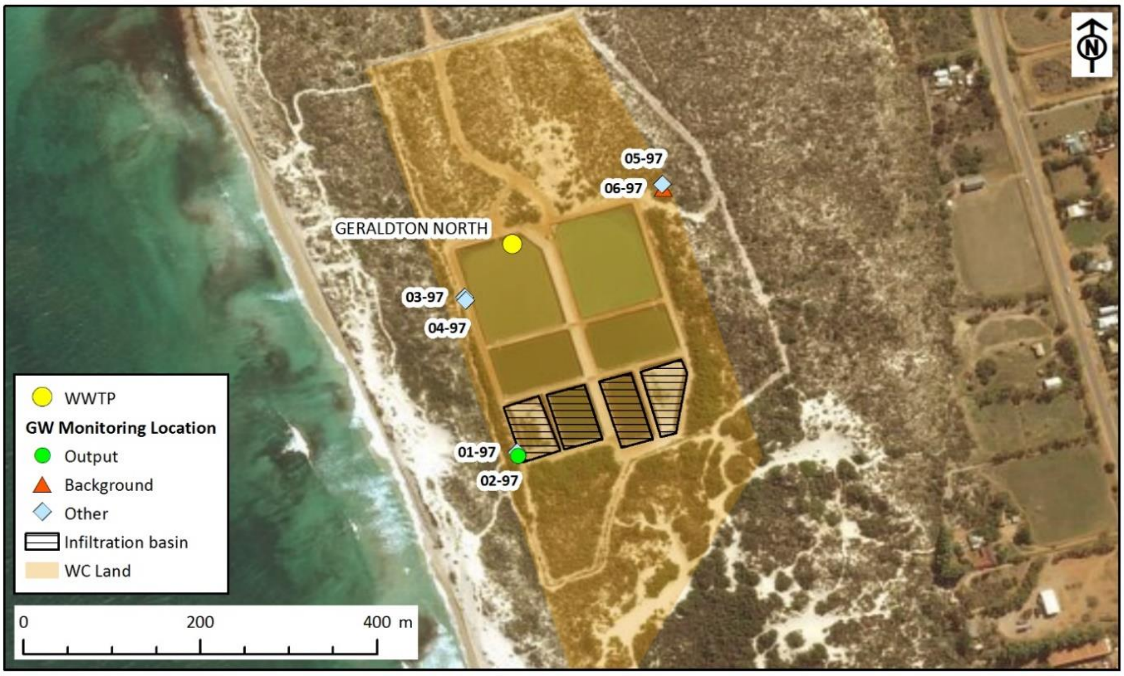


Figure 2. Geraldton North site map. WC Land: Water Corporation owned land.

Figure 3. Geraldton No. 2 site map. WC Land: Water Corporation owned land.

Table 2. Three TWW infiltration schemes selected for statistical analysis of *E. coli* removal efficiency.

Site Name	Geology	Observation Bore Name (Distance in m from Infiltration Scheme)	Background Groundwater Bore [1] Name (Distance in m From Infiltration Scheme)	Shown as
Geraldton North	Calcareous sands/Tamala Limestone	2/97 (6)	6/97 (200)	Figure 2
Geraldton No. 2	Calcareous sands/Tamala Limestone	6/94 (35), 10/94 (185)	4/94 (440)	Figure 3
Augusta	Sandy clays and lateritic gravels/fractured and karstic calcarenite	Bore 4 (90), Bore 6 (160)	na [2]	Figure 4

[1] Selected based on groundwater gradients and water quality data; [2] na = no suitable background bore.

