A QUANTITATIVE MICROBIAL RISK ASSESSMENT MODEL FOR TOTAL COLIFORMS AND *E. COLI* IN SURFACE RUNOFF FOLLOWING APPLICATION OF BIOSOLIDS TO GRASSLAND

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Abstract

The land application of treated municipal sludge ('biosolids') may give rise to surface runoff containing coliforms during episodic rainfall events, which may be potentially harmful to human health if not fully treated in a water treatment plant (WTP). This study used surface runoff water quality data generated from a field-scale study in which three types of biosolids (anaerobically digested (AD), lime stabilised (LS), and thermally dried (TD)) were spread on micro-plots of land and subjected to three rainfall events at time intervals of 24, 48 and 360 hr following application. Under the assumption that this water directly entered abstraction waters for a WTP without any grassed buffer zone being present, and accounting for stream dilution, die-off rate and modelling various performance scenarios within the WTP, the aim of this research was to conduct a human health risk assessment of coliforms (total and faecal), which may be present in drinking water after the WTP. Two dose response models for probability of illness were considered for total and faecal coliform exposure incorporating two different exposure scenarios (healthy populations and immuno-compromised populations). The simulated annual risk of illness for healthy populations was below the US EPA and World Health Organisation tolerable level of risk (10^{-4} and 10^{-6}, respectively). However, immuno-compromised populations may still be at risk as levels were greater than the tolerable level of risk for that subpopulation. The sensitivity analysis highlighted the importance of residence time in a stream on the bacterial die-off rate.

Keywords: biosolids, coliforms, risk, surface-runoff, water treatment

Introduction

The application of treated municipal sewage sludge ("biosolids") to agricultural land as a fertiliser can offer an excellent source of nutrients (nitrogen, phosphorus and potassium), increase organic matter and water absorbency, and reduce the possibility of soil erosion. It is also a cost-effective way to dispose of municipal waste and reduce over-reliance on landfill whilst cutting down on tipping fees. However, biosolids can also be non-point source contributors of heavy metals, human pathogens and xenobiotics (Clarke and Cummins, 2014; McCall et al., 2015; Peyton et al., 2016). Therefore, it is imperative that all biosolids are effectively treated to remove pathogens and contaminants to a "safe level" prior to being used as a land conditioner or fertiliser.

More than 10 million tonnes of sewage sludge was produced in the European Union (EU) in 2010 (Eurostat, 2014). Although EU policy favours the recycling of resources (COM, 2014), including sludge, national sludge recycling policy varies throughout Europe. In some countries, such as the Republic of Ireland, up to 80% of sludge is reused in agriculture (Eurostat, 2014), whereas in other countries, such as Germany, the land application of sludge is prohibited. This is due to the considerable public acceptance issues surrounding the reuse of treated sludge as a fertiliser. The main fear is that the presence of organic and inorganic contaminants in biosolids may accumulate in the food chain, or cause the contamination of soil and water (Clarke et al., 2015). The US Environmental Protection Agency (USEPA) Part 503 regulations classify biosolids according to Class A and Class B standard. Class A biosolids contain a faecal coliform density below 1000 most probable number (MPN)/ g of total solids (dry matter, DM), whereas Class B biosolids contain a geometric mean faecal coliform density of less than 2×10^6 MPN / g of total solids (DM) (USEPA 2006). In the USA, the land application of certain types of biosolids requires a class B designation, which must satisfy three different criteria, one of which includes faecal coliforms whose level cannot exceed 2×10^6 MPN/g (Pascual-Benito et al., 2015). In the EU, sewage sludge production is regulated by the Sewage Sludge Directive 86/287/EC. It does not specify limits for pathogens but instead specifies general land use, harvesting and grazing limits to provide protection against the risk of infection (Sobrados-Bernardos and Smith, 2012). A revision of the Sewage Sludge Directive (Working Document 3rd Draft) states that "the use of microbial indicators to evaluate the hygienisation of treated

sludge is based on fulfilling the limits of *E. coli* to achieve a 99.9% reduction and to less than 1×10^3 cfu/g dry weight, produce a sludge containing $< 3 \times 10^3$ spores of Clostridium perfringens/g (DM) and absence of salmonella. spp in 50 g (DM)" (EC 2000). Furthermore, the Working Document also states that sludge produced by conventional treatment shall at least achieve a 2 $\log_{10}$ reduction of *E. coli* (Mininni et al., 2014). European countries are allowed to include their own parameters in their national regulations (Pascual-Benito et al., 2015). For instance, in France the standards for maximum concentrations of pathogens in biosolids cannot exceed 8 MPN/10 g^{-1} DM for *salmonella,* whereas in Finland, the number of *E. coli must be* less than 1000 cfu and *Salmonella* must not be detected in 25 g of biosolids (Mininni et al., 2014). Meanwhile, in Ireland the standards for maximum concentrations must not exceed 1×10^3 MPN g^{-1} which is equivalent to Class B biosolids under the USEPA Part 503 regulation (Fehily Timoney and Company 1999).

Following land-spreading of biosolids, there are two main scenarios which can lead to human infection. First, pathogens may be transported *via* overland or sub-surface flow to surface and ground waters, and infection may arise *via* ingestion of contaminated water or accidental ingestion of contaminated recreational water (Jaimeson et al. 2002; Tyrrel and Quinton 2003). Alternatively, it is possible that viable pathogens could be present on the crop surface following biosolids application, or may become internalised within the crop tissue, where they are protected from conventional sanitization (Itoh et al. 1998; Solomon et al. 2002). In this case, a person may become infected if they consume the contaminated produce. Faecal coliform numbers in the stabilised biosolids can be high, up to 10^5 g^{-1} DM (Schwarz et al., 2014). Gerba and Smith (2005) reported general survival times for bacteria in soil to be 2-12 months, whilst Lang et al. (2007) reported survival times of enteric micro-organisms in sludge-amended soil varying between 24 hours to 2 years. The disparities in survival rates are difficult to define due to "knowledge gaps" with regards to decay mechanisms and the complex interactions between the environment and soil-specific factors that result in the decay of enteric bacteria (Schwarz et al., 2014). Therefore, it is critical to accurately determine the pathogen risk associated with land application of sewage sludge to fully understand the potential for environmental loss and consequently, human transmission.

Coliforms are bacteria that are always present in the digestive tract of animals including humans, and are found in their waste. They are also found in soil and plant material. Total coliform (TC) bacteria are common in the environment and, with a few exceptions, are generally harmless (USEPA 2013). They are typically used as an indication of other pathogens in drinking water. Faecal coliform bacteria are gram negative, non-spore forming rods that are found in the intestines and faeces of humans and other warm blooded animals. In general, human faecal waste gives rise to the highest risk of waterborne diseases (Odonkor and Ampofo, 2013). The predominant faecal coliform is *Escherichia coli* (USEPA 2006). *E. coli* is currently recognised by the World Health Organisation (WHO) as the best faecal indicator bacteria for monitoring faecal contamination of drinking water and faecal coliforms are suggested as an acceptable alternative (WHO 2011). *E. coli* is found in all mammal faeces at concentrations of 10^9 g^{-1}, but does not multiply significantly in the environment (Edberg et al., 2000). High levels of these bacteria indicate the presence of pathogens that cause waterborne diseases (Selvaratnam and Kunberger, 2004). Most coliform bacteria do not cause disease; however, some rare strains of *E. coli*, particularly O157:H7, can cause serious illness. As few as 10 cells can cause serious illness or even death (Liu et al., 2008). Diseases and illness that can be contracted in water with high faecal coliform counts include typhoid fever, hepatitis, ear infections (Oram, 2014), gastroenteritis and, dysentery (Gruber et al., 2014).

