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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

In Ireland, the land application of biosolids is the preferred option of disposing of municipal sewage waste. Biosolids provide nutrients in the form of nitrogen, phosphorus, potassium and increases organic matter. It is also an economic way for a country to dispose of its municipal waste. However, biosolids may potentially contain a wide range of pathogens, and following rainfall events, may be transported in surface runoff and pose a potential risk to human health. Thus, a quantitative risk assessment model was developed to estimate potential pathogens in surface water and the environmental fate of the pathogens following dilution, residence time in a stream, die-off rate, drinking water treatment and human exposure. Surface runoff water quality data was provided by project partners. Three types of biosolids, anaerobically digested (AD), lime stabilised (LS), and thermally dried (TD)) were applied on micro plots. Rainfall was simulated at three time intervals (24, 48 and 360 h) following land application. It was assumed that this water entered a nearby stream and was directly abstracted for drinking water. Consumption data for drinking water and body weight was obtained from an Irish study and assigned distributions. 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.

1. 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 Switzerland, the farm land application of sludge is prohibited (Evans, 2012). This is due to the considerable public acceptance issues surrounding the reuse of treated sludge as a fertiliser. The main concern 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).

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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 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). 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). 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 h and 2 years. The disparities in survival rates are difficult to define due to "knowledge gaps" 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 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).

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 subpopulation 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 mL^{-1} (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).

2. Materials and methods

2.1. 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 surface waters. 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. 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). Validation of the model is achieved through the implementation of relevant peer reviewed scientific experimental results and was 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).

2.2. Biosolid and soil characterisation

This study uses surface runoff water quality data generated from project partners. The treated biosolids investigated were anaerobically digested biosolids from the UK (AD-UK) (sourced from an EU-funded FP7 project (END-O-SLUDG, 2014)) and Ireland (AD-IRE), lime stabilised (LS) and thermally dried (TD). A soil-only control was also included. The sludge was applied to micro-plots and exposed to three continual rainfall events, applied using a rainfall simulator, at time intervals of 24, (RS1) 48 (RS2) and 360 (RS3) hr after application. Details regarding rainfall intensity/duration and the amount of runoff are provided in Peyton et al. (2016).

Three different scenarios (RS1, RS2 and RS3) were completed to account for the differences in time and surface runoff volumes. The mean and standard deviation of surface runoff volumes of TC and *E. coli*, as measured by Peyton et al. (2016) were calculated ($C_{surface-runoff}$) and are 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).

Riparian buffers are vegetated areas next to water resources that help to stabilise banks and protect water quality. Schueler (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. A pessimistic approach was adopted assuming that surface runoff following biosolid application entered an adjacent stream without any chance of buffering (e.g. contour buffer strips, vegetative barriers, filter strips).

It was assumed that the runoff effluent in stream water was then abstracted to a nearby drinking water treatment plant (DWTP). 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 stream 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 (in this case surface runoff) when predicting environmental concentrations of contaminants in receiving waters (EC , 2000). Therefore, a default dilution factor of 10 was applied to the data to calculate the predicted environmental concentrations in stream water (Eq.1).

$$PEC_{surface-water}(MPN/100\ mls) = C_{surface-runoff} / DF \quad (1)$$

Where $PEC_{stream-water}$ is the total concentration of coliforms (TC and *E. coli*) in stream waters following surface runoff from adjacent grassland, DF is the dilution factor, and ($C_{surface\ -runoff}$) (MPN 100 mL^{-1}) is the concentration in surface runoff.

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), it is also a function of grazing by protozoa (Zhang et al., 2010). 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. This has also been found in tropical areas, for example Ribolzi et al. (2016). The die-off rate in stream (D-off) was calculated according to Eq. (2):

$$Nt = N_0\, e^{(-kt)} \quad (2)$$

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

The k value was incorporated according to Schueler (2000), using a uniform distribution (values min 0.7 and max 1.5 d^{-1}). "*k*" values in this range mean that about 90% of the bacteria present will disappear from the water within 2–5 days. Therefore, it was assumed that water was abstracted for drinking water treatment from the stream to a nearby DWTP between 0 and 5 days after runoff into the receiving stream. To account for uncertainty, the time in stream "*t*" was fitted with a uniform distribution (min 0, max 5 day^{-1}). Figs. 1 and 2 provide a simulated time series from concentration in surface water to concentration post die-off and succeeding drinking water treatment.