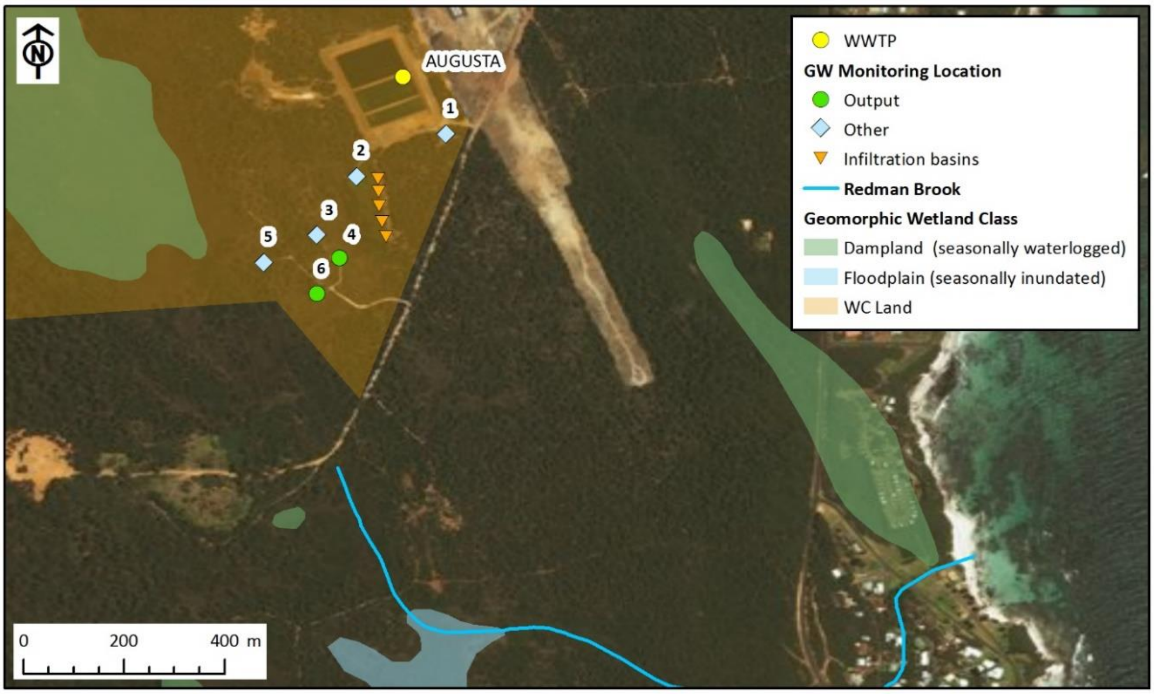


Figure 4. Augusta site map. WC Land: Water Corporation owned land.

The Geraldton North and Geraldton No. 2 WWTPs are both licenced and are located on calcareous sand overlying Tamala Limestone. The Geraldton North WWTP consists of two trains of primary and secondary ponds, followed by four infiltration basins (Figure 2). The second (western) train of treatment ponds and associated infiltration ponds were commissioned in 2008 [17]. The environmental receptor in close proximity to the site is the Indian Ocean (W). Groundwater flows to the coast in a WSW direction.

The Geraldton No. 2 WWTP consists of primary and secondary ponds followed by infiltration basins (Figure 3). Due to issues with clogging of the infiltration ponds, a high-rate irrigation disposal area was established in 2000 in the north of the site. The TWW was irrigated using overhead sprinklers on bare soil (see hatched area in Figure 3) and vegetation was managed—with no trees or crops grown. The environmental receptor in closest proximity to the site is the Indian Ocean. Groundwater flows to the coast in a WSW direction [17].

Augusta WWTP is licenced and consists of clay-lined primary, secondary and tertiary ponds [10] and a series of five small, ill-defined infiltration basins (Figure 4), located on ferruginous duricrust (laterite) potentially overlain by residual bleached quartz sand [18,19]. The environmental receptor in closest proximity to the site is the small stream, Redman Brook (SW, Figure 4). The headwaters of Redman Brook are 450 m from the infiltration basins, though there is likely to be connection in winter to the Dampland to the west of the site. Groundwater flows are likely to follow topography in a SW direction.