The WHO recommends that either *E. coli* or faecal coliforms be used as indicators of faecal contamination of water. The WHO guideline value for faecal coliforms (none detected in any 100 ml sample) is reflected in the standards of most OECD members and low-middle income countries (Bain et al., 2014). In their Guidelines for Drinking Water Quality, the WHO have developed a risk classification to prioritise interventions as higher levels of indicator organisms are generally indicative of greater levels of faecal contamination. The risk classification is based on the number of indicator organisms in a 100 ml sample which includes <1 ‘very low risk’, 1-10 ‘low risk’, 10-100 ‘medium risk”, > 100 ‘high risk’ or ‘very high risk’ (WHO, 2011).

During wastewater treatment, the sludge component of the waste becomes separated from the water component. As the survival of many microorganisms and viruses in wastewater is linked to the solid fraction of the waste, the numbers of pathogens present in sludge may be much higher than the water component (Straub et al. 1992). Although treatment of municipal sewage sludge

using lime, anaerobic digestion, or temperature, may substantially reduce pathogens, complete sterilisation is difficult to achieve and some pathogens, particularly enteric viruses, may persist. Persistence may be related to factors such as temperature, pH, water content (of treated sludge), and sunlight (Sidhu and Toze, 2009). Similarly, there is often resurgence in pathogen numbers post-treatment, known as the 'regrowth' phenomenon. Taskin et al. (2011) reported a sudden increase in *E. coli* density in anaerobically digested (AD) biosolids immediately after high speed centrifuge dewatering, a phenomena known as 'reactivation' and is separate from growth during the storage of dewatered biosolids cake. There are also links to contamination within the centrifuge, reactivation of viable, but non-cultural, organisms, storage conditions post-centrifugation (Zaleski et al. 2005), and proliferation of a resistant sub-population due to newly available niche space associated with reduction in biomass and microbial activity (McKinley and Vestal 1985). Iranpour and Cox (2006) observed reoccurrence of faecal coliforms in post-digested biosolids from thermophilic anaerobic digestion treatment. The explanations for reoccurrence may be linked to 1) incomplete destruction of the faecal coliforms during treatment, 2) contamination from external sources during post-digestion, or 3) a large drop of the post-digestion biosolids temperature to below the maximum for faecal coliform growth.

The European Drinking Water Directive 98/83/EC states that drinking water entering the distribution system should contain zero coliforms and zero *E. coli* in 100 mls (EC 2000). Despite advances in drinking water treatment, the WHO estimates that about 1.1 billion people globally drink unsafe water and the vast majority of diarrhoeal disease (88%) stem from unsafe water, lack of hygiene and sanitation (Ashbolt, 2004).

The objective of this work was to develop a quantitative microbial risk assessment (QMRA) model for coliforms in drinking water assuming the application of biosolids to agricultural land and resulting surface runoff entered abstraction waters for a water treatment plant (WTP).

Materials and methods

Model development

A quantitative drinking water treatment model was developed that was capable of predicting likely human exposure and resulting risk from TC and *E. coli* present in the drinking water without the possibility for attenuation to waters used for WTPs. This represents a pessimistic

scenario as, in reality, biosolids would not be spread to the edge of the field and that grassed buffer zones would be in place. The model was created in Microsoft Excel 2010 with the add-on package @Risk (version 6.0, Palisade Corporation, New York, USA). Uncertainty and variability can be accounted for in the model by means of probability density distributions and are represented in the model's equations by name (e.g. triangular, uniform). Data from peer reviewed scientific literature were incorporated at various steps of the drinking water treatment (i.e. coagulation and flocculation, sedimentation and disinfection). A process-based approach to modelling TC and *E. coli* fate and human exposure considers total concentration in surface runoff, dilution rate, bacteria die-off rate, drinking water treatment (primary, secondary and tertiary) and human consumption (adult and child).

Biosolid and soil characterisation

Three types of biosolids were investigated in this study. They were: anaerobically digested biosolids from the UK (AD-UK) and Ireland (AD-IRE), and lime stabilized (LS) and thermally dried (TD) biosolids. With the exception of ADUK, all biosolids originated from the same wastewater treatment plant (WWTP) in Ireland. The ADUK biosolids were sourced from the UK, and were used as part of an EU-funded FP7 project (END-O-SLUDG, 2014). The sludge was collected and land applied to small field plots at the maximum legal application rate in Ireland (Fehily Timoney and Company, 2014) and subjected to three successive rainfall events, applied using a rainfall simulator, at time intervals of 24, (RS1) 48 (RS2) and 360 (RS3) hr after application (Peyton et al., 2016). A soil-only control was also included in the experimental design.

Three different scenarios (worst case, xxx and yyy) were completed to account for the differences in time and surface runoff volumes. The mean and standard deviation of the surface runoff (Csurface-runoff) of TC and *E. coli*, as measured by Peyton et al. (2016), is shown in Table 1. Runoff results indicated that the AD-UK biosolids had significantly higher concentrations of *E. coli* in the RS1 and RS2 rainfall events, and exceeded the recommended standards of $> 1 \times 10^3$ MPN g^{-1} (Fehily Timoney and Company 2014). All of the reported Irish biosolids were some 10-fold below the Class A Irish standard (Peyton et al., 2016).

202 **Table 1.** Mean and standard deviation for total and faecal coliform in surface water.

Total coliforms			
Mean and standard deviation (n=15) (MPN/ 100 mls)			
	RS1	**RS2**	**RS3**
AD-UK	171840 ±158962	133516 ± 247832	134860.6 ± 119499
TD	299620 ± 511723.2	615760 ± 629487.1	980600 ± 822835.8
LS	15858 ± 27155.13	628400 ± 820378.8	492000 ± 614760.4
AD-IRE	155220 ± 163536.4	309934.4 ± 503104	197840 ± 190432.9
Control	158220 ± 121426	32850.4 ± 22214.2	470360 ± 506376
E. coli			
Mean and standard deviation (n = 15) (MPN/ 100 mls)			
	RS1	**RS2**	**RS3**
AD-UK	7055.4 ± 10283.15	4476 ± 5622	210.6 ± 419.6
TD	456 ± 804.3	114 ± 106	44.6 ± 94.23
LS	138.2 ± 21.5	358.2 ± 730.8	39 ± 61
AD-IRE	14.8 ± 21.4	271.6 ± 518.6	199.6 ± 440.7
Control	34.2 ± 47	30.4 ± 51.8	4 ± 8.9

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205 As a "worst case scenario" it was assumed that surface runoff following biosolid application
206 entered an adjacent stream without any chance of attenuation along the transfer continuum before
207 delivery to the surface water body. This is atypical in terms of grassland management. Schueler

et al. (2000) reported on the effectiveness of stream buffers and faecal coliform removal, and found that grass filter strips were effective in removing up to 70% of faecal coliforms. Similarly, Coyne et al. (1995) found that grass filter strips removed up to 74% of faecal coliforms from surface water. However, concentrations of faecal coliforms in surface water still exceeded minimum concentration standards for primary water.

