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Survival of Fecal Contamination Indicator Organisms in Soil

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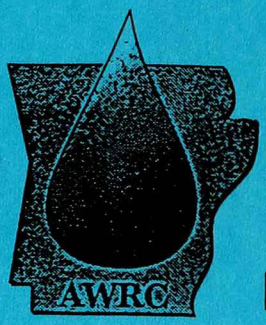

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SURVIVAL OF FECAL CONTAMINATION INDICATOR ORGANISMS IN SOIL

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ABSTRACT

Survival of Fecal Contamination Indicator Organisms in Soil

Soils amended with human or animal waste may result in pathogen contamination of ground and surface water. Because temperature has been shown to affect pathogen survival, two laboratory studies were conducted to evaluate the impact of extremes in temperature on bacterial and viral pathogen indicator die-off in soil. A Captina silt loam was amended with broiler litter (0.1 g/g dry soil), septic tank effluent, or *Escherichia coli* (ATCC 13706) culture (both at 0.04 and 0.1 mL/g dry soil in the two respective studies), incubated at 5 and 35°C, and analyzed over time to determine the number of fecal coliform, *E. coli*, and coliphage remaining. Pathogen indicator die-off rate constants (k) for all indicator-temperature-treatment combinations were determined by first-order kinetics. For all three pathogen indicators, die-off was significantly more rapid at 35°C than at 5°C. In both studies, fecal coliform die-off rates were not different from *E. coli* die-off rates across each temperature-treatment combination. Levels of these bacterial indicators appeared in a ratio of 1:0.94 with 95% confidence intervals at 0.89 and 0.99 in the *E. coli*- and litter-amended soils. Die-off of the viral indicator was significantly slower than the die-off of the bacterial indicators at 5°C in litter-amended soil. Die-off of the bacterial indicator, *E. coli*, in soil amended with *E. coli* culture was not significantly different than die-off in soil amended with broiler litter at 5 or 35°C in the two studies. Because the higher incubation temperature increased die-off rates for all three indicators, it is expected that the potential for contamination of ground and surface water decreases with increasing temperature.

K.A. Teague, D.C. Wolf, P.F. Vendrell

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ACKNOWLEDGEMENTS

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INTRODUCTION

Land application of municipal biosolids, animal manure, or inadequately renovated septic tank effluent can result in ground and surface water contamination. According to the 1992 *Water Quality Inventory Report*, over 330 miles of Arkansas water-body reaches exceed the 200 colony forming units (CFU) of fecal coliform/100 mL limit established as acceptable for primary contact (Arkansas Department of Pollution Control and Ecology, 1992). The principal origin of the pathogen indicator bacteria was given as non-point contamination.

The affected water bodies are located predominantly in the Northwest region of the state where agricultural use of poultry litter could be implicated as the major source of pollution. In 1993, over one billion commercial broilers were produced in Arkansas, the nation's top broiler-producing state (Arkansas Agricultural Statistics Service, 1994). It is estimated that 26.7 kg litter on a dry weight basis is generated daily per 1000 kg live weight (SCS, 1992). With current production rates, disposal of this litter, which is a mixture of manure and bedding materials such as woodchips, sawdust or rice hulls, is an escalating problem. In Northwest Arkansas, most of the broiler litter is recycled as an organic soil amendment which is applied to tall fescue (*Festuca arundinacea* Schreb.) and bermudagrass [(*Cynodon dactylon* (L.) Pers.] pastureland (Sims and Wolf, 1994).

Approximately 40% of Arkansas households utilize on-site septic systems to treat and dispose of their domestic wastewater (U.S. Department of Commerce, 1990). However, in areas with poor soil permeability or shallow bedrock, septic systems can provide contamination of ground and

surface waters. In fact, septic systems are reported to be a larger source of groundwater contamination than farming (Council on Environmental Quality, 1980).

The concern over fecal contamination of water supplies is due to the potential presence of pathogenic bacteria and viruses found in fecal waste. Between 1920 and 1990 in the United States, consumption of contaminated water resulted in over 450,000 reported cases of waterborne disease and 1,083 deaths (Craun, 1991). Because detection of pathogenic bacteria and viruses is dangerous, costly, and time consuming and requires special facilities, pathogen indicators are used to identify areas of fecal contamination. We can monitor soil and water with these indicators, which are usually not pathogenic themselves but signal the potential presence of fecal pathogens that cause diseases such as salmonellosis, infectious hepatitis, or polio.

Traditionally, total coliform have been the primary measure of the microbial quality of drinking water, and the presence of fecal coliform confirms human or animal fecal contamination (Federal Register, 1989). *Escherichia coli* make up approximately 90% or more of fecal coliform, and *E. coli* densities have been more closely related to cases of gastroenteritis than fecal coliform levels (Edberg et al., 1988; Federal Register, 1989). Because new colorimetric *E. coli* enumeration techniques provide rapid results, the USEPA currently allows either fecal coliform or *E. coli* to be used for confirmation of samples positive for total coliform (Federal Register, 1989). These bacterial indicators model pathogenic bacteria survival but may underestimate the potential for pathogenic viruses (Berg et al., 1978; Gallagher and Spino, 1968; McFeters et al.,

1974; Payment et al., 1985). Coliphages, viruses that replicate in coliform bacteria, have been suggested as viral pathogen indicators. They are also present in fecal waste and appear to exhibit survival behavior similar to that of pathogenic viruses (Yates et al., 1985).

