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Fecal Coliform and E. Coli In Surface Runoff and
subsurface tile drainage from manure and fertilizer
treated field plots

By:

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**FECAL COLIFORM AND E. COLI IN SURFACE RUNOFF AND
SUBSURFACE TILE DRAINAGE FROM MANURE AND
FERTILIZER TREATED FIELD PLOTS**

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Howard Y.F. Ng, Jennifer Sifton, Ramesh Rudra and John Whiteside

Abstract

The total coliform and *E.coli* density in surface runoff and in tile effluent were investigated in two field plots. One of the field plots was treated with fresh liquid manure at 10,000 L/ha while the other plot maintained no manure treatment. The cumulative coliform count in surface runoff from the manure treated plot was about 1.4 (9225/6716) times more than the non-manure treated plot. The cumulative *E. coli* count in surface runoff from the manure treated plot was about 1.9 (3472/192) times more than the non-manure treated plot. This implies that manure had potentially impacted the surface runoff.

The ratio of cumulative coliform between the manure treated plot and the non-manure treated plot in tile effluent is almost equal, being 1.1 (5071/4777). Similarly, the ratio of cumulative *E.coli* between the manure treated plot to the non-manure treated plot in tile effluent is 1.9 (234/125) suggesting that the manure treated plot promoted potential leaching of *E.coli* through soil medium to tile drainage water.

The reliability of test results for coliform and *E.coli* was assessed at 90% confidence limit. The results of assessment for coliform and *E.coli* in surface runoff are consistent on the manure treated plot, whereas coliform and *E.coli* in surface runoff in non-manure treated plot are being random occurrences.

The magnitude of the coliform and *E.coli* density in the tile effluent depended on the manure application rate. The results of this study further implied that other sources such as fecal deposits from birds and wildlife also can contribute significantly. This study showed that cropped land appeared to be attractive to wildlife and birds because of food sources it provided. Thus, increases in fecal deposits are inevitable.

Coliformes fécaux et *E.coli* dans les eaux de ruissellement et les eaux de drainage des parcelles traitées au lisier et à l'engrais chimique.

Howard Y.F. Ng, Jennifer Sifton, Ramesh Rudra et John Whiteside

Résumé

Nous avons déterminé la densité de coliformes totaux et d'*E.coli* dans les eaux de ruissellement et les effluents des drains de deux parcelles expérimentales. Une parcelle a reçu un épandage de lisier liquide frais (10 000 L/ha), tandis que l'autre n'a pas reçu d'épandage de lisier. Le nombre cumulé de coliformes était environ 1,4 (9225/6716) fois plus élevé dans les eaux de ruissellement de la parcelle traitée au lisier que dans celles de la parcelle non traitée au lisier. De même, le nombre cumulé d'*E.coli* était environ 1,9 (3472/192) fois plus élevé dans les eaux de ruissellement de la parcelle traitée que dans celles de la parcelle non traitée au lisier. Ces résultats semblent indiquer que le lisier a eu un impact sur les eaux de ruissellement.

Dans le cas des effluents des drains, le nombre cumulé de coliformes était presque égal dans les deux parcelles, présentant un rapport de 1,1 (5071/4777) entre la parcelle traitée au lisier et la parcelle non traitée au lisier; le nombre cumulé d'*E.coli* présentait quant à lui un rapport de 1,9 (234/125), ce qui laisse croire que le traitement au lisier favorise le lessivage des *E.coli* à travers le sol vers les eaux de drainage.

La fiabilité des résultats du test a été évaluée avec une limite de confiance de 90 % tant pour les coliformes que les *E.coli* des eaux de ruissellement. Les résultats de l'évaluation sont convergents dans le cas de la parcelle traitée au lisier, alors qu'ils présentent une distribution aléatoire dans le cas de la parcelle non traitée au lisier.

La densité des coliformes et des *E.coli* dans les effluents des drains dépend du taux d'épandage du lisier. Les résultats de la présente étude semblent également indiquer que d'autres sources telles que les déjections d'oiseaux et d'autres animaux sauvages peuvent contribuer de façon appréciable à cette densité. Les terres cultivées semblent attirer les animaux sauvages et les oiseaux en raison des sources de nourriture présentes dans ces lieux. De ce fait, une augmentation des déjections est inévitable.