It was assumed that the runoff effluent in surface-water was then abstracted to a nearby WTP. To account for TC and *E. coli* concentrations in surface water being discharged into the stream, this study used a dilution factor (DF), which is the ratio of concentration in the effluent to concentration in the receiving water after mixing in the receiving water (Colman et al., 2011). This assumes a homogenous distribution of the bacteria in the river and does not account for dispersion or advection. Dilution factors can vary between 1 (dry river bed in summer) up to 100,000. The EU Technical Guidance Document on Risk Assessment (2003) states that where there is a lack of specific data, a default dilution value of 10 is recommended for sewage from municipal WTPs when predicting environmental concentrations of contaminants in receiving waters (EC 2003). Therefore, a default dilution factor of 10 was applied to the data to calculate the predicted environmental concentrations in surface water (EQ.1).

$$PEC_{surface\text{-}water}\ (MPN/100\ mls) = C_{surface\text{-}runoff} / DF \qquad \text{Eqn. 1}$$

Where $PEC_{surface\text{-}water}$ is the concentration of coliforms (TC and *E. coli*) in surface waters receiving wastewater effluent, DF is the dilution factor, and ($C_{surface\ -runoff}$) (MPN/100 mls) is the concentration in surface water

The first order decay equation often used to describe bacterial die-off is expressed as Chick's Law, and is used to describe the survival (die-off rate) of TC and *E. coli* in soil, manure, streams and groundwater over time (Benham et al., 2006). Die-off is a function of temperature, nutrient levels, competing bacteria and solar radiation (Hrudey, 2004). The rate of bacterial "die-off" is greater in summer than winter due to higher temperatures and increased UV light (Murphy et al., 2015). Wilkinson et al. (1995) reported enhanced coliform concentrations in streams during high and rising flows following storm events. The die-off rate in stream (D-off) was calculated according to Eq. 2:

$$N_t = N_0\, e^{(-kt)} \qquad \text{Eqn. 2}$$

237 Where Nt is the number of coliforms at time t in surface-water (MPN/100 mls), N_0 is the original
238 number of coliforms following dilution in surface-water ($PEC_{surface\text{-}water}$) (MPN/100 mls), k is the
239 first order inactivation constant (d^{-1}), and t is the time in the stream (d^{-1}).

240 The k value was incorporated according to Schueler (2000), using a uniform distribution (values
241 min 0.7 and max 1.5 d^{-1}). k values in this range mean that about 90% of the bacteria present will
242 disappear from the water within 2 to 5 days. Therefore, it was assumed that water was abstracted
243 for drinking water treatment from the stream to a nearby WTP between 0 and 5 days. To account
244 for uncertainty, the time in stream "t" was fitted with a uniform distribution (min 0, max 5 day^{-1}).

245 **Drinking water treatment processes**

246 There are typically three stages to drinking water treatment (primary, secondary and tertiary)
247 (Figure 1). The three stages of drinking water treatment that were used were based on the Irish
248 Environmental Protection Agency's (EPA) best practice guidelines for drinking water treatment
249 manuals (EPA 1995, 2002, 2011). It is assumed that operations within the drinking water
250 treatment process are running efficiently or stable (C-opt). However, to account for inefficiencies
251 in treatment operations, a sub-optimal (CS-opt) and failure (C-fail) option were incorporated into
252 the model. Poor operation of filters and inadequate disinfection may pose a risk to human health.
253 In recent times, many WTPs have become automated.

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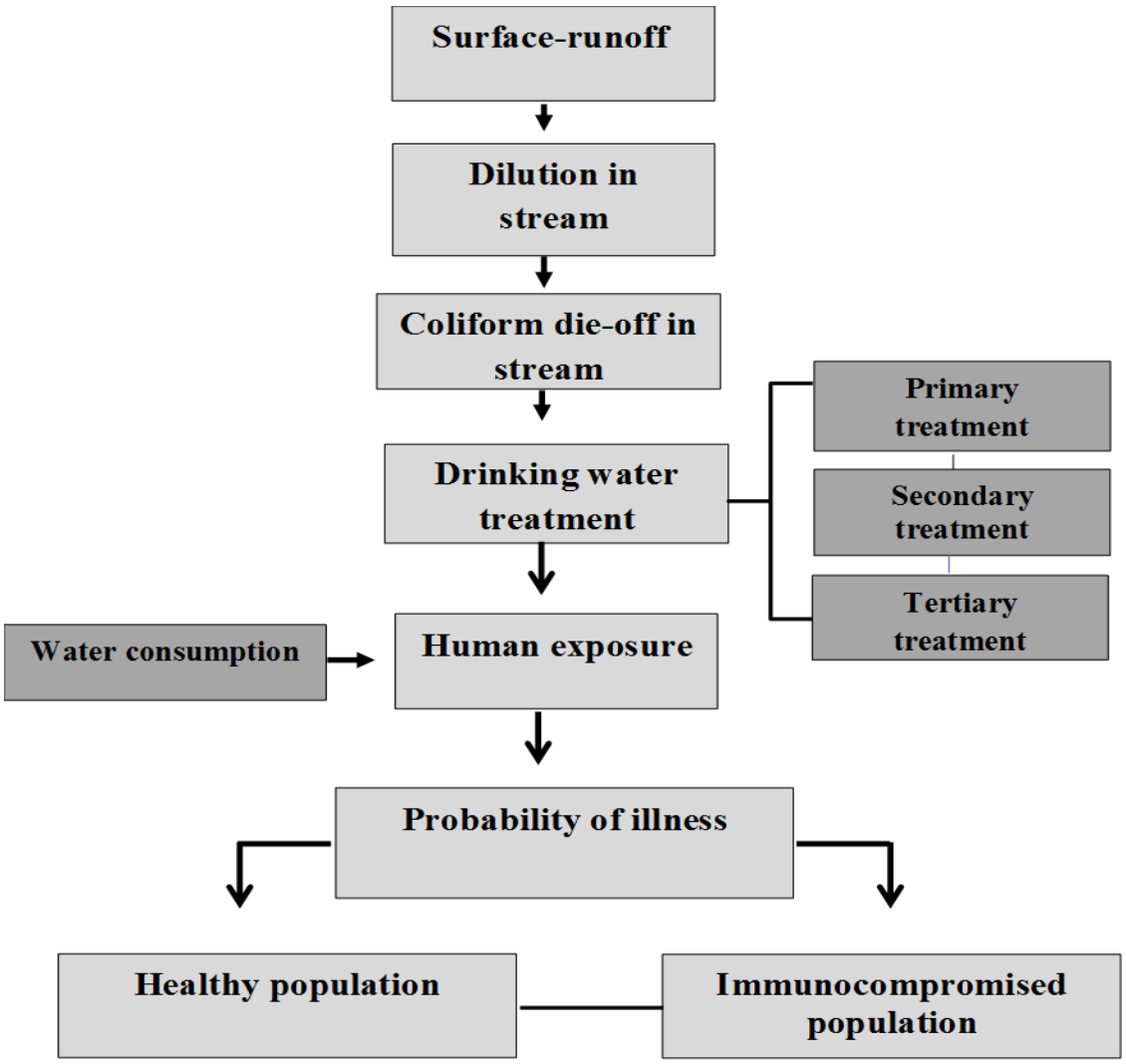

255

256 **Figure 1.** Flow diagram of the quantitative risk assessment drinking water model for coliforms
257 in biosolids applied to grassland.