The number of viable pathogens and indicators in ground and surface water is determined by their transport over and through the soil and by their ability to survive dynamic environmental conditions (Gerba et al., 1975). Survival is dependent upon environmental factors such as temperature, sunlight, soil moisture, pH, and organic matter content (Hurst et al., 1980; Reddy et al., 1981; Sorber and Moore, 1987; VanDonsel et al., 1967). Although the impact of temperature on bacterial indicator survival has been studied, there are insufficient data comparing bacterial and viral indicator die-off.

OBJECTIVES

Understanding the interrelationships between indicator organism populations, contamination source, and survival rates in soil at different temperatures is necessary for evaluating the potential for non-point source contamination of ground and surface waters. Consequently, the objective of this study was to evaluate the survival of fecal coliform, *E. coli*, and coliphages when incubated at 5 and 35°C in soil amended with broiler litter, septic effluent, or *E. coli* culture.

RELATED RESEARCH

The potential for fecal organisms to contaminate ground and surface water is regulated by their survival in soil and transport to water bodies. Studies are currently being conducted to evaluate transport of fecal bacteria in runoff from fields with land-applied poultry litter (Coyne and Blevins, 1995; Daniel et al., 1995). But, for fecal organisms to contaminate water bodies, they must survive in the soil environment long enough to be transported into water systems.

The effect of temperature on the survival of bacterial pathogens, bacterial indicators, and viral pathogens has been studied in soil and water systems (Reddy et al., 1981). VanDonsel and colleagues (1967) found that *E. coli* and *Streptococcus faecalis* survived longer in autumn and winter than in spring and summer in shaded and exposed outdoor soil plots. In a study of natural waters, there was a proportional decrease in *E. coli* survival as temperature increased from 5 to 15°C (McFeters and Stuart, 1972). Additionally, several temperature-dependent models have been developed that predict coliform die-off in lagoons and waste stabilization ponds (Mayo, 1995). Enteric viruses, such as poliovirus 1 and echovirus 1, have demonstrated increased survival at lower temperatures in groundwater and sludge-amended soils (Bitton et al., 1984; Sorber and Moore, 1987; Yates et al., 1985), and MS-2 bacteriophage has also shown increased survival in groundwater at low temperatures (Yates et al., 1985).

Enumeration techniques have been developed for coliphage in water samples (APHA, 1992; Grabow and Coubrough, 1986; Isbister et al., 1983; Kennedy et al., 1985; Wentsel et al., 1982). Though the incidence and

survival of coliphage have been evaluated in potable water (El-Abagy et al., 1988), in tropical waters (Hernández-Delgado et al., 1991), and in nonaerated liquid and semiliquid animal wastes (Pesaro at al., 1995), limited information is available on the survival of the viral pathogen indicators in soil systems.

The procedure for coliphage enumeration in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1992) describes equations for calculation of total or fecal coliform levels based upon the number of coliphages in a sample. These equations are derived from previous research that found correlations between the incidence of bacterial and viral indicators in natural and flood waters (Isbister et al., 1983; Kott et al., 1974; Wentsel et al., 1982). However, Hilton and Stotzky (1973) analyzed water samples taken from an area of the Hudson River with inputs of untreated sewage, and did not find a consistent relationship between coliform and coliphage levels. In a study on indicator populations in raw sewage, sewage lagoon effluent, and river water, Bell (1976) also found that the fecal coliform to coliphage ratio varied over time. He explained that characteristic ratios between bacterial and viral indicators cannot be determined due to the variable influence of temperature, chlorination, and sediment on survival of the different indicators.

MATERIALS AND METHODS

In two laboratory studies, a Captina silt loam (fine-silty, siliceous, mesic, Typic Fragiudult) was collected to a depth of 15 cm from a tall fescue pasture (Table 1). The pasture was located at the University of Arkansas Main Experiment Station in Fayetteville, Arkansas in an area that had not received prior waste application. The field-moist soil was sieved through a 2-mm screen and 10-g dry-weight equivalent portions were weighed into 150-mL dilution bottles.

Two waste sources, broiler litter and septic tank effluent, were surface-applied to evaluate pathogen indicator survival in this soil. The litter for the first study was taken from a broiler house at the University of Arkansas Farm where the chickens (*Gallus gallus domesticus*) were 3 to 4 weeks old in the first growout. The litter for the second study was taken from a George's Inc. farm west of Springdale, Arkansas where the broilers were 7 weeks old and in the second growout. The litter was applied at a rate of 0.1 g moist litter/g dry soil in both studies (Table 2). Effluent from a 4725-L combination septic tank was collected prior to secondary treatment from a residence located on Hwy 16 West in Fayetteville, Arkansas. The effluent was applied at a rate of 0.04 and 0.1 mL/g dry soil in the first and second study, respectively (Table 3). Two additional treatments were applied at the same rate as the septic effluent: 1) *E. coli* (ATCC 13706) culture as a positive bacterial control, and 2) sterile water as a negative control, which was used to determine background levels in the soil itself.