NWRI RESEARCH SUMMARY

Plain language title

NWRI and the School of Engineering, University of Guelph are studying the density of coliform and *E. coli* bacteria in runoff from the manure and the fertilizer treated field plot.

What is the problem and what do scientists already know about it?

Manure contains essential nutrients and organic matter to improve crop yields and to improve soil's ability to retain valuable nutrients. Animal manure contains pathogenic bacteria. Waterborne pathogenic bacteria pose a threat to sources of drinking water. Spreading of liquid manure on agricultural lands is a source of indicator bacteria such as fecal coliform, fecal streptococcus and *Escherichia coli* (*E. coli*), a subgroup of fecal coliform.

Why did NWRI do this study?

To find out the magnitude of transport of the indicator bacteria in surface runoff and in subsurface water after manure spreading on agricultural land.

What were the results?

The researchers found that manure treatment on agricultural land slightly increases both total coliform and *E. coli* transport to the tile drainage water. The magnitude of the total coliform and *E. coli* density in the tile effluent depended on the manure application rate. The study also found that other sources such as fecal deposits from bird and wildlife can also significantly contribute indicator bacteria.

How will these results be used?

These results supported the Great Lakes Water Quality Program for nutrient management practices that help farmers maximize manure utilization by crops while minimizing sources of pollution of drinking water sources.

Who were our main partners in the study?

School of Engineering, University of Guelph

Sommaire des recherches de l'INRE

Titre en langage clair

L'INRE et l'école de génie de l'Université de Guelph étudient la densité de coliformes et de bactéries *E. coli* dans les eaux de ruissellement de parcelles traitées au lisier ou à l'engrais chimique.

Quel est le problème et que savent les chercheurs à ce sujet?

Le lisier contient des nutriments essentiels et de la matière organique permettant d'augmenter le rendement des cultures et la capacité du sol à retenir des nutriments de valeur. Il renferme aussi des bactéries pathogènes. Or les bactéries pathogènes d'origine hydrique constituent une menace pour les sources d'eau potable. L'épandage de lisier liquide sur les terres agricoles fournit un apport de bactéries indicatrices telles que les coliformes fécaux, les streptocoques fécaux et les *Escherichia coli* (*E.coli*), un sous-groupe des coliformes fécaux.

Pourquoi l'INRE a-t-il effectué cette étude?

Détermination de l'importance du transport de bactéries indicatrices vers les eaux de ruissellement et les eaux souterraines après un épandage de lisier sur les terres agricoles.

Quels sont les résultats?

Les chercheurs ont constaté qu'un traitement des terres agricoles au lisier augmente légèrement le transport à la fois de coliformes totaux et de *E.coli* vers les eaux de drainage. La densité de coliformes totaux et de *E.coli* dans les effluents des drains dépend du taux d'épandage. De plus, l'étude montre que des sources additionnelles, comme les déjections d'oiseaux et autres animaux sauvages, influent également sur le taux de bactéries indicatrices.

Comment ces résultats seront-ils utilisés?

Ces résultats servent d'appui pour le Programme sur la qualité de l'eau des Grands Lacs en ce qui concerne les pratiques de gestion des nutriments qui aident les agriculteurs à maximiser l'utilisation du lisier par les cultures tout en réduisant au minimum la pollution des sources d'eau potable.

Quels étaient nos principaux partenaires dans cette étude?

École de génie, Université de Guelph.

**Fecal Coliform and *E.coli* in Surface Runoff and Subsurface Tile Drainage from
Manure and Fertilizer Treated Field Plots.**

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Abstract. The total coliform and *E.coli* density in surface runoff and in tile effluent were investigated in two field plots. One of the field plots was treated with fresh liquid manure at 10,000 L/ha while the other plot maintained no manure treatment. The cumulative coliform count in surface runoff from the manure treated plot was about 1.4 (9225/6716) times more than the non-manure treated plot. The cumulative *E. coli* count in surface runoff from the manure treated plot was about 1.9 (3472/192) times more than the non-manure treated plot. This implies that manure had potentially impacted the surface runoff.

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The magnitude of the coliform and *E.coli* density in the tile effluent depended on the manure application rate. The results of this study further implied that other sources such as fecal deposits from birds and wildlife also can contribute significantly. This study showed that cropped land appeared to be attractive to wildlife and birds because of food sources it provided. Thus, increases in fecal deposits are inevitable.

Keywords. Surface runoff, subsurface drainage, liquid manure, fecal coliform, *E. coli*

Introduction

Manure contains essential nutrients and organic matter to improve crop yields and soil's ability to retain valuable nutrients. Animal manure contains indicator bacteria. Indicator bacteria such as fecal coliform, fecal streptococcus, and *Escherichia coli* (*E.coli*), a subpopulation of fecal coliform, may originate from several sources such as deer, horses, dogs and birds (USDA 1997). It is difficult to ascertain the specific sources of the contamination in surface waters. Many waterborne microorganisms may cause a threat to sources of drinking water. Specifically, *E. coli* O157:H7 has been identified as a human pathogen (Riley et al., 1983; Tarr, 1995), and produces potent toxins that can cause severe illness in humans. *E. coli* O157:H7 may survive and grow in ovine or bovine feces under favorable environmental conditions (Hudva et al., 1998). Canadian drinking water guidelines specified the maximum acceptable concentration (MAC) for coliforms in drinking water as no sample should contain more than 10 total coliform organisms per 100 mL, and none of which should be *Escherichia coli* or thermo tolerant coliforms (Canadian Council of Minister of the Environment (CCME, 2001). Spreading of liquid manure on agricultural lands or the use of treated wastewater for irrigation are potential sources of these microorganisms.

In a field study, Culley and Phillips (1982) found that manure applications in winter resulted in significantly higher fecal coliform and streptococcus counts in surface runoff, and fecal streptococcus counts in subsurface discharge when compared with applications during other seasons. Warnemuende et al., (2001) reported that an increase in manure application rate is more likely to cause a greater increase in bacterial contamination in subsurface drainage with spring application than with fall application. Joy et al., (1998) investigated microbial contamination of subsurface tile drainage water and found that significant amounts of bacteria can reach surface water by infiltrating through the soil and travelling through the subsurface tile drains to receiving water. Rain shortly after manure application is suggested to be the most important indicator of bacteria contamination rather than spreading rate (Evans and Owens, 1972; Baxter-Potter and Gillian, 1988). Patni et al., (1984) measured bacterial water quality of surface runoff and tile drainage water from manure applied to fertilized cropland. They found higher concentrations of bacteria in both surface and tile drainage water after periods of high rainfall.

The purpose of this investigation was to compare coliform and *E.coli* density in surface runoff and in tile drainage water between two field plots. One of the field plots was manure treated while the other one was without manure treatment. The results may aid in determination of manure application rates, and the timing and methods to maximize nutrient utilization by plants from manure, while minimizing potential sources of water pollution.

Methods and Procedures

Field Plot

The study site comprised of two plots located at the Elora Research Station (43°38'02"N 80°24'50"W) in Elora, Ontario. Each plot had an area of 0.0836 ha (27.5 m x 30.5 m). The two field plots (Figure 1(A)), were under grass cover for more than five years before planting of corn in 2000, 2001 and 2002. A detailed description of the two field plots is given in Ng and Rudra (2001). For convenience, it is briefly repeated here. The soil type at each plot is predominately imperfectly drained Conestoga silt loam. There is a discontinuous sand layer at the level of the drain, which extends to a depth of 4 m at some locations. Below 4 m there is a tight hard till

which provides a low permeability barrier to seepage downward. The tiles for this design are 102 mm in diameter and are inter-connected in a closed loop design with one outlet at the corner of the plot.

Agronomy

Both plots were sprayed with Roundup at 7 L/ha on May 3 and again on May 7. Pre-emergence treatment with Prime Extra Magnum at 3.75 L/ha was applied on May 18, 2002. Both plots were implemented with no-tillage practice. Both plots were seeded with corn (*Zea mays* L.) at 63,000 seeds/ha at 75 cm between rows and at 51cm between plants. Fresh dairy liquid manure at 10,000 L/ha was spread on what will be referred to as Plot 3. The second plot that will be referred to as Plot 4 had 500 kg/ha of 0-20-20 (N-P-K) applied to it. The application rate of liquid manure on Plot 3 was about 18% of the maximum loading amount (56,000 L/ha) recommended by the Ontario Agricultural Code of Practice (Ontario Ministry of Agriculture and Food, 1976).

Sampling

A galvanized well with a cemented bottom (1.25 m diameter and 1.9 m deep) was established on each plot (Figure 1(A)) to receive tile drainage water (Ng and Rudra 2001). The inverted tile slope referenced to the outflow at the well was 0.008%. The spacing between cross-connected tiles was 9 m. The invert of tiles is 1.1 m below the ground surface. An ISCO model 2900 autosampler collects the tile flow samples. The autosampler contains 24-500 mL bottles and can be set to collect sample at desired time intervals. A water level sensor activates the autosampler. When the water level in the well rises to a preset level the autosampler is activated to start sample collection. The surface runoff sample was collected at a dugout of confluence from 3 perforated PVC pipes of 102 mm in diameter located at the north side of each plot. The pipe is 3.3 m in length. The PVC pipes are buried at half depth of its diameter size from soil surface. All three pipes are laid in a "T" shape layout.

Tile flow volume was measured using a calibrated sump pump. When the water level in the well rose to a preset level, the sump began to discharge water out of the well. When the water level returned to the preset level the sump stopped. The tile flow volume was calculated from the duration of the pump operation, multiplied by the pump discharge rate. The discharge rates of the sump pump were 1.1623 L/s and 1.1598 L/s for plot 3 and 4 respectively. An emergency discharge pump was installed at 0.41 m from the bottom of the well to prevent the sample well from becoming flooded. A rain gauge and a rainwater sampler (by Hohener Enterprises Ltd., Richmond Hill, ON) were established to measure the amount of rainfall and to collect rainwater for testing chemical compositions and biological counts for coliform and *E.coli*. Irrigation was administered during dry periods for the purpose of producing surface and tile drainage samples.

Background bacteria

One litre of fresh liquid manure sample was collected for bacterial background determination prior to spread on Plot 3. For each irrigation episode, a background sample was also taken for testing of coliform and *E. coli* in irrigation water. Duration of irrigation lasted between 3 to 5 hours. Irrigation water came from the Grand River. The intake was located in Fergus, Ontario and a pipeline carried the water to Elora site. Coliform and *E.coli* densities were also tested for rainwater samples.

Water sample treatment

Tests of coliform and *E. coli* densities for samples of rainwater, surface runoff, tile drainage, irrigation and groundwater were performed as soon as possible after being brought back to the laboratory from the field. The time lag between sample collection and sample testing varied between 4 to 24 hours depending on the time of occurrence of a rain event.

It is apparent that bacteria will begin to die off once in the environments of water and soil. However, those bacteria which are washed from surface or subsurface runoff and contact soil particulate and/or tile drainage sediments, becoming absorbed on the particles, can survive for lengthy period. Loeffler (1991) demonstrated an eighty day survival period of an antibiotic resistant *E.coli* in natural sediments of a marsh which received agricultural wastes. Depending on the number of samples, surface runoff and tile drainage samples were composited to reduce testing costs. One composite sample was prepared for analysis for each month during December to May, prior to manure application. From June onward and prior to harvest in October, two to five samples were collected each month from each rain event or irrigation episode.

Bacterial count procedures

The Coliplate test kit was used to test total coliform and *E. coli* in the water samples. The method is simple and results are available within 24 hours of sampling. This kit is enriched with a medium that enables the coliform bacteria to utilize the nutrients and reagents in the medium while inhibiting the growth of background bacteria. Each Coliplate contains 96 wells. Water samples were dispensed into the wells (one plate per sample) and placed in an incubator for 24 hours at 35°C and observed. The cells that turn blue are counted. The blue cells represented the total coliform response cells, while the colonies of *E. coli* were detected by fluorescence under a long wave ultra violet light. Quantifications of both total coliform and *E. coli* were referenced to a table of the Most Probable Number (MPN) of colony forming-units (CFU) per 100 mL of sample provided in the kits.

