Microbial contamination of subsurface tile drainage water from field applications of liquid manure

D.M . JOY[1], H. LEE[2], C.M. REAUME[2], H.R. WHITELEY[1] and S. ZELIN[1]

[1]School of Engineering and [2]Department of Environmental Biology, University of Guelph, Guelph, ON, Canada N1G 2W1; and [2]Solmax Geosynthetics, Etobicoke, Ontario, M8W 3S2. *Received 31 January 1997; accepted 18 March 1998.*

Joy, D.M., Lee, H., Reaume, C.M., Whiteley, H.R. and Zelin, S. 1998. **Microbial contamination of subsurface tile drainage water from field applications of liquid manure**. Can. Agric. Eng. **40**:153-160. Application of liquid manure to fields is a common practise to efficiently utilize and dispose of manure in many North American farm operations. Guidelines given to farm operators frequently focus on the amounts of manure required to provide certain nutrient requirements given the source of the manure and plant requirements. Under proper conditions, this is an efficient use of manure with few adverse environmental impacts. However, under adverse conditions, excessive application rates can result in significant ground and surface water contamination by bacterial and other contaminants in the manure. A key problem is that the pathways by which these contaminants reach surface waters are not well understood. Because of this, not enough information is available on the amounts which can be safely spread and those conditions to avoid in order to prevent inadvertent contamination by the spreading of liquid manure. The experiments described in this paper report on bacterial contamination at a single field site due to liquid manure spreading under accepted practises in Ontario over a two year period. A nalidixic acid resistant strain of *Escherichia coli* was used as a biotracer to quantify the degree of transport from the field level to nearby receiving waters. The results show that significant amounts of bacteria can reach surface water by infiltrating through the soil and travelling through sub-surface tile drains to the receiving water. Rain shortly after manure application is suggested to be the most important indicator of bacterial contamination rather than spreading rate (volume applied per unit area) or condition of the field prior to spreading.

L'application de purin dans les champs est une pratique communément utilisée en Amérique du Nord pour se défaire des déjections animales. Dans les normes d'application fournies aux agriculteurs, les dosages de purin sont calculés de manière à rencontrer les besoins nutritifs en certains éléments, selon la provenance du fumier et les besoins des plantes. Lorsque les conditions sont adéquates, cette façon de faire est efficace et a peu d'impacts environnementaux. Cependant, dans de mauvaises conditions, des applications excessives conduisent à la contamination des eaux de surface et souterraines par les bactéries et autres contaminants du fumier. Les voies empruntées par ces contaminants pour atteindre les eaux de surface sont mal connues. A cause de cela, on ne connaît pas les conditions et les quantités de purin qui peuvent être épandues de manière sécuritaire. Durant deux ans, on a étudié la contamination dues aux bactéries provenant d'un champ où les épandages avaient été faits selon les pratiques admises en Ontario. On a utilisé une souche de *Escherichia coli* résistante à l'acide nalidixique comme biotraceur afin de suivre le mouvement des bactéries entre le champ et les eaux de surface à proximité. Les résultats montrent que des quantités importantes de bactéries atteignent les cours d'eau en s'infiltrant dans les sols et en se déplaçant dans les tuyaux de drainage souterrain. Il semble que le meilleur indicateur de mouvement des bactéries soit les précipitations peu de temps après les épandages plutôt que le dosage (volume appliqué par unité de surface) ou les conditions du champ avant l'application.

INTRODUCTION

Land application of manure is the primary mode of manure disposal on many dairy, swine, and beef operations in Canada. Current guidelines recommend maximum application rates that strive to meet nutrient requirement needs without causing contamination of surface and groundwater. Documented incidents of beach closures (Dean and Foran 1991) and numerous cases of contaminated rural farm wells in which liquid manure application was identified as one of the key sources of contamination (Rudolph and Goss 1993) have raised questions as to the ability of the current guidelines to adequately protect surface and groundwater from contamination by this practise.

Verification of manure application rate guidelines has been limited due to a lack of field information. This is particularly true when it comes to including field and meteorological conditions which may affect the likelihood of contamination by liquid manure spreading. Thus, there has been a real need for field data documenting the levels of contamination that can be expected to result from liquid manure application and the factors, particularly environmental, which can lead to unintentionally high levels of contamination. This paper reports on the results of two years of field trials at an operational farm examining the movement of bacteria from field application of liquid manure to the surface water through tile drains.

BACKGROUND

The association of agricultural practices, and in particular the application of manure to fields, with subsequent surface and subsurface water pollution is well established. A recent survey of domestic wells in rural Ontario (Rudolph and Goss 1993) found that approximately 25% of the wells do not provide water that meets drinking-water standards for microorganisms and/or nitrates and concludes that agricultural practices were among the main contributors. The potential for pollution is recognized by the Ontario Agricultural Code of Practise (Ontario Ministry of Agriculture, Food and Rural Affairs 1976) which recommends maximum loading amounts (56,000 L/ha or

5.6 mm) for liquid manure application and as well suggests conditions under which spreading should be discouraged (e.g. when the ground is frozen). These recommendations are made with the objective of minimizing applications when the manure will most likely contaminant surface runoff.