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259 The first stage (primary treatment) considers the screening, storage, pre-conditioning and pre-
260 chlorination of the water. In the current study, primary treatment was assumed to have a
261 negligible impact on coliform removal. Secondary treatment involves the coagulation,
262 flocculation, sedimentation and filtration of the influent. Coagulation/flocculation, sedimentation
263 and filtration remove particles, including microorganisms (bacteria, viruses and protozoa) (WHO
264 2011). The commonest types of coagulants used are aluminium-based (e.g. aluminium sulphate
265 (alum) or polyaluminium chloride (PAC)). Both aluminium and ferric salts, either in monomer or
266 polymeric forms, have been reported as effective coagulants in treating wastewater (Kang et al.,

2003; Pang et al., 2009). When properly performed, coagulation, flocculation and sedimentation can result in 1-2 log removal of bacteria, viruses and protozoa (WHO 2004). In accordance with the Irish EPA's guidance manual (Ireland EPA 2002), the coagulant considered was aluminium sulphate ($Al_2 (SO_4)_3$ (referred to as alum) for both TC and *E. coli*.

As faecal coliforms are the indicator organism for *E. coli*, reductions in *E. coli* counts were used to account for variability and uncertainty in the data. Pritchard et al. (2010) compared the efficacy of alum sulphate to more natural coagulants and reported *E. coli* reductions of 89% using 30-50 mg L^{-1} of alum sulphate. Bulson et al. (1984) reported removal rates of *E. coli* of 99.99% following a dose of 15 mg L^{-1} of alum sulphate. A study conducted by Sarpong et al. (2010) showed that total coliform counts were reduced by 95% using a 5 ml dose of alum sulphate. Similarly, Bergamasco et al. (2011) reported a 99% reduction in total coliforms using a 15 ml dose of alum sulphate. Thus, a uniform distribution was used to model coagulation, flocculation and sedimentation incorporating a decimal reduction to account for variability and uncertainty in the data (min 0.89, max 0.99).

As a "worst case scenario", the model assumes a 90% probability of coagulation and flocculation occurring at an optimum stable run (Copt) and 5% probability for both sub-optimal (CS-opt) and failure (C-fail) (Table 2). When operating optimally, the model assumes a removal rate (uniform distribution min 0.89, max 0.99) (Table 3). When operating sub-optimally, the model assumes a removal of 50% of the optimal removal rate, and zero removal during failure events. It was assumed that aluminium sulphate was applied at an optimum dose of approximately 10 mg L^{-1}.

Table 2. Model inputs and distributions

Stage	Symbol	Description	Model /distribution	Units
		Effluent (Surface-runoff)		
	$C_{surface\text{-}runoff}$	Initial concentration in surface runoff	Lognormal (based on Table 1)	MPN/ 100mls
Dilution	DF	Dilution in stream	Dilution factor (10)	-
	$PEC_{surface\text{-}water}$	Concentration of coliforms in surface-water following dilution	$C_{surfacerunoff}$ / DF	MPN/100 mls

Stage	Symbol	Description	Model /distribution	Units
Die-off	K	First order inactivation constant	Uniform	d^{-1}
	t	Time in stream	Uniform (min 0, max 5)	d^{-1}
	D-off	Die-off rate in stream	$N = N_0 \ 〖\exp(-kt)〗$	MPN/ 100mls
Secondary treatment				
	C-opt	Coagulation/Flocculation and sedimentation optimum	0.90	Probability
	CS-opt	Coagulation/Flocculation and sedimentation sub-optimum	0.05	Probability
	C-fail	Coagulation/Flocculation and sedimentation fail	0.05	Probability
	Cr	Coagulation/Flocculation and sedimentation reduction	Uniform (min 0.89, max 0.99)	Decimal reduction
	F-opt	Filter optimum run	0.9	Probability
	FS-opt	Filter sub-optimum run	0.1	Probability
	Frd	Filter reduction (rapid sand)	Uniform (min 0.74, max 0.99)	Decimal reduction
Tertiary treatment				
	D	Disinfection	Uniform (min 0.97, max 0.99)	Decimal reduction
Output	Pstt	Post-secondary and tertiary treatment	Pstt =D-off × (1-Cr) × (1-Frd) × (1-D)	MPN/ 100mls
Human exposure				
Consumption	TWi	Tap water intake (adult)	Lognormal (mean 0.564,	L d^{-1}
Output	Vcc	Viable coliforms/ *E. coli* consumed	Pstt × Twi	MPN/ d^{-1}
Dose response				
Output	I(H)	Probability of illness (healthy)	1-EXP (- 0.0000005 × Vcc)	-
Output	I (Ic)	Probability of illness (immunocompromised)	1-EXP (- 0.01 × Vcc)	-

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291 The filtration process is the last treatment stages that can physically remove contaminants before
292 disinfection. One of the most popular filtration processes used in Ireland is the rapid gravity sand
293 process (Ireland EPA 1995). A study by Li et al. (2012) showed that direct rapid sand removal
294 can remove 0.6 - 1.5 log-units of total faecal coliform, depending on the loading rate and grain
295 size distribution. Mwabi et al. (2012) demonstrated that designing and building a bio-sand
296 filtration system was effective in removing 2 - 4 $\log_{10}$ of coliform bacteria. Koivunen et al.
297 (2003) showed that tertiary treatment by the rapid sand filtration process found, on average, a
298 97% reduction of faecal coliforms and total coliforms in four conventional wastewater treatment
299 plants in Helsinki, Finland. In keeping with the Irish EPA's filtration manual guidelines, rapid
300 gravity filtration was considered in the model. Filtration can be stable or unstable due to
301 optimum, sub-optimum or failure of the coagulation/flocculation process. As a "worst case
302 scenario" the model assumes a 90% probability of filtration operating at an optimum stable run
303 (Fopt) and 10% probability for sub-optimal run (FS-opt). To model rapid sand filtration under
304 optimum conditions and to account for uncertainty and variability in the data, a decimal
305 reduction uniform distribution was assigned (min 0.74 max 0.99) (Table 2). When operating sub-
306 optimally, the model assumes a removal of 50% of the optimal removal rate.

307 **Disinfection**

308 Disinfection is the process by which an organism's viability/infectivity is destroyed with a
309 specific percentage of the population dying over some time frame defined as a rate (Betancourt
310 and Rose, 2004). Worldwide, chlorine is the most commonly used disinfection in drinking water
311 treatment, although other alternatives are being increasingly introduced such as ozonation,
312 ultraviolet irradiation, ultrasonic vibration, ultra-filtration, silver, bromide and iodine, membrane
313 filtration and granular activated carbon (GAC). Chlorine is added to provide a disinfectant
314 residual to preserve the water in distribution, where the chlorine is in contact with the water for a
315 longer period of time compared to the pre-chlorination process in primary treatment (Irish EPA,
316 2011). The principal factors that influence disinfection efficiency are the disinfection
317 concentration, contact time, temperature and pH (depending upon the disinfection) (Cotruvo et
318 al., 2013). Chlorination has been found to remove *E. coli* between 97-99% (O' Connor and O'
319 Connor 2001). However, a report by Igunnnuugbemi et al. (2009) showed that water storage post

chlorination significantly reduced survival of *E. coli* and that the presence of *E. coli* following chlorination could undermine the effectiveness of chlorination. To account for uncertainty in the data, a uniform distribution (minimum 0.97, maximum 0.99) was assigned to model the inactivation attributed to the disinfection process.

Removal of coliforms and bacteria (TC and *E. coli*) was quantified in terms of a decimal reduction. The concentration of coliforms remaining after secondary and tertiary treatment in a WTP was calculated by multiplying the level present post primary treatment by the decimal reduction due to coagulation/ flocculation, sedimentation, filtration and disinfection. The equation is:

$$P_{STT} = \text{D-off} \times (1\text{-Cr}) \times (1\text{-Frd}) \times (1\text{-D}) \qquad \text{Eqn.3}$$

Where: P_{STT} is the coliform concentration post-secondary and tertiary treatment (MPN/100 mls), Cr is decimal reduction due to coagulation /flocculation and sedimentation, Frd is decimal reduction due to filtration, and D is the decimal reduction due to disinfection.

