Culturable *Escherichia coli* in Soil Mixed with Two Types of Manure

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ABSTRACT

We evaluated the impact of the manure type used in soil–manure mixtures on the detection of culturable *E. coli* as tested in water quality monitoring. A series of incubation experiments, lasting up to 200 d, allowed evaluation of the potential impact of manure $\times$ soil interactions on the augmentation of culturable *E. coli*. Two soil types (sandy loam and a silt loam), two manure types (liquid swine manure and solid beef cattle manure), and three temperature levels (4, 12, and 20°C) were investigated. The significance of the presence of competing microorganisms was estimated by comparing results from manure mixtures with sterile and nonsterile soils. Water content in the soil–manure mixtures was maintained close to field capacity to eliminate the specific impact of water availability. We found that culturability of the indicator organism, *E. coli*, changed with time and was dependent on the type of manure used and its interaction with soil. *Escherichia coli* could be cultured for a longer time from soils with liquid manure additions. Whereas *E. coli* numbers were initially higher from soils treated with solid beef cattle manure, their numbers decreased more rapidly and the duration of their apparent survival was shorter. Resilience of culturable *E. coli* was independent of their initial numbers in manure.

FOLLOWING LAND APPLICATION of manure several factors have a direct effect on the survival of enteric bacteria. Generally, availability of water overrides the impact of other factors (Gerba and Bitton, 1984; Mubiru et al., 2000). However Ritchie et al. (2003), found that the soil water potential had no effect on the survival patterns of laboratory maintained *E. coli* O157:H7. Survival in the environment is prolonged under cool temperatures (Wang et al., 1996); however lack of water can reverse this trend (Sjogren, 1994). Longer survival times observed in finer textured soils (Fenlon et al., 2000) are most likely due to the increased water retention capacity of clays (Gerba and Bitton, 1984; Rattray et al., 1992; Stotzky, 1985). While acidity can decrease survival of enteric bacteria (Cuthbert et al., 1950; Sjogren, 1994) the pH in most agricultural soils is not sufficiently extreme to have a significant impact on their die-off rates (Gerba and Bitton, 1984; Sjogren, 1994). Greater soil organic matter content enhances *E. coli* survival (Tamási, 1981). Enteric bacteria may be protected against damaging environmental factors within protozoa (Barker and Brown, 1994; King et al., 1988). Exposure to sunlight kills manure bacteria due to the direct impact of the UV rays,

visible light (Sinton et al., 1999), or by drying. However both these effects are usually limited to the surface of manure material (Kress and Gifford, 1984). Once enteric bacteria reach water bodies they can survive for considerable periods, especially when associated with sediments (Conboy and Goss, 2001; Davies et al., 1995; Sherer et al., 1992). In regions with winter snow cover, application of manure in the fall can lead to contamination with fecal bacteria during the spring melt, provided that bacteria survive over winter. Generally, the most important stress factor during the winter is exposure to freezing temperatures. Sporulating enteric bacteria can survive longer in the environment than nonsporulating species, the classic example being *Clostridium* spp. (gram-positive bacteria). Gram-negative bacteria, such as *E. coli*, can also adapt to environmental stress by modifying cell membrane chemistry. Typically, changes are observed in the ratios of saturated to unsaturated fatty acids, ratios of *trans*- to *cis*-monoenoic fatty acids, and the ratios of cyclopropyl fatty acids to their monoenoic precursors. Enteric bacteria in soils can also be protected from stress factors by occupying microhabitats, which are more common in finer textured soils (Heijnen and van Veen, 1991). The interaction of enteric bacteria with the soil environment can differ from that in the absence of manure components (Unc and Goss, 2004) and this can affect their retention and transport through the soils.