Table 1
Mean and standard deviation for total and *E. coli* in surface water.

Total coliforms			
Mean and standard deviation (n = 15) (MPN 100 mL^{-1})			
	RS1	RS2	RS3
AD-UK	171,840 ± 158,962	133,516 ± 247,832	134,860.6 ± 119,499
TD	299,620 ± 511,723.2	615,760 ± 629,487.1	980,600 ± 822,835.8
LS	15,858 ± 27,155.13	628,400 ± 820,378.8	492,000 ± 614,760.4
AD-IRE	155,220 ± 163,536.4	309,934.4 ± 503,104	197,840 ± 190,432.9
Control	158,220 ± 121,426	32,850.4 ± 22,214.2	470,360 ± 506,376
E. coli			
Mean and standard deviation (n = 15) (MPN 100 mL^{-1})			
	RS1	**RS2**	**RS3**
AD-UK	7055.4 ± 10,283.15	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

2.3. Drinking water treatment processes

The processes for conventional drinking water treatment include primary, secondary and tertiary. The Irish Environmental Protection Agency's (EPA) best practice guidelines for drinking water treatment manuals (IRELAND EPA, 1995; 2002, 2011a,b) were used as a guide to develop the drinking water treatment model. Drinking water supplies in Ireland are predominantly sourced from surface waters or groundwaters influenced by surface waters (IRELAND EPA, 2011a,b). It is assumed that operations within the drinking water treatment process are running efficiently or stable. Poor operation of filters and inadequate disinfection may pose a risk to human health. In recent times, many DWTPs have become automated.

The primary treatment stage comprises the screening, storage, pre-conditioning and pre-chlorination of the water. In the current study, it was assumed that primary treatment has a negligible

