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Risk Assessment of *E. coli* Survival Up to the Grazing Exclusion Period After Dairy Slurry, Cattle Dung, and Biosolids Application to Grassland

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Grassland application of dairy slurry, cattle dung, and biosolids offers an opportunity to recycle valuable nutrients (N, P, and K), which may all introduce pathogens to the soil environment. Herein, a temporal risk assessment of the survival of *Escherichia coli* (*E. coli*) up to 40 days in line with the legislated grazing exclusion time points after application was examined across six scenarios: (1) soil and biosolids mixture, (2) biosolids amended soil, (3) dairy slurry application, (4) cattle dung on pasture, (5) comparison of scenario 2, 3, and 4, and (6) maximum legal vs. excess rate of application for scenario 2 and 3. The risk model input parameters were taken or derived from regressions within the literature and an uncertainty analysis ($n = 1,000$ trials for each scenario) was conducted. Scenario 1 results showed that *E. coli* survival was higher in the soil/biosolids mixture for higher biosolids portion, resulting in the highest 20 day value of residual *E. coli* concentration (i.e., C_{20}, $\log_{10}$ CFU g^{-1} dw) of 1.0 in 100% biosolids or inoculated soil and the lowest C_{20} of 0.098 in 75/25 soil/biosolids ratio, respectively, in comparison to an average initial value of ~6.4 $\log_{10}$ CFU g^{-1} dw. The *E. coli* survival across scenario 2, 3, and 4 showed that the C_{20} value of biosolids (0.57 $\log_{10}$ CFU g^{-1} dw) and dairy slurry (0.74 $\log_{10}$ CFU ml^{-1}) was 2.9–3.7 times smaller than that of cattle dung (2.12 $\log_{10}$ CFU g^{-1} dw). The C_{20} values of biosolids and dairy slurry associated with legal and excess application rates ranged from 1.14 to 1.71 $\log_{10}$ CFU ha^{-1}, which is a significant reduction from the initial concentration range (12.99 to 14.83 $\log_{10}$ CFU ha^{-1}). The *E. coli* survival in un-amended soil was linear with a very low decay rate resulting in a higher C_{20} value than that of biosolids or dairy slurry. The risk assessment and uncertainly analysis showed that the residual concentrations in biosolids/dairy slurry applied soil after 20 days would be 45–57% lower than that of the background soil *E. coli* concentration. This means the current practice of grazing exclusion times is safe to reduce the risk of *E. coli* transmission into the soil environment.

Keywords: biosolids, dairy slurry, *E. coli*, decay, risk assessment, agriculture, soil

INTRODUCTION

Globally, provision of a circular economy safeguards against volatile fertilizer prices, global diminishing resources (e.g. synthetic fertilizers, fossil fuel) and an increased demand for food (Heffer and Prud'homme, 2013). In the European Union (EU), the Landfill Directive (EC, 1999) promoted a circular economy by targeting an 85% reduction in the disposal of sewage sludge to landfill by 2014 from 1995 levels. Such an ambitious target was aided by the Sewage Sludge Directive (EEC, 1986), which directed a major proportion of sewage sludge to land (Lucid et al., 2013; Fijalkowski et al., 2017). The standard management practice for dairy slurry and manure on dairy farms is land application without any necessary pathogen treatment. In contrast land application of treated sewage sludge (henceforth called "biosolids"), which typically involves pre-treatment has variable land application uptake across EU member states ranging from 0% (e.g., Belgium—Brussels and Flanders, Switzerland, and Romania) to >50% (e.g., Norway, Ireland, Spain, UK, France) with an average of 39% being reused in agriculture across the EU (Lucid et al., 2013; Healy et al., 2016a; Fijalkowski et al., 2017). By comparison, about 60% of biosolids in the USA, Canada, and Australia are recycled to agriculture (Tozzoli et al., 2016). The EU figures from 2010 suggest an 81.8% increase in sewage sludge production when compared to 5.5 million tons of dry solids (tds) produced in 1992, and this figure is expected to increase up to 13 million tds by 2020 (EC, 2010; Healy et al., 2017). The positives of land application include a source of nitrogen (N), phosphorus (P), potassium (K), other plant nutrients, and an increase in soil organic matter (Sharma et al., 2017). The negatives can be heavy metal bioaccumulation, runoff losses of nutrient, metal, enteric pathogens and emerging contaminants, and bio-transfer of persistent organic pollutants to the food chain (Healy et al., 2016a,b, 2017; Clarke et al., 2017, 2018; Fijalkowski et al., 2017).