2.2. Sampling and Analysis

Water quality was determined from grab samples collected at each infiltration site and recovered water from nearby monitoring bores—as required by the environmental licence conditions for each of the sites by Water Corporation system operators. Typical water quality parameters common to both TWW and groundwater include pH, electrical conductivity (EC), total dissolved solids, *E. coli*, total nitrogen, ammonium-N, nitrate-N and total phosphorus. Licence conditions for all sites stipulate the sampling frequency for TWW and groundwater quality as being typically either quarterly, six monthly or annually. However, higher frequency data was available for TWW. This study focuses on *E. coli* and the data used in this study were collected between 2006 and 2016, though this varied slightly between sites and between TWW and groundwater samples. According to the site licence conditions, Geraldton North TWW and groundwater were sampled quarterly (2008–2015), Geraldton No. 2 TWW quarterly and groundwater 6 monthly (2008–2015), and Augusta TWW and groundwater quarterly (2006–2015). However, additional ad hoc samples (e.g., monthly TWW data supplied for Augusta) were also included in the analysis (see Table 3 for sample numbers). Where background groundwater data was available the dilution of TWW in native groundwater was assessed using EC values.

Table 3. *E. coli* and EC data of the ambient groundwater, infiltrated TWW and observation bore for each TWW infiltration scheme.

Site Name	Bore Name	*E. coli* (*n*/100 mL)					EC (mS/m)			
		n	% n.d.	Mean	50th	95th	*n*	Mean	50th	95th
Geraldton North	TWW	43	0	7.7×10^4	1.8×10^4	3.0×10^5	63	260	250	350
	Obs. Bore 2/97	22	41	2700	8	2600	34	1440	550	5000
	Groundwater 6/97	22	68	2.3×10^6	<1	5600	34	930	860	1700
Geraldton No. 2	TWW	56	0	4.1×10^{10}	6.9×10^6	8.8×10^9	105	221	221	242
	Obs. bore 6/94	35	94	<10	<10	<10	46	261	259	308
	Obs. bore 10/94	34	58	2600	1.3	1000	41	268	265	337
	Groundwater 4/94	35	86	2100	<10	160	43	522	520	603
Augusta *	TWW	115	12	400	110	1600	189	108	105	157
	Obs. bore 4	19	52	68	4.2	200	35	148	140	237
	Obs. bore 6	28	100	<10	<10	<10	44	209	202	312

* No suitable background bore; *n* = number of samples; n.d. non-detects.

Water samples were maintained below 4 °C and delivered to the laboratory within 24 h according to procedures and storage times recommended in the Standard Methods for the Examination of Water and Wastewater [20]. The presence of *E. coli* was determined using the most probable number procedure [21]. Electrical conductivity was monitored in the observation bores to provide an indication of the degree of mixing between the TWW and the native groundwater. EC was determine using the standard electrode method (platinum electrode) as per Standard Methods (2510 B) [20].

2.3. Probabilistic Approach to Characterise Infiltration Pond Removal of E. coli

Assessment of infiltration pond treatment performance can be challenging, as *E. coli* concentrations are variable over time, and each infiltration system also varies as a function of several factors including hydraulic residence time, injectant water quality and temperature. Therefore, *E. coli* concentrations are represented as stochastic variables and described by probability density functions (PDFs). This approach has been previously used to determine the treatment performance in advanced water treatment plants (e.g., [22]) and was used to determine an input 'infiltrated' PDF and an output 'monitored' PDF. These infiltrated and monitored PDFs can then be used to derive theoretical infiltration treatment efficiency PDFs for each specific infiltration scheme.

Microbiological water quality data is commonly well-described by lognormal distributions [23]. Accordingly, the variability of *E. coli* numbers has been characterised by fitting observed data to lognormal PDFs. Removal efficiency (RE) can be obtained by the following:

$$RE_{(x)} = 1 - MON_{(x)}/INF_{(x)} \tag{1}$$

where $RE_{(x)}$ is the calculated value of the removal efficiency PDF at percentile x, and the $INF_{(x)}$ and $MON_{(x)}$ are values of the PDFs for the hazard at percentile, x, for the infiltrated TWW and monitored groundwater, respectively.

An advantage of this method is that such plots can be prepared even with the censored data included, where some of the data is below the detectable limit of quantification since the PDF can be plotted from the detectable results accounting for the percentage of non-detectable results of analyses. In all cases in the current study, the data was highly left censored and so not amenable to conventional summary statistical analysis.