Table 1. Physical and chemical properties of the Captina silt loam (fine-silty, siliceous, mesic, Typic Fragiudult) used in laboratory studies.

						Mehlich III Extractable					
pH	Carbon	Sand	Silt	Clay	NO_3-N	P	K	Ca	Na	Mg	Conductivity at 25°C
	%				mg/kg						dS/m
5.6	0.8	12.1	76.0	11.9	17	18	164	791	82	58	0.056

Table 2. Physical and chemical characteristics of broiler litter used in laboratory studies.

	Moisture	N	P	C
	---------- % ----------			
1st Study	32.3	ND*	ND	ND
2nd Study	20.4	5.34	1.30	29.27

* ND indicates no data because a power loss occurred in the storage freezer before the sample could be analyzed.

Table 3. Physical and chemical characteristics of septic tank effluent used in laboratory studies.

	pH	Solids		NPOC	NH_4-N	NO_3-N	Cl	Conductivity at 25°C
		Suspended	Total					
		mg/L						dS/m
1st Study	7.0	40	517	104	13.7	2.8	25.4	0.43
2nd Study	6.7	27	556	ND	15.4	0.5	29.1	0.44

ND indicates no data.

In the first study, all four treatments were replicated three times. In the second study, the broiler litter and septic treatments were replicated three times while the *E. coli* culture and the control treatments were replicated twice due to analytical constraints. Once treatments were imposed, the soil was adjusted to field capacity (-30 kPa or 18% gravimetric moisture) by the addition of sterile water. All bottles were covered with Saran Wrap® to prevent moisture loss but allow gas exchange.

The soils were arranged in a completely randomized block design, incubated at 5 and 35°C, and destructively sampled over time. Soils kept at the warmer incubation temperature were sampled more frequently because die-off was anticipated to be more rapid. In the first study, sampling times were at 0, 7, 14, 21, and 35 days for the 5°C incubation temperature, and soils incubated at 35°C were sampled after 0, 2, 5, 7, and 14 days. In the second study, sampling times were at 0, 14, 28, and 56 days and 0, 5, 14, and 28 days for the 5 and 35°C soils, respectively.

At each sampling time, 95 mL of phosphate buffer (APHA, 1992) and 5 glass beads were added to each dilution bottle to create a 10^{-1} dilution (Fig. 1). The bottles were placed on a horizontal shaker at 280 oscillations/min for 30 min, and then a serial dilution was performed by adding 5 mL to 45 mL phosphate buffer.

In the first study, the multiple fermentation tube technique with A-1 Broth was used so fecal coliform could be enumerated directly without a confirmation phase (APHA, 1992). Five tubes containing 10 mL A-1 Broth were inoculated with a 1-mL sample at appropriate dilutions and incubated at 35°C for 3 h then transferred to a 44.5°C waterbath for an additional