Field studies on the pollutant loadings to surface water from manured fields have been reported by a number of researchers. Field trials on manured fields of sandy clay loam were reported by Culley and Phillips (1982). They considered a range of application rates and times and found the highest bacterial concentrations in spring runoff, regardless of the time of application. Patni et al. (1984, 1985) measured the bacteriological quality of surface runoff and tile drainage water from manured fields. They found that the manured fields had higher bacterial concentrations in runoff than non-manured fields, that the greatest concentrations occurred in surface runoff (compared to the tile-drain water), and that higher concentrations occurred in both surface runoff and tile-drain water after periods of high rainfall.

Niemi and Niemi (1991) compared surface water bacterial levels in "pristine" and agricultural areas. Their results indicated that while agricultural areas had higher levels of bacterial contamination, often exceeding bathing standards of 1000 Colony Forming Units (CFU)/100 mL, pristine areas also had faecal indicators in at least half the samples taken. In addition, bacterial levels were higher for both types of areas after periods of high rainfall.

One problem with the studies to date is the difficulty of quantifying the relationship between the application of manure and the resulting water bacterial quality. Because of the multiplicity of sources of bacteria, it is difficult to ascertain what is the precise source of the contamination in surface waters. In addition, the reduction in contamination levels with time is difficult to determine. To avoid this problem, antibiotic-resistant strains of bacteria were investigated by Rahe et al. (1978) and McCoy and Hagedorn (1980) as a means of determining the transport rates of bacteria through ground water. These strains are similar to those present in faecal matter, rarely present in the environment, harmless, and thus make excellent biotracers to study the movement of bacteria from sources such as septic systems and field applications of manure. These biotracers have been used successfully by a number of researchers in studies on septic systems (e.g. Shadford et al. 1997).

Huysman et al. (1993) used an antibiotic-resistant strain of *clostridia* naturally present in swine manure spread on fields in Belgium. Because it was naturally present in swine manure, it did not need to be added as a tracer. They showed that the presence of the biotracer in groundwater and soil was highly correlated with the application of swine manure; whereas, the traditional indicators such as *E. coli* and faecal streptococci were not good indicators of where manure had been applied.

To study the transport of bacteria from liquid manure to tile drains, Dean and Foran (1991) and Fleming et al. (1990) added a strain of *E. coli* resistant to nalidixic acid (*E.coli* NAR) to liquid manure. This strain is not normally present in manures nor in the environment and thus the detection of its presence in surface or ground water after application showed the rate and degree of transport. Their results showed that, under the correct conditions, that movement of bacteria from the surface application to surface waters through tile drainage could occur quickly.

OBJECTIVES

The purpose of the study was two-fold:

1. To further investigate the use of *E.coli* NAR as a biotracer for studies of bacterial pollution from agricultural sources, and
2. To determine the extent of bacterial movement from manured fields and through tile drains to surface water under normal agricultural practices.

To do this, a series of four field studies were carried out over the course of two years, all at the same site using the *E. coli* NAR.

SITE DESCRIPTION

The experimental site is at the Elora Research Farm in Elora, ON. Fields at the farm are all systematically tile drained due to the imperfect internal drainage of the soils. The surface soil is predominately loam and slopes in the area are generally in the range of 4 to 9%. Watertable depths are typically 3 m at the top of slopes and less than 1 m in swales.

The field studied is actively farmed using conventional mouldboard plow tillage practices and has 100-mm diameter drain tiles of vitrified clay pipe at approximately 17 m spacing installed in 1967. It had been planted with corn for the 5 years leading up to 1994.

Figure 1 shows the 0.9 ha portion of the field that was used for the trials. Surface slopes are approximately 4% in this area as measured by a survey carried out for the study. To monitor water quality and quantity, three parallel tile lines flowing from east to west were equipped with access chambers consisting of vertical sections of 900 mm diameter plastic pipe (Fig. 2). Downstream of the access chambers, drainage water enters an interceptor which drains into a ditch approximately 20 m from the edge of the 0.9 ha area (Fig. 1).

