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Enteropathogenic bacterial contamination of a latosol following application of organic fertilizer

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ABSTRACT. Poultry manure is used as fertilizer *in natura*, but little is known about whether it contaminates the soil with pathogenic organisms. The aim of this study was to assess the effects of organic, organomineral and mineral fertilizers on soil contamination by enteric pathogens, using poultry manure as the organic fertilizer. Manure was applied in field experiments at rates of 7.0 ton. ha^{-1} (maize crop, 2008/2009), 8.0 ton. ha^{-1} (wheat crop, 2009) and 14 ton. ha^{-1} (maize crop, 2010/2011). Organomineral fertilizer was applied at the same rates but was comprised of 50% manure and 50% mineral fertilizer. At 30 and 70 days after fertilization, the organic fertilizer and the upper 0-5 cm layer of the soil were tested for the presence of helminth eggs and larvae and enteropathogenic bacteria. Fecal and non-fecal coliforms (*Escherichia coli* and *Clostridium perfringes*) were found in the organic fertilizer, but neither *Salmonella* spp. nor enteroparasites were detected. The population of enteropathogenic bacteria in the soil was similar among the treatments for all crops at both evaluation times. The population of thermotolerant coliforms in the organic fertilizer was larger than the maximum level allowed in Brazil, but neither the organic or nor the organomineral fertilizer contaminated the soil.

Keywords: soil contamination, soil quality, *Triticum aestivum*, *Zea mays*.

Contaminação de bactérias enteropatogênicas em um latossolo adubado com fertilizante orgânico

RESUMO. O esterco de poedeira *in natura* é utilizado como fertilizante, mas pouco se sabe se esta prática pode contaminar o solo com organismos patogênicos. O objetivo do trabalho foi o de avaliar o efeito da adubação orgânica, organomineral e mineral na contaminação do solo com estes organismos, utilizando o esterco de poedeira como fertilizante orgânico. Foram aplicadas 7,0 (milho 2008/2009), 8,0 (trigo 2009) e 14 t ha^{-1} (milho 2010/11) de esterco, em experimentos a campo. A adubação organomineral consistiu em 50% destas quantidades e 50% das quantidades aplicadas com fertilizantes minerais. Após 30 e 70 dias da adubação, avaliou-se a ocorrência de ovos e de larvas de helmintos e de bactérias enteropatogênicas no fertilizante orgânico e destes microrganismos na camada de 0-5 cm do Latossolo. Coliformes totais e fecais (*Escherichia coli* e *Clostridium perfringens*) foram constatados no fertilizante orgânico, enquanto *Salmonella* sp e os enteroparasitas não foram detectados. A população de bactérias enteropatogênicas do solo foi similar entre os tratamentos, em todas as culturas e ambas épocas de avaliações. A população de coliformes termotolerantes do fertilizante orgânico foi maior que o máximo permitido no Brasil, mas a adubação orgânica ou organomineral não contaminou o solo.

Palavras-chave: contaminação do solo, qualidade do solo, *Triticum aestivum*, *Zea mays*.

Introduction

The application of agricultural residues as organic fertilizer has grown in Brazil and worldwide. As a result of the increased generation of these residues, their application as fertilizer has increasingly been the subject of environmental (DELGADO et al., 2012; GALLOWAY et al., 2003; HOULBROOKE et al., 2004) and agronomic (MAGUIRE et al., 2011) studies. The generation of poultry manure is increasing as the egg industry grows in response to domestic and international markets, enabling the application of raw (untreated) manure as fertilizer for agricultural soils (DEMIR et al., 2010; FIGUEROA et al., 2012). Studies by Demir et al. (2010) and Figueroa et al. (2012) demonstrate the beneficial effects of the application of organic fertilizers including of the application of

poultry manure in high doses (tons per ha), which has a low concentration of nutrients compared to mineral fertilizers.