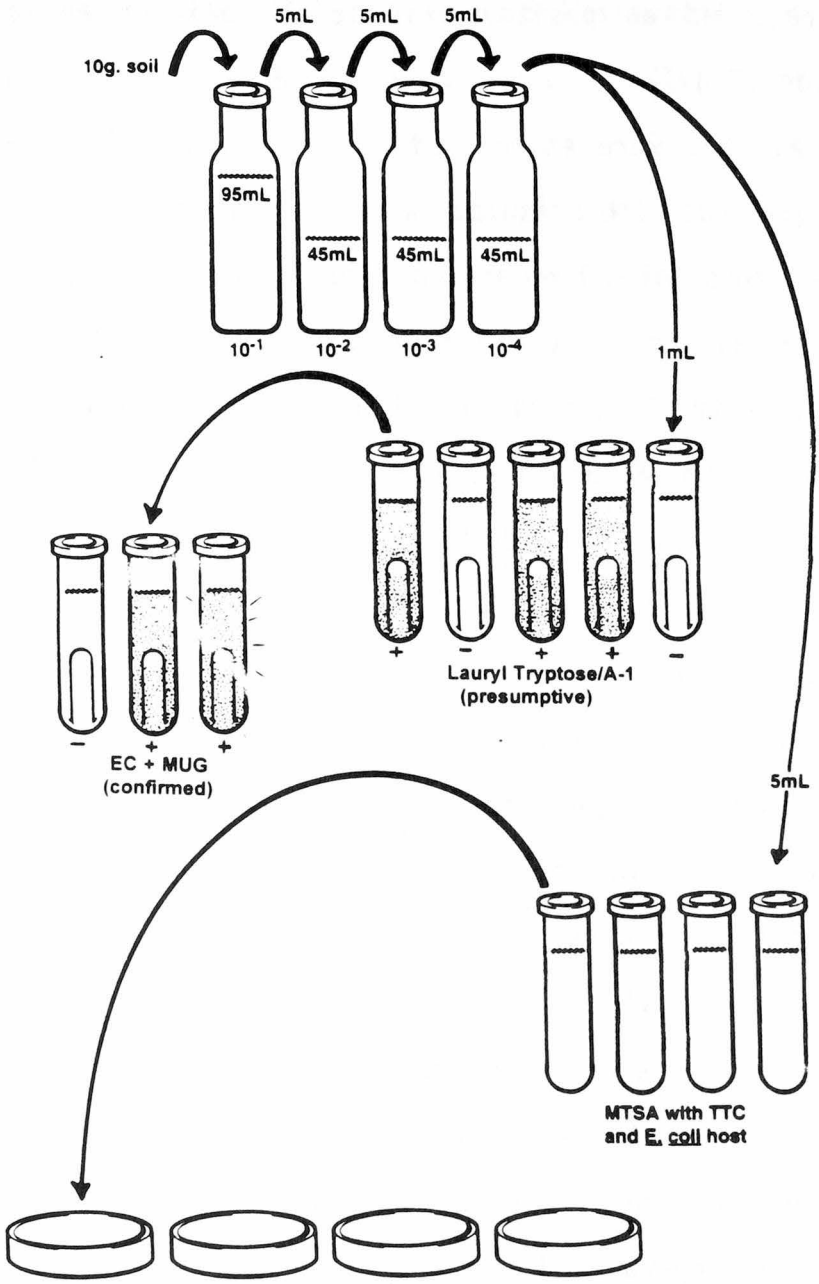

Fig. 1. Sampling flow for serial dilution and enumeration of pathogen indicators.

21 h. Positive tubes, those demonstrating turbidity and gas production, were regarded as positive for fecal coliform and recorded for each dilution (Fig.1).

E. coli were enumerated with the same multiple-tube fermentation technique, but with presumptive and confirmed phases. In the presumptive phase, 1-mL samples from appropriate dilutions were used to inoculate five tubes containing 10 mL Lauryl Tryptose Broth (LT). After incubating at 35°C for 24 to 48 h, positive LT tubes were confirmed by inoculating into tubes with 10 mL EC Broth + MUG (4-methylumbelliferyl-β-D-glucuronide) and incubating at 44.5°C for 24 h (Fig. 1). The EC + MUG tubes that fluoresced when exposed to long-wave U-V light, were considered positive for *E. coli* and counted across each dilution.

In the second study, both fecal coliform and *E. coli* were enumerated with the multiple-tube fermentation technique using Lauryl Tryptose and EC Broth + MUG for the presumptive and confirmed phases, respectively. The LT tubes that demonstrated turbidity and gas production were considered positive for total coliform and were subjected to the confirmed phase in EC Broth + MUG as described above. After the second incubation period, tubes that demonstrated turbidity and gas production under natural light were considered fecal coliform positive while tubes that fluoresced when exposed to long-wave UV light were considered positive for *E. coli*. In all the multiple-tube fermentation methods described above, the numbers of positive tubes across appropriate dilutions were applied to a statistical table to determine the most probable number (MPN) of fecal coliform or *E. coli*/g dry soil.

Coliphages were enumerated with techniques described in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1992). At appropriate dilutions, a 5.0-mL sample was inoculated into each of four tubes containing 5.5 mL Tryptic(ase) Soy Agar, 1.0 mL thawed *E. coli* (ATCC 13706) host, and 4 drops 2,3,5-triphenyl tetrazolium chloride (3% in ethanol). The contents of each tube were vortexed, poured into a sterile petri plate, inverted, and incubated for 7 h at 37°C (Fig. 1). Plaques, which appeared as clear zones in the *E. coli* lawn, were counted and averaged across the four plates as Plaque Forming Units (PFU)/g dry soil.

$$\text{PFU/g dry soil} = \frac{\text{Plate Count (total for four plates)}}{(20\text{ mL})\ (\text{Dilution})}$$

Pathogen indicator levels contained in the soil, broiler litter, septic effluent and *E. coli* culture at the start of each survival study are given in Table 4. All indicator levels determined during the incubations were converted to the natural logarithm, and control levels were subtracted from treatments to correct for background levels. Linear regression analysis was applied to the net level of indicators remaining over time for the individual replications. The die-off rate constants (k) were calculated by the rate equation:

$$A_t = A_o e^{-kt}$$

where: A_t = level of indicators remaining at a given time
A_o = initial level of indicators at start of die-off period
k = die-off rate constant (per day)
t = time (days)

and the slope of each regression line on the natural logarithm scale was given as the die-off rate constant (k) for a given temperature-treatment

Table 4. Initial pathogen indicator levels in Captina soil, broiler litter, septic effluent, and *E. coli* culture used in survival studies.

