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Assessing the probability of infection by *Salmonella* due to sewage sludge use in agriculture under several exposure scenarios for crops and soil ingestion

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HIGHLIGHTS

- Nine scenarios for evaluating *Salmonella* risk by using sewage sludge in soil
- Regrowth of *Salmonella* spp. in soil and internalization in vegetables
- Annual risk for consumers of vegetables and field workers
- Sanitary and health measures need to be implemented.

GRAPHICAL ABSTRACT

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ABSTRACT

A deeper understanding about the risks involved in sewage sludge practice in agriculture is required. The aims of the present study were to determine the annual risk of infection of consuming lettuce, carrots and tomatoes cultivated in soil amended with sewage sludge. The risk to agricultural workers of accidental ingestion of sludge or amended soil was also investigated.

A Quantitative Microbial Risk Assessment was conducted based on *Salmonella* concentrations from five WWTPs were used to estimate the probability of annual infection associated with crops and soil ingestion. The risk of infection was estimated for nine exposure scenarios considering concentration of the pathogen, sewage sludge dilution in soil, variation of *Salmonella* concentration in soil, soil attachment to crops, seasonal average temperatures, hours of post-harvesting exposure, *Salmonella* regrowth in lettuce and tomatoes, *Salmonella* inhibition factor in carrots, crop ingestion and frequency of exposure, sludge/soil ingestion by agricultural workers and frequency of exposure. Annual risks values varied across the scenarios evaluated. Highest values of annual

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risk were found for scenarios in which the variation in the concentration of *Salmonella* spp. in both soil and crops (scenario 1) and without variation in the concentration of *Salmonella* spp. in soil and variation in crops (scenario 3) ranging from 10^{-3} to 10^{-2} for all groups considered. For agricultural workers, the highest annual risks of infection were found when workers applied sewage sludge to agricultural soils (2.26×10^{-2}). Sensitivity analysis suggests that the main drivers for the estimated risks are *Salmonella* concentration and ingestion rate. These risk values resulted from conservative scenarios since some assumptions were derived from local or general studies. Although these scenarios can be considered conservative, the sensitivity analysis yielded the drivers of the risks, which can be useful for managing risks from the fresh products chain with stakeholders' involvement.

1. Introduction

Sewage sludge has been recognized as a suitable component for soil amendment, and its use has been intensified in recent decades worldwide (Egan, 2013). Moreover, its usage can minimize environmental pollution in line with a global trend of exploiting some residuals generated in wastewater treatment plants (WWTP). Although in Brazil the application of sewage sludge remains restricted, it has been seen as a promising alternative for managing this residue. Brazilian sludge generation totaled around 372,000 tons/year in 2001 with the Sao Paulo Metropolitan Region alone responsible for 274,000 tons/year, representing a major contributor to sewage sludge generation in Brazil (UN-Habitat, 2008).

Although sewage sludge usage in agriculture is recognized worldwide, some issues regarding its quality and impact on human health must be taken into account, such as the presence of pathogens, an issue previously reported by several authors (Gerba and Smith, 2005; Jiménez et al., 2007; Pepper et al., 2008; Navarro et al., 2009). Hence, this usage may represent a public health concern if not applied properly.

Salmonella spp. are among the pathogens typically found in sewage sludge and their presence has been well documented in several studies (Iranpour and Cox, 2006; Horswell et al., 2007; Sidhu and Toze, 2009; Viau et al., 2011). These reports reveal that sludge sewage can potentially disseminate the pathogen if the sludge is applied to agricultural fields without sanitary criteria or the establishment of barriers, since this bacterium can remain viable in soil at 20 °C to 30 °C for anything from 30 to 968 days (Heaton and Jones, 2008); under certain conditions, such as moisture and carbon availability, even regrowth can be observed (Eamens et al., 2006; Gibbs et al., 1997). Several outbreaks associated with vegetable consumption and *Salmonella* spp. presence have been reported (Brandl, 2006; Hirneisen et al., 2012).

According to Francis et al. (2012), people' eating habits are shifting toward a healthier lifestyle. These changes include a higher intake of vegetables. Furthermore, studies have shown that climate change, which can cause droughts, floods and higher temperatures, may also increase the capacity of *Salmonella* spp. to infect pre-harvest leafy green vegetables and even increase their internalization (Liu et al., 2013; Ge et al., 2012).

A deeper understanding about the risks involved in this practice is required. The aims of the present study were to better clarify this important issue and determine the annual risk of infection of consuming lettuce, carrots and tomatoes (three of the most consumed vegetables in Brazil) cultivated in soil amended with sewage sludge. The risk to agricultural workers of accidental ingestion of sludge or amended soil was also investigated.

2. Material and methods

In order to assess the risk of infection by *Salmonella* spp. in sewage sludge, nine different scenarios were devised representing the following conditions:

a) Consumption of lettuce, tomatoes and carrots:

- presence or absence of variation in *Salmonella* spp. concentration in amended soils;
- presence or absence of *Salmonella* spp. regrowth in crops.

b) Accidental ingestion of soil or sludge by agricultural workers:

- Ingestion of amended soil in presence or absence of variation in *Salmonella* spp. concentration in amended soils;
- Direct ingestion of sewage sludge.

Table 1 summarizes all nine scenarios devised.

2.1. Concentrations of Salmonella spp. in raw sludge (C)

Data on the identification and concentration of *Salmonella* spp. in the five WWTPs was drawn from the study carried out by Krzyzanowski et al. (2014) in which quantification and characterization was performed according to EPA Method 1682 (USEPA, 2006): *Salmonella* spp. in sewage sludge by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium.

Data on *Salmonella* concentrations were clustered due to the different concentration patterns found in the five WWTPs (Table 2). The clustering was based on significance tests, where WTTPs were considered as equivalent when differences in *Salmonella* concentrations were not significant at a level of 0.1. Due to the high occurrence of samples with left-censored data (concentrations below the detection limit — DL) and ties, which preclude the use of tests based on normal distribution assumptions, a signal test proposed by Putter (1955) was applied (*ANU* — asymptotic uniformly most powerful nonrandomized test) for its conceptual simplicity and good empirical results (Coakley and Heise, 1996). WWTPs 1, 3 and 5 were grouped together, while WWTPs 2 and

Table 1
Description of the different scenarios devised in this study for evaluating annual risk of infection by *Salmonella* through ingestion of crops or by accidental ingestion of soil/sewage sludge.

Scenario	Risk of infection related to	Variation in *Salmonella* concentration in soil	Regrowth of *Salmonella* in crops
1	Lettuce/tomatoes	Present	Present
2	Lettuce/tomatoes	Present	Absent
3	Lettuce/tomatoes	Absent	Present
4	Lettuce/tomatoes	Absent	Absent
5	Carrots	Present	Inhibition
6	Carrots	Absent	Inhibition
7	Ingestion of soil + sludge by agricultural workers[a]	Present	NA
8	Ingestion of soil + sludge by agricultural workers[a]	Absent	NA
9	Direct ingestion of sludge by agricultural workers[b]	NA	NA

NA: not applicable.
[a] Workers who directly handle amended soil.
[b] Workers who directly apply sewage sludge in soil to be amended.