2.4. Assessment of Other Sites with High Number of Censored Data

Due to the high number of censored data (79–100% of data less than the limit of detection, LOD) the remaining 15 sites could not be analysed using the probabilistic approach outlined above. For these 15 sites, summary statistics of *E. coli* numbers in the TWW were calculated using SigmaPlot 14.0© [24]. The median *E. coli* number was assumed as indicative of the typical TWW concentration. Utilising the entire TWW-impacted groundwater data set (censored and uncensored data) the median (50th percentile) falls within the censored data, i.e., <LOD despite not being able to use the approach in Section 2.3 to estimate the median value. As such, for the purposes of providing an estimate of *E. coli* removals for these sites, the median *E. coli* number in TWW-impacted groundwater was assumed to be zero. Indicative $\log_{10}$ *E. coli* removals were calculated by the following:

$$E.\ coli\ \text{removal}\ (\log_{10}) = \text{median}\ E.\ coli_{\ \text{TWW}} - \text{median}\ E.\ coli_{\ \text{GW}} \tag{2}$$

where *E. coli* $_{\text{TWW}}$ is the median in TWW, and *E. coli* $_{\text{GW}}$ is the median in groundwater (assumed to be zero).

3. Results

3.1. Overview of E. coli Numbers TWW and Groundwater

Figure 5 illustrates the variability in *E. coli* numbers in the TWW in relation to the minimum and maximum detection limits of 10 n/100 mL and 24,000 n/100 mL. Notably, each of the three sites with sufficient uncensored groundwater data to proceed with the probabilistic approach represent the minimum (110 n/100 mL), median (18,000 n/100 mL) and maximum (>24,000 n/100 mL) of median *E. coli* numbers in TWW across all sites.

Median *E. coli* numbers in TWW for the remaining wastewater infiltration schemes varied between 160 n/100 mL (Bremer Bay) and >24,000 n/100 mL (Esperance). Across all of these sites, there was a maximum of six detections (>10 n/100 mL) of *E. coli* in groundwater observation bores, with censored values representing 79% to 100% of the total *E. coli* measurements (n = 7 to 117) across the sites (see Table S1). Where *E. coli* was detected in the groundwater, the detections were likely from a combination of observation bore contamination due to improper sealing, sampling errors or laboratory errors. However, due to the large number of sites investigated, and the limited information on land use and site histories, it is not possible to be definitive as to the source of *E. coli* detections in groundwater. Thus, for the majority of sites, there was insufficient data to proceed with the probabilistic approach. For these schemes, the observation bores were between 14 m (Kwinana) and 145 m (Onslow) from the infiltration basins (Table 1). The low *E. coli* values suggest an aquifer treatment capacity of between 1 and 3 $\log_{10}$ for *E. coli* for the sites is not able to be assessed using the probabilistic approach.