Study	Source	Units	Indicator		
			Fecal Coliform	*E. coli*	Coliphage
		per	------ MPN ------		PFU
1st	Captina Soil	g dry soil	1.4×10^1	1.6×10^1	< 5
	Broiler Litter	g dry litter	1.6×10^3	4.7×10^2	2.2×10^4
	Septic Effluent	mL effluent	2.0×10^3	3.0×10^3	$< 5 \times 10^1$
	E. coli Culture	mL culture	2.4×10^6	6.8×10^6	0
2nd	Captina Soil	g dry soil	2.3×10^1	2.3×10^1	< 5
	Broiler Litter	g dry litter	4.2×10^4	3.2×10^4	3.4×10^5
	Septic Effluent	mL effluent	1.2×10^2	1.2×10^2	$< 5 \times 10^1$
	E. coli Culture	mL culture	7.0×10^7	7.0×10^7	0

combination. Although initial regrowth of bacterial indicators may have occurred, this could not be confirmed due to analytical limitations that dictated sampling times. Therefore, die-off was considered only from the sampling day with maximum levels through the first sampling day where minimum detection levels of 2.0 MPN fecal coliform or *E. coli*/g dry soil or 5.0 coliphage PFU/g dry soil were reached. A minimum of five data points over at least three sampling days was used to determine each die-off rate constant. Additionally, die-off was not evaluated if initial indicator levels were not high enough to see a three-log reduction over time. Based on these criteria, the days of incubation that were used to determine the die-off rate constant for each indicator-treatment-temperature combination varied. These rate constants were compared by the general linear models (GLM) procedure, so the effect of temperature and waste source on bacterial and viral pathogen indicator survival rate constants in soil systems could be evaluated.

RESULTS

The data were analyzed by organism and the rate constants (k), standard errors, and intercepts for the indicator die-off equations are given in Tables 5 and 6 for the first and second studies, respectively. The R^2 values describe the fit of each die-off line over the days of incubation given in the last columns.

In both studies reported here, survival of the three pathogen indicators was enhanced at 5°C while die-off was more rapid with the warmer incubation temperature. Trends can be identified when the die-off rate constants are compared across the two incubation temperatures within a given treatment. For example, in the first study, the coliphage die-off rate constant at 35°C for the broiler litter treatment was 0.10/day while at 5°C, the die off rate was 0.01/day (Fig.2). Even though the initial indicator levels were different for the two studies, the die-off trends were similar.

The fecal coliform and *E. coli* die-off rate constants for the waste-amended soils support rate constants reported in the literature. McFeters and Stuart (1972) evaluated the impact of temperature on *E. coli* survival in stream waters. Based on the half-lives reported, *E. coli* die-off rate constants were 0.15, 0.23, and 0.50/day at 5, 10, and 15°C, respectively.

By rearranging the first order rate equation and solving for time (t), the number of days required for a 99.9% reduction in indicators or a 3-fold decrease in $\log_{10}$ numbers were estimated for each temperature-treatment combination (Table 7). Based on the die-off rate constants calculated for coliphage in the broiler litter-amended soil, it would take 35 to 69 days at 35°C for a 3-$\log_{10}$ or 99.9% reduction in numbers.

Table 5. Rate equation information for pathogen indicator die-off in soils amended with broiler litter, *E. coli* culture, and septic effluent at 5 and 35°C during the first laboratory study.

Pathogen indicator	Incubation temperature	Treatment	Die-off rate constant	Standard Error	Intercept	R^2	Data points regressed	Sampling days used
	°C		per day		ln/g dry soil			
Fecal Coliform	5	Broiler Litter	0.11	0.04	8.40	0.43	12	7 - 35
		E. coli Culture	0.25	0.02	14.64	0.89	15	0 - 35
		Septic Effluent	0.16	0.03	6.28	0.47	12	0 - 35
	35	Broiler Litter	ND	ND	ND	ND	7	ND
		E. coli Culture	0.87	0.06	14.64	0.90	14	0 - 14
		Septic Effluent	0.26	0.07	6.28	0.41	13	0 - 14
E. coli	5	Broiler Litter	0.24	0.03	10.82	0.48	12	7 - 35
		E. coli Culture	0.24	0.03	14.20	0.94	14	0 - 35
		Septic Effluent	ND	ND	ND	ND	4	ND
	35	Broiler Litter	0.94	0.09	16.85	0.56	6	5 - 14
		E. coli Culture	0.94	0.09	14.49	0.86	13	0 - 14
		Septic Effluent	ND	ND	ND	ND	3	ND
Coliphage	5	Broiler Litter	0.01	0.01	10.15	0.23	15	0 - 35
		E. coli Culture	ND	ND	ND	ND	0	ND
		Septic Effluent	ND	ND	ND	ND	0	ND
	35	Broiler Litter	0.10	0.01	10.15	0.71	15	0 - 14
		E. coli Culture	ND	ND	ND	ND	0	ND
		Septic Effluent	ND	ND	ND	ND	0	ND

* ND indicates insufficient data to determine the die-off rate constant.

Table 6. Rate equation information for pathogen indicator die-off in soils amended with broiler litter, *E. coli* culture, and septic effluent at 5 and 35°C during the second laboratory study.