Tests for water quality monitoring seek to establish the presence of organisms indicative of fecal contamination. *Escherichia coli* is the most common of these indicators. While the persistence of various *E. coli* strains may vary within the environment (Topp et al., 2003), the water quality monitoring tests do not differentiate between strains, and isolation of viable *E. coli* is used to indicate possible fecal contamination. Land application of manure modifies the potential for such indicators to reach the water resources and thus act as a signal of contamination. Both *E. coli* added with the manure and the *E. coli* already present in the soils might lead to a contaminant signal. Therefore in this study we used non-strain-specific identification and enumeration methods to allow a broad evaluation of the importance of manure and soil characteristics for the detection of culturable *E. coli* as a possible signal of fecal contamination in receiving waters.

The specific impact of water availability was eliminated by maintaining a constant water content, while the importance of temperature was investigated together with intrinsic properties of manure and soils. Thus, the present study aimed specifically at evaluating the significance of the type of manure on the potential persistence of *E. coli* in soil–manure mixtures.

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Published in Soil Sci. Soc. Am. J. 70:763–769 (2006).

Soil Biology & Biochemistry

doi:10.2136/sssaj2004.0367

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Abbreviations: GLM, general linear model; MF, membrane filtration; MPN, most probable number.

MATERIALS AND METHODS

Experiment 1: Culturable *E. coli* in Incubated Manure–Soil Mixtures

Experimental Design

A multilevel factorial design was employed, with soil type (Conestogo silt loam [fine-loamy, mixed, superactive, mesic Typic Hapludalf] and Fox sandy loam [fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludalf]), soil sterility status (fresh and sterile), manure type (solid beef cattle manure and liquid swine manure), and incubation temperature (4, 12, and 20°C) as factors. The combination of factors ([2][2][2][3]) resulted in 24 treatments. All incubations were performed in laboratory under controlled conditions. Soil manure mixtures were incubated in individual sterile plastic vials that were covered with a perforated lid to allow gas exchange. The gravimetric water content of the soil–manure mixtures was adjusted to 0.3 g g⁻¹ for the Conestogo silt loam and 0.2 g g⁻¹ for the Fox sandy loam. These values corresponded approximately to the field capacity for the respective soils. During incubation, water was added weekly as necessary to maintain the soil moisture content nearly constant.

Initial levels of *E. coli* in each soil and manure were enumerated before mixing. The soil–manure mixtures were sampled, weekly over the first month, biweekly during the second and third months, and monthly toward the end of the experiment. Enumeration of *E. coli* in mixtures containing fresh soil continued until measured *E. coli* numbers in soil–manure mixtures dropped below those for the corresponding untreated soil. For mixtures with sterilized soil, enumeration continued until a steady value was attained and confirmed. Thus, in the fresh soil treatments, enumeration continued for about 100 d, while the mixtures with sterile soils were tested for up to 200 d.

Initially, at each sampling event three vials from each treatment were collected and destructively tested for the presence and enumeration of culturable *E. coli*. Thus, 72 evaluations (3 replications of 24 treatments) were carried at each sampling time. As the numbers of detectable *E. coli* declined the enumeration method was switched from the membrane filtration and incubation method to the most probable number (MPN) method. This MPN method was used only for the sterile soils treatments after about 80 d of incubation. At this stage the *E. coli* concentration reached an equilibrium level that changed little until the end of the trial. For this method, a composite sample of four randomly selected vials was tested for each treatment. Most probable number values have not been used in the statistical analyses, which focused on the first 7 wk of the trial. Full details of the method are presented below.

Soils

Samples of two typical Hapludalf soils, a Conestogo silt loam (sand = 0.3 g g⁻¹; silt = 0.5 g g⁻¹; clay = 0.2 g g⁻¹; organic matter = 0.048 g g⁻¹) and a Fox sandy loam (sand = 0.63 g g⁻¹; silt = 0.29 g g⁻¹; clay = 0.08 g g⁻¹; organic matter = 0.022 g g⁻¹) were collected from the surface 10 cm of the plowed layer in the fall of 2000. Soil textural and organic matter contents were determined by standard methods (Carter, 1993). The soils have not received organic amendments for at least 5 yr before sampling. Freshly collected soil was passed through a 2-mm sieve. A portion of each soil type was sterilized over 5 d by daily autoclaving at 121°C for 2 h. Between each autoclaving the soils were kept under aseptic conditions at room temperature to facilitate germination of spores. Sterile deionized water (<50 mL 1000 g⁻¹ soil) was added to the soil to compensate for