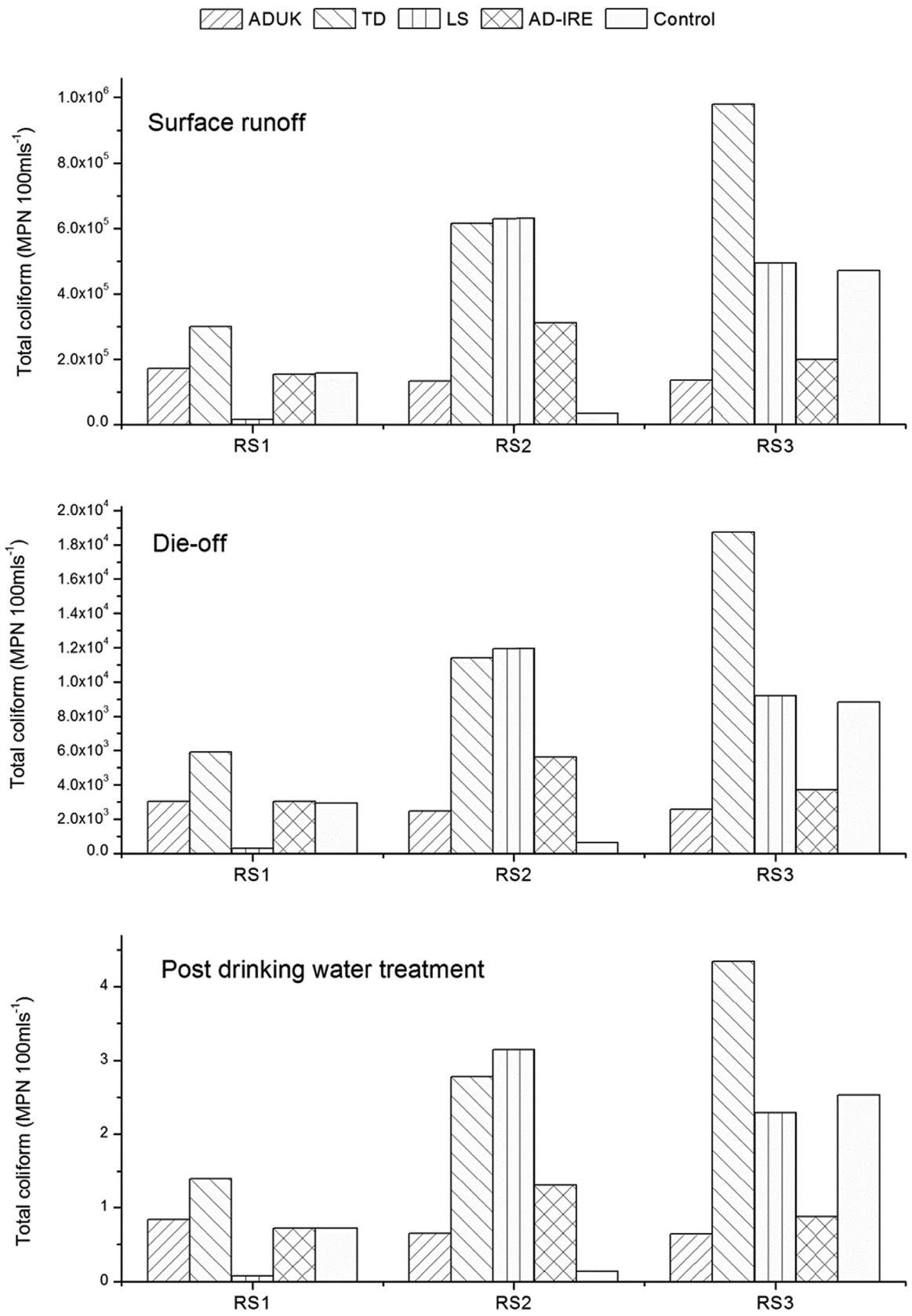

Fig. 1. Simulated time series for mean TC in surface runoff, die-off and post drinking water treatment.

impact on coliform removal. Secondary treatment involves the coagulation, flocculation, sedimentation and filtration of the influent. Coagulation/flocculation, sedimentation and filtration remove particles, including microorganisms (bacteria, viruses and