Pathogen indicator	Incubation temperature	Treatment	Die-off rate constant	Standard Error	Intercept	R^2	Data points regressed	Sampling days used
	°C		per day		ln/g dry soil			
Fecal Coliform	5	Broiler Litter	0.18	0.04	10.09	0.97	9	0 - 28
		E. coli Culture	0.18	0.04	17.25	0.80	7	0 - 56
		Septic Effluent	ND	ND	ND	ND	6	ND
	35	Broiler Litter	0.37	0.06	10.09	0.16	9	0 - 14
		E. coli Culture	0.37	0.06	17.25	0.97	8	0 - 28
		Septic Effluent	ND	ND	ND	ND	6	ND
E. coli	5	Broiler Litter	0.17	0.04	9.79	0.97	9	0 - 28
		E. coli Culture	0.17	0.04	17.06	0.80	7	0 - 56
		Septic Effluent	ND	ND	ND	ND	6	ND
	35	Broiler Litter	0.38	0.06	9.79	0.18	9	0 - 14
		E. coli Culture	0.38	0.06	17.06	0.95	8	0 - 28
		Septic Effluent	ND	ND	ND	ND	6	ND
Coliphage	5	Broiler Litter	0.04	0.07	13.00	0.54	12	0 - 56
		E. coli Culture	ND	ND	ND	ND	0	ND
		Septic Effluent	ND	ND	ND	ND	0	ND
	35	Broiler Litter	0.20	0.03	13.00	0.71	12	0 - 28
		E. coli Culture	ND	ND	ND	ND	0	ND
		Septic Effluent	ND	ND	ND	ND	0	ND

* ND indicates insufficient data to determine the die-off rate constant.

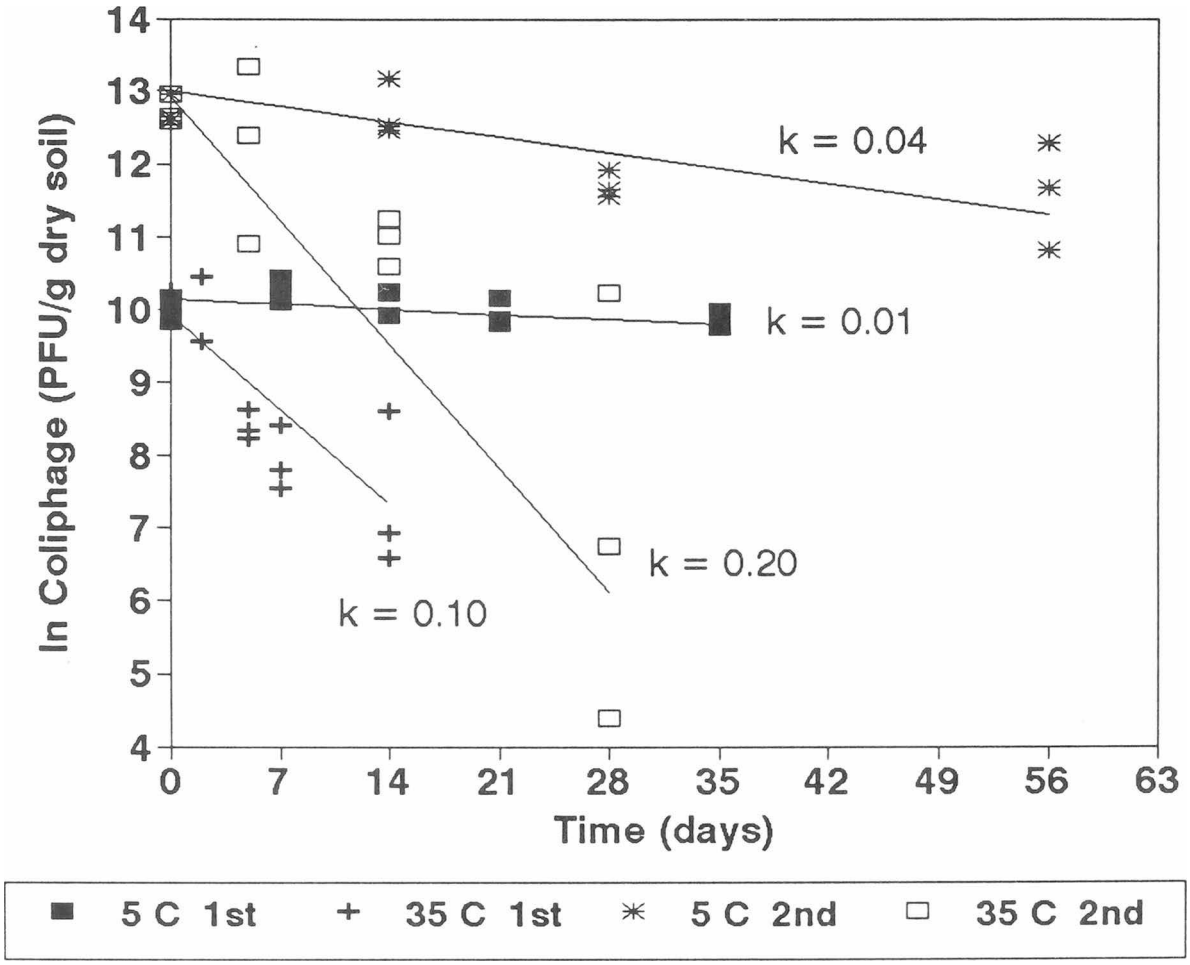

Fig 2. Coliphage die-off in broiler litter-amended Captina soil at 5 and 35°C in two laboratory studies.

Table 7. Time (in days) required for a 99.9% reduction in pathogen indicator levels.

	Temperature	Treatment	Indicator		
			Fecal coliform	*E. coli*	Coliphage
			days		
1st Study	5°C	Broiler Litter	63*	29	691*
		E. coli Culture	28	29	ND‡
		Septic Effluent	43*	ND	ND
	35°C	Broiler Litter	ND	7	69*
		E. coli Culture	8	7	ND
		Septic Effluent	27*	ND	ND
2nd Study	5°C	Broiler Litter	38	41	173*
		E. coli Culture	38	41	ND
		Septic Effluent	ND	ND	ND
	35°C	Broiler Litter	19	18	35*
		E. coli Culture	19	18	ND
		Septic Effluent	ND	ND	ND

* Indicates extrapolation beyond the length of the study.
‡ ND indicates insufficient data for determination of a die-off rate constant (k).

However, because the die-off rate constant was not significantly different than zero at 5°C, coliphage levels may not change over extended time periods. On the other hand, fecal coliform and *E. coli* levels would be reduced by 99.9% in ≤ 63 days at 5°C in both studies. VanDonsel and his associates (1967) found the time required for a 90% or one $\log_{10}$ reduction in fecal coliform levels in field plots ranged from 3.3 days in summer to 13.4 days in autumn.

The natural logarithm of the levels of *E. coli* in all soils amended with broiler litter and *E. coli* culture were plotted against those of fecal coliform. The regression line for each replication at every sampling time was $y = 0.94\ x + 0.81$ and the 95% confidence intervals for the slope of the line ranged from 0.89 to 0.99.

Table 8 summarizes die-off rate constants so that survival rates among the three pathogen indicators could be compared within a given temperature and treatment for each study. These rates were determined by analyzing the data by organism, but the comparisons among the indicators were made by analyzing the data by temperature. There were no significant differences in die-off rates between the two bacterial indicators, *E. coli* and fecal coliform, at both incubation temperatures in the litter and the *E. coli* culture treatments. The same was true for both studies even though the methodology for fecal coliform enumeration differed. With the direct method, counts for fecal coliform were available within 24 h, whereas the presumptive and confirmation phases of the EC + MUG method took up to 72 h. When studying survival rapid counts can be advantageous because during periods of rapid die-off, bacterial populations can drop to undetectable levels within 3 days. However, use of the A-1 method for