In Ireland, 98% of the biosolids (out of 53,543 tds year^{-1} produced) go to land (Irish Water, 2015; Clarke et al., 2018). The application rate is typically determined by pH, metal and nutrient content of the soil, and the nutrient and metal content of the biosolids as per limits recommended in the "Codes of Good Practice for the Use of Biosolids in Agriculture" (Fehily Timoney Company, 1999). The guideline relates to post-application of biosolids to grassland and restricts the livestock grazing period stating that "cattle should not be turned out onto pasture that has been fertilized with biosolids until 3–6 weeks after the date of application" (Fehily Timoney Company, 1999). There is growing concern on the survival of enteric *Escherichia coli* (*E. coli*) in biosolids and associated risk of transferring this fecal indicator organism (FIO) pollutant into the soil environment and subsequently, contamination of crops and nearby water sources, leading to the potential of spread of gastrointestinal disease (Greene et al., 2008; Ellis et al., 2018). The Sewage Sludge Directive 86/287/EC does not specify limits for *E. coli* counts as a fecal contamination indicator in biosolids, but specifies general land use, harvesting, and grazing limits to provide protection against the risk of infection (Sobrados-Bernardos and Smith, 2012). The revised version of the Sewage Sludge Directive (Working Document 3rd Draft), recommends that the *E. coli* in the biosolids needs to be less than 1×10^3 CFU g^{-1} dry weight (dw) and that the sludge must have limited spores of *Clostridium perfringens* ($<3 \times 10^3$ g^{-1} dw) with an absence of *Salmonella. spp* in 50 g (wet weight, ww) (EEC, 2000; Healy et al., 2017). This revised working document further states that *E. coli* concentration in biosolids needs to achieve at least a 2 Log_{10} reduction after conventional treatment. Therefore, it is critical to accurately determine the FIO pollution (herein *E. coli*) risk associated with land application of biosolids to fully understand the potential for environmental loss and consequently, human/animal transmission.

Survival patterns of biosolids-derived *E. coli* in the environment are complex, and a lack of a standardized approach to *E. coli* measurement makes quantifying their impact difficult. For example, Avery et al. (2005) spiked treated and untreated biosolids samples with a known concentration of *E. coli O157* to quantify the time taken to achieve a decimal reduction. The pathogen response was variable and ranged from 3 to 22 days, depending on sludge properties. Lang and Smith (2007)investigated indigenous *E. coli* survival in dewatered, mesophilic anaerobically digested (DMAD) biosolids, and in different soil types post DMAD biosolids application. Again, decimal reduction times proved variable, ranging from 100 days when applied to air-dried sandy loam, to 200 days in air-dried silty clay. When field moist soils were used this time decreased to 20 days, demonstrating the importance of water content in regulating survival behavior. Therefore, in order to quantify *E. coli* risk in a relevant, site-specific manner, it is necessary to incorporate both soil and biosolids characteristics in risk assessment modeling. This has been done previously by conducting soil, biosolids, and dairy slurry incubation studies where *E. coli* are often spiked to generate a survival response (Vinten et al., 2004; Lang and Smith, 2007; Moynihan et al., 2013). Pathogen decay rate (or death) is then calculated based on decimal reduction times, or a first-order exponential decay model previously described by Vinten et al. (2004), and has been shown to be highly contingent on soil type and biosolids or slurry combinations. Currently the Safe Sludge Matrix provides a legal framework for grazing animals and harvesting crops following land application of biosolids, and stipulates that a time interval of about 20 days (grazing exclusion period, and harvesting interval for grass and forage) and 10 months (harvesting interval for fruit, salads, vegetables, and horticulture) should be enforced to ensure safe practice, respectively (ADAS, 2001). However, further work is required to determine if these regulations are overly stringent, particularly in light of the comparatively larger pathogen concentrations reported for dairy slurries than biosolids. For example, *E. coli* concentrations ranged from 3×10^2 to 6×10^4 CFU g^{-1} in biosolids (Payment et al., 2001) compared to 7.5×10^4 to 2.6×10^8 CFU g^{-1} in fresh and stored dairy slurry, respectively (Hutchison et al., 2004). Recently, Healy et al. (2017) study pointed out that livestock exclusion times of more than 3 weeks after biosolids application (considering compliant application rates) may be overly strict with respect to the current exclusion criteria recommendation (e.g. 3–6 weeks in Ireland). Therefore, environmental losses of *E. coli* associated with biosolids application may not be as extensive as previously thought and further comparisons on pathogen risk should form the basis of future research.

The main objective of this study was to assess the risk of *E. coli* survival as an indication of the risk associated with land spreading biosolids to agricultural soils within the context of legislated grazing exclusion times. Herein, two exclusion time points at 20 and 40 days were considered in line with the exclusion criteria practice in the UK (i.e. Safe Sludge Matrix ∼20 days) and Ireland (i.e. Code of Good Practice for the Use of Biosolids in Agriculture ∼20–40 days). In particular, the objectives of the present study were to: (1) gather empirical data on *E. coli* concentration, and pathogen decay rate (k) for dairy slurry, cattle dung, and biosolids, and (2) conduct risk assessment modeling and uncertainty analysis of survival of *E. coli* at different time periods from application of dairy slurry, cattle dung, and biosolids to grassland up to the cattle exclusion time point (i.e. 20 and 40 days).