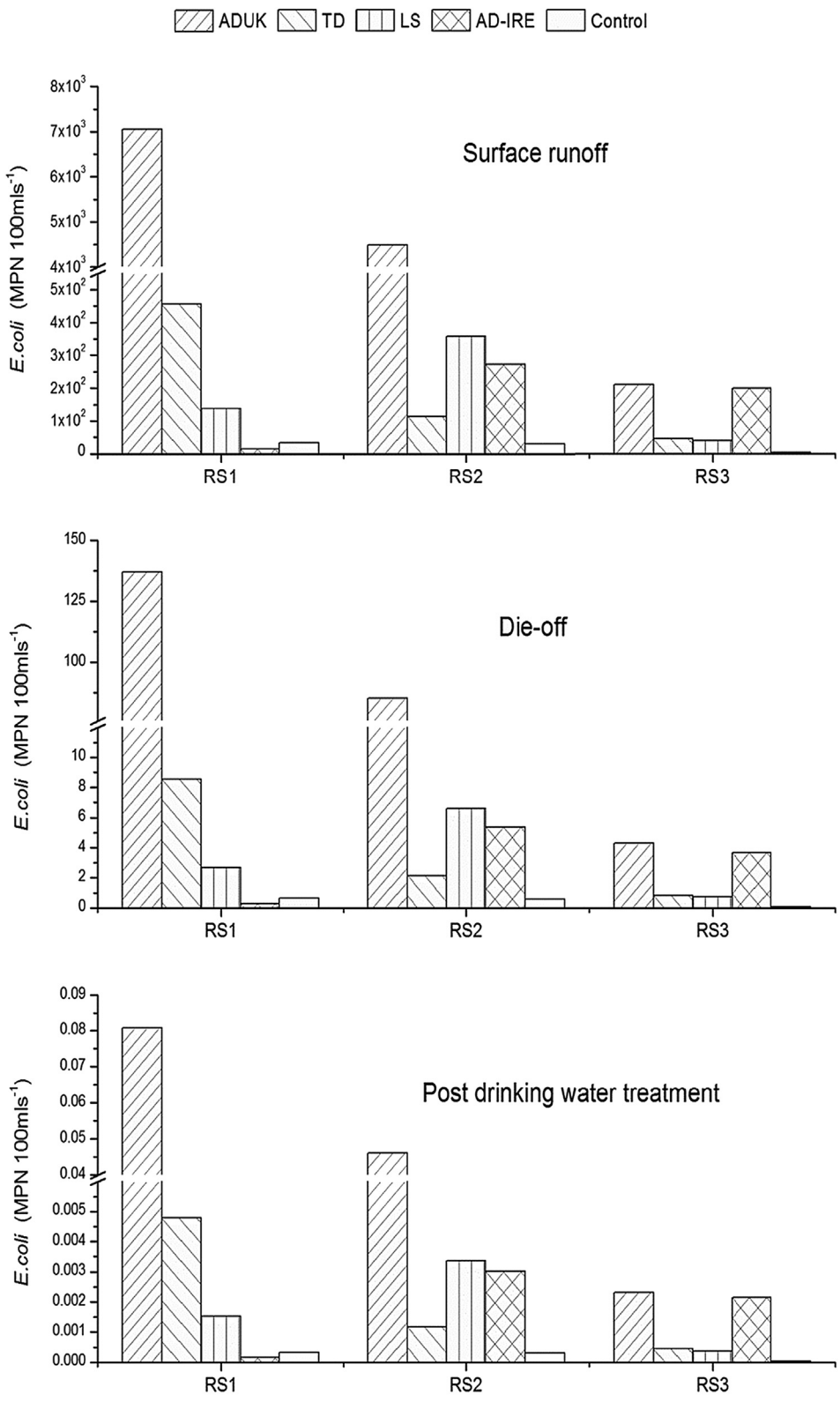

Fig. 2. Simulated time series for mean *E. coli* in surface runoff, die-off and post drinking water treatment.

protozoa) (WHO , 2011). The commonest types of coagulants used are aluminium-based (e.g. aluminium sulphate (alum) or polyaluminium chloride (PAC)). Both aluminium and ferric salts, either in monomer or 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*.

The presence of faecal coliforms indicates fecal contamination and the potential presence of enteric pathogens (disease causing organisms). An example of one group of fecal coliform bacteria is *Escherichia coli* or *E. coli*. Reductions in *E. coli* counts following drinking water treatment incorporating alum were obtained from the peer reviewed literature. Pritchard et al. (2010) compared the efficacy of alum sulphate to more natural coagulants. The study reported *E. coli* reductions between 89 and 99.8% using 30–50 mg L^{-1} concentration of alum sulphate. Bulson et al. (1984) reported removal rates of *E. coli* of 99.99% following a concentration of 15 mg L^{-1} of alum sulphate. A study conducted by Sarpong and Richardson (2010) showed that total coliform counts were reduced by 95% using a 5 ml L^{-1} concentration of alum sulphate. Similarly, Bergamasco et al. (2011) reported a 99% reduction in total coliforms using a 15 ml L^{-1} concentration 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). It was assumed that aluminium sulphate was coagulant used at the DWTP and was applied at an optimum concentration of approximately 10 mg L^{-1}.

The filtration process is the last treatment stages that can physically remove contaminants before disinfection. One of the most popular filtration processes used in Ireland is the rapid gravity sand process (IRELAND EPA, 1995). A study by Li et al. (2012) showed that direct rapid sand removal can remove 0.6–1.5 log-units of total faecal coliform, depending on the loading rate and grain size distribution. Mwabi et al. (2012) demonstrated that designing and building a bio-sand filtration system was effective in removing 2–4 $\log_{10}$ of coliform bacteria. Koivunen et al. (2003) showed that tertiary treatment by the rapid sand filtration process found, on average, a 97% reduction of faecal coliforms and total coliforms in four conventional wastewater treatment plants in Helsinki, Finland. In keeping with the Irish EPA's filtration manual guidelines, rapid gravity filtration was considered in the model. To model rapid sand filtration and to account for uncertainty and variability in the data, a decimal reduction uniform distribution was assigned (min 0.74 max 0.99) (Table 2).

