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ANDRESSA DE MATOS NASCIMENTO

APPLICATION OF DAIRY CATTLE WASTEWATER AS BIOFERTILIZER: QUANTITATIVE MICROBIAL RISK ASSESSMENT OF EXPOSURE TO BIOAEROSOLS

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Dissertação apresentada ao Programa de Pós-Graduação em Ecologia Aplicada ao Manejo e Conservação dos Recursos Naturais, da Universidade Federal de Juiz de Fora como parte dos requisitos necessários à obtenção do título de Mestre em Ecologia aplicada ao Manejo e Conservação dos Recursos Naturais.

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"APPLICATION OF DAIRY CATTLE WASTEWATER AS BIOFERTILIZER: QUANTITATIVE MICROBIAL RISK ASSESSMENT OF EXPOSURE TO BIOAEROSOLS"

Andressa de Matos Nascimento

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RESUMO

A reutilização ou reciclagem de águas residuárias fornece benefícios ambientais e econômicos, representando uma alternativa sustentável e circular para o gerenciamento de efluentes líquidos. No entanto, a aplicação de efluentes em culturas agrícolas por meio de pulverização cria uma situação potencialmente perigosa para indivíduos expostos a patógenos no ar. Este estudo usou ferramentas de Avaliação Quantitativa de Risco Microbiológico (AQRM) para avaliar quantitativamente os riscos ocupacionais e públicas de infecção associada a exposições a bioaerossóis em cenários de fertirrigação por pulverização de águas residuais não tratadas e tratadas. Análises de *Escherichia coli* (EC) e esporos de *Clostridium* spp. (CpSP) nos efluentes bruto e tratado, bem como relações patógeno / indicador da literatura foram usadas para estimar as concentrações de *Escherichia coli* O157:H7 (EC-O157:H7) e oocistos de *Cryptosporidium* spp (Crypto) no ar, e os resultados foram aplicados em um modelo de dispersão microbiológica atmosférica. A partir das concentrações de patógenos no ar, foram calculados os riscos infecciosos para os receptores a favor do vento. Os riscos de infecção por EC-O157:H7 para os trabalhadores a 10 m e 50 m de distância da fonte de emissão variaram entre $8{,}30 \times 10^{-1}$ e $3{,}35 \times 10^{-3}$ pppa, enquanto para os residentes a 100 m e 500 m variaram entre $6{,}37 \times 10^{-1}$ e $3{,}36 \times 10^{-4}$ pppa. Os valores de pico (percentil 95) dos riscos ocupacionais e públicos associados à exposição a Crypto foram de $3{,}29 \times 10^{-3}$ e $1{,}5 \times 10^{-3}$ pppa, respectivamente, e os riscos relacionados às exposições a CpSP foram inferiores a $1{,}41 \times 10^{-6}$ pppa. A digestão anaeróbia reduziu os riscos em aproximadamente uma ordem de magnitude. A distância da fonte foi inversamente proporcional ao risco de exposição. Recomenda-se que as águas residuais sejam tratadas antes de sua reutilização e adoção de métodos de aplicação com baixo potencial de aerossolização. Além disso, destaca-se a necessidade de os trabalhadores usarem equipamentos de proteção individual (EPI).

Palavras chave: Avaliação de risco. Dispersão microbiológica. Digestão anaeróbia. Fertirrigação.

ABSTRACT

The reuse or recycling of wastewater provides environmental and economic benefits, representing a sustainable and circular alternative for the management of liquid waste. However, the application of effluents to agricultural crops via spraying creates a potentially dangerous situation for individuals exposed to airborne pathogens. This study used Quantitative Microbial Risk Assessment (QMRA) tools to quantitatively assess the microbial risks of occupational and public exposures to bioaerosols in fertigation scenarios by spraying untreated and treated wastewater. Analyses of *Escherichia coli* (EC) and *Clostridium perfringens* (CpSP) in raw and treated effluents as well as pathogen / indicator ratios from the literature were used to estimate the concentrations of *Escherichia coli* O157:H7 (EC-O157:H7) and *Cryptospodirium* spp. (Crypto) in the air, and the results were applied to an atmospheric microbiological dispersion model. From the concentrations of pathogens in the air, infectious risks for downwind receptors were calculated. The risks of infection by EC-O157:H7 to workers at 10 m and 50 m away from the emission source ranged between 8.30×10^{-1} and $3,35 \times 10^{-3}$ pppy, whereas to residents at 100 m and 500 m ranged from 6.37×10^{-1} to 3.36×10^{-4} pppy. Peak values (95th percentile) of occupational and public risks associated with the exposure to Crypto were 3.29×10^{-3} and 1.5×10^{-3} pppy, respectively, and of risks regarding exposures to CpSP were lower than 1.41×10^{-6} pppy. Anaerobic digestion reduced risks by approximately one order of magnitude. The distance from the source was inversely proportional to the risk of exposure. It is recommended that wastewater is treated prior to its reuse and the adoption of application methods with low aerosolization potential. In addition, the need for workers to use personal protective equipment (PPE) is highlighted.

Keywords: Risk assessment. Microbiological dispersion. Anaerobic digestion. Fertigation.

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS AND ACRONYMS

CpSP	*Clostridium perfringens* spores
Crypto	*Cryptosporidium* spp. oocysts
EC	*Escherichia coli*
EC-O157:H7	*Escherichia coli* O157:H7
QMRA	Quantitative Microbiological Risk Assessment
pppy	per person per year
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

SUMMARY

1 INTRODUCTION

Livestock contributes to approximately 40% of the global value of agricultural output and supports the livelihoods and food security of almost a 1.3 billion people (FAO, 2017). Brazil has the second largest cattle herd in the world, with 213.5 million animals, and is considered the main meat exporter and the sixth largest producer of milk in the global ranking (IBGE, 2018). Intensive breeding of cattle has been continuously developed in a global scale (MAO et al., 2015). Dairy cattle wastewater, which is a combination of manure (faeces and urine), water used to wash milking parlours and feeding strips, can be considered as a valuable source of water and nutrients (DUNGAN, 2014).

The reuse or recycling of wastewater provides environmental and economic benefits, representing a sustainable, ecological and circular alternative for the management of liquid waste (ERTHAL et al., 2010; MACIEL et al., 2019; MAGRI et al.2019; TEIXEIRA et al., 2017). However, the lack of standards and/or regulations remains a limiting factor for wastewater reuse in many countries (DIAS et al., 2019). The presence of pathogens in wastewater, such as strains of *Escherichia coli* serovar O157:H7 (EC-O157:H7), *Clostridium perfringens* spores (CpSP) and *Cryptosporidium* spp. oocysts (Crypto), responsible for causing gastrointestinal infections in humans and other warm-blooded animals, may pose risks to public health (CHAIDEZ et al., 2005; BALDERRAMA-CARMONA et al., 2014; BERNAL, 2017).

The treatment of effluents through anaerobic digestion (AD) provided reductions of up to 2.5 $\log_{10}$ for *Escherichia coli* (QI et al., 2018; MARÉCHAL et al., 2019). However, characteristics such as gram positive, spore-forming and anaerobic make CpSP very resistant to this and other treatment processes (BAGGE et al., 2005; WATCHARASUKARN et al., 2009; VIAU et al., 2011; FROSCHLE et al., 2015; COSTA et al., 2017).

The detection of pathogens in environmental samples is complex, expensive and generates long delays due to the nature of the analytical test (GUZMAN et al., 2007). In addition, conventional bacterial indicators may not provide accurate information on the persistence of protozoa during treatment processes, due to the high resistance of these pathogens to environmental stress (PAYMENT; FRANCO, 1993). Spores of *Clostridium perfringens* have been proposed as alternative indicators of protozoan oocysts in water, wastewater, sludge and biosolids treatments (PAYMENT; FRANCO, 1993; ROSE et al., 2004; GUZMAN et al., 2007).

The irrigation of agricultural crops with untreated and treated wastewater is one of the possibilities of reuse adopted in more than 20 million hectares of land in the world (FAO, 2013). However, application methods that release liquid fertilizers into the air create a potentially dangerous situation as a consequence of the process of aerosolization and transport of enteric and pathogenic microorganisms through the atmosphere (USEPA, 1982; BROOKS; TANNER; JOSEPHSON; et al., 2005). Pathogenic bioaerosols carried to downwind receptors have the potential to cause infections if they are directly inhaled or, in the case of enteric pathogens, swallowed after being lodged in the upper respiratory tract (DUNGAN, 2014).

Preliminary studies at wastewater application sites have observed a decrease in the density of microorganisms in the air with increased time and distance from the source (SORBER et al., 1976; PARKER et al., 1977; TELTSCH et al., 1980; USEPA, 1980; CAMANN et al., 1988). The monitoring of microorganisms is a difficult and costly process, which leads to the adaptation of mathematical models of atmospheric dispersion to estimate the emission, transport and dispersion of bioaerosols. Modelled microbial concentrations in the atmosphere are depend on the rate of microorganisms transformed into aerosol, the fraction of bioaerosols that remain viable and their survival during the period of suspension in the atmosphere and the atmospheric dispersion factor (USEPA, 1982).

Dispersion data of bioaerosols during waste application events are generally subjected to a quantitative risk (BROOKS; TANNER; GERBA; et al., 2005; HARDY et al., 2006; BROOKS et al., 2012; DUNGAN, 2014; JAHNE et al., 2015; BURCH et al., 2017). However, the study of the probability of infection by exposure to inhalation of CpSP and Crypto in rural areas is somewhat innovative. In general terms, the risk of infections caused by enteric pathogens in the air depends on five factors: (i) the amount of pathogens emitted by the source per unit of time; (ii) meteorological effects, such as wind speed, influencing the dispersion process; (iii) inactivation of bioaerosols during the transport period in the atmosphere; (iv) the number of pathogens inhaled, depending on the respiratory rate and the fraction ingested / inhaled; and (v) the hosts' response as a function of the ingested dose, where one of the points considered is the harmfulness inherent to the pathogen (LEUKEN et al., 2016; USEPA, 2019).

The methodology used in the Quantitative Microbiological Risk Assessment (QMRA) approach depends on reliable information on the input variables. The adoption of stochastic models in QMRA allows the incorporation and propagation of uncertainties to the model through the use of input values that follow a certain frequency or probability distribution function (PDF) and Monte Carlo simulations, which perform successive and random sampling

based on the cumulative distribution function (CDF) of each input variable (VOSE, 2008; DIAS et al., 2019). The results are expressed by a curve of probability of infection, illness or death and the unit used is risk per person per year (pppy) (HAAS et al., 2014; WHO, 2016).

Withing this context, the aim of this study were to develop stochastic simulations, using empirical data, to estimate risks to occupational (direct) and public (indirect and incidental) exposure to such bioaerosols (EC-O157:H7, CpSP and Crypto) considering different scenarios during spray irrigation using untreated and treated dairy cattle wastewater. The probabilistic estimate of the concentration of pathogen in the air was obtained using the Gaussian plume model.

2 MATERIAL AND METHODS

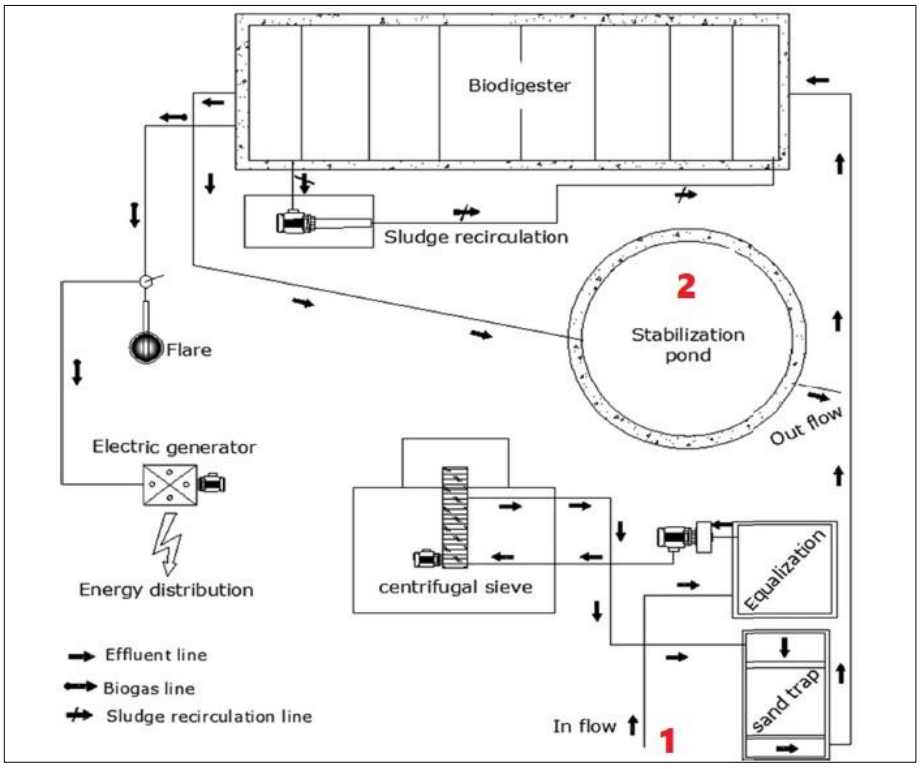
In this section the material and methods used for the development of the study were presented.

2.1 OBTAINING THE EMPIRICAL DATA

The case study was carried out at the experimental farm of the Embrapa Dairy Cattle (Brazilian Agricultural Research Corporation – Embrapa), located in the municipality of Coronel Pacheco, Minas Gerais state, Brazil (21° 33' 58" S; 43° 15' 21" W; 445 m altitude). The climate in the region is classified as humid subtropical (Cwa) in the Köppen & Geiger scale (CLIMATE, 2019), with an annual average temperature of 21,7°C, annual mean minimum and maximum temperatures of 15.6°C and 27.8°C, respectively, and an annual average rainfall of 1,516 mm (INMET, 2019).

The treatment system of the dairy cattle wastewater consists of the following steps: (i) equalization tank; (ii) preliminary treatment (centrifugal sieve to remove coarse solids and sedimentation tank to remove fine residues); (iii) secondary biological treatment in an anaerobic digester with sludge recirculation; and (iv) sedimentation tank (MENDONÇA et al., 2017). Samples of raw (inflow) (Sampling Point 1) and treated effluents (outflow) (Sampling Point 2) (Flowchart 1) were collected every two weeks between January and May 2019 for microbiological analysis (see section 2.2.1). In total, 10 samples were collected from each of the points assessed.

Flowchart 1- Flowchart of the dairy cattle wastewater treatment process and inflow (1) and outflow (2) sampling points.



Source: Mendonça *et al*. (2017).

A splash-plate applicator (Picture 1) with a capacity of 6,000 L used a pumping system to apply the biofertilizer to the BRS Capiaçu crop. Liquids were splashed against a metal plate, causing it to spread out in a "hand fan" shape, with a spray width of approximately 11 m. Based on (i) a 120-day cycle of the BRS Capiaçu crop and a nitrogen demand of the crop equal to 80 $kg.ha^{-1}.year^{-1}$ (PEREIRA et al., 2016), (ii) the characterization of the biofertilizer carried out by Gonçalves (2019) and (iii) the biofertilizer application system used, the fertigation procedure was repeated three times within the crop cycle, each procedure with a water application equal to 8.5 mm.

Picture 1 - Effluent application method.

Source: prepared by the author (2019)

2.1.1 Microbiological analysis

The enumerate spores of *Clostridium perfringens* (CpSP) dilution series (10^{-1} to 10^{-2}) were made in 0.1% (w/w) peptone saline solution. Then, samples were subjected to a water bath at a temperature of 75°C for 20 min to eliminate vegetative cells and activate the spores. One-mL aliquots were sown on to sterile plates and then 15 mL of Clostridial Agar (AC) medium (HIMEDIA, Mumbai, India) were added at 46-48°C. After the medium solidified, the cultures were incubated in an anaerobic chamber at 35°C for 72 h. The colonies were isolated for confirmatory tests: Gram stain, motility, lecithinase, lactose fermentation and gelatin liquefaction. For the enumeration of *Escherichia coli* (EC), dilution series (10^{-1} to 10^{-4}) were performed in 0.1% (w/w) peptone saline solution and then 0.1-mL aliquots were seeded onto Violet Red Bile Agar with Glucose and Lactose (HVB) (HIMEDIA, Mumbai, India). The plates were incubated at 35-37°C for 18-24 h. After this, red-pink colonies with brilliant precipitate were isolated in Eosyn Methylene Blue (EMB) (HIMEDIA, Mumbai, India) and subjected to the following confirmatory tests: Gram stain, indole, citrate and motility. The counts of CpSP and EC were expressed in colony-forming units per mL of sample (CFU mL^{-1}).

2.1.2 Meteorological data

The maximum and minimum daily values of wind speed for the year of 2019 (Graphic 1 – Appendix A) were obtained from an automatic meteorological station, located 200 m from the place where the experiment was carried out (INMET, 2019) and the average of the recorded values was used to calculate the atmospheric dispersion (D_d; see section 2.2.2) and aerosol speed (ad = wind speed ($m.s^{-1}$) / distance from source (m); see section 2.2.3). The maximum average speed was adopted to configure the worst scenario of public (residential) risks, as it implies low decay rates of bioaerosols in the air (USEPA, 1982); to estimate occupational risks, the minimum wind speed was considered, as they limit vertical mixing in the atmosphere, leading to higher surface concentrations (LEUKEN et al., 2016).

2.2 CONCENTRATION OF ORGANISMS IN THE ATMOSPHERE

The microbial concentrations in the air were estimated using a Gaussian microbiological dispersion model (CAMANN, 1980). The model takes into account three factors, described as follows and presented in Eq. 1 (adapted from USEPA (1982)): (i) number of microorganisms released per unit of time, determined by specific characteristics of the emission source; (ii) local environmental factors that affect aerosol dispersion; and (iii) decay of the organism during the period of transport and atmospheric dispersion (HARDY et al., 2006). In addition, in the case of *Escherichia coli*, the prevalence of the pathogenic strain *Escherichia coli* O157:H7 (EC-O157:H7) was considered in relation to the group of indicators *Escherichia coli*. For *Cryptosporidium* spp. (Crypto), spores of *Clostridium perfringens* (CpSP) were used as a model microorganism. The following sections describe how the input variables of Eq. 1 were obtained to determine the concentration of microorganisms in the atmosphere $C_{(x)}$.

$$\boldsymbol{C_{(x)} = Q_m \times D_{(x)} \times M_{(x)}} \qquad \text{Eq. 1}$$

Where: $C_{(x)}$ = density of the microorganism in the atmosphere ($cfu.m^{-3}$) at any distance downwind x; $D_{(x)}$ = atmospheric dispersion factor described by the Gaussian model ($s.m^{-3}$); Q_m = microorganism emission rate adjusted for loss of microbial viability during the spraying process ($cfu.s^{-1}$); $M_{(x)}$ = fraction of microorganisms that remains viable at a distance (x) from the source (dimensionless).

2.2.1 Pathogen emission rate (Q_m)

The potential of aerosolization of the microorganisms in the emission source is expressed in Eq. 2 (USEPA, 1982). The substitution of Q_m in Eq. 2 produces a microbiological dispersion model for aerosols from wastewater generated by spray irrigation (CAMMAN, 1980).

$$\boldsymbol{Q_m = W \times F \times E \times I \times R_{Pat/Ind}} \quad \text{Eq. 2}$$

Where: Qm = microorganism emission rate (cfu.s^{-1}); W = density of microorganisms in the wastewater (cfu.L^{-1}); F = application flow (L.s^{-1}); E = aerosolization efficiency (dimensionless); I = impact factor (dimensionless); $R_{Pat\ /\ Ind}$ = Pathogen / indicator relationship (dimensionless).

The values of concentrations (W) of EC and CrSP in untreated and treated effluents were obtained from the monitoring programme (Jan to May, 2019) and followed normal distribution. The application flow (F) adopted in this study was equal to 28 L.s^{-1}, resulting in an biofertilizer application of 8.5 mm in order to meet the nutritional requirements of the crop planted in the experimental area, taking into account the physic-chemical characterization of the biofertilizers (see section 2.1). The aerosolization efficiency (E), which expresses the fraction of microorganisms that were transformed into aerosols (USEPA, 1982), was defined through a range of values obtained from the literature in spray irrigation studies: 0.08% to 2.7% (SORBER et al., 1976; CAMANN, 1980; USEPA, 1980). From this, for EC, an aerosolization efficiency (E) between 0.01% and 3% was considered. As a consequence of high aerosolization potential of spores, as found for *Bacillus anthracis* (ANDERSON; BOKOR, 2012), it was assumed a aerosolization efficiency of 100% for CpSP and Crypto. The sprinkler impact factor (I) was disregarded, as previous research has shown little or no effect of pressure and type of spray plate on the concentrations of cultivable microorganisms after the sprinkler (DUNGAN, 2014).

Lack of data on pathogens may limit quantitative microbial risks assessment studies, which are, therefore, commonly performed based on indicator organisms (HOWARD et al., 2006; MACHDAR et al., 2013; YAPO et al., 2014; JAHNE et al., 2015; BURCH et al., 2017). For this, it is often applied a pathogen / indicator ratio in QMRA models.

Escherichia coli (EC) bacteria are used as a reliable indicator of enterohemorrhagic (EHEC) strains, being *Escherichia coli* O157:H7 (EC-O157:H7) one of the most important serotypes to public health (AITKEN et al., 2007). As only 8% of the total *Escherichia coli* population is estimated to be pathogenic (HAAS et al., 1999; HOWARD et al., 2006; MACHDAR et al., 2013; YAPO et al., 2014), the EC-O157:H7 (pathogen) / EC (indicator) ratio used to calculate the concentration of pathogenic EC-O157:H7 was considered to be equal to 0.08 in this study ($R_{Pat}/_{Ind} = 0.08$).

CpSP has proven to be a useful model organism for Crypto because it is a spore-forming bacteria highly resistant to environmental conditions (WHO, 2006). Based on a study performed by Rose et al. (2004), the pathogen / indicator ratio between Crypto and CpSP in wastewater was considered to be equal to 0.0001 in this study ($R_{Pat}/_{Ind} = 0.0001 = 10^{-4}$). In addition, the bacteria *Clostridium perfringens* type A is known to cause gastrointestinal infections due to their ability to produce an enterotoxin (LEE et al., 2016). Therefore, the term of the equation corresponding to the pathogen / indicator ratio for CpSP was considered to be equal to 1.0 ($R_{Pat/Ind} = 1.0$).

2.2.2 Atmospheric dispersion factor (D_d)

The atmospheric dispersion factor ($D_{d(x)}$) considered in this study is based on a Gaussian three-dimensional dispersion model (Eq. 3) based on atmospheric stability, downwind distance, wind speed and height of the aerosol plume (CAMMAN, 1980; HARDY et al., 2006). Aerosols released from a point source will reach an average plume height (H) and will be diffused in the horizontal (y-axis) and vertical (z-axis) directions during the course along the distance from the central line in the wind direction (axis x) (PETTERSON; ASHBOLT, 2005).

$$\boldsymbol{D_{d\,(X)}} = \frac{1}{2\pi u \sigma_y(x)\sigma_z(x)} exp\left(-\frac{y^2}{2\sigma_y}\right)\left[\exp -\frac{(z-H)^2}{2\sigma_z(x)} + \exp -\frac{(z+H)^2}{2\sigma_z(x)}\right] \quad \text{Eq. 3}$$

Where: $D_{d\,(x,\,y,\,z)}$ = atmospheric dispersion factor at a distance (x) from the emission source ($s.m^{-3}$); u = wind speed ($m.s^{-1}$); $\sigma_{y\,(x)}$ = horizontal diffusivity in the x coordinate; $\sigma_{z\,(x)}$ = vertical diffusivity in the x coordinate; H = height of aerosol emission.

Steady state conditions and spray application as a point source were assumed in this study as the area in the study was relatively small. The error introduced by these assumptions decreases with distance in the wind, because the geometry of all sources is increasingly similar to that of a point source (USEPA, 1982). To represent the people's breathing zone in the wind, 1.5 m for the z input was adopted (USEPA, 1982). Due to the type of application used in the present study, the emission height of the source (H) was assumed to be 1.5 m (GURIAN et al., 2012). In order to analyse the risks for the worst scenarios, the minimum average of the wind speeds (U_{min} = 0.3 m.s^{-1}) was considered for the occupational scenario, which implies in less dispersion and greater concentrations of the pathogens in the atmosphere for workers located near the source (x-axis of 10 m and 50 m). For public risk, it was considered a maximum average speed (U_{max} = 3.0 m.s^{-1}), in order to analyse the risks for residents located at greater distances from the source that bioaerosols would be able to disperse (x-axis of 100 m and 500 m). Further information on the models applied as well as calculations are available in the Supplementary Material.

2.2.3 Loss of viability of microorganisms in the atmosphere $M_{(x)}$

A simple first-order kinetic model, driven by a mortality rate to explain the inactivation of microorganisms with increasing aerosol age (an indirect measure of how long the microorganism remains in the atmosphere) is presented in Eq. 4 (CAMANN, 1980). The rate of deterioration of viability during the period of transport in the atmosphere varies according to the microorganism and two main factors: temperature and relative humidity, probably due to the drying process (HARDY et al., 2006).

$$\boldsymbol{M_{(x)} = e^{\lambda.ad}} \qquad \text{Eq. 4}$$

Where: M (x) = fraction of microorganisms that remains viable at a distance (x) from the source (dimensionless); λ = decay rate of viability (s ^{1}); ad = wind speed (m.s ^{1}) divided by the distance x (m).

To explain the inactivation of EC-O157:H7, the λ factor was adopted to vary uniformly between 0.6 and 0.185 considering temperature ranging from 20 to 40°C (POON, 1966). The uncertainty and limitations of the studies and the scarcity of data on the loss of viability of CpSP

in the air can overestimate (λ = 0.004) or underestimate (λ = 0.039) the risk (USEPA, 1982), which led to the adoption of a uniform distribution with minimum and maximum values equal to 0.01 and 0.10, respectively. For Crypto, the same decay behaviour in the air was assumed as its indicator microorganism due to similarities between them. As workers are exposed to bioaerosols soon after or even during emission and are very close to the source (between 10 and 50 m in this study) of aerosols, the decay of pathogens was not considered in the occupational exposure scenario (GURIAN et al., 2012). To determine the age of the aerosol (ad), the values of wind speed ($m.s^{-1}$) and distance from the source (m) were the same used in the calculation of the dispersion factor (D_d; see section 2.2.2).

2.3 DOSE

Having determined the concentration of pathogenic microorganisms in the atmosphere ($C_{(x)}$), it is possible to estimate the dose ingested by individuals exposed to irrigation events with wastewater by spraying. The simplest way to establish the dose is to assume that it is equivalent to the modelled concentration of pathogens. However, as not all pathogens are inhaled, variables such as breathing rate (br), fraction of inhaled pathogens to be ingested (ag) and duration of exposure (t) should be included for a more detailed and accurate estimate of the effective dose ingested, as expressed in Eq. 5 (BROOKS et al., 2012).

$$\boldsymbol{d = C_{(x)} \times br \times ag \times t} \qquad \text{Eq. 5}$$

Where: d = dose of pathogens per exposure event (org); Cd = concentration of pathogens in the air ($cfu.m^{-3}$); br = breathing rate ($m^3.h^{-1}$); ag = fraction of inhaled aerosol particles ingested (%); t = time of exposure (h).

2.3.1 Breathing rate (br)

Inhalation volumes per hour were obtained from the technical support document for exposure assessment and stochastic analysis (OEHHA, 2012), where specific respiration rates are shown for adults of different ages. The age group between 16 and 70 years was selected, as it covers a representative portion of the population exposed to the risk of the present study. In order to work with stochastic modelling, a lognormal probability density function (μ = 0.579; σ = 0.225) was assumed to the respiration rate (br) input variable.

2.3.2 Fraction of inhaled aerosol particles ingested (ag)

A portion of the inhaled aerosols can actually contribute to the intake dose, based on the size of the inhaled particles (HARDY et al., 2006). This is because a fraction of sufficiently large aerosolized particles (> 5 μm) that are deposited in the upper respiratory tract and ingested by the swallowing process (BROOKS et al., 2012). Based on data on the literature, and taking into account uncertainty and variability associated with this type of exposure and when evaluating microorganisms of different sizes (bacteria and bacterial spores), a uniform distribution in the reported range of 10 to 80% was used for this study for all organisms assessed (MEDEMA et al. 2004; HARDY et al., 2006; BURCH et al., 2017).

2.3.3 Exposure time (t)

Exposure time per event (application of waste water via spraying) was considered to be one hour (1 h) for workers – occupational risks (BROOKS et al., 2012) and eight hours (8 h) for residents in nearby locations – public risks (JAHNE et al., 2015).

2.4 RISK ASSESSMENT

The risks to human health due to exposure to airborne microbial pathogens (bioaerosols) generated from spraying (raw or treated) effluent application events were modelled using the Quantitative Microbiological Risk Assessment (QMRA) approach. The reference pathogens for this study, *Escherichia coli* O157:H7, *Clostridium perfringens* and *Cryptosporidium* spp were selected because they are responsible for outbreaks of gastrointestinal diseases in humans, are present in matrices contaminated by warm-blooded animal faeces and have persistence in the environment (MARA; HORAN, 2003; NAG et al., 2019). The exposure route of bioaerosols containing gastrointestinal pathogens is considered to be a combination of ingestion and inhalation, as inhaled pathogens deposited in the upper respiratory tract may be ingested (HARDY et al., 2006). For *Clostridium perfringens* and *Cryptosporidium* spp, the probability of infection is described by an exponential model (Eq. 6) when the host-microorganism interactions are constant and expressed by an r parameter (PETTERSON; ASHBOLT, 2005). For *Escherichia coli* O157:H7, the beta-Poisson model (Eq. 7) was chosen, characterized by the parameter r not as a discrete value, but as a distribution of values, specifically a beta-Poisson distribution (HAAS et al., 2014). The parameters $\alpha = 0.248$

and β = 48.80 were adopted for *Escherichia coli* O157:H7 considering the beta-Poisson dose-response model, and the parameters r_{cl} = 1.82 x 10^{-11} for *Clostridium perfringens* and r_{cr} = 0.00419 for *Cryptosporidium* spp were assumed considering the exponential dose-response model (HAAS et al., 2014; LEE et al., 2016).

$$\boldsymbol{P_i = 1 - exp(-d/r)} \quad \text{Eq. 6}$$

$$\boldsymbol{P_i = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}} \quad \text{Eq. 7}$$

Where: Pi = probability of infection due to exposure event (pppy); d = dose of pathogens ingested (org); α and r = pathogen "infective constants" (dimensionless); β = dependent parameter of mean infective dose (dimensionless).

The cumulative probability of infection based on the number of days or events, n, per year, assuming that no more than one land application event occurs daily was determined according to Eq. 8. Considering that the cycle between planting and harvesting of the BRS Capiaçu cultivar is repeated three times a year and that each cycle consists of three biofertilizer application events (three exposure events), for the present study nine annual exposure events were considered (n = 9).

$$\boldsymbol{P_a = 1 - (1 - P_i)^n} \quad \text{Eq. 8}$$

Where: P_a = Probability of annual infection; P_i = Probability of infection due to exposure event; n = number of events or days of exposure per year.

A summary of the exposure scenarios and the PDF assumed for each input variable of the models considered are presented in the Supplementary Material (Tables S5, S6 and S7).

2.4.1 Stochastic modelling

The factors involved in the estimation of microbiological risk involve great spatio-temporal variation (variability), such as meteorological data, in addition to possible measurement and sampling errors (uncertainty) (BURCH et al., 2017). To include the uncertainty and variability inherent in a potential risk assessment, some input parameters of the models used were expressed by ranges of values described by the probability distribution functions (PDF) (Tables S5 and S6), instead of discrete point estimates. The exposure analysis was performed from simulations with 10,000 iterations and Hypercube-Latin random sampling method, producing a complete distribution of the results and propagating the uncertainty and variability for the model output (PETTERSON; ASHBOLT, 2005; JAHNE et al., 2015). Simulations were performed using @Risk software, version 4.5 (Palisade Corporation, Newfield, United States of America). The annual risk results were expressed as per person per year (pppy).

2.5 STATISTICAL ANALYSIS

The values obtained from the microbiological analysis of the raw and treated effluents were subjected to the Mann-Whitney test with significant differences between the variances considered at the level of significance of 5% (p-value $\leq$ 0.05). Statistical analyses were performed using RStudio software, version 3.5.1 (RStudio, Boston, United States of America).

3 RESULTS AND DISCUSSION

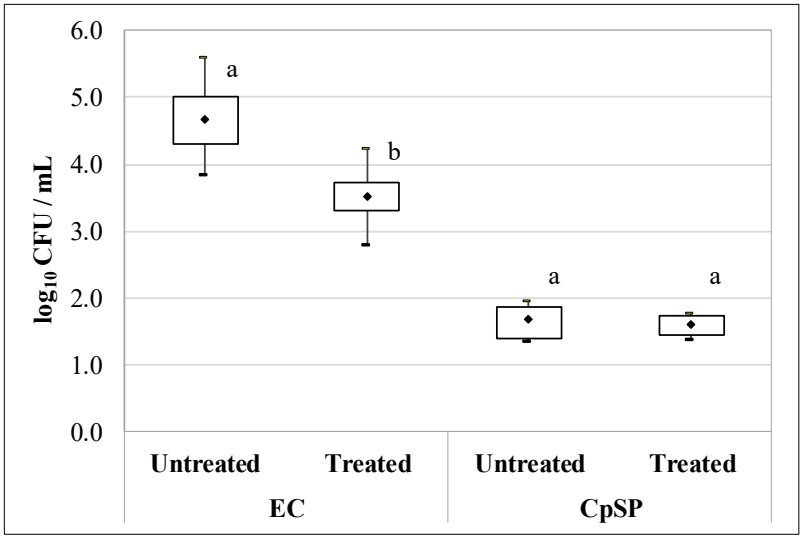
This section presented the results obtained in the study and the discussion.

3.1 QUANTIFICATION OF MICROORGANISMS IN EFFLUENTS

Faecal contamination indicators microorganisms *Escherichia coli* (EC) and *Clostridium perfringens* spores (CpSP) were quantified in untreated and treated wastewater samples, as shown in Graphic 1. No significant differences (Mann-Whitney test; p-value > 0.05) were identified between the concentrations of CpSP in the raw and treated effluents. For EC, a reduction of approximately 1.0 $\log_{10}$ was observed in the treatment system, with concentrations

in the raw wastewater significantly higher than in the treated effluent (Mann-Whitney test; p-value < 0.05).

Graphic 1 - Concentration of *Escherichia coli* (EC) and *Clostridium perfringens* spores (CpSP) in untreated and treated effluents.



Source: prepared by the author (2019)

For each organism, medians followed by the same letter showed no significant differences between them.

The average concentration of *Escherichia coli* recorded in the untreated effluent of this study (approximately 4.5 $\log_{10}$ CFU.mL^{-1}) was 1 to 2 orders of magnitude higher than the values found in other dairy cattle wastewater (DUNGAN, 2012) and similar to values detected in manure (JAHNE et al., 2015; QI et al., 2018; CHIAPETTA et al., 2019). However, Dungan (2014) justified the values obtained being lower than the expected concentrations for dairy cattle effluents to be a consequence of the dilution effect when the treated effluent is sent to storage ponds. The differences in the microbiological composition of the residues can be related to several factors, such as chemical characteristics of manure (for example, ammonium content), pH, dry matter, temperature, oxygen, microbial competition and moisture of these materials, as well as the diet and the health of the animal (MANYI-LOH et al., 2016).

The anaerobic digestion treatment had a significant effect at decreasing the levels of EC, providing an average reduction close to 1.0 $\log_{10}$ (Graphic 1). A similar result was found by Maréchal et al. (2019), who observed removal rates varying from 0.7 to 2.5 $\log_{10}$. Higher

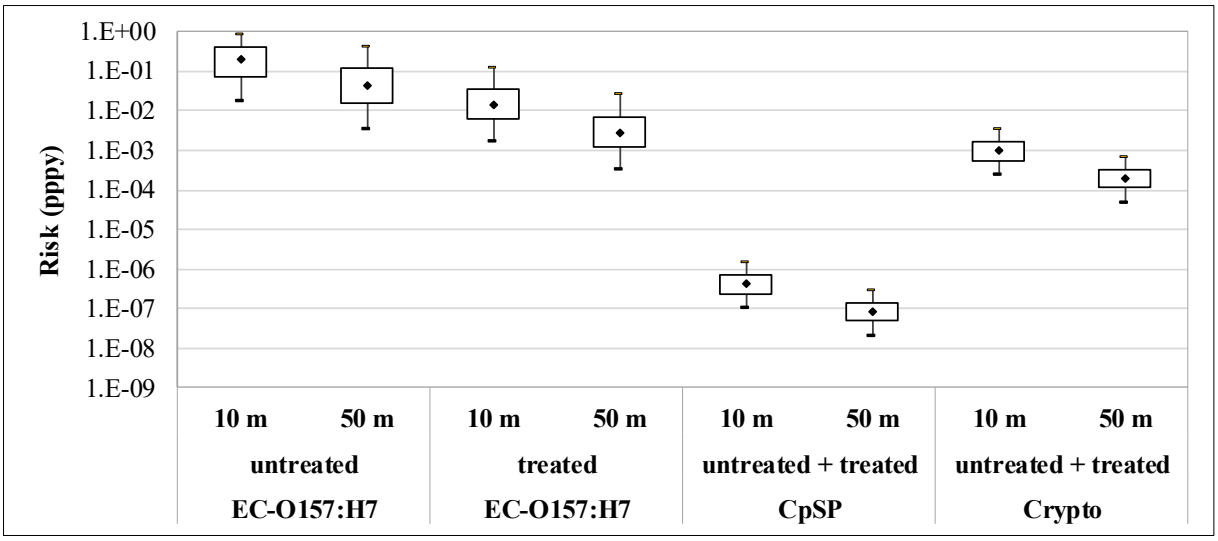
removal rates (4.9 $\log_{10}$) were obtained in the anaerobic digestion of manure (QI et al., 2018). The variation between the reported results can be attributed to differences in the chemical composition of the waste, environmental factors (temperature), type of digester used and operational conditions such as hydraulic retention time (MANYI-LOH et al., 2016).

The counts of CpSP remained practically stable after the anaerobic digestion. Low removal efficiencies were also observed by Maréchal et al. (2019), who found concentrations of spores of *Clostridium* spp. in manure similar to those quantified in treated compost. Concentrations of Clostridia ranging from 4.95 to 4.70 $\log_{10}$ CFU g^{-1} have been reported in untreated and treated cattle manure (COSTA et al., 2017). Huong et al., (2014) also did not detect any significant difference in the concentration between leachate and biodigester effluent. The high resistance of *Clostridium* bacteria to treatment processes can be explained by its ability to form spores (FROSCHLE et al., 2015).

3.2 OCCUPATIONAL RISK

Graphic 2 shows the annual occupational risks of infection by aerosolized *Escherichia coli* O157:H7 (EC-O157:H7), *Clostridium perfringens* spores (CpSP) and *Cryptosporidium* spp. (Crypto) during fertigation with untreated and treated dairy cattle wastewater, assuming the worst conditions (high concentrations of bioaerosols), characterized by minimum average wind speed, absence of pathogens decay in the air and the proximity to the source (10 m and 50 m). It was considered nine exposure events per year, of 1 h each. More detailed results are presented in Table S8 (Supplementary Material).

Graphic 2 - Probability of annual occupational risk of infection by aerosolized *Escherichia coli* O157:H7 (EC O157:H7), *Clostridium perfringens* spores (CpSP) and *Cryptosporidium spp*. (Crypto) during fertigation with dairy cattle wastewater.



Source: prepared by the author (2019)

The horizontal lines in the box plots, from bottom to top including the whisker caps, represent the 5th, 25th, 50th, 75th, and 95th percentiles.

The highest values obtained for annual risks are associated with EC-O157:H7 and untreated effluents, with median values equal to 1.9×10^{-1} pppy at 10 m and 4.4×10^{-2} pppy at 50 m, higher than acceptable level of risk for recreational water (32 illnesses/1000 swimmers/exposure event) (USDA, 2016). These results are similar to risks provided by the application of bovine manure in the soil (BROOKS et al., 2012). High concentrations combined with a low infectious dose of this pathogen may justify such findings (WESTRELL et al., 2004).

The risks of infection by aerosolised EC-O157:H7 from treated wastewater were lower than 1.16×10^{-1} pppy (95^{th} percentile), similar to those estimated for workers responsible for spreading sludge from municipal wastewater treatment plants on agricultural land (2.6×10^{-1} pppy) (WESTRELL et al., 2004). The anaerobic digestion of the raw effluent led to a risk reduction of approximately one order of magnitude. Substantial differences of risk of infection

were detected between aerosolised *Salmonella* spp. and *Listeria monocytogenes* during land application of manure and biosolids (BROOKS et al., 2012). Waste treatment can change the risk considerably, but the magnitude of the reduction depends on the effectiveness of the treatment and the resistance of the pathogen (GALE, 2005).

The risk outcomes for CpSP and Crypto varied widely (10^{-8} to 10^{-3} pppy; Graphic 2) and most of the values are within tolerable risk levels commonly adopted for drinking water (10^{-4} pppy or 10^{-6} DALY) (WHO, 2011; USEPA 2012). The difference in infectivity between these microorganisms proved to be a determining factor, as the concentrations of Crypto were calculated considering the number of CpSP present in the effluents and, therefore, was lower than the levels of spores of *Clostridium perfringens*. These results demonstrate the important role of CpSP as an indicator organism.

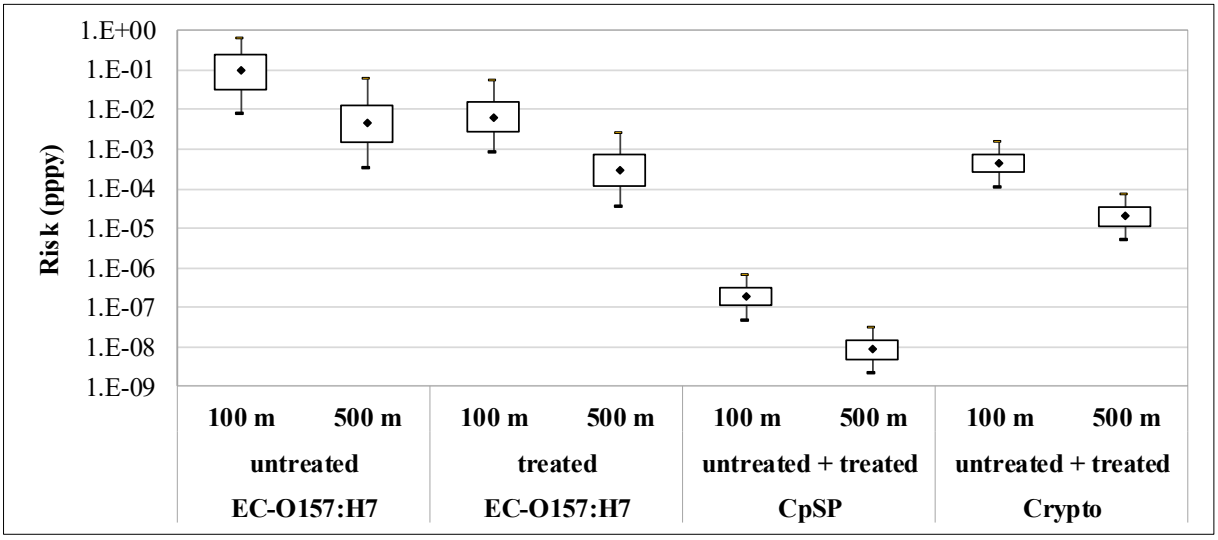
Medema et al. (2004) obtained an average annual probability of cryptosporidiosis infection for workers at a municipal wastewater treatment plant equal to $1{,}8\times10^{-1}$ pppy, higher than the maximum occupational risk found in this study (3.3×10^{-3} pppy – 95th percentile at 10 m from source; Graphic 2). Higher concentrations of microorganisms in the air in wastewater treatment plants may have led to higher risks. To my best knowledge, the QMRA study involving exposure to aerosolised CpSP reported in the present research is the first of its kind and does not present contemporary microbial comparisons in the literature.