MATERIALS AND METHODS

Empirical Data on *E. coli* Concentration and Decay Rate

The die-off patterns of *E. coli* in dairy slurry, cattle dung, and biosolids were analyzed from the published peer-reviewed literature to develop an overview of the *E. coli* concentration and decay rate (k) as presented in **Table 1**. In this case, 12 relevant papers were utilized to generate the data under five categories—(1) un-amended soil, (2) *E. coli* spiked soil, (3) biosolids, (4) dairy slurry, and (5) cattle dung. These studies were deemed relevant based on the availability or possibility of derivation of initial *E. coli* concentration and k value. The heterogeneous nature of the above five categorized materials and their diverse treatment conditions like moisture level, seasonality, application dose, and condition were also considered to cover the wide range of data set. Data were obtained from tables or log-linear regression equations where available (Himathongkham et al., 1999; Oliver et al., 2006; Lang and Smith, 2007; Martinez et al., 2013; Hodgson et al., 2016; Roberts et al., 2016); otherwise, data were extracted from digitized figures to derive log-linear regression equation by plotting $\mathrm{Log_{10}}$ CFU $\mathrm{g^{-1}}$ dw vs. Time (days) (Avery et al., 2004, 2005; Oliver et al., 2010; Schwarz et al., 2014; Biswas et al., 2018; Ellis et al., 2018). The die-off pattern of pathogens can be described by the first-order kinetics Equation (1), which upon integration gives the linear Equation (2) (Mubiru et al., 2000; Martinez et al., 2013). This natural logarithm based linear Equation (2) was converted to the base 10 logarithm (i.e., $\mathrm{Log_{10}}$) based Equation (3) and compared with a straight line equation (y=mx+c) to get the slope (m) and subsequently, the die-off or decay rate (k) values were obtained using Equation (4) (**Table 1**). The linear Equation (2) can be transformed to an exponential model (Equation 5) to assess the risk of *E. coli* content in soil after application of different organic residues like dairy slurry, sewage sludge, and cattle dung (Vinten et al., 2004).

$$\frac{d(C)}{dt} = -kC \tag{1}$$

where C is the *E. coli* concentration per unit of mass or volume and k is the die-off or decay rate.

$$\mathrm{lnC_t} = \mathrm{lnC_o} - k\mathrm{t} \tag{2}$$

Here, C_t is concentration of *E. coli* at time t in the soil, C_o is the concentration of *E. coli* at time zero in the soil, t is fixed time period (e.g. grazing period) (days), k is the die-off function of the *E. coli* ($\mathrm{day^{-1}}$).

$$\mathrm{Log_{10}C_t} = \mathrm{Log_{10}C_o} - \frac{k\mathrm{t}}{2.303} \tag{3}$$

$$\mathrm{Slope, m} = -\frac{k}{2.303} \tag{4}$$

$$\mathrm{C_t} = \mathrm{C_o}e^{-k\mathrm{t}} \tag{5}$$

Risk Assessment and Uncertainty Analysis

In this study, the exponential Equation (5) was used to quantify the concentrations of *E. coli* in the soil after any time period to be known following land application of the aforementioned organic materials. Traditionally, the burden of *E. coli* accumulation in soil from livestock feces or land spreading of dairy slurry is calculated by assuming the exponential decay pattern of *E. coli* survival over time (Oliver et al., 2009, 2010). A risk assessment of the survival of *E. coli* up to 40 days after application was examined across six scenarios (**Table 2**)—(1) soil and biosolids mixture, (2) biosolids amended soil, (3) dairy slurry application, (4) cattle dung on pasture, (5) comparison of scenario 2, 3, and 4, and (6) maximum legal vs. excess rate of application for scenario 2 and 3. The risk model input parameters i.e., initial *E. coli* concentration (C_0) and decay rate (k) were used from the **Table 1** as presented in **Table 2**.

In scenario 1, the values of C_0 (i.e., concentration of *E. coli* at day 0) and k were taken as the average for soil to sludge mixture matrix of un-amended soil (Lang and Smith, 2007), 100% soil (*E. coli* spiked) (Oliver et al., 2006; Ellis et al., 2018), 75% soil to 25% sludge (Ellis et al., 2018), 50% soil to 50% sludge (Ellis et al., 2018), 25% soil to 75% sludge (Ellis et al., 2018), and 100% sludge (Avery et al., 2005; Ellis et al., 2018). In scenario 2, C_0 was considered as the average of biosolids associated *E. coli* from five different studies and the k value was considered individually from the respective study and also, as an average value of those studies (**Table 1**, **2**). Similar to scenario 2, C_0 and k values (**Table 2**) were assigned to scenario 3 and 4 considering five different studies (as mentioned in **Table 1**) for dairy slurry and cattle dung, respectively. In scenario 5, the average value for C_0 and k was assigned as in scenarios 2, 3, with 4 used to provide a comparison among biosolid, dairy slurry and cattle dung treatments. Scenario 6 was considered to assess the risk of *E. coli* survival under estimated legal and excess application rate of biosolids and dairy slurry in grassland.

TABLE 1 | Concentration (*E. coli*, Log_{10} CFU g^{-1} dw) and decay rate (*k*, $days^{-1}$) for a variety of biosolids, dairy slurry, and cattle dung.