2.4. Disinfection

Disinfection is the "process by which an organism's viability/infectivity is destroyed with a specific percentage of the population dying over some time frame defined as a rate" (Betancourt and Rose, 2004). Worldwide, chlorine is the most commonly used disinfection in drinking water treatment, although other alternatives are being increasingly introduced such as ozonation, ultraviolet irradiation, ultrasonic vibration, ultra-filtration, silver, bromide and iodine, membrane filtration and granular activated carbon (GAC). Chlorine is added to provide a disinfectant residual to preserve the water in distribution, where the chlorine is in contact with the water for a longer period of time compared to the pre-chlorination process in primary treatment (Irish IRELAND EPA, 2011a,b). The principal factors that influence disinfection efficiency are the disinfection concentration, contact time, temperature and pH (depending upon the disinfection) (Cotruvo et al., 2013). Chlorination has been found to remove *E. coli* between 97 and 99% (O'Connor and O'Connor, 2001). Following chlorine treatment of drinking water, certain levels of residual chlorine known as free chlorine residual (FCR) remain in the water to prevent the regrowth of microorganism that may enter the water prior to entering the consumer's home. A study by Igunnugbemi et al. (2009) demonstrated how FCR < 40 mg L^{-1} may not prevent the survival of fecal coliforms in drinking water even though the World Health Organisation recommend FCR concentrations between 0.2 and 0.5 mg L^{-1} for chlorine treated piped drinking water. 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:

$$P_{STT} = D - off \times (1 - Cr) \times (1 - Frd) \times (1 - D) \quad (3)$$

Where: P_{STT} is the coliform concentration post-secondary and tertiary treatment (MPN 100 mL^{-1}), 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.

2.5. 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).

2.6. 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

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 100 mL^{-1}
Dilution	DF	Dilution in stream	Dilution factor (10)	–
	$PEC_{stream\text{-}water}$	Concentration of coliforms in stream-water following dilution	$C_{surfacerunoff}$/DF	MPN 100 mL^{-1}
Die-off	K	First order inactivation constant	Uniform (min 0.7, max 1.5)	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 100 mL^{-1}
Secondary treatment				
	Cr	Coagulation/Flocculation and sedimentation reduction	Uniform (min 0.89, max 0.99)	Decimal reduction
	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 100 mL^{-1}
Human exposure				
Consumption	TWi	Tap water intake (adult)	Lognormal (mean 0.564, SD 0.617)	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)	–

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:

$$P_i = 1 - \exp(-d \times r) \quad (4)$$

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:

$$P_{i\,(365)} = 1 - (1 - P)^{365} \quad (5)$$

2.7. Sensitivity analysis

A sensitivity analysis based on rank order correlation was performed to evaluate how the model's predictions are dependent on variability and uncertainty in the model input parameters. The entire model was constructed in Microsoft Excel 2010 (with the @Risk 6.0 add on) (V4.5, Palisade Corporation, Newfield, NY) using Monte Carlo simulation techniques and run for 10,000 iterations.

3. Results

The model produced several output distributions (TC and *E. coli* concentration in effluent post DWTP, viable coliforms consumed, and probability of illness) that were used to compare coliform concentrations detected in surface runoff and the potential risk to human health. Figs. 1 and 2 show a simulated time series of initial TC and *E. coli* concentrations detected in surface runoff, die-off and post drinking water treatment. Following drinking water treatment, reductions of 99% and between 87 and 99% were recorded for *E. coli and* TC, respectively. Pritchard et al. (2010) recorded reduction values of 97% for *E. coli* using alum and sand for coagulation and filtration; however no tertiary treatment (i.e. disinfection) was considered. The TD biosolid had the greatest concentration of TC (mean count values 1.3, 2.7 and 4.2 MPN 100 mL^{-1}) for corresponding rainfall simulation times (24 (RS1), 48 (RS2) and 360 h (RS3)), whilst *E. coli* concentration from ADUK biosolid were greater (mean count values 7.3×10^{-2}, 4.7×10^{-2} and 2.4×10^{-3} MPN 100 mL^{-1}) at each rainfall simulation time (RS1, RS2 and RS3).

The results for mean human exposure *via* drinking water consumption show that following drinking water treatment and based on human consumption patterns, the biosolids TD and LS produced the greatest concentration of TC that can be viably consumed combining the rainfall simulation times of 48 and 360 h ((RS2 and RS3) (mean viable total coliform values 16.83 and 26.75 MPN d^{-1}, respectively) Fig. 3). The greatest concentration of viable *E. coli* that can be consumed was for ADUK biosolids and rainfall simulation times of 24 and 48 h s (RS1 and RS2) (Fig. 3) mean viable *E. coli* consumed values 5.20×10^{-1} and 2.34×10^{-1} MPN d^{-1}, respectively. These results are in accordance with reported results for water quality in Ireland (IRELAND EPA, 2015).

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. Results show that for exposure to TC and immuno-compromised populations following surface runoff from the LS biosolid treatment (RS1), the mean risk of illness yr^{-1} was 9.92×10^{-1}. The mean probability of illness yr^{-1} 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

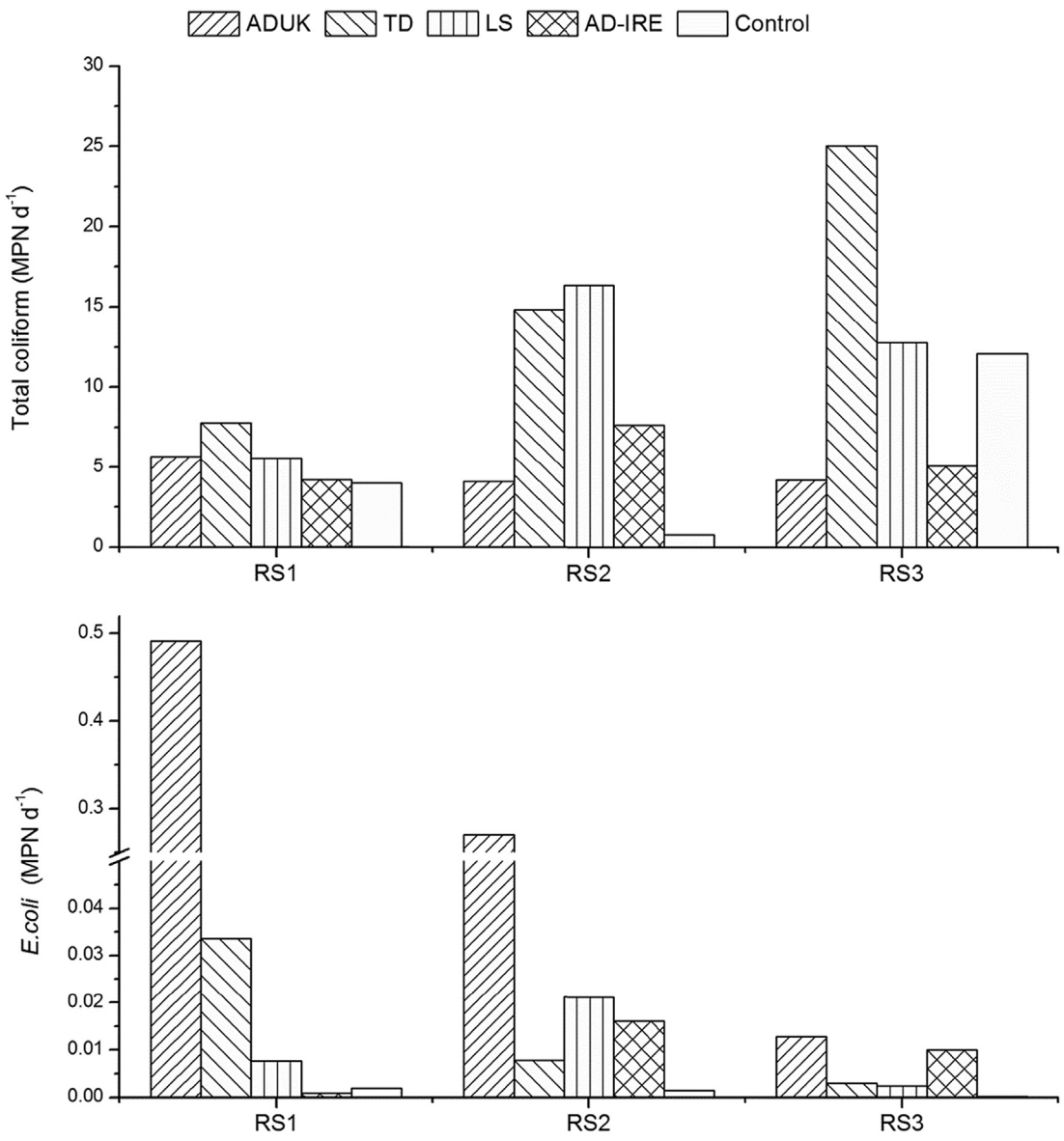

Fig. 3. Simulated mean TC and *E. coli* consumed.