Results and Discussion

Initial coliform and *E. coli* density in fresh manure

One litre of fresh manure sample was collected for determination of initial concentration of coliform and *E.coli*. Due to the Coliplate's detection limit (ranging from 5 to 5000 CFU/100 mL), the manure sample was serially diluted with distilled water in proportion to its original volume. The results of total coliform and *E. coli* counts for dilution factors of the fresh manure sample are given in Table 1 and plotted in Figure 2.

Table 1. Coliform and *E.coli* counts for fresh manure sample

Percentile	Fresh manure sample	
Dilution	Coliform count	<i>E. Coli</i> count
(%)	(CFU /100mL)	CFU /100mL
0.02	55	52
0.03	220	200
0.04	350	328
0.05	534	434
0.10	1174	938

The initial coliform and *E. coli* density in fresh manure was approximated by extrapolation (Figure 2) to the Y-axis intercept. The readings were approximately 38,640 CFU/100 ml (1400 x 27.6 mean dilution factor) for coliform and 34,500 CFU/ 100 ml (1250 x 27.6 mean dilution factor) for *E. coli*. The results suggested that fresh manure had about 89% of *E. coli* contained in the total coliform.

Background coliform and *E. coli* densities in irrigation water and rainwater

There were seven irrigation episodes initiated during the experimental period. Samples were collected for five out of seven of the irrigation episodes and for five rainfall events. Results of coliform and *E. coli* counts for the irrigation water and rainwater samples at 24-hr incubation period are given in Table 2. Table 2 shows that both irrigation water and rainwater contain coliform bacteria but almost negligible *E. coli*.

Background coliform and *E. coli* density on plots

Prior to manure field experiment, surface runoff and tile effluent samples collected from Plot 3 and Plot 4 were tested for coliform and *E. coli* density (Table 3 and Table 4). Coliform and *E. coli* show significant number of counts in surface runoff (Table 3) for 24 hours incubation time. The high bacteria counts were likely caused by bird and other wildlife feces. The coliform and *E. coli* counts in tile effluent samples (Table 4) from Plot 3 and Plot 4 were less than or equal to detection limit suggesting that bacteria transport to subsurface water were minimal.

Table 2. Background coliform and *E. coli* counts (CFU/100mL) for irrigation and rainwater samples

Sample date dd/mm/year	Irrigation water		Rainwater	
	Coliform	<i>E. coli</i>	Coliform	<i>E. coli</i>
24/07/2002	59	5		
14/08/2002	28	3	94	<3
29/08/2002	8	<3		
10/09/2002	28	<3		
25/09/2002	28	3	94	<3
04/10/2002			11	<3
18/10/2002			11	<3
31/10/2002			<3	<3
Mean	30	3	43	<3

<3 is taken as 3 in the calculation.

Table 3. Background coliform and *E. coli* densities in surface runoff samples prior to manure treatment on Plot 3

No. of samples: 5	Plot 3		Plot 4	
	24-hour incubation		24-hour incubation	
	Coliform	<i>E. coli</i>	Coliform	<i>E. coli</i>
Geometric mean	29	14	60	29
Maximum	694	619	794	794
Minimum	3	3	5	3
Standard deviation	267	245	296	306

Table 4. Background coliform and *E. coli* counts in tile effluent samples prior to manure treatment on Plot 3

No. of samples: 5	Plot 3		Plot 4	
	24-hour incubation		24-hour incubation	
	Coliform	<i>E.coli</i>	Coliform	<i>E.coli</i>
Geometric mean	4	7	3	3
Maximum	11	11	3	3
Minimum	3	3	3	3
Standard deviation	3	10	0	0

Comparison of coliform and *E.coli* density between manure treated and untreated plots

On May 30 2002, 10,000 L/ha fresh dairy liquid manure was spread onto Plot 3, and 500 kg/ha of 0-20-20 (N-P-K) was applied to Plot 4. During dry periods, irrigation was administered to both Plot 3 and Plot 4 in order to produce surface runoff and tile effluent. The surface runoff and tile effluent samples from Plot 3 and Plot 4 were tested for coliform and *E. coli*. The geometric mean, maximum, minimum and standard deviation of the test results for coliform and *E.coli* are presented in Tables 5 and 6. The results (Table 5 and 6) showed that the standard deviation is significantly lower or higher than the geometric mean, which can produce a mean with gross error.