Type of materials	Treatment	Concentration, $[C_0]$ (Log_{10} CFU g^{-1} dw)	Decay rate, *k* ($days^{-1}$)	D values (days)	R^2	References
Soil	Unamended sandy loam—moist	3.13	0.023	100	0.390	Lang and Smith, 2007
	Unamended sandy loam—air-dried	2.26	0.012	200	0.170	Lang and Smith, 2007
	Unamended silty clay—moist	0.79	0.007	333	0.150	Lang and Smith, 2007
	Unamended silty clay—air-dried	0.91	0.014	167	0.130	Lang and Smith, 2007
E. coli spiked soil	100% soil (inoculated)	5.93	0.131	18	0.918	Ellis et al., 2018
	Intact soil+*E.coli* (dry: 25% moisture)	6.89	0.088	26	0.974	Oliver et al., 2006
	Intact soil+*E.coli* (wet: 50% moisture)	6.18	0.069	33	0.805	Oliver et al., 2006
	Repacked soil+*E.coli* (dry: 25% moisture)	6.92	0.076	30	0.994	Oliver et al., 2006
	Repacked soil+*E.coli* (wet: 50% moisture)	6.61	0.096	24	0.950	Oliver et al., 2006
Biosolids	soil to biosolids 75/25	6.17	0.208	11	0.960	Ellis et al., 2018
ADD sludge cake	soil to biosolids 50/50	6.51	0.155	15	0.959	Ellis et al., 2018
ADD sludge cake	soil to biosolids 25/75	6.28	0.126	18	0.987	Ellis et al., 2018
ADD sludge cake	100% biosolids	6.44	0.049	47	0.826	Ellis et al., 2018
Sewage sludge	Sewage sludge waste (SSW)	7.31	0.145	16	0.872	Avery et al., 2005
DMAD Biosolids	Amended sandy loam—moist	5.14	0.115	20	0.880	Lang and Smith, 2007
DMAD Biosolids	Amended sandy loam—air-dried	5.16	0.023	100	0.330	Lang and Smith, 2007
DMAD Biosolids	Amended silty clay—moist	5.12	0.115	20	0.930	Lang and Smith, 2007
DMAD Biosolids	Amended silty clay—air-dried	4.25	0.012	200	0.210	Lang and Smith, 2007
ADD Biosolids	Amended loamy sand to sandy soil	7.82	0.087	27	0.888	Schwarz et al., 2014
Class B Biosolids	Surface applied sandy loam (Culture)	6.00	0.290	8	–	Roberts et al., 2016
Class B Biosolids	Surface applied clay loam (Culture)	6.00	0.060	38	–	Roberts et al., 2016
Dairy Slurry*	Dairy slurry	7.27	0.198	12	0.889	Avery et al., 2005
	Repacked soil+slurry (dry: 25% moisture)	6.18	0.054	43	0.939	Oliver et al., 2006
	Repacked soil+slurry (wet: 50% moisture)	6.43	0.094	25	0.987	Oliver et al., 2006
	Shallow Injection (May application)	6.10	0.110	21	–	Hodgson et al., 2016
	Surface broadcast (May application)	6.10	0.230	10	–	Hodgson et al., 2016
	Shallow Injection (July application)	5.86	0.023	100	–	Hodgson et al., 2016
	Surface broadcast (July application)	5.86	0.097	24	–	Hodgson et al., 2016
	Shallow Injection (October application)	6.15	0.029	79	–	Hodgson et al., 2016
	Surface broadcast (October application)	6.15	0.036	64	–	Hodgson et al., 2016
	Fresh manure slurry	6.09	0.106	22	0.910	Himathongkham et al., 1999
	Old manure slurry	6.40	0.060	38	0.810	Himathongkham et al., 1999
	Dairy slurry	6.30	0.098	23	0.398	Biswas et al., 2018
Cattle dung	Repacked soil+feces (dry: 25% moisture)	6.06	0.054	43	0.985	Oliver et al., 2006
	Repacked soil+feces (wet: 50% moisture)	6.24	0.058	39	0.942	Oliver et al., 2006
	Surface applied sandy loam (Culture)	6.00	0.050	46	–	Roberts et al., 2016
	Surface applied clay loam (Culture)	6.00	0.071	32	–	Roberts et al., 2016
	Dung-pats on pasture	7.13	0.042	55	0.688	Oliver et al., 2010
	Cattle feaces on pasture	5.36	0.061	38	0.732	Avery et al., 2004
	Cowpats on grazing lands	6.14	0.048	48	–	Martinez et al., 2013

DMAD, dewatered mesophilic anaerobically digested; ADD, Anaerobically digested dewatered; dw, dry weight.
*D value indicates the time required for 90% pathogen reduction; $[C_0]$, initial E. coli concentration; *values presented as wet weight basis (Log_{10} CFU ml^{-1}) assuming 1 ton = 1 m^3 slurry.*

The estimation of a legal application rate for biosolids and dairy slurry was based on the required P application rate of 40 kg ha^{-1} for pasture establishment at a low Morgan's P Index soil (e.g. P Index 2 equivalent to Morgan's P of 3.1–5.0 mg l^{-1}) (Peyton et al., 2016; Teagasc Greenbook, 2016). In general, P is the limiting factor for estimating legal application rate of waste derived organic fertilizers such as biosolids and dairy slurry (Lucid et al., 2013). The legal maximum application rate of biosolids was estimated to be in the range of 3.0 to 5.2 ton ha^{-1} by Lucid et al. (2013) based on the P Index of the soil, the legal limits of N, P, and metal concentration of the soil, the dry matter content, and the nutrient and metal concentration of the biosolid amendment. The estimated legal application rate of biosolids and dairy slurry is presented in **Table 3** and these

TABLE 2 | Scenario and parameters used for risk assessment modeling and Monte Carlo uncertainty analysis.

Scenario	Description	Model parameters	
		$[C_0]$*, Log_{10}CFU g^{-1}	k, day^{-1}
1	Soil and biosolids mixture		
	Un-amended soil	1.77	0.014
	100% soil (inoculated)	6.51	0.092
	Soil to biosolids: 75/25	6.32	0.208
	Soil to biosolids: 50/50	6.32	0.155
	Soil to biosolids: 25/75	6.32	0.126
	100% biosolids	6.88	0.097
2	Biosolids amended soil	6.48	0.066; 0.087; 0.121; 0.134; 0.145; 0.175
3	Dairy slurry application	6.43	0.074; 0.083; 0.088; 0.098; 0.108; 0.198
4	Cattle dung on pasture	6.16	0.042; 0.048; 0.053; 0.056; 0.06; 0.061
5	Comparison of scenario 2, 3 and 4		
	Biosolids	6.48	0.121
	Dairy slurry	6.43	0.108
	Cattle dung	6.16	0.053
6	Estimated maximum legal application rate vs. excess rate of application	Biosolids: 12.99; 13.69 Dairy slurry: 14.13; 14.83	Biosolids: 0.121; Dairy slurry: 0.108

**Values presented as dw basis except for dairy slurry (wet weight basis assuming 1 ton = 1 m^3 slurry).*

TABLE 3 | Biosolids and dairy slurry landspreading rate for risk assessment model and Monte Carlo uncertainty simulation.