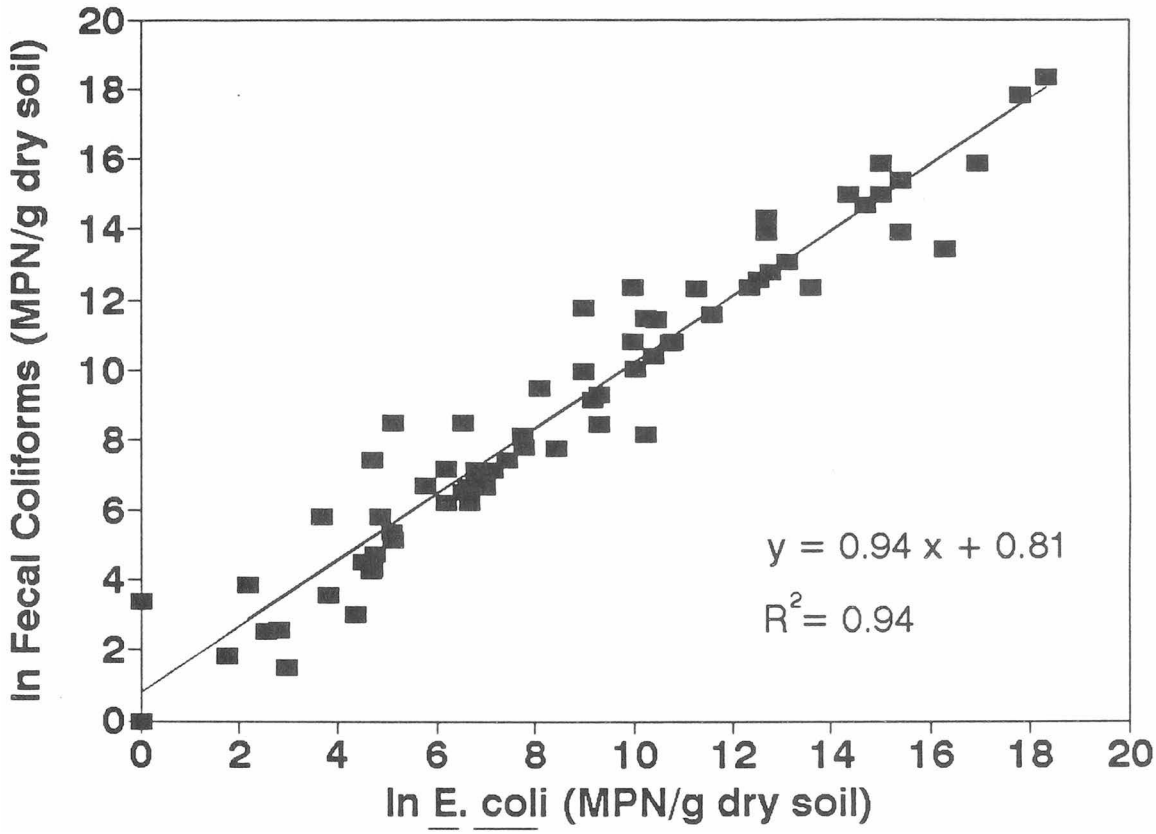

Fig. 3. Relationship of *E. coli* to fecal coliform in Captina soil amended with broiler litter or *E. coli* culture in two laboratory studies.

Table 8. Pathogen indicator die-off rate constants (k) for fecal coliform, *E. coli*, and coliphage in two laboratory studies.

		Indicator					
		Fecal Coliform		*E. coli*		Coliphage	
Study	Treatment	5°C	35°C	5°C	35°C	5°C	35°C
		k values (per day)					
1st	Broiler Litter	0.11a*D‡	ND	0.24aB	0.94aA	0.01bB§	0.10bA
	E. coli Culture	0.25aC	0.87aA	0.24aB	0.94aA	ND	ND
	Septic Effluent	0.16BD	0.26B	ND	ND	ND	ND
2nd	Broiler Litter	0.18aB	0.37aA¥	0.17aB	0.38aA	0.04bB§	0.20aA
	E. coli Culture	0.18aB	0.37aA	0.17aB	0.38aA	ND	ND
	Septic Effluent	ND	ND	ND	ND	ND	ND

* For a given treatment and temperature within a study, rates with the same lower case letter are not significantly different at $p \geq 0.05$.

‡ For a given indicator ***and*** treatment ***or*** temperature in the 1st study, rates with the same capital letter are not significantly different at $p \geq 0.05$.

ND Indicates insufficient data for determination of a die-off rate constant.

§ Die-off rate constant is not significantly different than 0.

¥ For a given indicator and treatment or temperature in the 2nd study, rates with the same capital letter are not significantly different at $p \geq 0.10$.

enumeration of fecal coliform included disadvantages such as the need for increased incubation tube and media preparation, separate inoculations, and additional room in the 44.5°C waterbath. These needs dictated a limited number of dilutions that could be analyzed for the bacterial indicators, resulting in missing data. Since the liabilities proved to be too great, fecal coliform and *E. coli* were enumerated simultaneously with EC Broth + MUG during the second study.

The bacterial die-off rate constants were also compared to those of coliphage within the litter-amended soils. At 35°C, in the first study, the die-off rate was 0.94/day for *E. coli* while the die-off rate for coliphage was 0.10/day in soil amended with broiler litter. At 5°C, in the same study, coliphage survived at least 10 times longer than the bacterial indicators, supporting the idea that fecal coliform and *E. coli* may not adequately indicate the potential for die-off of pathogenic viruses.

Significant differences among treatments or temperatures can also be compared within each study in Table 8. Die-off rates for the bacterial indicator, *E. coli*, were not different in soil amended with *E. coli* culture than in soil amended with broiler litter. This may be surprising because the *E. coli* in the wastes had already been subjected to harsh environmental conditions in the broiler house or the septic tank, whereas, those in the *E. coli* culture amendment were grown under optimal conditions in the laboratory where a carbon source and other nutrients were readily available. Most importantly, however, for each indicator, the die-off rates at 35°C were found to be significantly more rapid than at 5°C in all treatments supporting previous findings that lower temperatures enhance pathogen indicator survival (Reddy et al., 1981; Yates et al., 1985).

CONCLUSIONS

In the two laboratory studies, die-off of both bacterial and viral pathogen indicators in soil was significantly more rapid at 35°C than at 5°C, where indicator survival was enhanced. In fact, in the first study, the coliphage die-off rate was ten times more rapid when the incubation temperature increased from 5 to 35°C. In soil amended with broiler litter, the viral pathogen indicator persisted ≥ 10 times longer than the bacterial indicators at 5°C, in the first study. The die-off of *E. coli* in the broiler litter was not significantly different from the die-off of the *E. coli* indicator in the *E. coli* culture treatment at either temperature. Furthermore, the relationship between fecal coliform and *E. coli* levels was 1:0.94 with 95% confidence intervals at 0.89 and 0.99 in soils amended with broiler litter or *E. coli* culture. Increased survival at lower temperatures suggests that potential contamination of ground and surface water escalates with decreasing temperature.

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