4.6×10^{-5}, respectively).

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 counts of *E. coli*, in drinking water, therefore, a sensitivity analysis was conducted for the annual probability of illness for both biosolid treatments. The 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) (Fig. 4). This highlights the importance of residence time of bacteria in a 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 counts 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.

4. Discussion

Concentrations of TC and *E. coli* in surface runoff were quantitatively assessed following the spreading of biosolids on grassland. The aim was to study the fate of TC and *E. coli* in drinking water treatment, 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, also known as overland flow to hydrologists, 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 a combination of the rate of rainfall, rainfall

Table 3
Mean 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

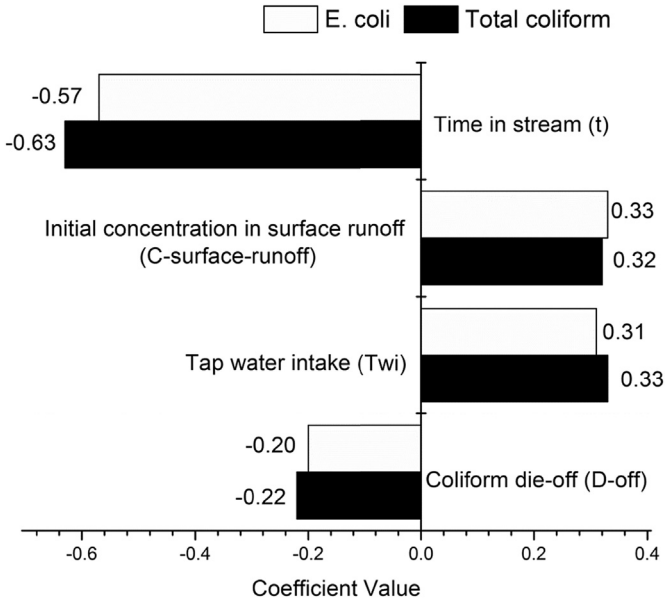

Fig. 4. Sensitivity analysis for TC annual probability of illness and TD biosolid treatment and *E. coli* annual probability of illness and ADUK biosolid treatment.

intensity and duration along with the soil surface crusting and vegetation covering the degree of soil saturation. Concentrations of TC and *E. coli* in surface runoff in this study are comparable to concentrations reported by Schreiber et al. (2015) who reported minimum count values of <10 CFU 100 mL^{-1} for *E. coli* in surface runoff following an investigation into various land uses. Similarly, Wallace et al. (2014) reported a large variability in bacterial counts following low and high filtered biosolid applications with a poor relationship ($r^2 = 0.44$) in runoff faecal CFU levels. All TC and *E. coli* counts had decreased by the third rainfall event (RS3; 360 h) and may be attributed to the desiccation of the pathogens in soil following the application of the biosolids. However, Lang and Smith (2007) suggest that "predation processes are likely to be an important pathogen inactivation mechanism in temperate biosolids amended agricultural soils". Furthermore, in temperate soil conditions, survival times of enteric bacteria supplied in bulky types of biosolids (i.e. dewatered sludge cake) may be shorter in moist soils and prolonged by dry conditions.

The mean concentration of TC after the DWTP 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 counts of TC 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 sludge retention time of about 10 days.

The mean concentrations of *E. coli* post drinking water show that the ADUK biosolids had the greatest counts 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 counts 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 EU states that there should be 0 in 100 ml of coliform bacteria and *E. coli* following drinking water treatment.

The mean viable consumption of TC and *E. coli* in drinking water showed the same trends as mean TC and *E. coli* counts 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 LeChevallier 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 concentration 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. It is important to note that in this study the amount of water consumed was based on actual drinking water consumed (i.e. not used for cooking, or hot drinks) rather than the recommended 2 L as proposed in many risk assessment guidelines. Therefore, the exposure results represent more realistic bacterial exposure estimates. 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 risk of illness recorded in this study is far lower than those recorded in previous studies. For example, 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.

5. 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 counts 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 and 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.

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