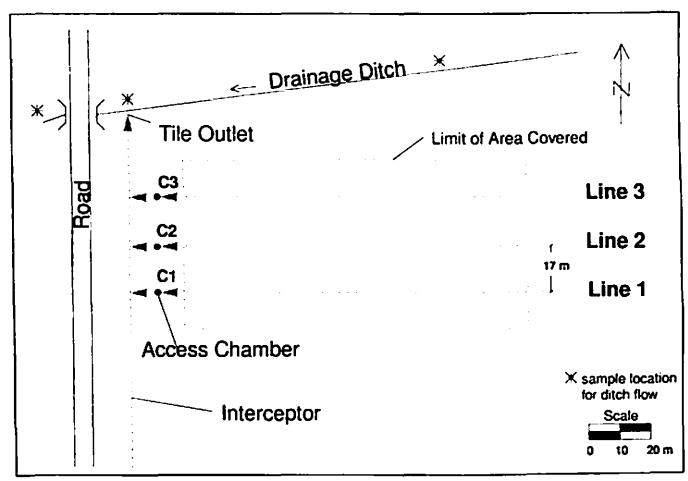

Fig. 1. Tile drainage schematic for the Elora Site showing sampling locations, subsurface drainage lines, and access chambers.

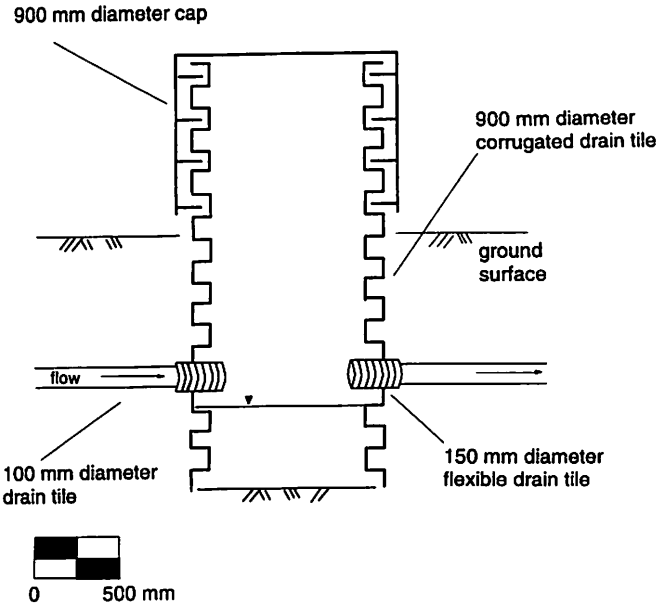

Fig. 2. Tile drain access chamber cross-section.

The typical frequency of liquid manure application to this field is twice per year. Spring application is done before planting and fall application after the crop is removed. The liquid manure comes from a dairy facility a short distance from the field. It is stored in underground tanks until spreading at which time it is agitated, pumped into a tanker for transport to the field, and applied by gravity from the back of the tanker using a deflector on the outlet nozzle.

MATERIALS and METHODS

Biotracer

The biotracer used for the experiments was a naturally, although rarely, occurring nalidixic acid resistant strain of *E. coli* (*E. Coli* NAR). Background levels of nalidixic acid resistant *E. coli* in the manure samples or field plots were not detectable, making *E. coli* NAR an ideal tracer. *E. coli* NAR is a Gram negative, rod-shaped bacterium having a size of approximately 1x2 μm. This strain exists singly or in pairs. It is a facultatively anaerobic chemoorganotroph, sensitive to ultraviolet light.

Use of *E. coli* NAR as a biotracer has been shown to be an effective means of determining bacteriological water quality (e.g. Rahe et al. 1978). Earlier work (Joy et al. 1992) has shown that *E. coli* NAR has similar growth and decay rates to other *E. coli* strains. Resistance of this strain to the antibody nalidixic acid allows it to be selectively isolated from water and soil samples to provide reliable information on the movement phenomena of this type of bacteria. The biotracer used in this experiment was obtained from G. Palmateer, Ontario Ministry of Environment and Energy, London, Ontario.

Experimental procedure

Preparation of the biotracer began two days before anticipated manure application. Stock culture of the biotracer was used to provide one loopful of *E. coli* NAR from a Trypticase Soy Agar plate supplemented with nalidixic acid. This was transferred to 25 mL of sterile Trypticase Soy Broth (TSB) in a 125-mL Erlenmeyer flask and allowed to incubate at 20 °C for 24 h with gyratory shaking at 200 rpm. The culture was then used to inoculate a 2-L Erlenmeyer flask containing 1.5 L of TSB. Incubation was continued by magnetic stirring at 20 °C for 18 hours.

Prior to transport to the field, the broth was transferred to sterile 250-mL centrifuge bottles and centrifuged at 400 x g for 20 minutes. The supernatant was decanted and the cells resuspended into 2 L of 0.1 M phosphate buffer, pH 7.5. The inoculum was then transported to the field on ice for introduction into the liquid manure.

Four experiments were conducted at the field site between the fall of 1992 and spring of 1994. All experiments were conducted in essentially the same manner consisting of some initial field measurements, inoculation of liquid manure with biotracer, spreading of manure, and subsequent measurements.

Prior to manure spreading, background levels of both indigenous *E.coli* and *E.coli* strains resistant to nalidixic acid were determined from soil cores taken at depths down to 900 mm. The samples were taken from three locations in the field. Cores were taken with a 20-mm diameter hand corer to each of the depths above. