THE PERSISTENCE OF INDICATORS AND PATHOGENS IN WASTEWATER BIOSOLIDS-AMENDED SOIL

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Summary

Nutrient rich -organic residuals from wastewater treatment, also known as “biosolids”, are widely used as soil conditioner and fertilizer for plant growth. These biosolids contain enteric pathogens, such as viruses, bacteria, fungi and protozoa (i.e., biosolids-associated pathogens, hereafter) which may pose risks to human during land application of biosolids. This chapter aims to summarize best data for decay of biosolids-associated pathogens in biosolids-amended soil from field-related studies for comparing their decay patterns. This type of information is currently not available easily.

Scientific publications and field reports were reviewed to obtain information on: (1) persistence of biosolids-associated pathogens in soil after land application of biosolids (expressed in terms of T90 value, i.e., time required for 90% reduction of microorganisms) and (2) factors affecting their survival in soil. A literature review of studies indicated that most of the studies have focused on understanding fate of biosolids-associated bacteria in soil. However, very few studies are available which focus on obtaining information of fate of viruses, helminths and protozoa in biosolids-amended soil.

Overall, there is a need for conducting more field studies for determining T90 values for different pathogens in biosolids-amended soil. Following knowledge gaps were identified: (i) Lack of information on combined effect of different factors on pathogen survival, (ii) Lack of information on decay patterns of viruses, helminths, protozoa in biosolids-amended soil under field conditions.

1.0 Introduction

This chapter aims to summarize best data for decay of pathogens, such as bacteria, viruses, protozoa, helminths, in biosolids-amended soil using field-related studies for comparing their decay patterns. These groups of pathogens have been mentioned in the Global Water Pathogen Project (GWPP, 2018) and are principle pathogens-of-concern in biosolids (Pepper et al., 2006). The compilation of information will be useful in policy making for application of biosolids to soil.

Nutrient rich -organic residuals from wastewater treatment, also known as “biosolids”, are widely used as soil conditioner and fertilizer for plant growth. These biosolids contain enteric pathogens which can spread diseases include viruses, bacteria, and protozoa (Lewis et al., 2003; Gerba and Smith, 2005; Lu et al., 2012). These biosolids might improve soil properties but introduce pathogens in soil (Pourcher et al., 2007). Land application of biosolids introduces enteric pathogens which are difficult to inactivate (Sidhu and Toze, 2009). Pathogens, present in biosolids (i.e., biosolids-associated pathogens; BAPs) might pose health risk (Eisenberg et al., 2008) through different exposure pathways (Figure 1). Land applied sludge needs proper treatment for removing pathogens before it can be applied on land. Levels of treatment required for different types of biosolids depend on pathogen concentration in biosolids and final allowable values of BAPs in soil after land application activities. For example, the USEPA Class A and Class B biosolids limits need to be followed before applying to land in the USA. Similarly, EPA Victoria has classified biosolids as Contaminant grade and Treatment grade and has different management rules for land application (EPA Australia). There are around 150 microbial pathogens which have been identified from all animal species that can be transmitted and, can be harmful for human (USEPA, 1998).

Figure 1. Schematic of possible interlinking of pathogens in sludge to biosolids treatment methods, land application activities and human exposure pathways Gdw-groundwater (Gerba and Smith, 2005; Gale, 2005; Eisenberg et al., 2008; Gurian et al., 2012; Lu et al., 2012)

Various studies have focused on determining concentrations of bacteria, viruses, protozoa in soil and surface and groundwater to under potential health risk. To understand decay behaviours of different BAPs in biosolids-amended soil, information related to decay of indicator microorganisms and pathogens in biosolids and biosolids-amended soil was compiled from different published field studies. Different keywords were entered in the Science Direct database and studies/papers relevant to our present context, i.e., decay of pathogen in biosolids-amended soil in field-based studies was compiled. This approach ensured the consideration of all relevant published papers from the database. Figure 2 shows keywords searched in science direct and number of studies obtained. Numbers of studies, found after entering different keywords, are presented in Figure 2. It also indicates number of those studies which were found to be relevant to our area of focus, i.e., “pathogen decay in biosolids amended soil”. It also includes some studies which were selected from the database of “Google Scholar”. Laboratory studies have been conducted to understand decay patterns of pathogens in mixtures of biosolids and soil, but there have been very few field-based studies. Data of field-based studies were compiled. Findings of laboratory studies were also included for those pathogens where not enough data on field studies was available.

Figure 2. Trend of published scientific articles over time in various areas-related to biosolids-amended soils Numbers in bracket represent studies which satisfied
our area of focus "pathogen decay in soil amended with biosolids". Data obtained from the Science Direct database on June 2nd, 2018 using keywords mentioned in legends of this figure.

Information on persistence of pathogen in biosolids-amended soil, expressed as T₉₀ (i.e., time required for 90% reduction of pathogen) was compiled along with experimental conditions (example: soil type, temperature and moisture, method of application of biosolids, etc.). T₉₀ values reported in published studies were used as-is. Studies where T₉₀ values were not reported, “GetData” software was used to extract points from time versus concentration plots. The extracted x and y values were exported to an Excel 2016 spreadsheet for plotting a graph and estimating T₉₀ values. Results of concentration values versus time were plotted on a base 10 semi-logarithmic graph and a linear regression was performed on the transformed data. The first order decay model (i.e., model #1: \( \log(C/C_0) = (-kt) \)) was used where C is the concentration at a given time point, \( C_0 \) is the initial concentration, k is the decay rate, and (t) is time. This approach was used for those studies where no time-lag (i.e., termed as \( \lambda \), hereafter) was observed during decay of pathogens. For studies, where time-lag in decay of pathogen was reported, the X-axis parameter (i.e., exposure duration (t)) was transformed to \( (t-\lambda) \) (model #2: \( \log(C/C_0) = (-k\ (t-\lambda)) \)). The slope value was taken as the inactivation rate constant and the inverse of slope was estimated to be the \( T_90 \) value (log (0.1) = -kt*) if no time-lag in decay of pathogens and \( t^*=(t-\lambda) \) if time delay happens in pathogen decay. A summary of numbers of published studies, used in directly obtaining or calculating values of \( T_90 \) of decay of bacteria, viruses, helminths and protozoa in biosolids-amended soil is presented in Table 1.

### Table 1. Summary of numbers of published studies, used in directly obtaining or calculating values of \( T_{90} \) of decay of bacteria, viruses, helminths and protozoa in biosolids- amended soil

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Field Studies</th>
<th>Lab Studies</th>
<th>Field Studies</th>
<th>Lab Studies</th>
<th>Field Studies</th>
<th>Lab Studies</th>
<th>Field Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Published Values</td>
<td>Useful for Getting ( T_{90} )</td>
<td>( T_{90} ) Given</td>
<td>( T_{90} ) Calculated (Model 1)</td>
<td>( T_{90} ) Calculated (Model 2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
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<tr>
<td></td>
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</table>

*Lab: laboratory; Model #1: \( \log(C/C) = (-k t) \); Model #2: \( \log(C/C) = (-k (t-\lambda)) \) *

Following sections presents a compilation of \( T_{90} \) values of bacterial pathogens and indicators, viruses, protozoa and helminths in biosolids-amended soils and discuss factors affecting their decay patterns. The last section documents identified knowledge gaps and suggest possible actions for filling these knowledge gaps.

### 2.0 Persistence of bacterial pathogens and indicator bacteria in biosolids-amended soil

This section documents \( T_{90} \) values of bacterial pathogens and indicators in biosolids-amended soil and studies effect of soil temperature, soil moisture and soil type on persistence (i.e., \( T_{90} \) values) under field- and laboratory-conditions. Information on experimental conditions of different laboratory- and field-based studies and decay of bacterial indicators and pathogens (i.e., Faecal coliforms, \( E.coli \), \( S. Enterica \), \( Salmonellae \), \( Listeria monocytogenes \)) in biosolids-amended soils are presented in Tables 2 and 3, respectively. These tables present information on biosolids type used and application method, climatic region, experimental conditions (soil type, temperature and moisture, biotic stress, \( T_{90} \) value and decay rate). Following decay of microorganisms in biosolids-amended soil.

### Table 2. Summary of findings of field-based studies on persistence of bacterial indicators and bacterial pathogens in biosolids- amended soil

<table>
<thead>
<tr>
<th>Country/ Climatic Region</th>
<th>Method of Application</th>
<th>Experimental Conditions</th>
<th>Indicator Microorganisms/Pathogen Studied</th>
<th>Study Period (Days)</th>
<th>( T_{90} ) (Days)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobically-digested Sludge</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Australia, Sub-tropical</td>
<td>Injection method (depth=30 cm), rate=1.5 t/ha</td>
<td>Sandy soil; soil temperature = 12 to 20°C; soil moisture = 1 to 22%</td>
<td>Pseudomonas aeruginosa</td>
<td>210</td>
<td>89</td>
<td>Gibbs et al., 1997</td>
</tr>
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<td>Australia, Sub-tropical</td>
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</tr>
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<td>Sandy soil; soil temperature = 12 to 20°C; soil moisture = 1 to 22%</td>
<td>Pseudomonas aeruginosa</td>
<td>210</td>
<td>89</td>
<td>Gibbs et al., 1997</td>
</tr>
</tbody>
</table>
References

Temperate Composted Sludge with Green Waste (CPT)

France, United Kingdom, Temperate Australia, Mediterranean Spain Tropical USA, Mediterranean Spain Temperate Warm and Country/Region
temperature:
- Northern temperate forests: (0 to 50 cm); rainfall = 45 cm; mean annual rainfall = 388.4 mm
- Young stand of pine forest. 40 Mg·ha⁻¹ (dry weight, dw)).
- Methane production in forest soils.
- Open slot trial, 10 cm depth of sludge to surface incorporation 10 t/ha; 30 cm; rate= 10 t/ha)
- Spring period (February, 1990 to May, 1996)
- Clay loam soil; soil moisture 5%; rewetting to 60% moisture.
- Injection method
- E. coli
- Autumn/Winter

Table 3. Summary of findings of laboratory-based studies on persistence of bacterial indicators and bacterial pathogens in biosolids-amended soil

<table>
<thead>
<tr>
<th>Country/Climate Region</th>
<th>Method of Application</th>
<th>Experimental Conditions</th>
<th>Indicator or Pathogen Studied</th>
<th>Study Period (Days)</th>
<th>Tₐ₀ (Days)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaerobically-digested Sludge</td>
<td>Soil from surface and subsurface</td>
<td>Spring and autumn</td>
<td>Coliforms</td>
<td>120</td>
<td>111</td>
<td>Pepper et al., 1993</td>
</tr>
<tr>
<td>Spain Mediterranean climate</td>
<td>5 different soil sites; Sandy loam soil (40-50 cm)</td>
<td>Spring season (September 1999-April 2000)</td>
<td>Faecal coliforms</td>
<td>120</td>
<td>84</td>
<td>Zubieta et al., 2005</td>
</tr>
<tr>
<td>Australasia</td>
<td>Sandy soils</td>
<td>Surface Application</td>
<td>Enterococci</td>
<td>720</td>
<td>283</td>
<td>Gondim-Porto et al., 2016</td>
</tr>
<tr>
<td>Australasia</td>
<td>Sandy soils</td>
<td>Surface Application</td>
<td>Enterococci</td>
<td>720</td>
<td>230</td>
<td>Gondim-Porto et al., 2016</td>
</tr>
<tr>
<td>Australia, Desert climate</td>
<td>Surface Application</td>
<td>Spring and summer</td>
<td>Enterococci</td>
<td>720</td>
<td>90</td>
<td>Gondim-Porto et al., 2016</td>
</tr>
<tr>
<td>Australia, Desert climate</td>
<td>Surface Application</td>
<td>Spring and summer</td>
<td>Enterococci</td>
<td>720</td>
<td>90</td>
<td>Gondim-Porto et al., 2016</td>
</tr>
<tr>
<td>Dewatered Anaerobically Digested Sludge (DADDS)</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>Trial 1: 20°C</td>
<td>E. coli</td>
<td>89</td>
<td>35</td>
<td>Ngole et al., 2006</td>
</tr>
<tr>
<td>United Kingdom, Temperate</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>Trial 2: 0°C</td>
<td>E. coli</td>
<td>119</td>
<td>31</td>
<td>Ngole et al., 2006</td>
</tr>
<tr>
<td>United Kingdom, Temperate</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>Trial 3: 0°C</td>
<td>E. coli</td>
<td>84</td>
<td>21</td>
<td>Baxley et al., 2003</td>
</tr>
<tr>
<td>United Kingdom, Temperate</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>Trial 4: 0°C</td>
<td>E. coli</td>
<td>14</td>
<td>24</td>
<td>Baxley et al., 2003</td>
</tr>
<tr>
<td>United Kingdom, Temperate</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>Trial 5: 0°C</td>
<td>E. coli</td>
<td>14</td>
<td>24</td>
<td>Baxley et al., 2003</td>
</tr>
<tr>
<td>Activated sludge</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>E. coli</td>
<td>60</td>
<td>40</td>
<td>Baxley et al., 2003</td>
<td></td>
</tr>
<tr>
<td>Camposted Sludge with Green Waste (CPW)</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>E. coli</td>
<td>60</td>
<td>37</td>
<td>Baxley et al., 2003</td>
<td></td>
</tr>
<tr>
<td>Thermally Dried Digested Product (TDP)</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>Trial 1: 20°C</td>
<td>E. coli</td>
<td>89</td>
<td>59</td>
<td>Lang et al., 2003</td>
</tr>
<tr>
<td>Thermally Dried Digested Product (TDP)</td>
<td>Injection to depth of 30 cm (0 to 5 cm); sandy loam soil</td>
<td>Trial 2: 9°C</td>
<td>E. coli</td>
<td>119</td>
<td>111</td>
<td>Lang et al., 2003</td>
</tr>
<tr>
<td>Anaerobically-digested Sludge</td>
<td>Botswana soil</td>
<td>Injection method</td>
<td>Faecal coliforms</td>
<td>120</td>
<td>84</td>
<td>Pepper et al., 1993</td>
</tr>
<tr>
<td>Botswana, Semi Arid</td>
<td>Falls season</td>
<td>Surface Application</td>
<td>Faecal coliforms</td>
<td>120</td>
<td>113</td>
<td>Cass, 2009</td>
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<tr>
<td>USA, Tropical climate</td>
<td>Site-reduced method</td>
<td>Clay loam soil; soil moisture 5%; rewetting to 60% moisture</td>
<td>Salmoella</td>
<td>107</td>
<td>10</td>
<td>Zubieta et al., 2005</td>
</tr>
<tr>
<td>USA, Tropical climate</td>
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<td>Salmoella</td>
<td>107</td>
<td>10</td>
<td>Zubieta et al., 2005</td>
</tr>
</tbody>
</table>

NA: Not available; NE: Not estimated; NR: Not reported; * Time after which bacteria reaches non-detectable levels (used if T₀ is unavailable); †T₀ values calculated from model described in methodology.
2.1. Soil temperature and soil moisture

This section documents effect of soil temperature on $T_{90}$ values of bacterial pathogens and indicators in biosolids-amended soil and compares $T_{90}$ values of E.coli of different biosolids types in soil. Studies have reported effect of variation of temperature on decay of bacterial indicator and pathogens in biosolids-amended soil. Concentrations of E.coli and Salmonellae were observed to increase in soil in temperate region (Ascot, UK) during autumn-winter (temperature range: 0.7-13°C) and spring-summer (temperature range: 13-25°C) because of favourable conditions of temperature and moisture for microbial growth and decline during summer due to dry conditions (Lang et al., 2007). Similarly, another study reported undetectable concentrations of faecal coliforms and Salmonellae during the summer period and high concentrations of these microorganisms under favourable conditions (Gibbs et al., 1997). Concentrations of indigenous E.coli were observed to decrease in soil-amended with enhanced treated biosolids than in control soil (Lang et al., 2007). About 90% reduction in Salmonella concentration was observed in 21 days in a mature stand of trees and 77 days in a young stand (closed, mature tree stand and open, young tree stand forest canopy in spring and autumn/winter) with the longest survival interval in winter (Horswell et al., 2007). In another study, $T_{90}$ value of faecal coliform decay was observed to be 3.3 days (in summer) and 13.4 days (in winter) (Van Donsal et al., 1967). A comparison of $T_{90}$ values of decay of E.coli of different biosolids in silty loam or clay loam soil in temperate region (10-20°C) (Figure 3) indicated that E.coli of biosolids types such as anaerobically digested sludge, mechanically dewatered biosolids or DMAD survive for shorter time period (Figure 3) than that of biosolids type, such as composted sludge, thermally dried digested biosolids or dewatered raw sludge. Overall, these observations indicate that consideration of ambient temperature during land application of biosolids is important as it can increase or decrease decay of bacterial indicator or bacterial pathogens in biosolids-amended soil. These microorganisms are likely to survive more in winter weather than in summer weather, indicating the importance of tracking their survival patterns in different seasons.

Figure 3. A comparison of $T_{90}$ values of decay of E.coli in different environmental conditions

This section documents effect of soil moisture on decay of bacterial pathogens and indicators in biosolids-amended soil. Moisture content has been shown to affect survival of bacteria (Burge et al., 1987; Ngole et al., 2006). For example, moisture content equivalent to field capacity was observed to increase number of E.coli in spring/autumn season and accounted for variation of 11-25% in $E_{coli}$ numbers (DMAD, sandy loam soil, Mean soil moisture:12.5-21%; Lang et al., 2007). This study reported that rewetting-drying soil conditions affected decay of pathogens of enhanced-treated biosolids. They further noticed the increase in E.coli numbers for 2-3 months in soil-amended with conventionally-treated biosolids and attributed to effect of soil wetting. In this regard, some studies have also reported an increase in bacteria concentration after rain event and attributed to the effect of increased moisture content on re-colonization of bacteria (Dehydrated anaerobic digested sludge, Moisture content: 5.1-22.8%, Temperature: 5-20°C, silty sandy soil; Estrada et al., 2004). For example, re-colonization of Salmonella and faecal coliform was observed after the rain event (Zaleski et al., 2005). Another study reported an increase in numbers of faecal coliforms and Salmonella after rainfall events (soil temperature = 12-30°C; soil moisture =1 to 22%, sandy soil) (Gibbs et al., 1997). In general, bacterial pathogen and indicator bacteria die more in reduced moisture content. However, some studies have reported a different trend of decay of indicator bacteria with soil moisture content (Chandler and Craven, 1980; Lang et al., 2007; Lang and Smith, 2007; Cass, 2009). For example, a UK-based study (Location: Ascot, UK) reported a greater decay of E.coli of biosolids amended in moist soil ($T_{90}=20$ days) than in dry soil ($T_{90}=100-200$ days) and attributed to the effect of competition of E.coli with other indigenous soil-microorganisms for nutrients and moisture (DMAD, sandy loam soil; Mean soil moisture: 12.5-21%; Table 2; Lang et al., 2007). The above mentioned discrepancies in relationship of survival of bacteria with soil moisture content from two different groups of studies (Burge et al., 1987; Ngole et al., 2006; Zaleski et al., 2005; Estrada et al., 2004; Chandler and Craven, 1980; Lang et al., 2007; Lang and Smith, 2007; Cass, 2009) indicate the need for incorporating the effect of interaction of soil moisture content with other factors on survival of bacteria under field conditions.
2.2 Soil Type

This section documents effect of soil type (sand, clay and silt) and soil texture (fine versus coarse) on decay of bacterial pathogens and indicators in biosolids-amended soil. Sometimes, mixing of biosolids with soil might result in a soil environment, not conducive for bacteria growth. For example, higher rate of survival of fecal coliforms was reported in fine textured soil (i.e., Pima soil) than in coarse textured soil (i.e., Brazito soil) (Pepper et al., 1993). Studies have also reported effect of presence of clay in soil on survival of bacteria (Ngole et al., 2006; Lang and Smith, 2007). For example, survival rates of E. coli were observed to be higher in fine texture of the vertisol (with high clay content) than in luvisols and arenosol (Ngole et al., 2006). In spring period, ribotype 027 spores decreased in loam soil with no change in numbers in sandy loam soil (Xu et al., 2016). In addition, the presence of organic matter in soil has also been reported to affect survival of bacterial indicator and bacterial pathogens (Sessitsch et al., 2001). Organic matter in soil aids in formation of soil aggregates where bacterial pathogens can reside and remain shielded from predators. Above observations indicate that soil type and soil characteristics (texture, presence of organic content) can influence the survival of bacteria in biosolids-amended soil.

2.3 Biotic Stress

This section documents effect of biotic stress (due to background microbial community) on decay of bacterial pathogens and indicators in biosolids-amended soil. Predation processes are likely to be an important pathogen inactivation mechanism in biosolids-amended soil. Predation and parasitism are considered to be one of the major factors affecting persistence (Roper and Marshall, 1978). For example, a UK study reported that both the indigenous soil and sludge microbial communities affected decay of E. coli in biosolids-amended soil (Lang et al., 2007). Decay of indicator organisms in soil has also been linked to change in biotic stress. Indigenous microbial population restricts regrowth and re-colonization of bacteria (Zaleski et al., 2005). Background E. coli, present in environment, has been shown to affect decay of enteric bacteria. For example, persistence of Salmonella was found to be affected by microbial antagonism of indigenous soil microbial community (You et al., 2006). Protozoa has been found to graze bacteria, with predation of gram negative bacteria (Rogers and Smith, 2007). For example, a reduction in S. typhimurium numbers was observed after addition of protozoa (Mallory et al., 1983).

2.4 Combined effect of different environment factors on bacterial survival

This section documents effect of interaction amongst different factors, such as soil temperature, soil moisture, soil type (sand, clay and silt), soil texture (fine versus coarse) on decay of bacterial pathogens and indicators in biosolids-amended soil. Although decay of bacteria in biosolids-amended soil depends on different factors, very few studies have studied combined effect of different factors, such as temperature, moisture content, soil type and characteristics, biotic stress (Lang et al., 2007; Cass, 2009). An UK study focused on studying combined effect of temperature and moisture content on decay of E. coli and Salmonella in biosolids-amended soil in temperate condition and reported that temperature and moisture had little effects on bacterial decay (Lang et al., 2007). Another study reported that high number of microorganisms were present at high temperature as contradictory to other studies (Temp: 15.5°C, mean annual rainfall: 388.4 mm, Table 2; Gondim-Porto et al., 2017) and attributed to the combined effects of other factors, such as solar radiation, type of soil in mediterranean climate, etc. Effects of type of biosolids, time from amendment and some other physical properties (i.e., water content of the samples, daily average temperature and air humidity) were found to have 44.5% variation in bacterial population with dose and time being the major affecting factors. More field studies, similar to those of Lang et al. (2007) and Cass (2009), need to be conducted to identify combined effect of different factors on survival of bacteria in field-related conditions.

3.0 Persistence of viral pathogens in biosolids-amended soil

This section documents persistence of viral pathogens and indicators in biosolids-amended soil and studies effect of soil temperature, soil moisture and soil type on persistence (i.e., T90 values) under field- and laboratory-conditions. A review of literature indicates that there have been very few studies which focused on persistence of viruses in biosolids-amended soil. Most of the studies have focused on persistence of enteric viruses in biosolids only but not in soil. Tables 4 and 5 present summary of findings of field- and laboratory-based studies on decay of viruses in biosolids-amended soil. The decay times for viruses in biosolids-amended soil were found to be smaller than 100 days and dependent on factors, such as soil temperature, soil moisture, and soil characteristics. Following sections discuss effects of these factors on decay of viruses.
3.1. Soil temperature and soil moisture content

This section documents effect of soil temperature and soil moisture content on persistence of biosolids-associated viruses and viral indicators in biosolids-amended soil. Desiccation and temperature have been shown to affect decay of viruses in soil. For example, a study reported that poliovirus or echovirus was not detected in soil after 8 days in fall condition whereas these viruses were found to survive for 35 days in wet summer (13.6 cm rainfall, temperature: 23.5-29°C; Bitton et al., 1984). Another study reported that poliovirus could survive up to 170 days at temperature of 3-10°C (Bagdasaryan, 1964). The viral indicator, MS2, was found to decay more in dry conditions than in humid conditions (-3.3-25°C; Choi et al., 2004).

The $T_{90}$ value of adenovirus-2 was observed to be 35 days at 20°C and 132 days at 4°C (Ogorzaly et al., 2010). Cox A9 of activated sludge-based biosolids was observed to survive more at 15°C ($T_{90} = 3.5$ days) than at 30°C ($T_{90} = 1$ days) on loess soil (65% sand, 10% silt, and 25% clay) (Nasser et al., 2002) (Table 4). Similar type of trend was observed for MS2 (Nasser et al., 2002) (Table 4). Some studies have also reported the effect of different depths of soil on $T_{90}$ values of viruses. For example, $T_{90}$ values were observed to vary from 1 to 30 days (in soil samples collected from 0-5 cm depth) and from 12 to 56 days (in soil samples collected from 5-15 cm depth) (minimum $T_{90}$ value=1 day (hot summer); 56 days (winter)) (Sorber and Moore, 1987). In a microcosm study, $T_{90}$ values of decay of AdV-GFP and MNV-1 in sediment were found to be 7.49 and 6.4 days, respectively under natural condition and 27.4 and 18.6 days, respectively in dark condition (Elmahdy et al., 2018). More inactivation of viruses in sediment was observed under sunlight and high temperature conditions.
3.2. Soil type

This section documents effect of soil type and soil conditions on persistence of biosolids-associated viruses and viral indicators in biosolids-amended soil. Adsorption of viruses to soil has been observed to decrease their decay rate in soil. Factors, such as soil pH, soil characteristics (clay content, exchangeable phosphorous, exchangeable aluminium content) affect decay of viruses in biosolids-amended soil (Hurst et al., 1980). Soil pH has been shown to affect adsorption of viruses on soil. Low soil pH increases adsorption of viruses on soil and thus it decreases their numbers in soil. An increase in soil pH has been shown to increase virus decay rate, i.e., decrease in virus survival (Hurst et al., 1980). Clayey soil has been shown to increase virus adsorption as compared to sandy soil (Straub et al., 2006). Clayey soil has been observed to increase her adsorption on soil and thus it decreases their numbers in soil. An increase in soil pH has been shown to increase virus decay rate, i.e., decrease in virus survival (Hurst et al., 1980). Clayey soil has been shown to increase virus adsorption as compared to sandy soil (Straub et al., 1992) and thus, virus survive more in sandy soil than in clayey soil. For example, two virus types (PhiX-174, vMC) were observed to survive more in sandy soil than in clayey soil (Fongaro et al., 2017). T90 values of Phix-174 were observed to be 10.5 ± 0.6 days in clayey soil than 21.2 ± 1.1 days in sandy soil. Similarly, T90 values of vMC, were observed to be 12.4 ± 1.3 days in clayey soil than 43.4 ± 1.3 days in sandy soil (Fongaro et al., 2017). Poliovirus of anaerobically-digested sludge was observed to survive more in clayey loam soil (T90 =16days) than in sandy soil (T90 =11 days) (temperature=150°C; desert climate) (Straub et al., 1992; Table 4). Further, virus number concentration has been observed to decrease more in unamended soil than in biosolids-amended soil. For example, a field-based study (Schwarz et al., 2013) reported that both MS2 and adenovirus survived more in unamended soil than in biosolids-amended soil (Table 5). These observations indicate that soil type affects survival of viruses and need to be considered for deciding setback time for minimizing contact of humans with virus particles from biosolids-amended soil.

4.0 Persistence of protozoa in biosolids-amended soil

This section documents effect of soil temperature and soil moisture content on persistence of biosolids-associated protozoa in biosolids-amended soil using information from small number of available studies. Not much research has been carried out for understanding persistence of protozoa, such as Giardia and Cryptosporidium spp. in biosolids-amended soil. Data related to decay of protozoa in actual field conditions have been lacking and studies have used laboratory conditions for studying decay patterns of protozoa in biosolids-amended soil. For example, sentinel chambers replicating soil environmental conditions were used to study survival of oocysts (Jenkins et al., 1999). Factor, such as temperature, pH, and ammonia had been found to affect inactivation of oocysts. A decline of oocysts has been reported for temperature ranging between 35°C and 50°C within 70 days. High temperature was observed to affect inactivation rate of protozoan cysts, making them less resistant to heat than other pathogens (Pepper et al., 2006). Decay of Cryptosporidium parvum in biosolids-amended soil was observed to increase with high temperature and soil desiccation (Nasser et al., 2007). Overall, a review of small number of available studies indicated that temperature affects inactivation of oocysts. However, effect of other factors on inactivation of protozoa has not be studied in details. More efforts in this direction is required so that a complete understanding on decay of biosolids-associated protozoa in biosolids-amended soil can be developed.

5.0 Persistence of helminths in biosolids-amended soil

This section documents effect of soil temperature and soil type on persistence of helminths in biosolids-amended soil using information from small number of available studies. Helminths, such as Ascaris lumbricoides, Trichuris trichiura, Taenia saginata, Taenia solium, Necator americanus, and Hymenolepis nana are also present in biosolids and may pose exposure risks to human after land application activities. Tables 6 and 7 present summary of findings of field- and laboratory-based studies on decay of Ascaris in biosolids-amended soil, respectively. Ascaris eggs have been used as an indicator for Helminths ova and have been mostly studied. Survival of helminths ova has been observed to be dependent on soil temperature, soil type and soil conditions (Maya et al., 2010; de Faria et al., 2017). Following sections present relation of different factors on persistence of helminths in biosolids-amended soil.

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Table 6. Summary of findings on field-based study on persistence of Ascaris in anaerobically-digested biosolids-amended soil

<table>
<thead>
<tr>
<th>Country/Climate Region</th>
<th>Soil Type</th>
<th>Application Type</th>
<th>Experimental Condition</th>
<th>Study Period (Days)</th>
<th>T90 (Days)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil, Tropical climate</td>
<td>Sandy soil; w/ Eucalyptus</td>
<td>Injection; rate=40 t/ha</td>
<td>temperature = 15 to 30°C; soil moisture=80%</td>
<td>364 days</td>
<td>49*</td>
<td>de Faria et al., 2017</td>
</tr>
</tbody>
</table>

*Time after which Ascaris reaches non detectable levels
Table 7. Summary of findings of a laboratory-based study (Williams et al., 2012) on persistence of *Ascaris spp.* in dewatered, mesophilic, anaerobically digested sludge biosolids-amended soil; Tucson, Arizona (USA); Hot semi-arid climate; Application type=Soil-biosolids mixture; temperature =2.8 to 34.3°C; exposure time= 30 days

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>T&lt;sub&gt;90&lt;/sub&gt; (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sandy loam</td>
<td>15</td>
</tr>
<tr>
<td>clayey soil</td>
<td>19</td>
</tr>
</tbody>
</table>

5.1. Soil temperature

This section documents effect of soil temperature on persistence of helminths in biosolids-amended soil. Effect of climate on survival of helminths has been studied extensively. In temperate climate regions, ova have been found to remain viable for up to 7 years in the soil (Smith et al., 1999), indicating a chance of exposure long after the application of biosolids on soil. Average time for persistence of viable *Ascaris spp.* ova in biosolids-amended soil was observed to be about 7 weeks under humid temperature (70% moisture, 20°C, conventional aerobically-digested activated sludge; de Faria et al., 2017). Two Brazilian studies (Thomaz-Soccol et al. (1999) and Souza et al. (2008) reported that helminths ova survived longer for 180 days in biosolids-amended soil in colder region than 3 weeks in other studied regions of Brazil.

5.2. Soil type

This section documents effect of soil type on persistence of helminths in biosolids-amended soil. Effect of soil type on survival of *Ascaris* ova has also been reported. Ova incubated in biosolids-amended clay loam was observed to survive more than ova incubated in sandy loam (Williams et al., 2012). For example, *Ascaris spp.* of dewatered, mesophilic, anaerobically digested sludge was observed to survive longer in clayey soil (T<sub>90</sub> =19 days) than in sandy loam soil (T<sub>90</sub> =15 days) (Williams et al., 2012; Table 7). For temperature ranging between 20 and 38°C, survival times for *Ascaris* in clay soil were observed to be greater than 90 days due to higher retention of moisture by clayey particles. Ova incubated at 25°C was observed to have a lower inactivation rate than that at 37°C. Temperatures above 50°C had been shown to shorten the inactivation duration of *Ascaris* to 2 hours (Kato et al., 2003).

6.0. Identified knowledge gaps and recommendations for future studies
A compilation of information related to decay of biosolids-associated indicator microorganisms and pathogens in soil was carried out with an emphasis of use of field-related information and some laboratory-related information. A general trend of decay of biosolids-associated bacteria and viruses in biosolids-amended soil with respect to soil temperature, soil moisture content, soil type, organic matter is shown in Figures 4 and 5, respectively. These figures represent the probable trends of decay of BAPs in different environmental conditions as observed from the studies reported. An exact trend of pathogen decay with environmental factors cannot be developed from little data available for some of the pathogens. A comparison of variations of $T_{90}$ with temperature for tropical and temperate climatic regions indicated that $T_{90}$ values decrease with temperature (Figure 4). Viruses also show similar type of trend in all climatic conditions. In case of bacterial pathogens, variation of $T_{90}$ with moisture content was observed to vary between two climatic conditions. $T_{90}$ value was observed to be high at high moisture content for tropical condition and low for temperate condition (Figure 5). The variation of decay behaviour under different climatic conditions also depend on other factors, like biotic stress, organic matter content, time period, etc. (Figures 4 and 5). 3D scatterplot has been shown to show effect of temperature and moisture content on persistence of $E. \text{coli}$ (bacteria), MS2 (Virus), $Ascaris$ (Helminth) (Figure 6). This plot (Figure 6) shows persistence of bacteria is longer than virus and helminth in given temperature and moisture condition keeping soil and biosolid same. Similar types of plots in different environmental condition (Temperature, moisture, soil type, biosolids type) would help in understanding factors affecting persistence in better way.

**Figure 4.** General trends showing effect on decay of biosolids-associated bacteria (shown in terms of $T_{90}$) due to: (a and b) temperature; (c and d) moisture content, (e) soil texture, biotic stress

![Figure 4](image1)

**Figure 5.** General trends showing effect on decay of biosolids-associated viruses (shown in terms of $T_{90}$) due to: (a) temperature; (b) moisture content, (c) extent of adsorption on soil or clay content

![Figure 5](image2)

**Figure 6.** Effect of persistence of biosolids-associated pathogens ($E. \text{coli}$, MS2, and $Ascaris$) as a function of temperature and moisture content in sandy soil amended with anaerobic digested sludge

![Figure 6](image3)
Table 8 presents a list of field-related important identified knowledge gaps which need to be addressed immediately. More fields-related studies are required for obtaining these types of information to make decisions using realistic field-based information with respect to biosolids application on soil.

<table>
<thead>
<tr>
<th>Gap Type</th>
<th>Impact of Identified Gaps</th>
<th>Possible Actions (References)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined effect of different factors on pathogen survival</td>
<td>Factors (i.e., soil pH, temperature, moisture content, biotic stress, competition for nutrients, etc.), interact with amongst each other and affect pathogen survival; findings of studies focusing on one factor-at-a-time do not provide complete information on survival when multiple factors simultaneously affect pathogen survival; information is useful in determining decay rate under different environmental condition (Simultaneous effect of temperature, moisture content, soil type, etc.)</td>
<td>Conduct more field studies (Lang et al., 2007; Cass, 2009; Gondim-Porto et al., 2017)</td>
</tr>
<tr>
<td>Decay patterns of viruses helminth, protozoa in biosolids-amended soil</td>
<td>Studies are available on decay behaviours of bacteria in biosolids-amended soil, but this type of information is not available extensively for viruses and protozoa. As biosolids carry both viruses and protozoa, chances of their exposures from biosolids-amended soil exist; information is useful in deciding setback distance and setback time using decay patterns information of all types of BAPs as $T_{90}$ values of different BAPs vary widely</td>
<td>Conduct more decay studies for different types of pathogens (Jenkins et al., 1999; Schwarz et al., 2013; de Faria et al., 2017)</td>
</tr>
<tr>
<td>Decay patterns based on field-related data</td>
<td>Information on decay rates for enteric pathogens in biosolids-amended soil is valuable more under laboratory conditions than under field conditions. Field studies present monitoring challenges as soil has varied physiochemical properties which could affect decay of pathogens differently; $T_{90}$ values obtained from field-related studies can be used for framing land application of biosolids guidelines</td>
<td>Conduct more field-related studies (Pepper et al., 1993; Gibbs et al., 1997; Lang et al., 2003; Lang et al., 2007; Zaleski et al., 2005; Eamens et al., 2006; Pourcher et al., 2007; Horswell et al., 2007; Elmahdy et al., 2018)</td>
</tr>
</tbody>
</table>

*BAP-biosolids associated pathogens

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References


The Persistence of Indicators and Pathogens in Wastewater Biosolids-amended Soil


