Use of biosolids in maize cultivation in small rural farms in Northeastern Brazil: Quantitative Microbial Risk Assessment Approach

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Abstract: In the northeastern region of Brazil, maize is extensively cultivated providing food security and income for small farmer families. The objective of this study was the application of the Quantitative Microbiological Risk Assessment (QMRA) methodology to evaluate the risks of using class B biosolids as an organic fertilizer in maize cultivation in a small rural property in this region of Brazil. For the calculations, it was considered information available in the scientific literature and laboratory data. During the hazard identification stage, the risk of infection was evaluated considering the pathogenic microorganisms: *Salmonella*, rotavirus, *Campylobacter*, *Cryptosporidium*, *Escherichia coli and Ascaris lumbricoides*. The results showed that the risks are higher during the application of biosolids. Regarding the maize crop cultivation, from 30 days of incorporation of biosolid into soil and taking into account the microbial decay in tropical soils, the calculated risks were tolerable according to the World Health Organization (WHO) recommendations. Special attention should be given to preventing the risk of *Ascaris lumbricoides* infection. Considering the low level of formal education of these farmers and the endemic presence of helminths in northeastern Brazil, it is imperative to implement technical assistance and risk prevention actions as the use of personal protective equipment (PPE), good hygiene practices and the construction of fencing to restrict children access to the maize crop area.
Keywords: agriculture, biosolids, microbial risk, low-income farms

1. Introduction

Initiatives for the valorization of sewage sludge in Brazil have been growing over the years due to the increase of its production and the restrictions of its final disposal in landfills, imposed by the current environmental legislation. After being submitted to processes of stabilization and disinfection, the sewage sludge is called biosolids and can be used for agricultural purposes, if it meets specific criteria in order to minimize the risk of environmental contamination and of causing problems to public health. In Brazil, the Directive 375/2006 of the National Environmental Council (CONAMA) [1] defines the criteria and procedures for the use of biosolids in agriculture. According to this legislation, biosolids are classified in classes A or B with specific requirements as to microbiological quality for each of these classes. For class A, the microbiological characteristics must meet the following criteria: i) thermotolerant coliforms $<10^3$ NMP per gram TS; (ii) viable helminth eggs < 0.25 egg.g^{-1} TS; iii) absence of *Salmonella* in 10g TS and iv) virus < 0.25 PFU.g^{-1} TS. For class B, the requirements are: (i) thermotolerant coliforms $<10^6$ MNP.g^{-1}TS and (ii) viable helminth eggs < 10 eggs per gram of TS. In addition, it is determined that class B biosolids cannot be applied to pasture and cultivation of olive groves, tubers and roots, and flooded crops, as well as other crops in which the edible part comes into contact with the soil. This Directive 375/2006 is supported by American and British legislation. However, on closer inspection, it should require epidemiological studies and risk analysis to justify the limits imposed. Especially in the case of helminth eggs, since for the less restrictive class it establishes a limit value of 10 eggs.g^{-1} TS while standards prevailing in other countries do not determine a specific value, mainly when considering that the climatic conditions in Brazil are less favorable to the survival of these pathogens in the soil.

One option to evaluate the risks is through the Quantitative Microbiological Risk Assessment (QMRA), a method that estimates the inherent risks of infection, disease or death of individuals considering scenarios of exposure to the pathogenic organisms, through dose-response models. The dose response model provides a quantitative relationship between the likelihood of adverse effects and the level of microbial exposure.

Regarding biosolids, the most common exposure scenarios include the risk to the consumer through food fertilized with biosolids, and the risk to the population from accidental ingestion of biosolid particles. This last scenario includes children who frequently put their hands into their mouths, and adults, especially farmers who work with the application of the biosolids while managing the soil-plant system. Varghese et al. [2] conducted a study in India to estimate the risks of Adenovirus contamination and concluded that the estimated risk was so negligible that after 30 days of soil contact it could be considered null. The authors then pointed out the need to consider soil microbial decay in agricultural risk studies of biosolids.

In Brazil, agriculture conducted on small family farms is the only source of food and income for most of the poor rural population and biosolids could be an affordable source of nutrients to ensure soil fertilization and increased production, and therefore, income, besides food security to these individuals. Hence, it is justified the need of studies that foment a discussion about the restrictions and the criteria imposed by the current Brazilian legislation in order to stimulate the safe practice of the use of biosolids in this country.

This study aimed to apply the QMRA to evaluate the microbiological risk of the use of biosolids classified as class B according to the CONAMA Directive 375/2006 [1] on the cultivation of maize in a family farming property located in the northeastern region of Brazil.

2. Methods

Initially, the QMRA methodology proposed by the USEPA [3] was used to qualitatively determine the risks of using biosolids (derived from class B) in agriculture. At this stage, the Problem Planning, the Scope and the Formulation were divided into two main items: i) characterization of the concern with infectious diseases and ii) characterization of the host or population that can be affected.

A representative scenario of the rural area of a small municipality in the *Bahia* State, Brazil was elaborated (Figure 1). The population data and the typology of small family farms were obtained from a Brazilian agricultural census published by the Brazilian Institute of Geography and Statistics [4]. The data show that approximately 84% of the rural properties in *Bahia* State are small family farms and it was adopted the concept of family agriculture indicated by the Federal Law 11326/2006 [5].

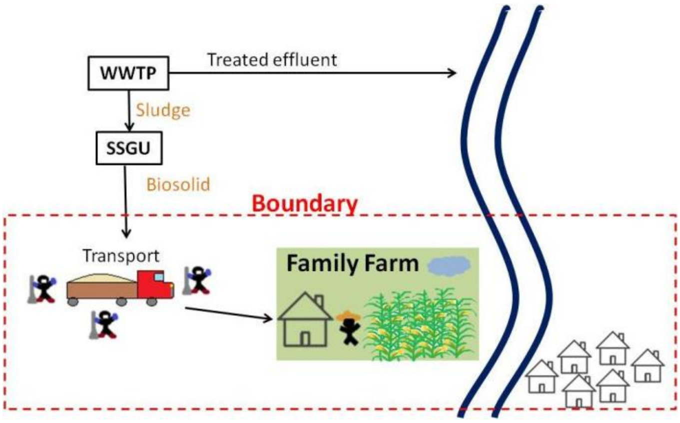

Fig 1. Scenario of the use of biosolids in small farms in northeastern Brazil

The maize crop was chosen because it is very widespread and distributed throughout the Brazilian northeast region and represents 53% of the temporary crops in the *Bahia* State. It can be stated that the cultivation of maize is indispensable to guarantee the food security of these families. The Brazilian legislation classifies this crop as temporary and according to data from the agricultural census cited above [4] the area of a small family farm occupied by temporary agriculture is equal to 4.42 hectares (average value). However, the area devoted to the construction of housing was subtracted and it was considered that the farmers could use part of this area to plant other crops such as legumes and cassava, for example. Therefore, it was admitted that the maize crop occupies 0.8 hectares of this small farms. Other assumptions used to elaborate the scenario are described in Table 1.

Table 1. Assumptions for application of biosolids in small family farms

Assumption	Reference
The product class B derived from sewage sludge (biosolids) came from an industrial facility called Sewage Sludge Generating Unit (SSGU). The raw sludge was discarded from an Upflow Anaerobic Sludge Blanket Reactor (UASB) and the steps of sludge treatment were dewatering, natural drying in beds and composting.	[1]
Appropriate trucks that avoid losses carried out the transport of biosolids between the SSGU and small farms.	[1]
The application rate of biosolids should be equal to 6 tons per hectare.	[6]
Maize cultivation cycle between 50 and 60 days (planting occurred during summer).	[1]
Avoid planting crops or other land management for a minimum period equal to 30 days after the application of biosolids.	[1]
Biosolid application must occur at least six months before manual harvest.	[1]
Use of mechanical biosolids spreaders, i.e. attachment to a tractor, for example.	[1]
Farmers must wear appropriate gloves and footwear during contact with the soil cultivated with biosolids.	[1]

The minimum removal for the pathogen concentration was considered in order to match the values provided by Directive 375/2006 [1]. the concentrations were defined as: 10 *Salmonella*.$(gTS)^{-1}$; 10 rotavirus.$(gTS)^{-1}$; 10 *Campylobacter*.$(gTS)^{-1}$; 10 oocysts of *Cryptosporidium*.$(gTS)^{-1}$. The value considered for *Campylobacter* was established according to the same value for *Salmonella*, due to the lack of data regarding its concentration in the sludge, and knowing that its removal can be of up to 5 log.
In addition to the proposed scenario and according to the variables necessary for the application of the QMRA, it was assumed that the exposure would result from the following activities: i) application to the soil resulting in accidental ingestion of biosolid particles at a given value of 50 $mg.d^{-1}$; and soil particles with the presence of biosolids starting from an average value of 50 $mg.d^{-1}$; ii) management of the soil-plant system, in which regarding the accidental soil particles intake, it was assumed that the management frequency would be equal to 2 days per week; that same frequency was established as the maximum limit of days that one child could be in contact with the environment, that is, 105 days a year.
It was also considered that the harvest takes place after a minimum period of 80 days and a maximum of 120, in both harvests – considering that the maize crop takes about 50 to 60 days, and that the Directive 375/2006 [1] establishes 30 days between the application of the biosolid and any manual work or handling. In addition, calculations were performed considering only one day between application and sowing; 30 days, which is the interval established for sowing; maximum time of cultivation of the maize crop of 60 days, in order to observe the variation of the risks on account of the number of days.
Considering the area of exposure (0.8 ha), in which the depth was equal to 20 cm and the soil density was approximately equal to 1.5 $g.cm^{-3}$, the dilution factor was equal to (0.002 or 1:500). It is necessary to take into account the difficulty of obtaining microbial decay data under climatic conditions similar to those found in Brazil, which results in uncertainties and approximations. Hence, the value of the *E. coli* decay constant (k = 0.012 $log.d^{-1}$) was obtained by Magalhães [7] during experiments conducted in Brazil. It was assumed the value of the constants decay for rotavirus established by Hurst et al. [8].
The mathematical model of beta-Poisson was used to calculate the risk associated with a single exposure to bacteria, viruses and helminths. The exponential model was applied to evaluate the same risk of exposure to protozoa. The values of the parameters (α, β and r) of the dose-response models were obtained in USEPA [3] and Bastos et al. [9]. Table 2 shows the information and parameters that were used for risk modeling and calculation.

Table 2. Considerations and values of the parameters applied during the QMRA of maize cultivation in the small farms located in the northeastern Brazil

Microorganism	Pathogens concentration ($org.gTS^{-1}$)	Dilution factor	Microbial decay in soil		Biosolids ingestion (adults)	Biosolids ingestion (children)	One-year exposure frequency (days)
			k ($log_{10}.d^{-1}$)	Time (days)			
Salmonella spp.	10	1:500	0.246				
Campylobacter jejuni	10	1:500	0.63				
Rotavirus	10	1:500	0.11	a; b			
Cryptosporidium parvum	10	1:500	0.076		0.05 g	0.5 g	105
E. coli	1.2×10^2	1:500	0.012				
Ascaris Lumbricoides	0.25	1:500	-				

Legend: a - The interval between application of biosolids and sowing was equal to 180 days (recommendation published in the CONAMA Directive 375/2006); b - Three scenarios were elaborated to perform the calculations. First, it was considered only one day between biosolids application and sowing; this interval was equal to 30 days in the second scenario and, finally, 60 days, which is the cycle of maize cultivation

It is necessary to emphasize it was not considered the soil dilution factor and the microbial decay for the calculation of the risk associated with the accidental ingestion of biosolids. These factors are only relevant for a scenario that considers the application of biosolids.

3. Results and Discussion

The daily and annual estimated risk of infection associated with accidental ingestion of biosolid particles during the application was higher for rotavirus contamination (7.3×10^{-1} pppa). The annual risks took into account that the application would happen twice a year (for two crops). As the numbers refer to multiple exposures, the values obtained are higher since they provide the information that the individual will be in contact with that pathogen twice a year, which may cause an infection. In addition, all values are above the value considered to be tolerable by WHO (10^{-6} pppa) [10] and USEPA (10^{-4} pppa) [11]. However, it should be emphasized that the proposed scenario considered direct contact, in which workers would be without personal protective equipment (PPE), which interferes with the results obtained for adults; and that there would be no restriction of access to the public, as fences, for example, implying the possible presence of children in the area. In addition, even hand washing reduces the risks associated with inadvertent ingestion of particles.

On the other hand, considering the requirements of the Directive 375/2006, for the management of the soil-plant system, the results were significantly lower than that found for the application (1.0×10^{-14} pppa). Certainly, these results are justified by the adoption of an interval between the application of biosolids in the soil, and sowing which was equal to 180 days. The result of the daily risk estimates of E. coli infection (4.4×10^{-4} pppa) was higher than that of the other pathogens. However, these values are considered tolerable by WHO (10^{-6}) [10].

However, it should be noted that these values represent multiple exposures, that is, the risk calculation is performed considering that during all 105 days in which the soil management for maize planting and harvest occurs, the farmer has direct contact with the pathogenic microorganisms present in the biosolids. For the presence of helminths, the value of the risk is higher than that calculated for all other pathogens. This may have occurred because no decay of helminths in the soil was considered. However, only the annual risks are above the limits established by USEPA [11].

Therefore, safety and preventive measures such as hygienic practices, the use of PPE, the construction of fences and the security signage of the area fertilized with biosolids are essential measures to minimize risks. When considering the current level of information and environmental education of these Brazilian small farmers, it becomes evident the demand for public policies of technical assistance and education to promote the practice of using biosolids in family agriculture.

Table 3 shows the risks of infection associated with the accidental ingestion of soil particles with biosolids during the management of maize planting and harvesting, reducing the time interval between fertilizer spreading and sowing recommended by Directive 375/2006.

Table 3. Daily and annual risk of infection (pppa) estimated for time intervals ≤ 60 days

Microorganism	Interval	Daily risk Adults	Daily risk Children	Annual risk Adults	Annual risk Children	Reference value (WHO)
Salmonella spp.	1 day	$1.3x10^{-5}$	$1.3x10^{-4}$	$1.4x10^{-3}$	$4.7x10^{-2}$	
	30 days	$9.8x10^{-13}$	$9.8x10^{-12}$	10^{-10}	10^{-9}	$1.0x10^{-6}$
	60 days	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	
Campylobacter jejuni	1 day	$4.4x10^{-6}$	$4.4x10^{-5}$	$4.6x10^{-4}$	$1.6x10^{-2}$	
	30 days	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	$3.1x10^{-4}$
	60 days	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	$<10^{-14}$	
Rotavirus	1 day	$4.8x10^{-4}$	$4.8x10^{-3}$	$4.9x10^{-2}$	$8.2x10^{-1}$	
	30 days	$3.1x10^{-7}$	$3.1x10^{-6}$	$3.3x10^{-5}$	$3.3x10^{-4}$	$7.7x10^{-4}$
	60 days	$1.6x10^{-10}$	$1.6x10^{-9}$	$1.6x10^{-8}$	$1.6x10^{-6}$	
Cryptosporidium	1 day	$3.5x10^{-5}$	$3.5x10^{-4}$	$3.6x10^{-3}$	$3.6x10^{-2}$	
parvum	30 days	$2.2x10^{-7}$	$2.2x10^{-6}$	$2.3x10^{-5}$	$2.3x10^{-4}$	$2.2x10^{-3}$
	60 days	$1.2x10^{-9}$	$1.2x10^{-8}$	$1.2x10^{-7}$	$1.2x10^{-6}$	
E. coli	1 day	$5.9x10^{-5}$	$5.9x10^{-4}$	$6.2x10^{-3}$	$6.0x10^{-2}$	
	30 days	$2.7x10^{-5}$	$2.7x10^{-4}$	$2.8x10^{-3}$	$2.7x10^{-2}$	$1.0x10^{-6}$
	60 days	$1.2x10^{-5}$	$1.2x10^{-4}$	$1.2x10^{-3}$	$1.2x10^{-2}$	
Ascaris Lumbricoides	1 day	-	-	-	-	
	30 days	$5.9x10^{-5}$	$5.9x10^{-4}$	$6.2x10^{-3}$	$6.0x10^{-2}$	$1.0x10^{-6}$
	60 days	-	-	-	-	

It can be stated that for a period of more than 30 days of the spreading and mixing of biosolids with the soil, the daily risk of infection was lower than the values recommended by WHO [10]. Only the risk associated with infection by *E. coli* exceeded the maximum value allowed in a log house. However, when considering the values recommended by the USEPA [11], this risk is tolerable.

Although the values of the microbial decay constants may have been overestimated, it should be remembered that in this study the value of the amount of biosolids accidentally ingested was higher than the values usually used to calculate the risk. Lagoy [12] established that the maximum daily value of accidental ingestion of soils by children was equal to 250 $mg.d^{-1}$ and for adults, 50$mg.d^{-1}$. Oliveira et al.

[13] evaluated the persistence of pathogenic microorganisms for 258 days in a tropical clayey soil amended with human class B biosolids and cultivated with sugarcane. The authors concluded that after 35 days, no enterovirus was detected in any sample and demonstrated that the persistence time for viable *Ascaris* spp. eggs in soil was less than 41 days.

4. Conclusions

Regarding the risks associated with the maize cultivation in soils amended with biosolids class B in the Brazilian northeast, the QMRA approach allowed to conclude that:

- The risks are more significant during spreading and incorporation of biosolids into the soil. On the other hand, the risks are insignificant during the subsequent steps (planting and harvesting).
- In order to mitigate the risks, it is necessary to encourage the use of personal protective equipment, good hygiene practices and mechanized application of biosolids, as recommended by the CONAMA Directive 375/2006. Considering the current stage of information for small farmers in northeastern Brazil, the adoption of these practices is a great challenge.
- For the safe dissemination of the practice of application of biosolids class B in the cultivation of maize in small agricultural properties in the Brazilian northeast it is necessary to obtain values of decay constants of microorganisms taking into take into account the soil characteristics and climatic conditions at local level.

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