any detected evaporative loss (measured gravimetrically). The effectiveness of the autoclaving to kill microbes was evaluated by plating dilutions of aliquots of autoclaved soil onto plates of tryptic soy agar. No growth was detected after 48 h with either soil type. Thus, for experimentation, autoclaved (sterile) and nonautoclaved (fresh) soils of each soil type were used.

Manure

Solid beef cattle manure from an in-barn manure pile (dry matter content 0.2 g g⁻¹) and fresh liquid swine manure from a storage tank (dry matter content 0.01 g g⁻¹) were used in the tests. The soil–manure mixtures were prepared by mixing 1.2 g manure into 13.8 g soil (dry weight), placed in a 100-mL polypropylene incubation vial (i.e., 8 parts manure to 92 parts dry weight soil). This was approximately equivalent to incorporating a manure application of 50 Mg ha⁻¹ into the top 5 cm of soil.

E. coli Extraction and Enumeration

To extract the *E. coli* cells, the soil–manure mixtures were added to a known volume of water (10 g fresh mixture to 95 mL sterile water) and mechanically shaken for 20 min at 200 rpm (Wollum, 1986). Subsequent decimal dilutions were prepared for the enumeration phase. Initially, enumeration was done through membrane filtration (MF, 0.45-μm Millipore filter [Millipore Inc. Billerica, MA]) followed by incubation on absorbent pads saturated with m-ColiBlue24 broth (Hach Co., Loveland, CO; method no. 10029 [Grant, 1997]). This substrate allows simultaneous enumeration of both *E. coli* (blue colonies) and total coliforms (red colonies) (Grant, 1997). It was found that the use of nitrocellulose filters resulted in a change in the color of coliform colonies from red to various hues of brown. Therefore only nylon filters were used to avoid confusion.

As the numbers of bacteria in the soil–manure mixtures declined, suspended soil particles interfered with the plate count and subsequently enumeration was continued by broth incubation using the MPN method with five tubes per dilution (Clesceri et al., 1989). The MPN method was only required for the sterile soil treatments and only after the *E. coli* concentration reached an equilibrium value around MPN 10². Five dilutions were prepared, from 10⁰ to 10⁻⁵. The results from the most appropriate three dilutions were considered for the MPN evaluation (Clesceri et al., 1989; Roussanov et al., 1996). The presumptive (lauryl tryptose broth) and confirmatory phases (brilliant green lactose bile broth) for coliforms (Clesceri et al., 1989) were followed by confirmation of *E. coli* in EC broth with added MUG fluorogenic enzyme substrate (4-methylumbelliferyl-β-D-glucuronide). The MPN tests were only required at values lower than 10³ cfu (colony-forming units).

About 10% of *E. coli* positive samples from both the MF and the MPN tests were randomly confirmed for *E. coli* on EC-MUG agar.

Experiment 2: Impact of Freezing on Culturable *E. coli* in Soil–Manure Mixtures

Experimental Design

A second experiment investigated the impact of one time short-term freezing on the detection of culturable *E. coli*. Such events are common in Ontario in the spring or fall during manure application seasons. A multilevel factorial design was employed, with soil type (Conestogo silt loam and Fox sandy loam), soil sterility status (fresh and sterile), manure type (solid beef cattle manure and liquid swine manure), and freezing pattern (immediate freezing and delayed freezing) as factors.

Table 1. Time variability of the factor significance for the measured *E. coli* concentration in the incubated soil–manure mixtures (general linear model [GLM] results).