Materials	Typical total P content[c] (kg ton^{-1})	Average *E. coli* concentration[d] (CFU g^{-1})	P application rate (kg ha^{-1})	Estimated maximum legal application			Estimated excess application[e]		
				Application rate (ton ha^{-1})	Estimated *E.coli* (CFU ha^{-1})	Estimated *E.coli* (Log_{10} CFU ha^{-1})	Application rate (ton ha^{-1})	Estimated *E.coli* (CFU ha^{-1})	Estimated *E.coli* (Log_{10} CFU ha^{-1})
Biosolids[a]	12.3	3.01×10^6	40	3.25	9.80×10^{12}	12.99	16.26	4.90×10^{13}	13.69
Dairy slurry[b]	0.8	2.71×10^6	40	50	1.35×10^{14}	14.13	250	6.77×10^{14}	14.83

*[a]Values presented as dw basis; [b]Values presented as wet weight basis assuming 1 ton = 1 m^3 slurry; [c](Teagasc Greenbook, 2016); [d]**Table 1**; [e]5 times higher than the legal application rate.*

values are comparable with those of commonly used application rate in previous studies (e.g. Brennan et al., 2012; Lucid et al., 2013).

In order to reflect the variability of the model input parameters for a particular soil type, organic material, *E. coli* concentration (C_0) and die-off rate (k) across time, we applied a Monte Carlo simulation (run of 1,000 times per scenario) to compute the probability density distributions for the final concentration in the soil. For the analysis we assumed a uniform distribution of C_0, k, and time as in **Table 2**.

RESULTS AND DISCUSSION

E. coli $[C_0]$ and *k*

The empirical data on initial concentration (C_0) and k values of *E. coli* are presented in **Table 1**. Results show that these parameters vary widely across each type of material ranging from 0.79–3.13 (unamended soil), 5.93–6.92 (inoculated soil), 4.25–7.82 (biosolids), 5.86–7.27 (dairy slurry) and 5.36–7.13 (cattle dung) for C_0 (log_{10} CFU g^{-1}), and 0.007–0.023 (unamended soil), 0.069–0.131 (inoculated soil), 0.012–0.290 (biosolids), 0.023–0.230 (dairy slurry), and 0.042–0.071 (cattle dung) for k (day^{-1}) values, respectively. The treatment nature and condition of each type of material is largely heterogeneous (e.g. soil type, soil to biosolids ratio, sludge type, slurry moisture, slurry age, dung condition) across and within the incorporated reference studies, which can reasonably explain such variability for C_0 and k values. However, it was observed that the mean value of both C_0 (log_{10} CFU g^{-1}) and k (day^{-1}) when compared among inoculated soil ($C_0 = 6.5 \pm 0.44$, $k = 0.092 \pm 0.024$), biosolids ($C_0 = 6.0 \pm 0.99$, $k = 0.115 \pm 0.079$), dairy slurry ($C_0 = 6.2 \pm 0.37$, $k = 0.095 \pm 0.064$), and cattle dung ($C_0 = 6.1 \pm 0.52$, $k = 0.055 \pm 0.010$) is not statistically different at the 95% significance level as determined by one-way ANOVA [$F_{(3,32)} = 0.665$, $p = 0.579$ for C_0 and $F_{(3,32)} = 1.477$, $p = 0.239$ for k). This means the empirical range of the C_0 and k values of *E. coli* for three major organic residue based fertilizers (biosolids, dairy slurry, and cattle dung) as presented in **Table 1** are suitable for risk assessment modeling. The wide data set of C_0 and k values will provide a variability range for the risk assessment and a prediction of uncertainty through the probability distribution.

E. coli Survival Pattern Across Six Scenarios

In scenario 1, the different combinations of soil and biosolids in the incubation experiment produced different k values and therefore different distributions of *E. coli* concentrations over time in soil i.e. potential losses in runoff. The *E. coli* survival pattern in 100% inoculated soil and 100% biosolids is similar, and *E.coli* concentration reduction of ~5.69 log_{10} CFU g^{-1} dw was observed leading to the 20 day concentration (C_{20}) of ~1.0 log_{10} CFU g^{-1} dw (see **Figure 1A**). The survival is the lowest in the soil to biosolids mixture ratio of 75/25 and after 20 days the concentration was 0.098 compared to 0.282 and 0.509 log_{10} CFU g^{-1} dw in 50/50 and 25/75 equivalents, respectively. In comparison to the inoculated soil and biosolids or soil/ biosolids mixture, the survival pattern in un-amended soil was linear with a very low decay rate (0.014 day^{-1}) resulting in the highest C_{20} concentration of 1.34 log_{10} CFU g^{-1} dw. After 40 days, the *E. coli* concentrations (log_{10} CFU g^{-1} dw) were: 0.166, 0.0015, 0.0126, 0.0409, 0.1436 for the 100% soil (inoculated), 75/25 soil/biosolids, 50/50 soil/biosolids, 25/75 soil/biosolids ratios,

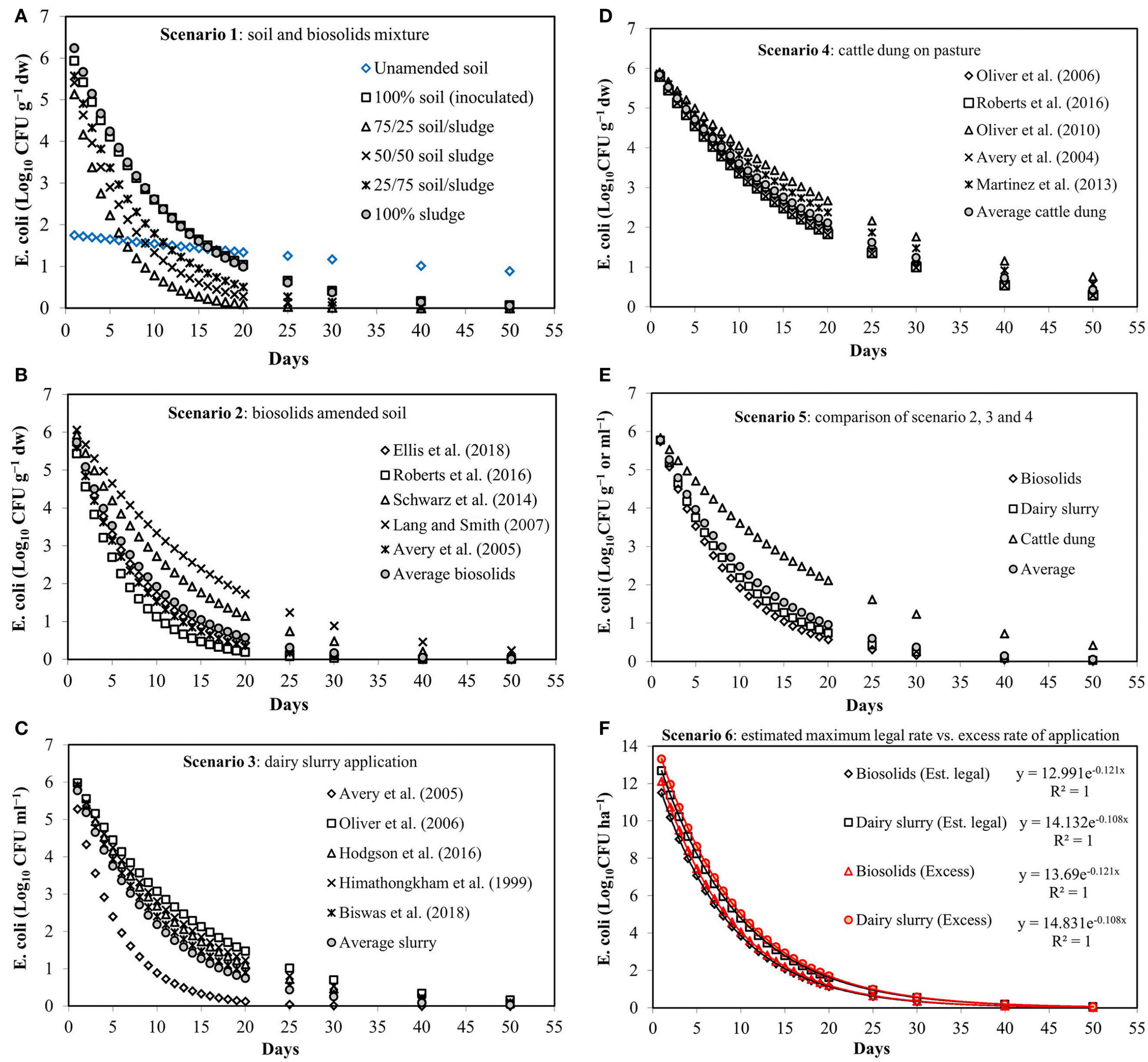

FIGURE 1 | Concentration of *E. coli* over time based on k values and different scenarios as presented in **Table 2**. **(A)** scenario 1, **(B)** scenario 2, **(C)** scenario 3, **(D)** scenario 4, **(E)** scenario 5, and **(F)** scenario 6.

and 100% biosolids, respectively, compared to the C_{40} value of 1.02 for un-amended soil. These results likely reflect that *E. coli* populations in un-amended soil are more adaptive than the imported *E. coli* and can survive as natural soil microflora under favorable soil conditions (e.g. soil texture and structure, pH, moisture, temperature, UV radiation, and nutrient and oxygen availability). For example, *E. coli* was observed to survive in control soils for more than 9 years, particularly, as becoming naturalized in the low-temperature environments of temperate maritime soils (Brennan et al., 2010a,b).

In scenario 2, the *E. coli* survival trend in biosolids amended soil was assessed based on the empirical data (**Tables 1**, **2**) from five reference studies as shown in **Figure 1B**. The *E. coli* concentration ($\log_{10}$ CFU g^{-1} dw) after 20 days was ≤0.57 from an initial value of 6.48 for the average biosolids and three study references (Avery et al., 2005; Roberts et al., 2016; Ellis et al., 2018), except for Schwarz et al. (2014) ($C_{20} = 1.14$) and Lang and Smith (2007)($C_{20} = 1.72$). The C_{40} value ranged from 0.006 to 0.46 $\log_{10}$ CFU g^{-1} dw for all five reference studies.

In scenario 3, *E. coli* survival pattern in dairy slurry application associated soil was assessed based on the empirical data (**Tables 1**, **2**) from five reference studies as shown in **Figure 1C**. In this case, the C_{20}, $\log_{10}$ CFU ml^{-1} concentrations were 0.12, 1.47, 1.12, 1.23, 0.90, and 0.74 compared to the initial value of 6.43 from Himathongkham et al. (1999), Avery et al. (2005), Oliver et al. (2006), Hodgson et al. (2016), and Biswas et al. (2018). The C_{40} concentration ranged from 0.002 to 0.34 $\log_{10}$ CFU ml^{-1} for all five reference studies.

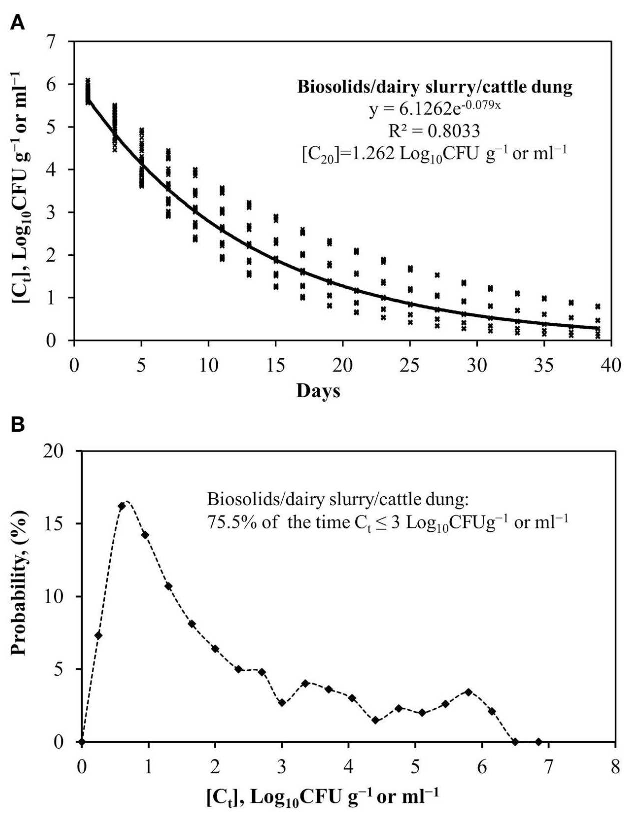

FIGURE 2 | Monte Carlo analysis of **(A)** soil *E. coli* concentrations over time and **(B)** probability distribution of [C_t] after biosolids/dairy slurry/cattle dung application to land.

In scenario 4, cattle dung associated *E. coli* survival pattern was assessed based on the input data from five reference studies as shown in **Figure 1D**. In this scenario, the C_{20} and C_{40} concentrations ranged from 1.82 to 2.67 and 0.54 to 1.16 $\log_{10}$ CFU g^{-1} dw, respectively, compared to the initial value of 6.16 $\log_{10}$ CFU g^{-1} dw for all five reference studies. A comparison of *E. coli* survival patterns in biosolids, dairy slurry and cattle dung can be seen from scenario 5 (**Figure 1E**). In general, the C_{20} value of biosolids (0.57 $\log_{10}$ CFU g^{-1} dw) and dairy slurry (0.74 $\log_{10}$ CFU ml^{-1}) was 2.9–3.7 times smaller than that of cattle dung (2.12 $\log_{10}$ CFU g^{-1} dw). The C_{40} value was < 1.0 $\log_{10}$ CFU per unit mass or volume for any of this material when compared to the same in un-amended soil. However, the results of actual survival patterns in cattle dung studies under natural field conditions differ from studies that use first-order die-off approximations (Van Kessel et al., 2007; Soupir et al., 2008; Muirhead, 2009; Oliver et al., 2010). The reason for such discrepancies could be the potential of *E. coli* “re-growth” which were not considered when using first-order decay model. Instead a constant decay rate (*k*) value was used. In reality, *E. coli* growth and re-growth phases in deposited dung-pats can be highly interactive with environmental conditions such as: temperature, UV radiation, soil type, and rainfall events (Oliver et al., 2010). For example, the *E. coli* growth magnitude was observed to vary from 0.5 to 1.5 $\log_{10}$ CFU g^{-1} dw due to different environmental factors (Sinton et al., 2007; Van Kessel et al., 2007; Oliver et al., 2010). This means the estimation of *E. coli* risk from cattle dung on pasture by single *k* value based first-order decay model can potentially underestimate the growth potential and provides a conservative indication of fecal indicator organism accumulation over time. The modification of first-order decay equation by incorporating growth factor can improve the model predictability under field conditions. Therefore, the results of the present study represent scenarios without regrowth considerations.

In scenario 6, biosolids and dairy slurry were considered as the most commonly applied organic fertilizer for agricultural landspreading with two estimated application rates (ton ha^{-1}): maximum legal and excess as shown in **Table 3**. The *E. coli* survival pattern in this case is presented in **Figure 1F**. The C_{20} values of biosolids associated with legal and excess application rates are 1.14 and 1.21 $\log_{10}$ CFU ha^{-1}, respectively, in comparison to 1.63 and 1.71 $\log_{10}$ CFU ha^{-1}, respectively, for dairy slurry associated application. The C_{40} values in this case were less than ≤0.2 $\log_{10}$ CFU ha^{-1} when compared to C_0 ($\log_{10}$ CFU ha^{-1}) values of biosolids (12.99–13.69) and dairy slurry (14.13–14.83), respectively (**Figure 1F**).

Uncertainty and Probability Distributions of *E. coli* Concentration

The uncertainty analysis (**Figure 2**) indicated that soil *E. coli* concentrations would be at least 3.5 $\log_{10}$ CFU g^{-1} or ml^{-1} lower than the C_0 range of 6.2 to 6.5 $\log_{10}$ CFU g^{-1} or ml^{-1} in about 75.5% of the time (i.e. $C_t \leq 3$ $\log_{10}$ CFUg^{-1} or ml^{-1})

after application of either biosolids or dairy slurry or cattle dung to land (**Figure 2B**). Considering the variability of C_0 and k values due to the material type and study references (scenario 5, **Table 2**), the predicted *E. coli* concentration at any time can be estimated from $y = 6.1262e^{-0.079x}$ [similar to exponential Equation (5)] as developed from Monte Carlo simulation of 1,000 trials (**Figure 2A**). Accordingly, the C_{20} value can be expected as 1.262 $\log_{10}$ CFU g^{-1} or ml^{-1} which is comparatively lower than that of un-amended soil in this study, pointing toward the remaining *E. coli* after 20 days of application as being soil indigenous *E. coli*. The Monte Carlo analysis of biosolids (for scenario 2) provides the predictive exponential equation $y = 6.3097e^{-0.112x}$ with a probability distribution of $C_t \leq 3$ $\log_{10}$ CFU g^{-1} dw for 82% of the time (Figure S1). Similarly, dairy slurry (scenario 3) and cattle dung (scenario 4) based analysis provide regressions of $y = 6.459e^{-0.123x}$ and $y = 6.1179e^{-0.049x}$, respectively, with a probability distribution of $C_t \leq 3$ $\log_{10}$ CFU g^{-1} of 83 and 61.5% of the time, respectively (Figures S2, S3). The predicted C_{20} ($\log_{10}$ CFU g^{-1} or ml^{-1}) values of biosolids and dairy slurry associated *E.coli* was 0.672 and 0.552, respectively, while the equivalent for cattle dung was 2.296, indicating a higher risk associated with longer survival of *E. coli* in cattle dung on pasture. For the estimated legal and excess application rate of biosolids or dairy slurry (scenario 6, Figures S4, S5), the predictive exponential equations developed were $y = 13.497e^{-0.113x}$ and $y = 14.169e^{-0.113x}$, respectively, with a probability distribution of *E. coli* concentration remaining ≤ 3 $\log_{10}$ CFU ha^{-1}, 63% of the time. While the C_{20} ($\log_{10}$ CFU ha^{-1}) concentration for scenario 6 ranged from 1.408 to 1.478, the C_{40} value was almost negligible (0.147–0.154 $\log_{10}$ CFU ha^{-1}).

The outcomes of the uncertainty analyses depended on the distribution of the model variables and the associated parameters of these distributions. In other words, if different distribution parameters had been assumed, different outcomes may have been expected. For the scenarios in this study (**Table 2**) the distributions of the data are based on a range (maxima and minima) of empirical data collected from the literature (**Table 1**). In absence of detailed information on the probability density distributions of these variables, we employed the uniform distribution as the most parsimonious distribution.

CONCLUSIONS

An empirical database of dairy slurry, cattle dung and biosolids associated *E. coli* concentration and decay rate (k) was developed to assess the risk of *E. coli* survival up to a legislated grazing exclusion period. The use of a traditional exponential *E. coli* decay model and Monte Carlo uncertainty analysis showed that soil *E. coli* concentrations at 20 days would be at least 3.5 $\log_{10}$ CFU g^{-1} lower than the initial range of 6.2 to 6.5 $\log_{10}$ CFU g^{-1} or ml^{-1} in 75.5% of simulations after application of either biosolids, dairy slurry or cattle dung to land. The predicted C_{20} value was 1.262 $\log_{10}$ CFU g^{-1} or ml^{-1}, which is lower than that of un-amended soil in this study, indicating that the majority of *E. coli* 20 days after application would be mainly indigenous soil *E. coli*. For the estimated legal and excess application rates of biosolids or dairy slurry, the probability distribution of *E. coli* concentration remained at ≤ 3 $\log_{10}$ CFU ha^{-1} 63% of the time. The predicted C_{20} concentration for the estimated legal to excess application rates was 1.408–1.478 $\log_{10}$ CFU ha^{-1}, while the C_{40} equivalent ranged from 0.147 to 0.154 $\log_{10}$ CFU ha^{-1}. This indicates 40 days as safer than 20 days for a grazing exclusion period. However, considering the decay period of *E. coli* in un-amended soil, the 20 day exclusion period seems safe to reduce the risk of *E. coli* transmission into the soil environment and subsequently, negating the risk of contamination of crops and nearby water sources. The finding of this study supports the current practice of grazing exclusion times in the UK and Ireland.

AUTHOR CONTRIBUTIONS

SMA, OF, and KR contributed to the study conception and design of the study. SMA developed the empirical database, performed risk assessment modeling and uncertainly analysis with the assistance of OF. SMA wrote the manuscript, OF provided feedback and revised where necessary. SE, ST, and BG reviewed the manuscript and provided comments for improvement. Finally, all authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fsufs.2018.00034/full#supplementary-material

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