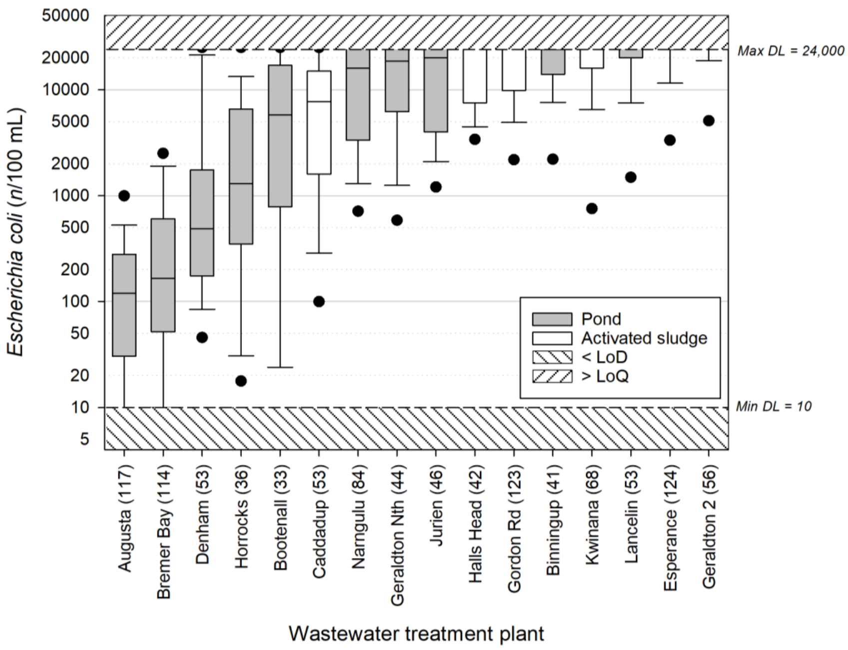


Figure 5. Summary of *E. coli* numbers in treated wastewater (TWW). Boxes represent 25th to 75th percentiles with line shown at 50th percentile, the whiskers represent the 10th and 90th percentile values and black circles show the 5th and 95th percentile values. The number of samples are shown along with the treatment plant name. LOD is the limit of detection and LOQ is the limit of quantification.

3.2. *E. coli and EC at Three Infiltration Sites Analysed with Probabilistic Approach*

The results of monitoring *E. coli* and electrical conductivity (EC) measurements for TWW and groundwater at Geraldton North, Geraldton No. 2 and Augusta are given in Table 3. The summary statistics presented in Table 1 are typically from the same lognormal fitted curves, used to subsequently determine the removal efficiency for each parameter. The exceptions are the *E. coli* numbers in groundwater bore 6/94, 4/94 (Geraldton No. 2) and bore 6 (Augusta), where data could not be fitted due to there being too few detections; here, summary statistics of measured data are reported.

In fitting the data, a number of potential distributions were evaluated which included normal and lognormal distributions. The data did not allow good discrimination between the potential distributions, and so lognormal was eventually selected based on the best fits and previous results [25].

The calculated median *E. coli* numbers in observation bores varied between 1 and 8 *n*/100 mL, all below the analytical limit of detection (10 *n*/100 mL). The median groundwater *E. coli* was similar at these three sites, despite four orders of magnitude variation in the median TWW numbers.

3.3. *EC as an Indicator of Mixing at Infiltration Sites*

Lognormal probability plots for the TWW and observation and background bores for EC for each of the infiltration sites are presented in Figure 6. Electrical conductivity (EC) is commonly monitored in the observation bores, and provides an indication of the degree of mixing between the TWW and the native groundwater. For the Geraldton WWTPs, the TWW is fresher than the native groundwater, so any observed increase in salinity will indicate potential dilution of TWW-impacted groundwater with native groundwater. For Geraldton North, TWW represents 51% of groundwater at 2/97, while for Geraldton No.2, TWW represents 87% and 85% of groundwater at 6/94 and 10/94, respectively. This indicates that a higher proportion of TWW is represented in these bores and that dilution does not play a large role in decreasing *E. coli* at these sites. No suitable background bore was located for the Augusta site, therefore the proportion of mixing with ambient groundwater could not be assessed. Notably, the background bore salinity in proximity of the Geraldton WWTPs is variable, indicating the vulnerability of groundwater in the unconfined aquifers to various influences on water quality.

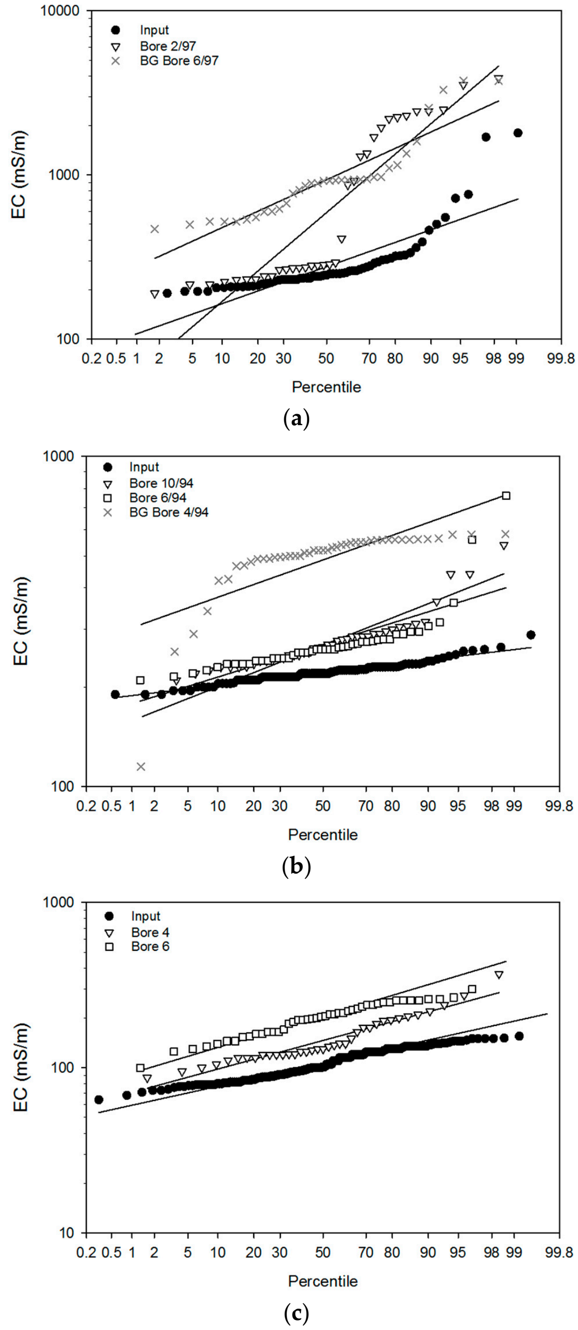
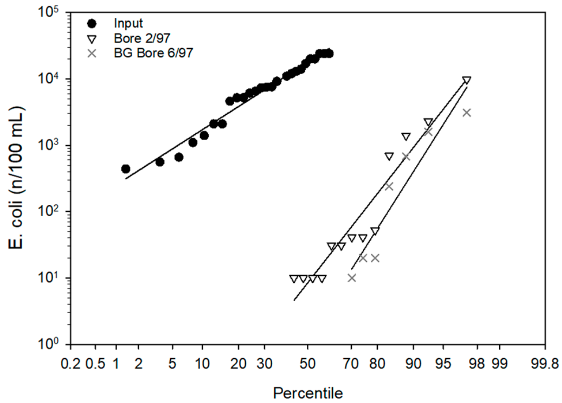


Figure 6. Lognormal probability plots for electrical conductivity (EC) for (**a**) Geraldton North, (**b**) Geraldton No. 2 and (**c**) Augusta.

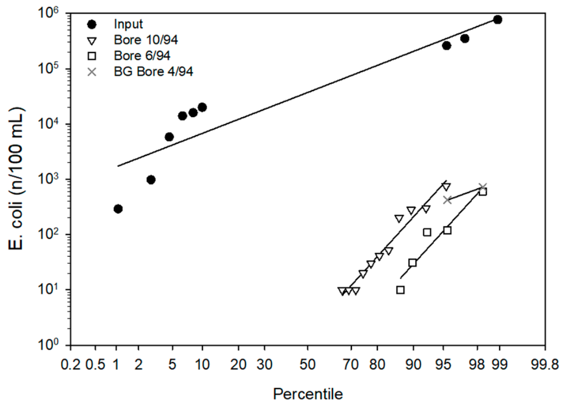
For the remaining 14 sites, the calculation of mixing fractions was not possible at 8 sites due to the lack of background monitoring locations, either without a bore or the infiltration basins being situated on a groundwater divide. Of the remaining sites, TWW represented 4% to 100% of groundwater in the observation bores. However, the locations of both background and observation bores were generally not situated on ideal flow paths, thus the variability may be higher than expected for ideal bore placement. Between and within site variation in TWW disposal rates also impacts the assessment of the mixing fraction. These issues are inherent in the compliance monitoring data set used and are too varied to discuss in detail herein.

3.4. E. coli Removal Across Three Infiltration Sites

Lognormal probability plots for the TWW and observation and background bores for *E. coli* for the infiltration schemes are presented in Figure 7. For *E. coli*, which was the most measured microbial indicator, median treated wastewater concentrations ranged from 110 *n*/100 mL to >24,000 *n*/100 mL. Figure 3 shows that there was a substantial removal of *E. coli* over the long-term operation at each of the infiltration schemes. Both Geraldton sites show some evidence of faecal indicator contamination in the assigned background bores that may warrant further investigation; highlighting the vulnerability of shallow groundwater to various contamination sources.



(**a**)



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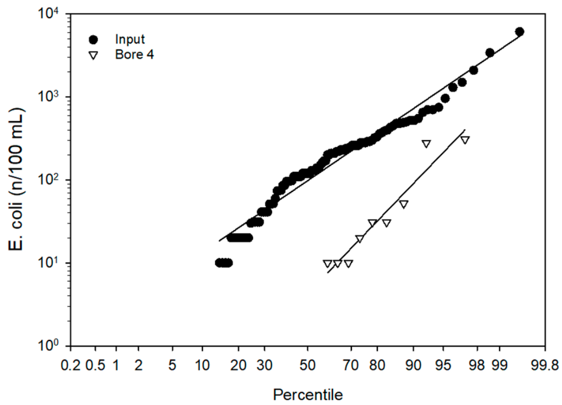

(**c**)

Figure 7. Lognormal probability plots for *E. coli* at (**a**) Geraldton North, (**b**) Geraldton No. 2 and (**c**) Augusta.

3.5. Removal Efficiency of *E. coli* during TWW Infiltration

The lognormal fitted *E. coli* and EC data values shown in Table 4 were then used to calculate the percentage removal of *E. coli* during infiltration according to Equation (1). This removal may represent inactivation, filtration and attachment processes, as well any reduction in *E. coli* numbers due to dilution (as indicated by EC). The results of the Monte Carlo calculations for removal efficiency are shown in Table 4.

Table 4. The calculated mean, median and 95th percentile removal efficiency (RE) for *E. coli* and EC.

Site Name	Bore Name	*E. coli* RE			EC RE		
		Mean	50th	95th	Mean	50th	95th
Geraldton North	Obs. Bore 2/97	0.68	>0.99	>0.99	−4.8	1.2	−0.77
Geraldton No. 2	Obs. bore 6/94	n.d. ‡	n.d. ‡	n.d. ‡	−0.18	0.17	−0.03
	Obs. bore 10/94	0.86	>0.99	>0.99	−0.22	0.20	−0.07
Augusta	Obs. bore 4	−0.70	0.96	>0.99	−0.46	0.35	−0.31
	Obs. bore 6	n.d. †	n.d. †	n.d. †	−1.1	0.93	−0.06

n.d. not determined; ‡ 2 detections in groundwater (n = 35); † 0 detections in groundwater (n = 30).

For *E. coli* the median removal efficiency was 96% at Augusta Bore 4 and >99% at Geraldton North and Geraldton No. 2 bore 10/94. Similar removals have also been observed for treated wastewater infiltration studies conducted at the Halls Head WWTP, where *E. coli* concentrations were ≤1 n/100 mL [26] and in the Floreat Infiltration Galleries [27].

4. Discussion

4.1. Fate of *E. coli*

Median *E. coli* removal efficiency was quantified at 96% or above at three sites equating to a 1.4 $\log_{10}$ to >2 $\log_{10}$ reduction in *E. coli* in groundwater. For nine of the remaining 14 sites with median *E. coli* numbers < LoQ, and using a groundwater *E. coli* count of 10 n/100 mL, a log removal of between 1.2 $\log_{10}$ and 3.4 $\log_{10}$ can be claimed—though these cannot be statistically proven. If a more sensitive detection limit for *E. coli* in groundwater of <1 MPN or n/100 mL had been adopted, higher removal efficiencies could be calculated for more sites; potentially increasing log removal by up to 1 $\log_{10}$. More sensitive detection limits would also provide greater opportunities for reuse in Western Australia, e.g., *E. coli* < 1 MPN or cfu/100 mL would allow high-exposure risk level reuse, such as non-potable residential use (Table 5), greatly decreasing the demand on valuable potable water resources.