Assessment of reliability of test results

There are a number of methods for assessing the acceptance or rejection of the questionable results. The most common method is based on Q-test (Nollet, 2000). The Q-test is performed at 90% confidence level, which is considered an appropriate limit for the test. The rejection quotient is given as follows:

$$Q = (X_n - X_{n-1}) / (X_n - X_1)$$

Where X_n is the questionable results in a set of results in ascending order X_1, X_2, \dots, X_n . The value obtained from the calculation of Q (experimental) is compared with a table of critical values of Q (critical at 90 % confidence limit). The results of Q (experimental) had two values out of eight are being unreliable for coliform, and five values out of eight for *E.coli* in surface runoff samples from Plot 3, where for Plot 4, all the Q (experimental) values are being unreliable for coliform and *E.coli* in surface runoff. This may suggest that the occurrence of coliform and *E.coli* in surface runoff is consistent on Plot 3 compared to coliform and *E.coli* in Plot 4, which are a random occurrence.

The results of Q (experimental) had three values out of twenty one results for both coliform and *E.coli* designated as unreliable in tile drainage samples from Plot 3, whereas in Plot 4, the results of Q (experimental) also had three values out 21 results being unreliable for coliform, but all values being unreliable for *E.coli* in tile drainage samples. The results of Q (experimental) test imply that there is a positive impact from coliform and *E.coli* on tile drainage with surface applied manure.

Table 5. Results of coliform and *E. coli* counts (CFU/100 mL) in surface runoff after application of manure on Plot 3 and of fertilizer on Plot 4.

No. of samples: 8	Plot 3		Plot 4	
	24-hour incubation		24-hour incubation	
	Coliform	<i>E.coli</i>	Coliform	<i>E.coli</i>
Geometric mean	962	162	375	60
Maximum	2424	1794	2424	854
Minimum	534	11	3	3
Standard deviation	751	570	718	316

Table 6. Results of coliform and *E. coli* counts (CFU/100 mL) in tile effluent after application of manure on Plot 3 and of fertilizer on Plot 4.

No. of samples: 21	Plot 3		Plot 4	
	24-hr		24-hr	
	Coliform	<i>E.coli</i>	Coliform	<i>E.coli</i>
Geometric mean	31	5	38	4
Maximum	2280	104	2424	33
Minimum	3	3	3	3
Standard deviation	498	22	517	8

Because the measured values of the coliform and *E. coli* differed widely, the use of descriptor of geometric mean (Tables 3, 4, 5 and 6) appeared to be more appropriate. The geometric mean was calculated as the n th root of the product of n data points or equivalently by the use of logarithms. The standard deviation (Tables 3, 4, 5 and 6) was for arithmetic mean of the data points around a central value to describe the dispersion of a normal population, and that is from the range of a set of measurements. The range is the difference between the largest and smallest value in a set.

The cumulative coliform and *E. coli* counts for 24-hour incubation in surface runoff after manure application are shown in Figures 3 and 4. The cumulative coliform count in the manure treated plot was about 1.4 (9225/6716) times more than the non-manure treated plot. The cumulative *E. coli* count in the manure treated plot was about 1.9 (3472/192) times more than the non-manure treated plot. This implies that manure had potentially impacted the surface runoff.

The ratio of cumulative coliform between the manure treated plot and the non-manure treated plot in tile effluent is almost equal, being 1.1 (5071/4777). Similarly, the ratio of cumulative *E. coli* between the manure treated plot to the non-manure treated plot in tile effluent for 24-hour incubation is 1.9 (234/125) suggesting that the manure treated plot promoted potential leaching of *E. coli* through soil medium to tile drainage water. Dean and Foran et al. (1990) have demonstrated conclusively that when the tiles are running, liquid swine or cattle manure spread on tiled fields results in the significant contamination of the tile drainage by fecal indicator. It has been suggested that macro-pores in the soil and plant root channels allowed the transport of nutrients and bacteria through the soil to the field tile drainage below. It should also be noted that *E. coli* always exists on the plot surface whether or not that manure has been applied.

Summary and Conclusion

This study showed that manure treatment on agricultural land increased both coliform and *E.coli* transport to the tile drainage water slightly. The reliability of results for coliform and *E.coli* was assessed at 90% confidence limit. The results of assessment for coliform and *E.coli* in surface runoff and in tile effluent are consistent on Plot 3, whereas coliform and *E.coli* in surface runoff and in tile effluent in Plot 4 are being a random occurrence.