Treatment type	Factor	Probability that the difference between factor levels is 0 ($P_H = 0$) for each incubation period†			
		Week 1 and 2	Week 3 and 4	Week 5 and 6	Week 7 and after
Fresh soils/manure	Soil type	0.962	0.689	0.107	0.578
	Manure type	0.014	0.366	0.227	0.584
	Incubation temperature, °C	0.624	0.000	0.004	0.031
	R^2 (adjusted R^2)	22.6 (11.1)	39.8 (32.7)	32.5 (24.7)	29.6 (15.6)
Sterile soils/manure	Soil type	0.008	0.002	0.051	0.499
	Manure type	0.098	0.056	0.055	0.139
	Incubation temperature, °C	0.000	0.025	0.060	0.015
	R^2 (adjusted R^2)	82.2 (75.7)	67.5 (58.3)	52.9 (41.2)	27.3 (17.3)

Separate analyses have been performed for four periods of the incubation.

The significance of the factors was evaluated employing a GLM with three factors (i.e., $y_{ijkl} = m + ai + bj + ck + el[ijk]$). The Tukey test was used to obtain the $P_H = 0$ values.

† Analyses have been carried using the values presented in Fig. 2 and 3 (cfu values obtained by membrane filtration and m-ColiBlue 24 substrate [Grant, 1997]).

The combination of factors ([2][2][2][2]) resulted in 16 treatments. All experiments were carried in the laboratory under controlled conditions. After mixing the soil and manure, the mixtures were kept at room temperature (20°C) for about 16 h. Subsequently, half of the vials from each treatment were frozen at –15°C for 4 d (immediate freezing treatment). The rest of the vials were kept at 4°C for 4 d before they too were put into the freezer at –15°C for another 4 d (delayed freezing treatment).

Soils and Manure

Soil and manure mixtures were prepared as described for the first experiment.

E. coli Extraction and Enumeration

After the freezing cycle the soil–manure mixtures were thawed for about 10 h at 4°C, before enumeration. *Escherichia coli* were extracted and enumerated in the soil–manure mixtures before and after the freezing. The same methodology was used to extract *E. coli* cells as described for the first experiment.

Background enumeration of *E. coli* in soil and manure and enumeration in the soil–manure mixtures was carried by the MPN method with five test tubes per dilution using the approach described for the first experiment. Four composite samples obtained each from four vials for each treatment were used.

Data Analysis

The $\log_{10}$ -transformed counts expressed per 100 g dry weight soil–manure mixtures were analyzed statistically. The significance of factors on the magnitude of *E. coli* in the soil manure mixtures was evaluated through a general linear model (GLM) employing the Tukey test for the significance of means. The role of the soil type, manure type and incubation temperature have been evaluated separately for the fresh soils and sterile soils treatments ($y_{ijkl} = m + ai + bj + ck + el[ijk]$). These tests have been carried for several segments of the trial period (see Table 1). The significance of levels of the same three factors on the persistence of *E. coli* in fresh soil–manure mixtures above the initial values in the fresh soils have been evaluated through a multifactorial multiple regression analysis. Data analysis was carried with the Minitab 14 statistical software (Minitab Inc., State College, PA).

RESULTS AND DISCUSSION

The two experiments described the dynamics of *E. coli* numbers following the addition of manure to soils when

the water content of the mixtures was maintained close to field capacity. This practically eliminated the differences in the water availability between the two soils; a sandy loam and silt loam. Laboratory-grown labeled bacteria were not added, as their behavior might not necessarily be similar to the natural bacteria in the manure. For the two soils, the use of heat sterilization allowed the assessment of the importance of abiotic soil properties.

Impact of Factors on the Culturable *E. coli* Signal

For the mixtures containing fresh soils (Fig. 1), the period of increased counts of culturable *E. coli* from the manure was considered to be the period commencing at the mixing of the soil with manure and ending once the *E. coli* numbers in the mixtures decreased below the initial numbers in the soil (i.e., 5.1 $\log_{10}$ cfu for the silt loam soil, and 4.5 $\log_{10}$ cfu for the sandy loam soil, for each 100 g soil dry matter). This variation over time approach is similar to most monitoring practices used on agricultural fields and water courses where no parallel controls are employed. The initial numbers of *E. coli* in the two fresh soils was within the range of values reported by other researchers (e.g., Entry et al., 2000). Incubation of the fresh soil–manure mixtures resulted in an increase in the number of *E. coli* in the soil beyond

Fig. 1. Culturable *E. coli* associated with manure addition to fresh soils. The bars represent the period (d) following manure addition to fresh soils for which the concentration of *E. coli* in the mixtures was larger than the initial concentration in the fresh soils.

Fig. 2. Changes in the number of culturable *E. coli* over time in fresh soils after manure amendment. Two soil types, sandy loam and silt loam, were amended with (C) liquid swine manure and (Δ) solid beef cattle manure (8 parts fresh manure to 92 parts dry weight soil). Number of *E. coli* in unamended soils was $5.1 \log_{10}$ cfu in silt loam and $4.5 \log_{10}$ cfu in sandy loam. Bars represent one standard deviation from mean.

the sum of the initial numbers in the two materials (Fig. 2). As *E. coli* was present in soil before addition of manure it is possible that both soil and manure *E. coli* contributed to this increase. The contribution of manure *E. coli* to the postapplication increase was confirmed by the similar pattern of increase in numbers that occurred after the manure addition to the sterile soils (Fig. 3).

Statistical tests were carried on the values presented in Fig. 1. Thus, the period with increased numbers of culturable *E. coli* was longer for liquid swine manure, in sandy loam soil, and at cooler temperatures, but shorter for *E. coli* from solid beef cattle manure, in silt loam soil and at warmer temperatures. The results showed that all three factors, soil type, manure type, and incubation temperatures, were of importance ($P_{H=0} < 0.001$ for all three factors, adjusted $R^2 = 90.5$) (Fig. 1). In all treatments with sterile soils *E. coli* was still detected at an MPN concentration of about 10^1 to 10^2 100 g⁻¹ dry matter at the cessation of the experiment about 200 d from inoculation (data not presented). Other researchers also have noted extended survival of *E. coli* added with solid cattle manure (Lau and Ingham, 2001; Ingham et al., 2004). Our results suggest that such a phenomenon may also occur when other manure types are added to soil (i.e., liquid swine manure).

Detection Patterns of Culturable *E. coli*

Analysis of *E. coli* numbers with time, in the fresh soil treatments, used the estimated numbers that were in excess of the initial amount in soils before addition of

manure. Hence, for each combination of factors, the interval considered for this analysis varied. For the sterile soils, the same analysis was performed using the *E. coli* counts obtained before they reached an equilibrium level. These values, obtained using the MF method, are presented in Fig. 3. Equilibrium levels for all the treatments containing sterile soils were attained around Day 80 and they hovered around MPN 10^2 until Day 200 when the trial was stopped; these values are not presented in Fig. 3. At the start of the incubation the total number of culturable *E. coli* (Fig. 2 and 3) increased above the initial level indicating a change in the microbial kinetics. However, whereas in fresh soils the increase was up to one order of magnitude (Fig. 2), it was two to three orders of magnitude in sterile soils (Fig. 3). In fresh soils this initial increase depended on manure type (Table 1).

The variation of *E. coli* numbers in fresh soil and manure mixtures was only partially described by the three factors considered. Manure type had a significant impact on the *E. coli* numbers (greater for the solid beef cattle manure, Fig. 3) for the first 2 wk after the start of the incubation (Table 1). However, the temperature was the only significant factor for the variation in numbers of *E. coli* in fresh soil over the rest of the incubation period (Table 1). While the significance of the factors varied between the fresh and sterile soil treatments, the GLM analysis (R^2) has shown that the three factors (soil, manure, and incubation temperature) better accounted for the variation in numbers of *E. coli* during incubation of sterile soils than fresh soils (Table 1).