In all scenarios analysed, the increase in the distances from the source from 10 m to 50 m resulted in a decrease of less than one order of magnitude of the risks due to the inhalation of bioaerosols. Similar reductions were observed during the application of biosolids to individuals close to the source and exposed to low wind speeds in relation to the risks associated with *Salmonella* spp., enterovirus, adenovirus and norovirus (VIAU et al., 2011). The pattern observed in both studies may be explained by the low dispersion of microorganisms in the atmosphere in such small distances, exposing individuals to similar doses of pathogens.

3.3 PUBLIC RISK

Graphic 3 shows the annual public risks of infection by aerosolised pathogens generated due to fertigation with untreated and treated dairy cattle wastewater, assuming favourable conditions for the dispersion of bioaerosols (maximum average wind speed). It was considered nine exposure events per year, of 8 h each. More detailed results are presented in Table S9 (Supplementary Material).

Graphic 3 - Probability of annual public risk of infection by aerosolized *Escherichia coli* O157:H7 (EC O157:H7), *Clostridium perfringens* spores (CpSP) and *Cryptosporidium spp*. (Crypto) during fertigation with dairy cattle wastewater.



Source: prepared by the author (2019)

The horizontal lines in the box plots, from bottom to top including the whisker caps, represent the 5th, 25th, 50th, 75th, and 95th percentiles.

The median risk of infection (9.37×10^{-2} pppy; Graphic 3) by aerosolised EC-O157:H7 from untreated dairy cattle effluent to residents located at 100 m from the emission source was approximately two orders of magnitude greater than the risks found from bovine manure application to land (JAHNE et al., 2015). A possible explanation for such disparities between the results of both studies is associated to different methods were used to estimate exposure: the dispersion of pathogens in the atmosphere was determined using Gaussian dispersion modelling in this study, whereas Jahne et al., (2015) were based on empirical observations. The median risk obtained in this study was also higher than tolerable risk levels commonly adopted for drinking water (10^{-4} pppy or 10^{-6} DALY) (WHO, 2011; USEPA, 2012).

A single event of dairy cattle wastewater application as fertilizer caused risks to residents located at 1 km from the irrigation site (DUNGAN, 2014) similar to annual public risks to residents at 500 m from the source in this study. Therefore, in the study performed by

Dungan (2014), multiple exposure scenarios probably would implicate in higher risks compared to the findings in the present study, even involving higher distances from the emitting source.

The estimated median risk of infection by EC-O157: H7 in irrigation events with treated effluents was 6.5×10^{-3} pppy for residents at 100 m from the application site, value close to tolerable risk levels commonly adopted for drinking water (WHO, 2011; USEPA, 2012). Working with similar exposure scenarios (i.e., atmospheric stability, wind speed and distance from the emission source), Viau et al. (2011) obtained risks of infection by aerosolised *Salmonella* spp., enterovirus and adenovirus, approximately 5, 3 and 1 orders of magnitude lower than the results of this studys. Two factors may be related to the disparities between the values found: different rates of treatment removal, leading to higher exposure doses; and discrepancies in the infectivity of the pathogens involved.

Crypto inhalation exposure caused public risks between 1.5×10^{-3} and 1.1×10^{-4} pppy to residents located at 100 m from a municipal wastewater treatment plant (STELLACCI et al., 2010). The risks from manure irrigation is generally between the acceptable risk levels for drinking water and recreational water (USDA, 2016). Microbial risk assessment studies involving the dispersion of CpSP bioaerosols were not found in the literature, but thermotolerant clostridia were considered a good indicator of the presence of pathogens in the air in events of application of biosolids (DOWD et al., 1997).

Dispersion of pathogens in the atmosphere as a function of distance clearly plays a key role in reducing the concentrations of microorganisms in the air (DUNGAN, 2014). The average risks at 100 m from the source aerosolized EC-O157:H7 (raw and treated effluent), CpSP and Crypto were approximately 10^{-2}, 10^{-3}, 10^{-7}e 10^{-4} pppy, respectively. The increase in distance (500 m) led to a decrease in risks by one order of magnitude. The effect of the dispersion of microorganisms is even more relevant when analysing greater distances. Dungan (2014) detected a reduction three times greater when comparing the risks at distances of 1 km and 10 km from the source.

3.4 OCCUPATIONAL RISK *VERSUS* PUBLIC RISK

Occupational exposures resulted in greater risks when compared to public ones. This result is in line with the observations by Brooks et al. (2012), who analysed the risks for workers and residents in areas close to the application of manure and biosolids. Three factors that characterize the scenarios may have caused these differences: the inclusion of a factor associated with the microbial decay in the atmosphere (public risk); the different distances

analysed and consequently different dispersions; and the adoption of different wind speeds. Occupational risks emphasize the importance of using personal protective equipment (PPE), responsible for reducing direct contact with pathogens, the inhaled/ingested dose and consequently the risks. Tanner et al. (2008) analysed the effect of using PPE and found a reduction of an order of magnitude of risks as a consequence of lower inhalation / ingestion doses of pathogens.

Inactivation of pathogens may have limited the risks to residents in relation to occupational risks. The isolated analysis of this parameter provided risks of about 6 to 7 orders of magnitude higher with reduction of the microbial deterioration factor (DUNGAN, 2014). The analysis of public risks involved distances greater than those adopted for occupational risks. The permanence of microorganisms in the atmosphere for longer periods intensifies the effect of environmental stressors (USEPA, 1982). Meteorological factors such as temperature, solar radiation and humidity can affect the viability of microorganisms and, consequently, their concentration in the air and their capability of initiating an infection (DUNGAN, 2014). For a given wind speed, an increase in the distance from the emission source from 5 to 1000 m was responsible for a decrease of approximately 1.5 $\log_{10}$ in risks of infection by enterovirus (VIAU et al., 2011). The adoption of higher speeds for public scenarios may also have influenced the exposure to lower risks. The increase in wind speed accentuates the dispersion effect, providing lower concentrations of pathogens in the air for a given distance. Changing the speed from 1.5 to 20 $m.s^{-1}$ led to a 75% dose decrease and a 2 $\log_{10}$ reduction in risks of infection by enterovirus (VIAU et al., 2011).

Although there are no specific guidelines for the risk associated with bioaerosols, only the average risks of exposure to aerosolised CpSP and Crypto to receptors at 500 m from the source were below the tolerable risk level recommended by the WHO (10^{-6} DALY) and the USEPA (10^{-4} pppy) for drinking water (WHO, 2011; USEPA, 2012).

3.5 VARIABILITY AND UNCERTAINTIES INHERENT IN RISK ANALYSIS

As in most risk simulations, there is variability and uncertainty associated with many parameters, such as concentration of pathogens, viability, infectivity and dose, dose-response models, health status of exposed populations and workers, environmental conditions and time of exposure (DUNGAN, 2014). Consequently, configurations of the exposure scenarios and the input variables of the model in this study were chosen through careful data selection (from

empirical data of this case study and the literature). From this, it was generated probability distribution functions (PDF) for most of the factors involved in the exposure estimates (Tables S5 and S6) and the estimated risks from 10,000 combinations of these input parameters. The adoption of PDFs in stochastic models incorporate uncertainties around the input parameters of the model and, consequently, the output variable (VOSE, 2008; DIAS et al., 2019).

Despite efforts to ensure reasonable and as realistic estimates of the risk as possible, validation of the model is not possible due to little or no epidemiological evidence of the health effects of exposure to bioaerosols from dairy cattle wastewater. In addition, there is a lot of uncertainties associated with inhaling pathogens in the air and their ability to cause infection after subsequent ingestion (DUNGAN,2014; VIAU,2011). Therefore, there is a need for mores studies involving factors such as the diversity of pathogens in wastewater, including the viability / infectivity of bioaerosols under various environmental conditions, assessing the susceptibility in a representative population of humans to pathogenic microorganisms in the air (DUNGAN,2014; VIAU,2011).

4 CONCLUSIONS

The results obtained in this study demonstrated that fertigation events with wastewater from dairy cattle by spraying may pose risks for both workers and residents close to the application sites. Occupational risks were greater than public risks, which emphasize the importance of the use of personal PPE by workers. On the other hand, the increase in the distance from the source of bioaerosol emissions was a factor that reduced the risks residents exposed to bioaerosols generated during fertigation.

Effluent treatment played an important role for reducing risk in both occupational and public scenarios. Thus, the adoption of treatment processes that effectively remove pathogens from wastewater can be a strategy for reducing exposure to airborne pathogens. Additionally, dilution of effluents in clean water may also reduce concentrations of pathogens in the biofertilizer and, consequently, decrease risks.

It should be noted that this microbial risk assessment is specific to a specific wastewater application site and the results should be used with caution. However, the methodology used is likely to be replicated for other analyses of exposure to bioaerosols in other scenarios.

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APPENDIX A – Atmospheric dispersion factor (D_d)

Atmospheric dispersion factor (D_d) based on Gaussian model:

$$D_{d\,(X)} = \frac{1}{2\pi u \sigma_y\,(x)\sigma_z(x)} exp\left(-\frac{y^2}{2\sigma_y}\right)\left[exp - \frac{(z-H)^2}{2\sigma_z(x)} + exp - \frac{(z+H)^2}{2\sigma_z(x)}\right]$$

Where: $D_{d\,(x,\,y,\,z)}$ = atmospheric dispersion factor at a distance (x) from the emission source ($s.m^{-3}$); u = wind speed ($m.s^{-1}$); $\sigma_{y\,(x)}$ = horizontal diffusivity in the x coordinate; $\sigma_{z\,(x)}$ = vertical diffusivity in the x coordinate; H = height of aerosol emission.

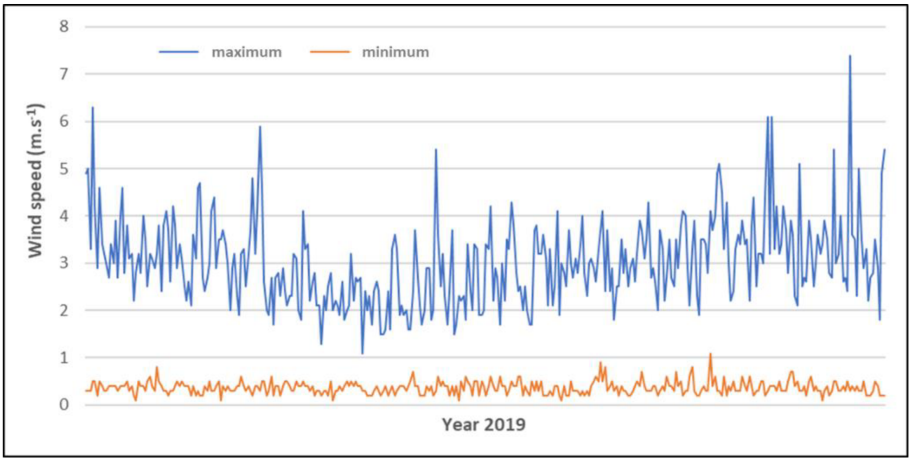
Steady state conditions and spray application as a point source were assumed in this study as the area in the study was relatively small. The error introduced by these assumptions decreases with distance in the wind, because the geometry of all sources is increasingly similar to that of a point source (USEPA, 1982). To represent the people's breathing zone in the wind, 1.5 m for the z input was adopted (USEPA, 1982). Due to the type of application used in the present study, the emission height of the source (H) was assumed to be 1.5 m (GURIAN et al.,2012). For occupational risks, it was considered a minimum average speed equal to 0.3 $m.s^{-1}$ (U_{min} = 0.3 $m.s^{-1}$) and distances (x-axis) of 50 m and 100 m from the source (Figure S1). For public risk, it was considered a minimum average speed equal to 3.0 $m.s^{-1}$ (U_{max} = 3.0 $m.s^{-1}$) and distances (x-axis) of 100 m and 500 m from the source (Figure S1). Horizontal and vertical diffusivity in the x coordinate ($\sigma_{y\,(x)}$ and $\sigma_{z\,(x)}$, respectively) considering information provided in Tables S1 and S2.

The application of the Gaussian model is only reliable for estimating the dispersion of pathogens over distances greater than 100m. Thus, for the occupational risk scenario, the formula used to calculate the dispersion factor was described in the following equation:

$$D_d\,(x) = \frac{1}{u\,H\,(max\,(W_{spray},\,(2 * X_{FW}))}$$

Where: Dd (x, y, z) = atmospheric dispersion factor at a distance (X_{FW}) from the emission source ($s.m^{-3}$); u = wind speed ($m.s^{-1}$); H = aerosol emission height (m); Wspray = application width (m); X_{FW} = worker distance from the emission source (m)

Graphic 1 - Maximum and average daily speeds for 2019



Source: INMET,2019

Table 1 – Pasquill Stability Classes

Wind speed ($m.s^{-1}$)	**Solar Radiation**		
	Strong	**Moderate**	**Weak**
0	A	A	B
1	A	B	B
2	A	B	C
3	B	B	C
4	B	B	C
5	B	C	D
6	C	C	D
7	C	D	D

Source: SEINFELD,1986

Table 2 - Rural dispersion parameters by Briggs.

Pasquill Classification	**σ_y* (m)**	**σ_z *(m)**
A	$0,22x \times (1+ 0,0001x)^{-0.5}$	0,20x
B	$0,16x \times (1+ 0,0001x)^{-0.5}$	0,12x
C	$0,11x \times (1+ 0,0001x)^{-0,5}$	$0,08x \times (1+ 0,0002x)^{-0,5}$
D	$0,08x \times (1+ 0,0001x)^{-0,5}$	$0,06x \times (1+ 0,0015x)^{-0,5}$

Source: BRIGGS, 1973

*for distances between 100 and 10.000m

Table 3- Calculation of the dispersion factor for the public scenario.

PUBLIC SCENÁRIO				
Dispersion Parameters				
Wind speed ($m.s^{-1}$)	**Stability class**	**Distances (m)**	**σ_y^* (m)**	**σ_z (m)**
3.0	B	x = 100 x = 500	$0{,}16x \times (1+ 0{,}0001x)^{-0.5}$	0,12x
Atmospheric dispersion factor (Dd)				
π	**y (m)**	**z (m)**	**H (m)**	
3.1415	0	1,5	1,5	

Source: prepared by the author (2019)

Table 4- Calculation of the dispersion factor for the occupational scenario.

OCCUPATIONAL SCENARIO			
Atmospheric dispersion factor (Dd)			
Wind speed ($m.s^{-1}$)	**H (m)**	**Wspray (m)**	**Distances (m)**
0.3	1.5	11	XFW =10m XFW = 50m

Source: prepared by the author (2019)

APPENDIX B – Concentration of organisms in the atmosphere ($C_{(x)}$)

Table 5 – Summary of the input parameters to determine the concentration of organisms in the atmosphere ($C_{(x)}$).

Variable	Distribution	Parameter(s) ; Value(s)
W = density of microorganisms in the wastewater [org.L^{-1}]	EC (untreated effluent) NORMAL DISTRIBUTION	μ = 94220000 σ = 0.54384
	EC (treated effluent) NORMAL DISTRIBUTION	μ = 6.49972 σ = 0.39198
	CpSP (untreated + treated effluents) NORMAL DISTRIBUTION	μ = 4.61985 σ = 0.19047
F = application flow [L.s^{1}]	EC, CpSP and Crypto CONSTANT VALUE	28.0
E = aerosolization efficiency [dimensionless]	EC EXPONENTIAL DISTRIBUTION	Min = 0.0012458 λ= 0.0040085
	CpSP CONSTANT VALUE	1.0
	Crypto CONSTANT VALUE	1.0
I = impact factor [dimensionless]	EC, CpSP and Crypto CONSTANT VALUE	1.0
$R_{Pat/Ind}$ = Pathogen / indicator relationship [dimensionless]	EC-O157:H7 / EC CONSTANT VALUE	0.08
	C. perfringens type A / CpSP CONSTANT VALUE	1.0
	Crypto / CpSP CONSTANT VALUE	0.0001
λ = decay rate of viability [s^{1}]	EC EXPONENTIAL DISTRIBUTION	Min = 0.033143 λ= 0.058422
	CpSP UNIFORM DISTRIBUTION	Min =0.01 Max = 0.1
	Crypto UNIFORM DISTRIBUTION	Min =0.01 Max = 0.1
ad = wind speed [m.s^{1}] divided by the distance x [m]	Occupational risk CONSTANT VALUE	0.03 for 10 m from source 0.006 for 50 m from source
	Public risk CONSTANT VALUE	0.03 for 100 m from source 0.006 for 500 m from source

Source: prepared by the author (2019)

Density of the microorganism in the atmosphere (C(x)) based on model $\boldsymbol{C_x = Q_m \times D_x \times M_x}$; where: $C_{(x)}$ = density of the microorganism in the atmosphere (cfu.m^{-3}) at any distance downwind x; $D_{(x)}$ = atmospheric dispersion factor described by the Gaussian model (s.m^{-3}); Q_m = microorganism emission rate adjusted for loss of microbial viability during the spraying process (cfu.s^{-1}); $M_{(x)}$ = fraction of microorganisms that remains viable at a distance (x) from the source (dimensionless); Pathogen emission rate (Q_m) base on the model $\boldsymbol{Q_m = W \times F \times E \times I \times R_{Pat/Ind}}$; where: Q_m = microorganism emission rate (cfu.s^{-1}); W = density of microorganisms in the wastewater (cfu.L^{-1}); F = application flow (L.s^{-1}); E = aerosolization efficiency (dimensionless); I = impact factor (dimensionless); $R_{Pat/Ind}$ = Pathogen / indicator relationship (dimensionless).

Loss of viability of microorganisms in the atmosphere $M_{(x)}$ based on the model $\boldsymbol{M_{(x)} = e^{\lambda . a_d}}$; where: $M_{(x)}$ = fraction of microorganisms that remains viable at a distance (x) from the source (dimensionless); λ = decay rate of viability (s ^{1}); ad = wind speed (m.s ^{1}) divided by the distance x (m).

APPENDIX C – Ingestion dose (d), single exposure infectious risks (P_i) and annual risks (P_a)

Table 6 – Summary of the input parameters to determine the pathogens' ingestion dose (d), single exposure infectious risks (P_i) and annual risks (P_a).

Variable	Distribution	Parameter(s) ; Value(s)
br = respiration rate [$m^3.h^{-1}$]	EC, CpSP and Crypto LOGNORMAL DISTRIBUTION	μ = 0.579 σ = 0.225
ag = fraction of inhaled aerosol particles ingested [dimensionless]	EC, CpSP and Crypto UNIFORM DISTRIBUTION	Min = 0.1 Max = 0.8
t = time of exposure [h]	Occupational risk CONSTANT VALUE	1.0
	Public risk CONSTANT VALUE	8.0
Dose-response models (P_i)	EC BETA-POISSON MODEL	α = 0.248 β =48.80
	CpSP EXPONENCIAL MODEL	$r = 1.82\times10^{-11}$
	Crypto EXPONENCIAL MODEL	r = 0.00419
Annual risk (P_a)	Occupational risk CONSTANT VALUE	n = 9.0
	Public risk CONSTANT VALUE	n = 9.0

Source: prepared by the author (2019)

Dose (d) based on the model $\boldsymbol{d = C_{(x)} \times br \times ag \times t}$; where: d = dose of pathogens per exposure event (org); Cd = concentration of pathogens in the air ($cfu.m^{-3}$); br = respiration rate ($m^3.h^{-1}$); ag = fraction of inhaled aerosol particles ingested (%); t = time of exposure (h).

Infectious risk to one exposure event based on the exponential dose-response model $\boldsymbol{P_i = 1 - exp(-d/r)}$; where: P_i = probability of infection due to exposure event; d = dose of pathogens ingested (org); r = pathogen "infective constants" (dimensionless);

Infectious risk to one exposure event based on the beta-Poisson dose-response model $\boldsymbol{P_i = 1 - \left(1 + \frac{d}{\beta}\right)^{-\alpha}}$; where: P_i = probability of infection due to exposure event; d = dose of pathogens ingested (org); α = pathogen "infective constants" (dimensionless); β = dependent parameter of mean infective dose (dimensionless).

Annual infectious risk base on model $\boldsymbol{P_a = 1 - (1 - P_i)^n}$; where: P_a = probability of annual infection (pppy); P_i = probability of infection due to one exposure event; n = number of events or days of exposure per year.

Exposure scenarios

Table 7– Summary of the adopted occupational and public risk exposure scenarios.

Occupational risk scenarios					
Effluent	**Wind speed**	**Pathogens**	**Decay in the air**	**Distances form the source**	**Exposure time**
Untreated and Treated	Average low	*E. coli* O157:H7; *C. perfringens*; *Cryptosporidium*	Not considered	10 m and 50 m	1 h
Public risk scenarios					
Effluent	**Wind speed**	**Pathogens**	**Decay in the air**	**Distances form the source**	**Exposure time**
Untreated and Treated	Average high	*E. coli* O157:H7; *C.perfringens*; *Cryptosporidium*	Considered	100 m and 500 m	8 h

Source: prepared by the author (2019)

APPENDIX D – Results of annual occupational and public risks

Table 8– Values obtained for annual occupational risks.

Parameter	EC-O157:H7		EC-O157:H7		CpSP		Crypto	
	untreated		treated		untreated + treated		untreated + treated	
	10 m	50 m	10 m	50 m	10 m	50 m	10 m	50 m
MIN	1.96E-04	3.92E-05	8.85E-05	1.77E-05	1.20E-08	2.40E-09	2.76E-05	5.53E-06
5%	1.66E-02	3.35E-03	1.65E-03	3.30E-04	1.05E-07	2.10E-08	2.41E-04	4.82E-05
10%	2.79E-02	5.68E-03	2.71E-03	5.44E-04	1.43E-07	2.86E-08	3.29E-04	6.57E-05
25%	7.22E-02	1.51E-02	6.07E-03	1.22E-03	2.43E-07	4.85E-08	5.59E-04	1.12E-04
50%	1.93E-01	4.38E-02	1.42E-02	2.87E-03	4.27E-07	8.55E-08	9.84E-04	1.97E-04
75%	4.28E-01	1.18E-01	3.44E-02	7.03E-03	7.26E-07	1.45E-07	1.67E-03	3.34E-04
90%	6.99E-01	2.63E-01	7.39E-02	1.55E-02	1.11E-06	2.22E-07	2.55E-03	5.10E-04
95%	8.30E-01	3.95E-01	1.16E-01	2.49E-02	1.43E-06	2.86E-07	3.29E-03	6.59E-04
MAX	9.99E-01	9.86E-01	7.49E-01	3.05E-01	4.88E-06	9.76E-07	1.12E-02	2.24E-03
AVG	2.80E-01	9.79E-02	3.06E-02	6.60E-03	5.55E-07	1.11E-07	1.28E-03	2.55E-04
SD	2.57E-01	1.40E-01	4.81E-02	1.21E-02	4.56E-07	9.12E-08	1.05E-03	2.10E-04
SKEWNESS	1.0369	2.6681	4.6075	5.4856	2.3133	1.0615	2.5228	2.5588
KURTOSIS	3.0618	11.3833	38.215	49.2843	13.1056	10.0632	147598	15.8967

Source: prepared by the author (2019)

Table 9 – Values obtained for annual public risks.

Parameter	EC-O157:H7		EC-O157:H7		CpSP		Crypto	
	untreated		treated		untreated + treated		untreated + treated	
	100 m	500 m	100 m	500 m	100 m	500 m	100 m	500 m
MIN	1.25E-04	5.67E-06	4.32E-05	1.96E-06	1.01E-08	4.59E-10	2.33E-05	1.06E-06
5%	7.38E-03	3.36E-04	7.76E-04	3.52E-05	4.70E-08	2.14E-09	1.08E-04	4.92E-06
10%	1.28E-02	5.83E-04	1.24E-03	5.64E-05	6.36E-08	2.89E-09	1.46E-04	6.66E-06
25%	3.37E-02	1.56E-03	2.67E-03	1.21E-04	1.10E-07	5.00E-09	2.53E-04	1.15E-05
50%	9.37E-02	4.54E-03	6.51E-03	2.96E-04	1.94E-07	8.82E-09	4.47E-04	2.03E-05
75%	2.38E-01	1.30E-02	1.60E-02	7.31E-04	3.19E-07	1.45E-08	7.35E-04	3.34E-05
90%	4.68E-01	3.28E-02	3.48E-02	1.61E-03	4.97E-07	2.26E-08	1.14E-03	5.20E-05
95%	6.37E-01	5.72E-02	5.49E-02	2.58E-03	6.51E-07	2.96E-08	1.50E-03	6.81E-05
MAX	9.95E-01	6.25E-01	4.07E-01	2.65E-02	2.95E-06	1.34E-07	6.77E-03	3.09E-04
AVG	1.74E-01	1.41E-02	1.46E-02	6.91E-04	2.49E-07	1.13E-08	5.73E-04	2.60E-05
SD	2.02E-01	3.15E-02	2.46E-02	1.27E-03	2.04E-07	9.28E-09	4.70E-04	2.14E-05
SKEWNESS	1.7505	7.742	5.9889	9.4216	2.1695	2.5911	2.364	2.4005
KURTOSIS	5.6755	95.5993	63.1861	189.831	10.663	16.7289	14.1563	14.3512

Source: prepared by the author (2019)