The magnitude of the coliform and *E.coli* densities in the tile effluent depended on the manure application rate. The results of this study further suggested that other sources such as fecal deposits from birds and wildlife can also contribute significantly. The results of this study suggested that cropped land appeared to be attractive to wildlife and birds because of food sources it provided. Thus, increases in fecal deposits are inevitable.

Acknowledgement

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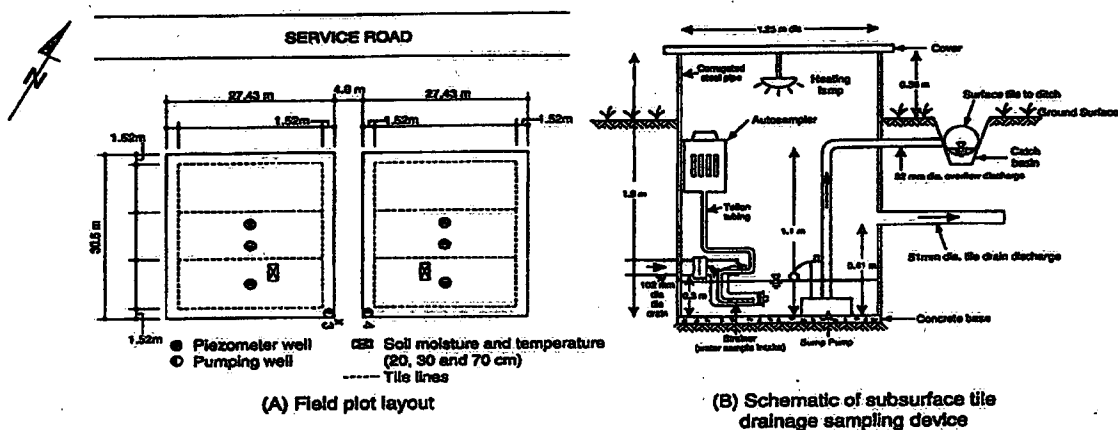
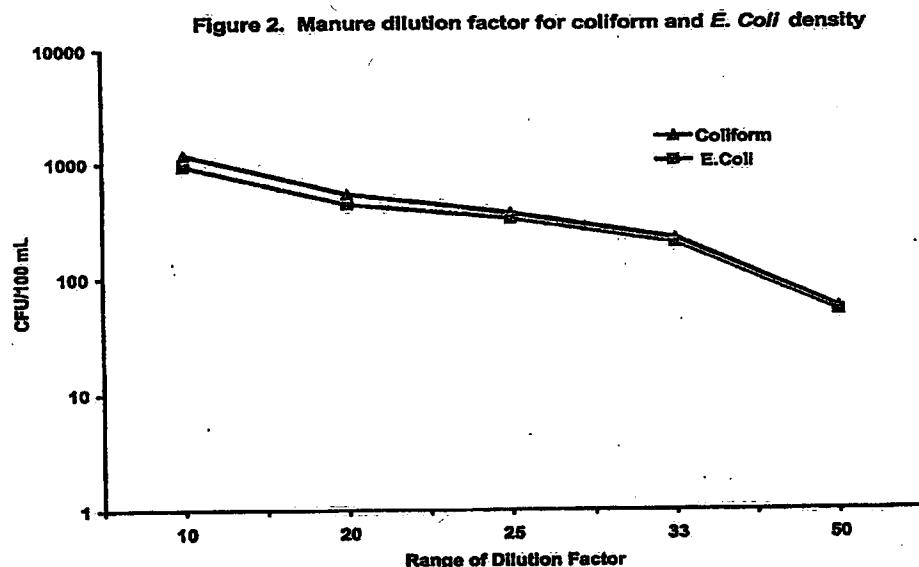
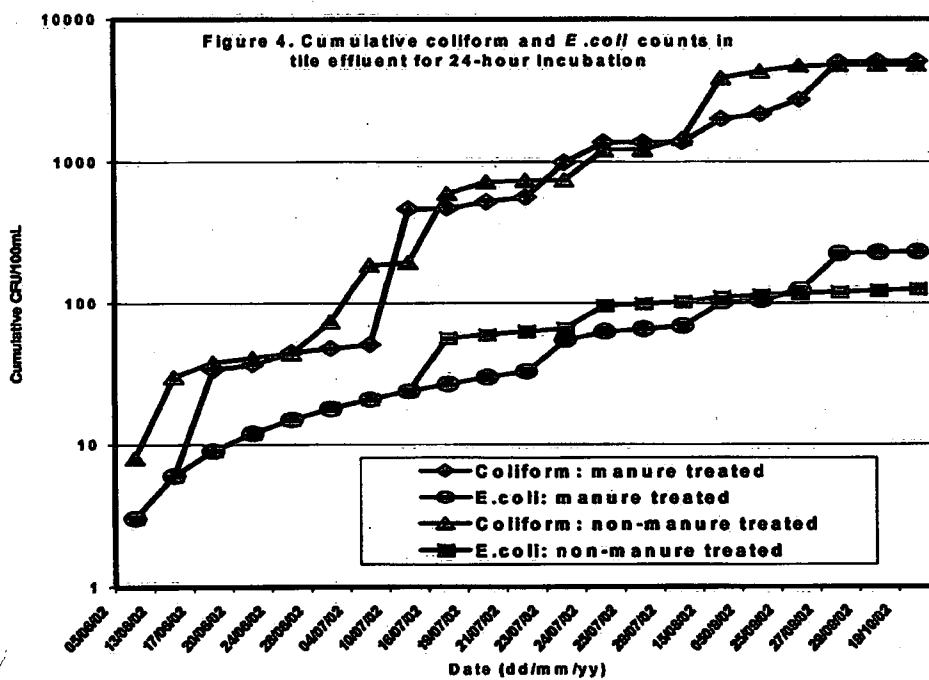
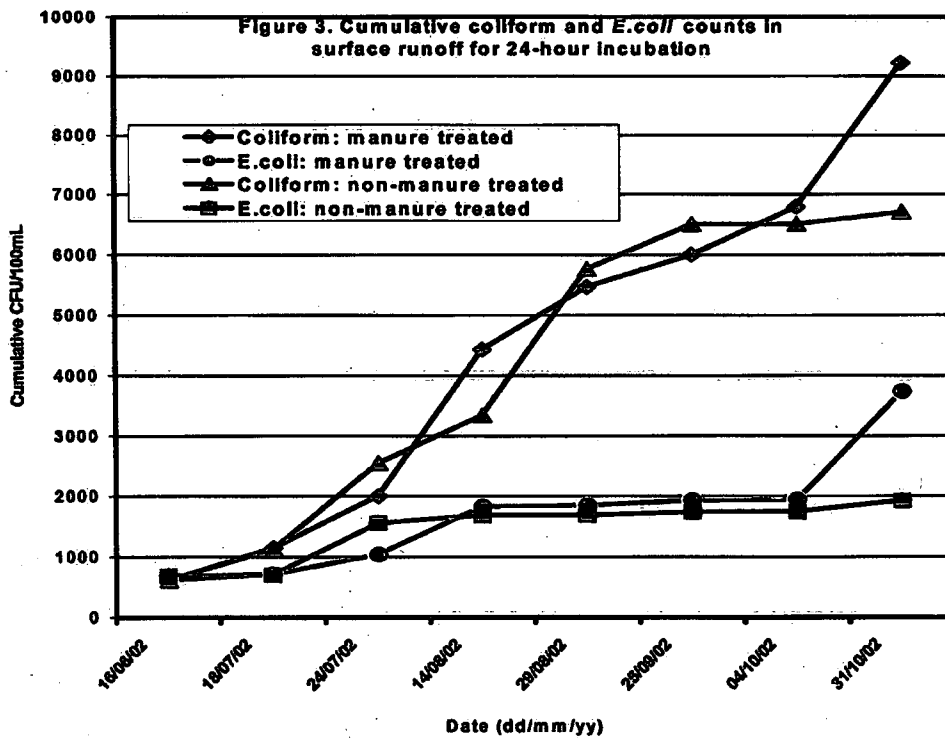


Figure 1. Field plot design and tile drainage sampling device





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