Fig. 3. Changes in the number of culturable *E. coli* over time in sterile soils after manure amendment. Two soil types, sandy loam and silt loam, were amended with (C) liquid swine manure and (Δ) solid beef cattle manure (8 parts fresh manure to 92 parts dry weight soil). Changes presented here were measured before stabilization of *E. coli* numbers (10^1 to 10^2 cfu 100 g^{-1} dry matter content of soil-manure mixtures) at 11 to 12 wk into the treatment. These stable levels were maintained for at least the 200 d of monitoring. Bars represent one standard deviation from mean.

Application of animal manure adds more enteric microorganisms to soils but it also provides supplementary available nutrients (Liang et al., 1996). If the amount of these nutrients is sufficiently large the competitive pressure between organisms may decrease temporarily and thus possibly allowing increased persistence of added *E. coli* (Tamási, 1981).

Most of the nutrients in solid manure are only mineralized over time (Kofoed et al., 1986). Hence after the initial augmentation in nutrient availability, reflected in the increased *E. coli* numbers, competitive pressure may have increased as readily available substrates are consumed. While predation and die-off may have occurred, the decrease in the number of culturable *E. coli* was found to occur in both the presence (i.e., fresh soils) and absence of native soil organisms (i.e., sterile soils) suggesting that substrate depletion played a role.

The only obvious difference between the sterile treatments and the fresh soil treatments was the absence of competitors, and possibly increased nutrient availability in the sterile soils due to lysis of soil microorganisms following soil autoclaving (Katznelson, 1940; de Boer et al., 2003). The different significance level for the soil type in sterile soil relative to fresh soil treatments (Table 1) suggests that, in the absence of competition or predation interactions with soil biota, increased nutrient availability in the silt loam (Fig. 3) was a likely cause. Both increased substrate availability as well the lack of competition or predation (Recorbert et al., 1992; Satoshi

et al., 1998) could explain the greater increase in the numbers of *E. coli* observed with the solid manure treatment on the sterile soils. On the other hand, significance of manure type also was close to the 0.05 probability level for variation in *E. coli* numbers over a long period in the sterile soil treatments, while it rapidly lost its significance after the first 2 wk incubation of the mixtures with fresh soils (Table 1). Soil type and thus their organic matter content was not important for the number and the persistence of *E. coli* in the fresh soils (Table 1). This suggests a role for the interaction of manure types with the activity of native soil organisms that are still active in the fresh soils.

Specific research is required to determine the impact of manure type on the activity of soil predators. Indirectly related research has shown that even relatively resistant microorganisms may be killed in the presence of organic materials containing excessive N (e.g., microsclerotia of *Verticillium dahlia*; Tenuta and Lazarovits, 2002).

Most of the N in liquid swine manure is in mineral form, and includes significant amounts of ammonia in solution. Ammonia and anionic organic compounds found in liquid manure were found to be major sources of toxicity (Gupta et al., 1997). While long-term experiments indicate that ammonia-oxidizing (nitrification) activity is enhanced by use of pig slurry (Ceccherini et al., 1998), immediately after application excess nitrite and nitrate can be toxic to bacteria (Tamási, 1981). Such a reason might explain the smaller numbers of *E. coli*

persisting in the liquid manure treatments over the duration of the experiment. However the duration of culturable *E. coli* recovery was lengthened when liquid manure was used (Fig. 1). The factors leading to this prolonged recovery are not known. Liquid swine manure may have had a negative impact on the indigenous soil microorganisms, therefore favoring the survival of manure bacteria. More research is needed before a proper answer can be given to these questions.

Explanation of some phenomena described here is more difficult, as many previous papers have concentrated on long-term, multi-annual impacts of manure application on soil biological activity and the effects described here are occurring over relatively shorter, periods of time.