However, the application of high doses of organic fertilizers has the potential to contaminate soil and water with enteropathogenic microorganisms and enteroparasites (HAWKE; SUMMERS, 2006; MACIOROWSKIA et al., 2004; UNC; GOSS, 2004). Although contamination by these organisms is of concern given the risk associated with the transmission of infectious and parasitic diseases through the food chain (MACIOROWSKIA et al., 2007; SILVA et al., 2005), other studies show that enteric organisms either do not survive or have a high rate of decline in fertilized soil (GAGLIARDI; KARNS, 2002; GARCÍA-ORENES et al., 2007; TALLON et al., 2007; ZIEMER et al., 2010). The discrepancies in the literature about enteropathogenic bacterial contamination of soil are related to the many different factors that affect the survivability of enteric organisms in the soil, including the type of organic fertilizer; fertilized crop, management and dosage; the use of mitigation practices; and environmental factors such as soil type, pH, humidity and temperature (MACIOROWSKIA et al., 2004; FORSLUND et al., 2011). However, the survival of pathogenic organisms in latosols fertilized with poultry manure has not been studied in Brazilian soils and climate conditions, and there are only a few reports from other countries (McMURRY et al., 1998). This lack of data makes predicting the environmental impacts of fertilization with poultry manure difficult; however, these impacts are of great concern (MACIOROWSKIA et al., 2004; SANTOS et al., 2010).

The aim of this study was to assess the effects of organic and organomineral fertilization with raw poultry manure on soil contamination by enteropathogenic organisms.

Material and methods

The study was conducted at the University of Passo Fundo (Universidade de Passo Fundo) and the field experiments were located in the municipality of Coxilha, Rio Grande do Sul, Brazil (28° 10.475' S, 52° 20.840' W). The region has an average altitude of 676 m, an average annual rainfall of 1,786 mm and an average annual temperature of 17.5ºC. The soil in the study area is a dystrophic Red Latosol and has been managed as a no-till farming system for 19 years.

Prior to the experiment, soil was sampled and analyzed according to Tedesco et al. (1995). Soil attributes were as follows: 29 and 172 mg dm^{-3} extractable P and K (Mehlich-I), respectively; 0.2, 6.1 and 2.5 $cmol_c$ dm^{-3} exchangeable Al, Ca and Mg, respectively; 26 mg dm^{-3} exchangeable Mn (potassium chloride, 1 mol L^{-1}); 5.3 pH in water (soil suspension:extractor 1:1) and 5.5 SMP index (soil suspension:extractor 1:1.5); 17 mg dm^{-3} available S (calcium phosphate, 500 mg P L^{-1}); 0.7 mg dm^{-3} available B (hot water); 0.9 and 2.4 mg dm^{-3} available Zn and Cu, respectively (hydrochloric acid, 0.1 mol L^{-1}); 31 g dm^{-3} organic matter (sulfochromic solution method and spectrophotometric quantification of reduced chromium); and 470 g dm^{-3} clay (densimeter).

The manure used as organic fertilizer was generated for five days on mats inside a poultry house and stored in the poultry house until being transported to the study area. Prior to fertilizer application, the fertilizer material was stored in stacks on the field. A 2.0 dm^{-3} sample of the fertilizer was collected from the stacks and analyzed for pathogens and physicochemical soil properties. Subsamples were taken from five locations in the stacks at a depth of 0.40 m using a shovel. The subsamples were pooled and packaged in sterile plastic bags inside Styrofoam boxes prior to being transported to the laboratory. The analyses of pathogenic organisms began on the day of sample collection.

To test the manure for the presence of viable helminth eggs, 4 g of wet manure sample were processed according to the Willis-Mollay Flotation Technique using a saturated NaCl solution with a density of 1,182 (m v^{-1}). The analysis of parasite larvae was performed using the Barmann method with an optical microscope with a 10x eyepiece and a 3.2x objective. The multiple-tube method was used for the analysis of total coliforms and thermotolerant coliforms, and the most probable number (MPN) was determined. The initial dilution was 1:10 (10^{-1}) in 10 g of wet sample diluted in 90 mL of 0.1% peptone water. Subsequent dilutions went up to 10^{-5}, using five tubes per dilution (EATON et al., 2005). For the *Salmonella* spp. assay, a 10 g aliquot of the wet sample was homogenized in 90 mL of 1% peptone water and incubated at 36±1°C for 18 to 24 hours. After pre-enrichment, the sample was inoculated in selective broths and plated onto selective and differential agars; genetically compatible colonies were confirmed through biochemical and serological tests (DOWNES; ITO, 2001).

The population of thermotolerant coliforms found in the manure was compared to the limits set

by the Normative Instruction no. 27/2006 of the Department of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento - MAPA) (BRASIL, 2006). Assays to test for the presence of total coliforms and *Salmonella* spp. were performed to complement the information from the other contamination indicators. Total coliforms and *Salmonella* spp. have been used as indicators of fecal contamination (GARCÍA-ORENES et al., 2007; MACIOROWSKIA et al., 2004). *E. coli* and *C. perfringes* were also tested since for because they are commonly found in poultry manure (SCHOCKEN-ITURRINO et al., 2010).

For the physicochemical analyses, the samples of organic fertilizer were dried at 65°C in a convection oven for 16 h, ground and sieved (10 mesh) and then analyzed in duplicates according to Tedesco et al. (1995). Organic C was quantified by the Walkley-Black method with external heating. Total N, P, K, Ca and Mg were determined by acid digestion (H_2O_2 + H_2SO_4) and a digestion mixture. Mineral N content was extracted with 1 mol L^{-1} KCl. Sulfur (S) and micronutrient (Zn, Fe, Mn and Cu) content were determined using nitro-perchloric digestion, except for B content, which was extracted through dry combustion in a muffle furnace at 600°C for 1 hour (Table 1).

The field treatments included three types of fertilization: organic, organomineral and mineral fertilization (Table 2). Fertilizer dosage was based on the concentration of total N in the organic fertilizer, the organic matter content of the soil, the expected grain yield of the fertilized crop and the type of plant residue from the previous crop (CQFS, 2004).

Table 1. Total concentration of some physicochemical properties in poultry manure used as organic fertilizer for grain crops. The results are expressed in wet mass of fertilizer.

Property	Maize (2008/2009)	Wheat (2009)	Maize (2010/2011)
Organic C (g kg^{-1})	120.0	87.1	68.0
Total N (g kg^{-1})	19.75	16.0	10.0
Mineral N (g kg^{-1})	1.61	0.50	0.90
P_2O_5 (g kg^{-1})	11.5	8.0	7.0
K_2O (g kg^{-1})	7.3	4.0	7.0
Ca (g kg^{-1})	24.7	17.2	25.5
Mg (g kg^{-1})	1.2	1.1	1.2
S (mg kg^{-1})	0.8	0.3	0.6
Zn (mg kg^{-1})	72.42	42.10	73.21
Fe (mg kg^{-1})	305.66	428.80	314.56
B (mg kg^{-1})	32.28	17.49	30.53
Mn (mg kg^{-1})	61.4	58.33	67.20
pH in $CaCl_2$	8.1	8.7	8.3
Water (g kg^{-1})	670.0	741.0	739.5

The treatments were applied to two crops of maize (*Zea mays*) and one crop of wheat (*Triticum aestivum* L.) planted in succession. After the wheat crop, soybean (*Glycine max* (L.) Merrill) was planted, followed by radish (*Raphanus sativus* L.). The fertilization treatments were not applied to the soybean and radish crops. After the radish cultivation, the treatments were reapplied to a second crop of maize.

Table 2. Fertilizer type and content of nitrogen (N), phosphorus (P_2O_5) and potassium (K_2O) for fertilizers applied to grain crops grown in a red latosol.

Fertilizer/Nutrient	Mineral	Organomineral		Organic
	Maize cultivation (2008/2009)			
	Sowing			
Fertilizer	NPK 08-25-20	NPK 08-25-15	Manure	Manure
Fertilizer (kg ha^{-1})	250	125	3,500	7,000
N, P_2O_5 and K_2O (kg ha^{-1})	20, 62 and 50	10, 31 and 25	69, 32[1] and 26	138, 64[1] and 51
	Top-dressing			
N (kg ha^{-1} as urea)	120	60		0
	Sowing + top-dressing			
N, P_2O_5 and K_2O (kg ha^{-1})	140, 62 and 50	139, 63 and 51		138, 64 and 50
	Wheat cultivation (2009)			
	Sowing			
Fertilizer	NPK 15-25-15	NPK 15-25-15	Manure	Manure
Fertilizer (kg ha^{-1})	200	100	4,000	8,000
N, P_2O_5 and K_2O (kg ha^{-1})	30, 50 and 30	15, 25 and 15	64, 26[1] and 16	128, 51[1] and 32
	Top dressing			
N (kg ha^{-1} as urea)	90	41		0
	Sowing + top-dressing			
N, P_2O_5 and K_2O (kg ha^{-1})	120, 50 and 30	120, 51 and 31		128, 51[1] and 32
	Maize Cultivation (2008/09)			
	Sowing			
Fertilizer	DAP and KCl	DAP and KCl	Manure	Manure
Fertilizer (kg ha^{-1})	170 and 164	85 and 82	7,000	14,000
N, P_2O_5 and K_2O (kg ha^{-1})	31, 78 and 98	15.5; 39 and 49	64, 26[1] and 16	140, 78[1] and 98
	Top-dressing			
N (kg ha^{-1} as urea)	109	60.5		0
	Sowing + top-dressing			
N, P_2O_5 and K_2O (kg ha^{-1})	140, 78 and 98	140, 78 and 98		140, 78[1] and 98

[1]Amount of available P_2O_5 as estimated with the efficiency index of 0.8.

The experimental design consisted of randomized blocks with four replicates. The area of the plots was 240 m^2 (20 x 12 m) with 2.5-m alleys between the plots and a 1.25-m border strip at either end of each plot. The amount of organic fertilizer varied with crop type, corresponding on a wet basis, to 7.0 t ha^{-1} (maize 2008-2009), 8.0 t ha^{-1} (wheat) and 14.0 t ha^{-1} (maize 2010-2011). The quantities of mineral fertilizer used in the mineral and organomineral fertilizer treatments were designed to match the amount of N, P and K applied in the organic fertilizer treatments (Table 2). The manure was applied with a truck equipped with broadcast spreader with a 12-m wide swath. Fertilizer application was performed with a seeder fertilizer machine in the planting furrows (P and K on maize) or manually by top-dressing (Urea on both maize and wheat, and P and K on this last crop).

At 30 and 70 days after fertilizer application, soil samples were taken for the enteropathogenic bacteria assays. Because of the limited vertical mobility of these bacteria in clayey soils (TALLON et al., 2007) and because the fertilizer was applied by top-dressing without incorporation into the soil, a shallow soil layer (0-5 cm) was sampled. The indicators of fecal contamination for the soil samples were also used to test for contamination in the organic fertilizer, as there is no specific national legislation indicating which microorganisms should be monitored in soils contaminated with animal manure.

Results and discussion

Enteroparasites and enteropathogenic bacteria in organic fertilizers

No helminth larvae or eggs were observed in the organic fertilizers applied to any of the crops (Table 3). The results shown in Table 3 are consistent with the quality standards for organic fertilizers, which allow a maximum of one viable egg per 4 g of dry matter (BRASIL, 2006). These results differ from those of a study by Santos et al. (2010), who noted the occurrence of helminth eggs in poultry fertilizers. The inconsistency between the results of this study and the results reported by Santos et al. (2010) may be related to temporal variability in parasite colonization of poultry manure or to characteristics of the fertilizer sample used by these authors. In the Santos et al. (2010) study, the organic fertilizer was stored for 120 days in compost heaps, whereas in the experiment in this study, the manure was deposited on the soil a few days after it was produced. Furthermore, Santos et al. (2010) warn that the presence of helminth eggs in their manure samples should be interpreted with caution because an error could have resulted from a flaw in their sampling procedures. The samples most likely represented the outer layers of the compost heap, which remain cooler in temperature than the central layers and are therefore less damaging to enteropathogenic organisms.

Table 3. Presence or absence of helminth viable eggs and larvae in poultry manure prior to the application of the manure as an organic fertilizer for three grain crops.

Crop (agricultural year)	Total solids	Eggs	Larvae
	(%)		
Maize (2008/2009)	33.0	Absent	Absent
Wheat (2009)	18.0	Absent	Absent
Maize (2010/2011)	26.1	Absent	Absent
Mean	25.7	Absent	Absent

The MAPA normative instruction no. 27/2006 restricts the population levels of enteropathogenic microorganisms and prohibits the occurrence of enteroparasites in organic fertilizers (BRASIL, 2006). Unlike helminth eggs and larvae, enteropathogenic bacteria were found in the samples of the organic fertilizers applied to the three grain crops (Table 4). Among the detected bacteria, there was a predominance of total coliforms and thermotolerant coliforms, with the latter group representing the majority of the coliforms. The presence of coliform bacteria is typical in animal manure, which is why thermotolerant coliforms are the preferred indicators of enteric organisms of homeothermic animals in organic fertilizer analyses. In general, thermotolerant coliforms comprise over 90% of total coliforms in animal manure, including poultry manure (SCHOCKEN-ITURRINO et al., 2010).

The most probable number (MPN) of thermotolerant coliforms in the organic fertilizers ranged from 3.3 x 10^5 (maize cultivation, 2008/2009) to > 9.0 x 10^7 (wheat cultivation, 2009), with an estimated mean of 3.3 x 10^5 per gram of dry matter (DM). These values are higher than the maximum values for organic fertilizers allowed by the MAPA in Brazil (BRASIL, 2006) and were found in all of the samples (Table 4). Although these MPN values were expected, the values are considered to be high because they exceed 30% of the maximum allowed value (Table 4). Moreover, these values are substantially higher than the reference value of quality for Class 1 watercourses in Brazil, which is less than 200 MPN for a 100 mL water sample.

Table 4. Enteropathogenic bacteria in poultry manure[1] used as organic fertilizer for grain crops.

Crop (year)	Total coliforms	Thermotolerant coliforms	*Escherichia coli*	*Salmonella* spp.	*Clostridium perfringes*
	.....MPN[3] g^{-1}.....			In 10 g	CFU[4] g^{-1}
Maize (2008/2009)	5.2×10^6	3.3×10^5	6.1×10^2	Absence	6.4×10^3
Wheat (2009)	$> 9.0 \times 10^7$	$> 9.0 \times 10^7$	7.5×10^3	Absence	1.7×10^6
Maize (2010/2011)	1.1×10^7	6.7×10^6	8.4×10^3	Absence	8.8×10^4
Mean	$> 3.5 \times 10^7$	$> 3.2 \times 10^7$	5.5×10^3	Absence	5.9×10^5
MVA[2]	-	1.0×10^3	-	Absence	-

[1]Results expressed as dry mass of organic fertilizer. Water content (kg t^{-1}): 670 (maize, 2008/2009), 741 (wheat, 2009) and 739.5 (maize, 2010/2011). [2]Maximum value allowed in organic fertilizer according to Normative Instruction no. 27/2006 (BRASIL, 2006). [3]Most probable number. [4]Colony-forming unit.

E. coli and *C. perfringes* were also found in all of the analyzed samples (Table 4). The populations of these two species were smaller that the populations of thermotolerant coliforms, but had relatively high mean values (MPN $g^{-1} = 5.5 \times 10^3$ and 5.9×10^5 for *E.coli* and *C. perfringes*, respectively). *E. coli* is one of the main specie in the coliform group and is typically used in the control of drinking water quality. This bacterium is found in the intestinal tract of birds and rarely propagates in other environments. In contrast, *Salmonella* spp. was the only species absent from all of the analyzed samples, which is acceptable under the MAPA standard (Table 4).

Enteropathogenic bacteria in soil

The MPNs of thermotolerant coliforms in the organic fertilizers were higher than the maximum value allowed by the MAPA (Table 4). Thus, these fertilizers should be treated before application to agricultural soils to avoid risk of environmental contamination (TIQUIA; TAM, 2002; SANTOS et al., 2010). However, poultry manure has been applied *in natura* to grain crops and is necessary to determine the survivability of enteropathogenic bacteria that are transferred to soils. The soil analyses in this study indicated that thermotolerant coliform populations were significantly smaller in the fertilized soils compared to the organic fertilizers (Table 4) for all three crops at both evaluation times (Tables 5 and 7).

Although thermotolerant coliforms and *E. coli* were found in the organic fertilizers, these bacteria were not detected (MPN $< 1.8 \times 10^2$ g^{-1}) in the soil samples collected from any of the three crops at either evaluation time (Tables 5 and 7). The bacteria did not survive in the fertilized soil, even in the wheat plots, which received an application of organic fertilizer with a higher density of thermotolerant coliforms (MPN $> 9.0 \times 10^7$ g^{-1} dry matter [DM]) than the organic fertilizer applied to maize (MPN $\leq 6.7 \times 10^6$ g^{-1} DM) (Table 4).

In addition to high levels of thermotolerant coliforms, the organic fertilizer used on the wheat crop contained a higher amount of colony-forming units of *C. perfringes* than the fertilizer applied to maize (Table 4). However, at 30 days after fertilization (DAF), the difference in population density between the soils fertilized with organic fertilizer or organomineral fertilizer (CFU $< 2{,}0$ g^{-1}) and the soils treated with mineral fertilizer was negligible and was determined as not significant by a t test. The same result was observed for total coliforms at 70 DAF (Table 6). *Salmonella* was not found in the soil samples from any of the treatments, which was expected given the absence of *Salmonella* in the organic fertilizer (unlike the other bacteria in this study, which were present in the organic fertilizer) (Table 4).

Among the bacteria that were tested for, only total coliforms and C. perfringes were detected in the soil samples. The MPNs of these organisms were low and generally did not differ among fertilization treatments for the three crops and the two evaluation times. The population of total coliforms decreased during the period from 30 DAF to 70 DAF, and C. perfringes was not detected in the samples at 70 DAF (Tables 5, 6 and 7).

Table 5. Enteropathogenic bacteria in a latosol 30 and 70 days after the application of different types of fertilizer to maize (agricultural year 2008/2009).

Type of fertilization	Total coliforms	Thermotolerant coliforms	*Escherichia coli*	*Salmonella* spp.	*Clostridium perfringes*
	.....MPN[1] g^{-1}.....			In 10 g	CFU[2] g^{-1}
		30 days after fertilization			
Organic	$11.5 \times 10^{4\,ns}$	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$1.8 \times 10^{2\,ns}$
Organomineral	6.4×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	1.9×10^2
Mineral	3.2×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	1.9×10^2
		70 days after fertilization			
Organic	$0.5 \times 10^{4\,ns}$	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$
Organomineral	1.4×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$
Mineral	3.0×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$

[ns]Non-significant difference between means in the column when comparing two treatments by a t test ($p < 0.05$). [1]Most probable number. [2]Colony-forming unit.

Table 6. Enteropathogenic bacteria in a latosol 30 and 70 days after the application of different types of fertilizer to wheat (agricultural year 2009).

Type of fertilization	Total coliforms	Thermotolerant coliforms	*Escherichia coli*	*Salmonella* spp.	*Clostridium perfringes*
	.....MPN[1] g^{-1}.....			In 10 g	CFU[2] g^{-1}
		30 days after fertilization			
Organic	4.1×10^4 ns	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	2.0×10^2 a
Organomineral	6.7×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	2.2×10^2 a
Mineral	7.1×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	0.2×10^2 b
		70 days after fertilization			
Organic	1.2×10^4 a	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$<1.0 \times 10^1$
Organomineral	0.3×10^4 b	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$<1.0 \times 10^1$
Mineral	0.3×10^4 b	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$<1.0 \times 10^1$

ns, a, b Non-significant (ns) or significant (a, b) difference between means in the column when comparing two treatments by a t test ($p < 0.05$). [1]Most probable number. [2]Colony-forming unit.

Table 7. Enteropathogenic bacteria in a latosol 30 and 70 days after the application of different types of fertilizer to maize (agricultural year 2010/2011).

Type of fertilization	Total coliforms	Thermotolerant coliforms	*Escherichia coli*	*Salmonella* spp.	*Clostridium perfringes*
	.....MPN[1] g^{-1}.....			In 10 g	...CFU[2] g^{-1}...
		30 days after fertilization			
Organic	5.4×10^4 ns	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$
Organomineral	1.6×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$
Mineral	3.3×10^3	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$
		70 days after fertilization			
Organic	1.6×10^4 ns	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$
Organomineral	1.0×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$
Mineral	0.3×10^4	$< 1.8 \times 10^2$	$< 1.8 \times 10^2$	Absence	$< 1.0 \times 10^1$

nsNon-significant difference between means in the column when comparing two treatments by a t test ($p < 0.05$). [1]Most probable number. [2]Colony-forming unit.

The absence of thermotolerant coliforms, E. coli and Salmonella demonstrate that the organic and organomineral fertilizers did not contaminate the soil. These results indicate that the enteropathogenic bacteria in the organic fertilizer either did not survive in the fertilized soil (such as in the case of the thermotolerant coliforms and *E. coli*) or had a high rate of decline (such as in the case of the total coliforms and *C. perfringes*), regardless of the simultaneous use of mineral fertilizer.

Many studies suggest that the enteropathogenic bacteria in organic fertilizers cannot compete with native microbiota and have low survival rate in soils fertilized with organic residues (GARCÍA-ORENES et al., 2007; MACIOROWSKIA et al., 2004; TALLON et al., 2007). This may be due to the adaptation of the enteropathogenic bacteria to an environment that is very different from the soil, as animal intestines that are characterized by low redox potential, high moisture content and high nutrient concentrations relative to soil (MACIOROWSKIA et al., 2004). Furthermore, the soil attenuation capacity was sufficient to prevent the survival or growth of the enteropathogenic organisms added to the soil in the organic fertilizer. Attenuation processes in soil include filtration, in which bacteria are retained in micropores, and adsorption, in which microorganisms are held to the surface of soil colloids through electrostatic charge and surface tension mechanisms (TALLON et al., 2007). These processes are maximized in soils with clayey texture (AISLABIE et al., 2011; GAGLIARDI; KARNS, 2000; UNC; GOSS, 2004). Gagliardi and Karns (2000) found a decrease of > 90% in E. coli and Cryptosporidium parvum Oocysts populations in clayey soil, which they attributed to the retention of these organisms on soil solids. The decline of enteropathogenic bacteria in soils treated with organic fertilizers is also favored by top-dressing (AISLABIE
et al., 2011) because this form of application exposes pathogens to UV radiation and desiccation, decreasing the population density of fecal bacteria (MACIOROWSKIA et al., 2004). It is likely that these attenuation processes occurred in the latosol soil in this experiment, given that the clay content was 470 g kg-1 and the organic fertilizer was applied by top-dressing without incorporation into the soil.

Conclusion

Organic and organomineral fertilizers made with poultry manure did not contaminate the soil with enteropathogenic bacteria after 30 days following application.

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