Classical colloid filtration theory [21] predicts that *E. coli* transport in most aquifers will be limited to distances of metres to tens of metres under typical infiltration pond operational conditions in a sandy soil [26]. The processes of major importance for the removal of *E. coli* are attachment to the aquifer sediments and decay [22,23]. The decay or dying-off of bacteria during transport depends on many factors such as pH, temperature, redox conditions, predation and attachment [11,13,15,28,29]. Similarly, processes of bacterial attachment are strongly linked to a wide variety of physicochemical, biological, and hydrodynamic factors. The exact mechanism of *E. coli* removal needs to be investigated on a site-by-site basis due to the wide difference in these parameters and is a matter for further investigation.

4.2. Aquifer Treatment Barrier in Water Recycling

The quantitative assessment of potential risk from microbial hazards in recycled water schemes relies on the measurement of individual pathogens. Given the large number of pathogenic hazards in source waters for MAR, three reference pathogens have been identified within the Australian Guidelines for Water Recycling to represent bacterial, viral and protozoan risks; *Camplylobacter*, rotavirus and *Cryptosporidium* [30,31]. Since the TWW infiltration schemes are regulated as disposal schemes, pathogen numbers are not currently required to be measured. However, the measured *E. coli* numbers can be considered in the context of the current Western Australian guidelines for the non-potable uses of recycled water, which instead applies a qualitative risk assessment [32]. Furthermore, should the infiltration schemes be altered to include re-use, then they will be assessed under the Australian Guidelines for Water Recycling [32] in accordance with Western Australian state policy [33].

Table 5. Validation and verification monitoring requirements for *E. coli* in Western Australian guidelines for the non-potable uses of recycled water (after [32]).

Exposure Risk Level (Level of Human Contact)	Potential End-Use	*E. coli* Compliance Value (MPN or cfu/100 mL)
High	• Multi-unit dwellings internal use and external surface irrigation • Agricultural irrigation of unprocessed foods • Urban irrigation with unrestricted access and application • Communal use, such as toilets	<1
Medium	• Urban irrigation with some restricted access • Fire fighting • Water features, such as fountains • Industrial use with potential human exposure • Dust suppression	<10
Low	• Communal subsurface irrigation • Urban irrigation with enhanced restricted access and application • Agricultural irrigation of non-edible crops	<1000

Following risk assessment and management, validation and verification monitoring is used to ensure the recycled water scheme continues to comply with water quality objectives. Microbial indicators, such as *E. coli*, play an important role in validation and verification monitoring programs for water recycling. The *E. coli* compliance values for high, medium and low risk exposures are summarized in Table 5. The median *E. coli* numbers in groundwater impacted by TWW infiltration (Table 5) meet the compliance value of <10 MPN or cfu/100 mL for medium risk level exposure. A more sensitive detection limit of <1 MPN or cfu/100 mL is required to assess the suitability of groundwater for higher exposure risk levels.

5. Conclusions

Characterisation of three pond infiltration systems that dispose of TWW via a non-potable aquifer showed −2.0 log improvements in microbiological water quality (measured as *E. coli*). The median *E. coli* numbers in groundwater impacted by TWW infiltration meet the compliance value of <10 MPN or cfu/100 mL for medium risk level exposure. Natural treatment systems—such as aquifers—can aid in the management of TWW by improving water quality. In Western Australia, the aquifer has been recognized as a treatment barrier in water recycling for non-potable use (e.g., Geraldton); however, the goal remains for this treatment capacity to be more widely recognised.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/1/173/s1, Table S1. Summary of *Escherichia coli* (*E. coli)* samples of treated wastewater (TWW), ambient groundwater and observation bores collected at wastewater treatment plants in Western Australia.

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Abbreviations

The following abbreviations are used in this manuscript:

EC	electrical conductivity
E. coli	*Escherichia coli*
MAR	managed aquifer recharge
MPN	most probable number
PDF	probability density function
RE	removal efficiency
TWW	treated wastewater
WWTP	wastewater treatment plant

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