Impact of Freezing

The initial number of culturable *E. coli* in the delayed freezing treatment was assessed after 4 d incubation at 4°C, but before freezing. An increase in the number of culturable *E. coli* was observed over this period in all treatments most noticeable for the mixtures of manure with sterile silt loam. While environmental isolates of *E. coli* may have the capability to grow even at low temperatures (Conboy, 1998) no significant growth was expected to occur under our experimental conditions. Freezing is considered to extend the survival of manure bacteria (Wang et al., 1996). However the 4-d deep freeze treatment generally reduced the numbers of culturable *E. coli* (Fig. 4). Larger losses were detected in the treatments with sandy loam soil where solid beef cattle manure had been added. Postfreezing comparison of results from the immediate freezing with the delayed freezing treatment showed no significant effect on the numbers of culturable *E. coli*. The sterility of the soils was also not important (Fig. 4).

Fig. 4. Changes in the number of culturable *E. coli* in soils amended with manure after freezing at -15°C for 4 d. For delayed freezing, soils amended with manure were incubated at 4°C for 4 d and then frozen. For immediate freezing, soils were frozen immediately after addition of manure. Changes in the numbers of culturable *E. coli* in the manure-amended soils are calculated by subtracting the number of culturable *E. coli* after freezing from the number of culturable *E. coli* before freezing. Bars represent 95% CI; $N = 4$ for each treatment.

Nevertheless, there was an increase in the detected *E. coli* numbers in the soils where freezing followed immediately after the liquid swine manure was added, an observation similar to that from a field experiment reported by Gessel et al. (2004). In our experiment this occurred in both sterile and fresh soils that were immediately frozen, suggesting that it was not a function of the interaction with soil microbes or the possible availability of nutrients following autoclaving. Manure type or soil type do not individually explain this result. Increased recovery of initially viable but not culturable organisms could be a possibility. Manure *E. coli* are not expected to be active at -15°C and little growth can be expected during the short period at 4°C as soils thawed. Thus while growth is marginally possible under the presented protocol it is questionable if it could have led to significant growth. The increased recovery of *E. coli* could more likely be due to changes in the culturability status of the cells originally present in the mixtures. Such changes were of significance as they occurred only where liquid manure was added to mixtures. Other researchers have found that presence of inimical microflora increased resistance of *Salmonella typhimurium* to freeze-thaw injuries (Aldsworth et al., 1998). No decline in bacterial numbers following freezing was noted when swine manure was applied to the field (Gessel et al., 2004), which may suggest the association of this phenomenon with swine manure. For the culturable *E. coli* monitored in our experiment on delayed freezing such a phenomenon only occurred in the sandy loam soil, which suggests that the properties of the finer textured soil may also have been important particularly as they may effect changes in the environmental chemistry and availability of water to freezing.

CONCLUSIONS

Results suggest that the source of organic residues containing *E. coli* can impact postapplication persistence of these organisms. Following the addition of solid beef cattle manure to soils the number of detected *E. coli* increased by up to three orders of magnitude, but persisted at large concentrations for a relatively short period. Addition of liquid swine manure to soils resulted in a smaller number of *E. coli* than for the solid beef cattle manure, but increased the period during which they could be recovered from the soil manure mixtures. Solid manure shortened the period for which *E. coli* was detected at large concentrations for all incubation temperatures considered including freezing. The impact of freezing when liquid manure was used varied with soil type, but immediate freezing led to increases in the postfreezing detected numbers. The potential resilience of culturable of *E. coli* in sterile soil, in the absence of water stress, was shown to be at least 200 d and was not dependent on the initial concentration of culturable *E. coli* in the applied manure, the incubation temperature, or the type of soil or manure. The type of manure applied to land and the soil properties can lead to variability in the strength of the *E. coli* signal that may reach water resources from manure-treated land. The practical rele-

vance and detailed understanding of biological, chemical, and physical mechanisms by which the different manure types affect the persistence and detection of associated bacteria need further research.

ACKNOWLEDGMENTS

This work was funded by the Ontario Ministry of Agriculture and Food. Many thanks to the editor and anonymous reviewers whose comments greatly helped improve the presentation of this manuscript.

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