Human Exposure

Water consumption in Ireland for adults was modelled using a lognormal distribution with a mean and standard deviation value of 0.564 ± 0.617 L d^{-1} according to a survey on adult consumption patterns conducted by the Irish Universities Nutrition Alliance (IUNA) which was based on 1274 consumers. The same survey was used to model variation in adult body weight (males and females) and a normal distribution with a mean and standard deviation value of 78 ± 16.5 kg was used (IUNA, 2011).

Dose response model

In order to assess the risk to human health from coliforms and *E. coli* associated with water consumption, the potential exposure to the organism(s) in the daily drinking water intake was estimated. Exponential models are widely used in microbial risk assessment (Teunis et al., 2004). The exponential model assumes that pathogen-host interactions can describe the pathogen-host survival probability by a discreet value (Haas et al., 2000). Two dose response models were considered for TC and *E. coli* exposure incorporating two different exposure scenarios (healthy populations and immuno-compromised populations). Immuno-compromised individuals include

patients on active anti-cancer drugs, HIV/AIDS and other chemotherapies. Allen et al. (2013) defines an immuno-compromised individual as having a haematology profile showing abnormal values for gamma globulins, white blood cells, red blood cells and liver function. The dose response model estimated the probability of illness resulting from a certain level of exposure. An exponential dose-response model was used for probability of illness, integrating an "r" value of 0.01 for immuno-compromised populations (I(Ic)) and an "r" value of 0.0000005 for healthy population (I(H)) as proposed by Gale (2005). As a "worst case scenario", the illness model was parameterized with the assumption that the virulence of the pathogen is similar to *E. coli* O157:H7. The *E. coli* O157:H7 strain is a particular serotype of the group referred to as verocytotoxigenic *E. coli* (VTEC). VTECs produce verotoxins or shiga-like toxins that are closely related to the toxin produced by *Shigella dysenteriea* (Cassin et al., 1998). The USEPA have proposed an acceptable benchmark of 10^{-4} annual infection/illness probability per person per year for *Shigella* (Grant et al., 2012). The WHO use the metric DALY (disability-adjusted life year) to estimate severity and duration of a disease. The 10^{-6} DALY tolerable burden of disease may be considered unrealistic and there have been proposals to introduce a less stringent burden of risk such as the upper limit for excess lifetime risk of cancer of 10^{-5} or a 10^{-4} limit in line with the USEPA limit (WHO 2011). Crockett et al. (1996) reported that ingestion of only 10-100 Shigella cells can lead to infection. The probability of illness per day can be expressed by:

$$P_i = 1 - \exp(- d \times r) \qquad \text{Eqn. 4}$$

Where P_i is the probability of illness (d^{-1}), d is the dose and 'r' represents an exponential parameter. The annual individual risk is calculated as:

$$P_{i\,(365)} = 1 - (1 - P)^{365} \qquad \text{Eqn. 5}$$

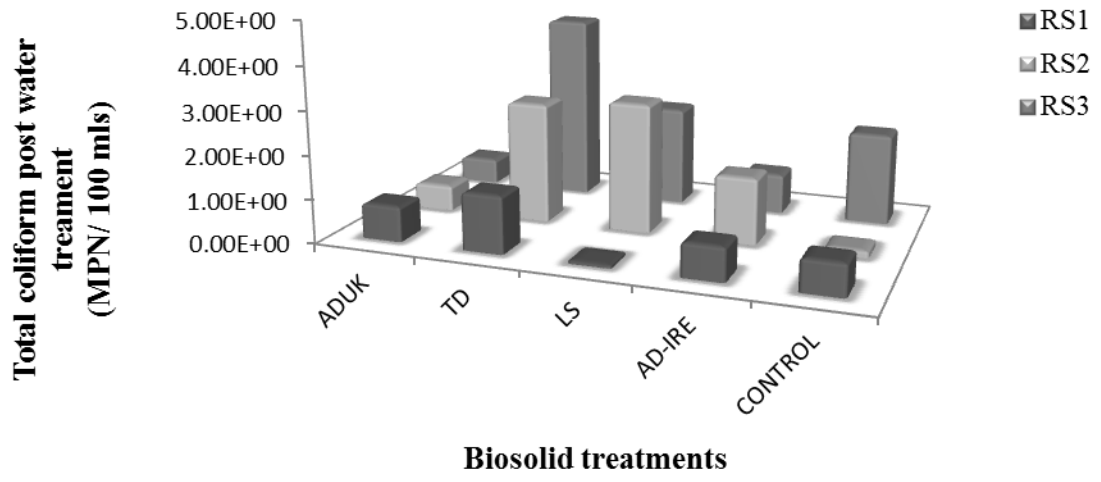
Sensitivity analysis

A sensitivity analysis, based on rank order correlation, was carried out to assess how the model's predictions are dependent on variability and uncertainty in the model input parameters. Sensitivity analysis assesses how the model predictions are dependent on variability and uncertainty in the model's inputs. Monte Carlo simulation performs risk analysis by building

models of possible results by substituting a range of values—a probability distribution—for any factor that has inherent uncertainty or variability (Kavcar et al., 2009). It then iterates the results using a different set of random values from the probability functions. Ten thousand iterations were performed for each simulation.

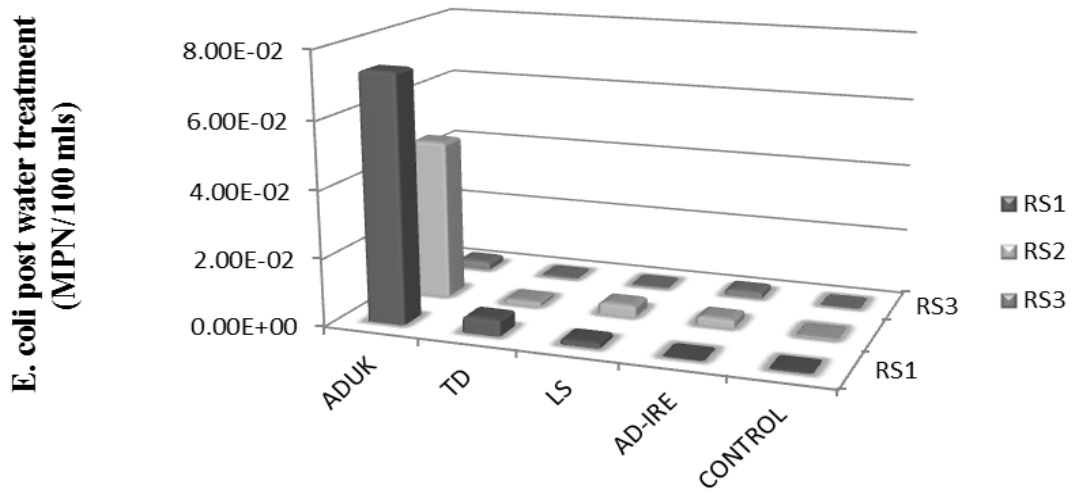
Results

Figures 2 and 3 give the predicted results of TC and *E. coli* remaining following drinking water treatment under the scenarios considered. The model produced several output distributions (TC and *E. coli* concentration in effluent post WTP, viable coliforms consumed, and probability of illness) that can be used to compare the concentration of coliforms that were detected in surface runoff and their potential risk to human health. The model predicted that surface runoff arising from the land spreading of TD biosolids and ADUK biosolids produced the highest concentrations of TC and *E. coli*, respectively, in drinking water. The modelled mean TC and *E. coli* concentration in drinking water was highest when the surface runoff concentrations from the TD and ADUK, respectively, biosolids at each rainfall simulation time (24, 48 and 360 hr) were used as input into the model (mean concentration values 1.3, 2.7 and 4.2 MPN/100 mls for TC and TD biosolid treatment (Figure 2), and 7.3×10^{-2}, 4.7×10^{-2} and 2.4×10^{-3} for *E. coli* and ADUK biosolid treatment (Figure 3).



395 **Figure 2.** Simulated mean total coliforms remaining following drinking water treatment

396



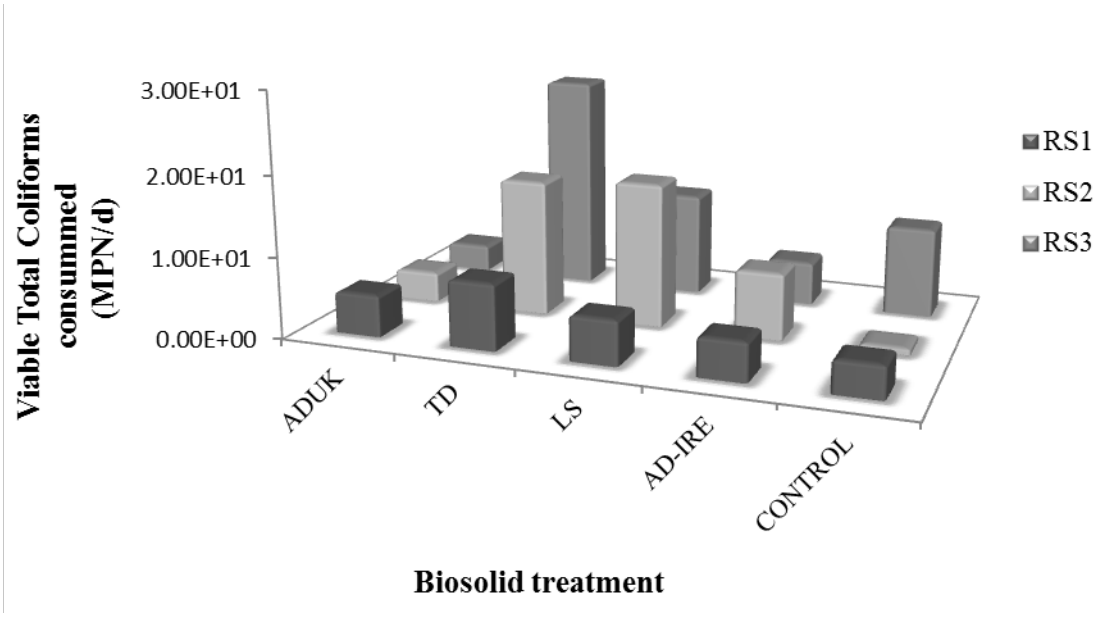
397

398 **Figure 3.** Simulated mean *E. coli* remaining following drinking water treatment

399

400 The EU states that there should be 0 in 100 mls of coliform bacteria and *E. coli* following
401 drinking water treatment. The results for mean human exposure *via* drinking water consumption
402 show that for TC the greatest viable coliforms consumed was for the biosolid TD and LS
403 combining rainfall simulation times of 48 and 360 hr (RS2 and RS3), respectively (Figure 4)
404 (mean viable total coliform values 16.83 and 26.75 MPN d^{-1}, respectively) for TD (mean viable
405 total coliform values 17.74 and 12.82 MPN d^{-1}, respectively) for LS biosolids. The results for *E.*
406 *coli* show that the greatest viable coliforms consumed was for ADUK biosolids and rainfall
407 simulation times of 24 and 48hrs (RS1 and RS2) (Figure 5) mean viable faecal coliforms
408 consumed values 5.20×10^{-1} and 2.34×10^{-1} MPN d^{-1}, respectively.

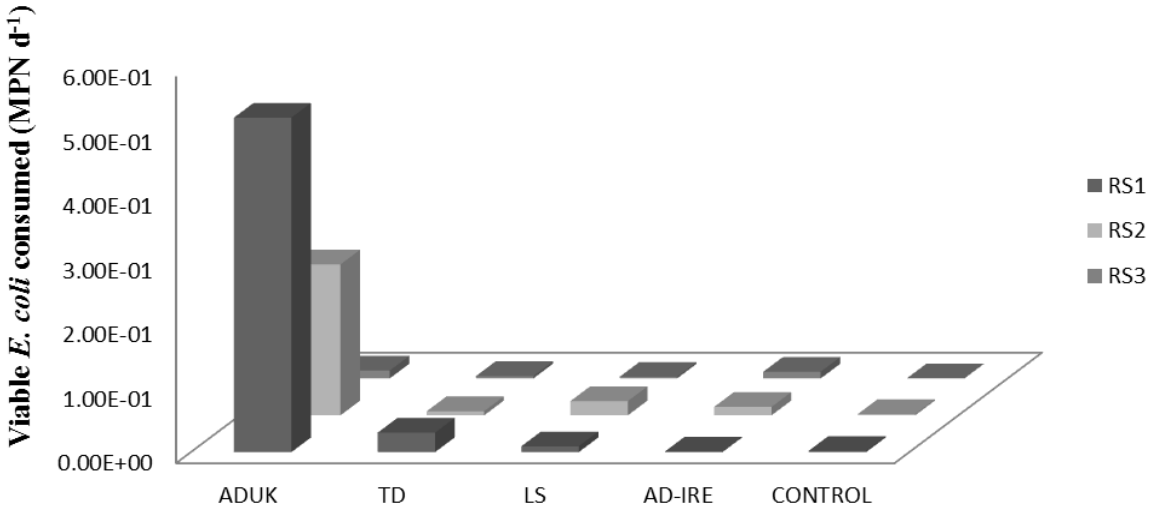
409



410

411 **Figure 4.** Simulated mean viable total coliforms consumed

412



413

414 **Figure 5.** Simulated mean viable *E. coli* consumed

415

The results for probability of illness (healthy and immuno-compromised) are displayed in Table 3. For each scenario (healthy and immuno-compromised), the risk assessment model produced a simulated probability of illness per day and per year. Compared to the healthy population, the immuno-compromised population are more at risk of illness with mean annual values for TC and immuno-compromised (9.92×10^{-1}) and LS biosolids treatment (RS1), (7.24×10^{-1} and 7.87×10^{-1}) for the TD biosolids treatment incorporating the RS2 and RS3 time frames, compared to mean annual values for TC and healthy population for the same biosolid treatments and time frames (1.01×10^{-3}) incorporating the LS biosolids treatment and RS1 time frame, mean annual values for healthy population and TD biosolids treatment (2.77×10^{-3} and 4.27×10^{-3} respectively), incorporating the RS2 and RS3 time frames. The mean annual values for *E. coli* and immuno-compromised populations show that the ADUK biosolids and the RS1 and RS2 time frames had the greatest probability of risk (values 2.1×10^{-1} and 1.7×10^{-1}, respectively). This is comparable to the healthy population for the same biosolid treatment and time frames (mean annual values of 7.0×10^{-5} and 4.6×10^{-5}, respectively).

Table 3. Probability of illness for healthy and immuno-compromised populations

Biosolid treatment	Probability of illness							
	Healthy population				Immuno-compromised population			
	(d^{-1})		(yr^{-1})		(d^{-1})		(yr^{-1})	
	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*	TC	*E. coli*
				RS1				
ADUK	2.57E-06	1.71E-07	8.90E-04	7.0E-05	2.81E-02	3.68E-03	5.62E-01	2.1E-01
TD	4.09E-06	1.46E-08	1.35E-03	4.2E-06	3.67E-02	2.86E-04	5.85E-01	4.1E-02
LS	2.76E-06	5.29E-09	1.01E-03	1.3E-06	5.22E-02	1.03E-04	9.92E-01	1.9E-02
AD-IRE	2.38E-06	3.94E-10	7.89E-04	1.4E-07	2.44E-02	7.87E-06	5.38E-01	2.5E-03
CONTROL	2.00E-06	9.51E-10	7.18E-04	3.9E-07	2.60E-02	1.90E-05	5.62E-01	6.2E-03
				RS2				
ADUK	1.86E-06	1.23E-07	6.34E-04	4.6E-05	2.05E-02	2.1E-03	4.65E-01	1.7E-01
TD	8.41E-06	3.46E-09	2.77E-03	1.0E-06	6.73E-02	6.9E-05	7.24E-01	1.5E-02
LS	8.86E-06	9.26E-09	2.71E-03	3.4E-06	6.32E-02	1.8E-04	7.10E-01	3.5E-02
AD-IRE	4.11E-06	6.35E-09	1.41E-03	3.0E-06	3.87E-02	1.3E-04	5.94E-01	2.9E-02
CONTROL	3.91E-07	8.69E-10	1.42E-04	3.2E-07	6.83E-03	1.7E-05	3.43E-01	5.2E-03
				RS3				
ADUK	1.66E-06	6.1E-09	5.99E-04	2.6E-06	2.30E-02	1.2E-04	5.31E-01	2.3E-02
TD	1.34E-05	1.2E-09	4.27E-03	1.2E-06	9.31E-02	2.4E-05	7.87E-01	6.7E-03
LS	6.41E-06	1.2E-09	2.20E-03	3.9E-07	5.47E-02	2.3E-05	6.82E-01	6.4E-03
AD-IRE	2.70E-06	5.1E-09	9.38E-04	2.3E-06	2.97E-02	1.0E-04	5.80E-01	2.3E-02
CONTROL	5.56E-06	1.1E-10	1.93E-03	4.6E-08	5.35E-02	2.2E-06	6.89E-01	8.6E-04

Sensitivity analysis was performed to investigate how variability of the outputs can be apportioned quantitatively to different sources of variability in the inputs. The analysis indicated that the LS and TD biosolids produced the highest concentration post WTP of TC, and ADUK produced the highest concentration of *E. coli*, in drinking water, therefore, a sensitivity analysis was conducted for the annual probability of illness for both biosolid treatments. Results for TC and *E. coli* show that the parameter of importance that affected the variance in model predictions was time in the stream (correlation coefficient -0.63 and -0.57, respectively) (Figures 6 and 7). This highlights the importance of residence time of bacteria in stream. The longer the bacteria are in the stream, the more likely the bacteria are subject to factors such as temperature, pH and photolysis, which may in-turn influence the growth or die-off rate of bacteria in a stream. The other parameters of importance were the tap water intake and initial concentrations in surface runoff (correlation coefficients 0.33 and 0.31, respectively, for Twi and 0.32 and 0.33, respectively, for C-surface-runoff). The die-off rate in the stream (-022 for TC and -0.20 for *E. coli*) was also of importance. The die-off rate is related to the residence time in the stream and is associated with sub-optimum conditions in the stream that influence bacterial growth.

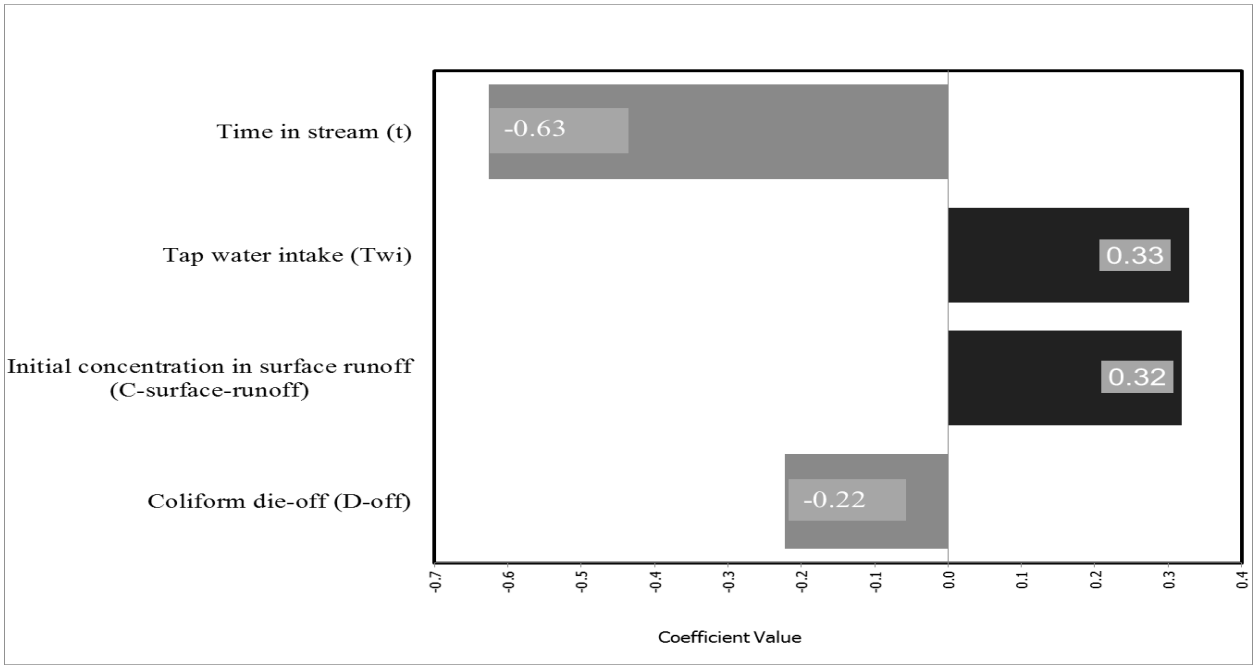


Figure 6. Sensitivity analysis for TC annual probability of illness and TD biosolid treatment

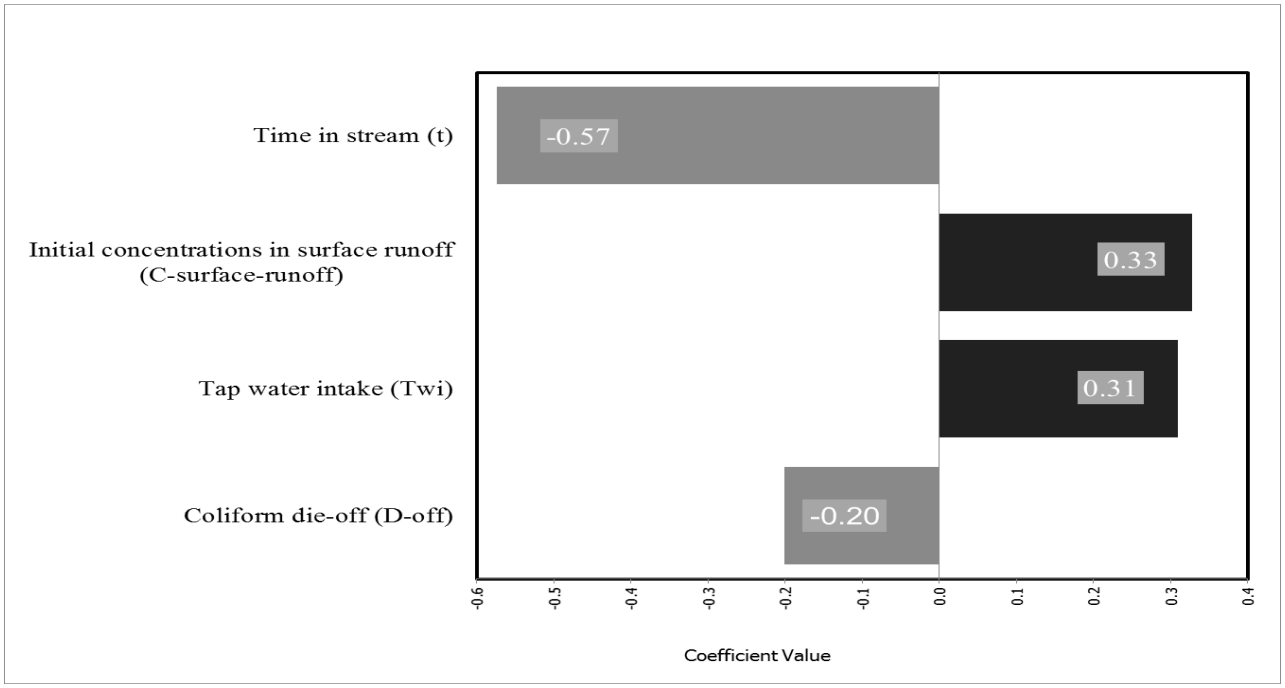


Figure 7. Sensitivity analysis for *E. coli* annual probability of illness and ADUK biosolid treatment

Discussion

Concentrations of TC and *E. coli* in surface runoff following the spreading of biosolids on grassland were quantitatively assessed to study their fate in drinking water treatment and subsequent consumption and human health effects. Initial concentrations of *E. coli* in surface runoff were above the recommended standards of $> 1 \times 10^3$ MPN g^{-1} and were equivalent to class B microbial matter under the USEPA Part 503 regulations. Surface runoff is distinguished from other types of runoff in that it does not pass through the soil. Therefore, typical soil-pathogen reactions (desiccation, photolysis, temperature and nutrients) may be by-passed depending on the rate off rainfall. Concentrations of TC and *E. coli* in surface runoff in this study are comparable to concentrations reported by Le Chevalier et al. (1991) and Schreiber et al. (2015). All TC and

E. coli concentrations had decreased by the third rainfall event (RS3; 360 hr) due to desiccation of the pathogens in soil following the application of the biosolids.

The mean concentration of TCs after the WTP showed that the TD biosolids (RS2 and RS3) and LS biosolids (RS2 and RS3) with the highest concentration of TC. This was attributed to initial concentrations of TCs in the influent and the time in stream combined with the removal rates associated with secondary treatment (e.g. coagulation/flocculation and sedimentation and filtration). Thermal drying is recognised as more effective in pathogen removal than mesophilic digestion and can achieve the time-temperature requirement for Class A biosolids (Iranpour and Cox, 2006). However, regrowth of pathogens can occur in thermally dried biosolids (Zaleski et al. 2005). Lloret et al. (2013) showed that the reduction in sludge retention time may be responsible for presence of coliforms post treatment. Lloret et al. (2013) reported that a minimum time of more than 10 days under thermophilic conditions is required to achieve appropriate sanitation of sludge. Similarly, Iranpour and Cox (2006) reported the presence of faecal coliforms after thermal drying, and attributed the reason to be the relatively short slude retention time of about 10 days.

The mean concentration of *E. coli* post drinking water show that the ADUK biosolids had the greatest concentration of *E. coli* for RS1 and RS2 only. This was also attributed to the initial concentration and the time in the stream of *E. coli* in the influent and associated drinking water treatment removal rates. Although initial concentrations of TC and *E. coli* in surface water were high, the effect of drinking water treatment significantly reduced overall TC and *E. coli* concentrations with a 99.9% reduction across all treatments and time frames.

The mean viable consumption of TC and *E. coli* in drinking water showed the same trends as mean TC and *E. coli* concentrations post drinking water treatment. Safe drinking water is a human right and in developed countries it has become an “entitlement”. Water consumers rely on the efficacy of drinking water treatment to produce a product that is pathogen free, odourless and clear. However, indicator bacteria are known to regrow in finished drinking water. This was highlighted in a report by Le Chevalier et al. (1991). The authors reported various factors attributed to the occurrence of coliforms in drinking water including disinfectant residuals, filtration and temperature. Bacterial growth can occur on any surface that is constantly wet, so the internal surface of water distribution pipes is normally coated with a biofilm (Gray, 2010).

Although the concentrations of coliforms post drinking water treatment in this study were significantly reduced, inefficiencies in drinking water treatment due to operational defects that promote the regrowth of coliforms and other pathogens can be a cause of concern for drinking water management.

Ideally water intended for human consumption should be pathogen free. However, in practice, this is an unachievable goal. A consequence of variable human susceptibility to pathogens is that exposure to drinking water of a particular quality may lead to health problems in different populations (WHO 2011), particularly the very young and immuno-compromised. Enteric pathogens are among the many agents that take advantage of the impaired or destroyed immune system; therefore, sensitive populations are considerably more vulnerable and may need special protection from waterborne microorganisms (Gerba et al., 1996). As *E. coli* is used as an indicator that faecal matter is present, it may indicate the presence of pathogens that cause waterborne diseases. The risk of illness for healthy populations was deemed negligible based on the tolerable risk guidelines set by the USEPA and the WHO for *Shigella*. However, based on the same guidelines, immuno-compromised populations may be at risk. Individuals who are truly immuno-compromised would follow medical advice regarding food and water intake, thus reducing the risk of illness.

The Sewage Directive has yet to address the bacteriological quality of treated water. The current European legislation requires that the sludge be subjected to a process of stabilisation before land application. With future demography increases and growing demand for water, the use of reclaimed water will rise; therefore efforts to assess the treatment efficacy are vital.

Conclusions

Application of biosolids on grassland and subsequent simulated rainfall over three time frames resulted in TC and *E. coli* counts in surface runoff. The concentrations of *E. coli* exceeded the recommended standards being some 10-fold below the Class A Irish standard. This prompted the need to investigate human exposure. Further analysis which included simulated dilution and die-off rate in a stream, drinking water treatment, and human exposure following consumption of the treated water resulted in a very low probability of illness based on the USEPA and the WHO

threshold of acceptable risk (10^{-4} and 10^{-6}, respectively) for healthy populations. However, the risk of illness for immuno-compromised populations exceeded the thresholds of acceptable risk by a factor of 3 for TC and a factor between 1-3 for *E. coli*. It is noted in such cases, susceptible populations would be subject to medical advice regarding food and water intake, thus reducing the risk of illness. The sensitivity analysis identified that the time in stream is an important parameter as the longer the bacteria are in the water and being exposed to ultraviolet light, varying temperature and pH, the greater the influence on bacterial growth. The risk assessment model developed in this study may be of importance to local authorities or regulatory agencies to evaluate the likely risk of *E. coli* entering potable water following biosolid application on agricultural land. As this study only focused on coliforms, future studies are needed in order to assess other compounds of concern e.g. pharmaceutical contaminants that may be present in biosolids.

Acknowledgements

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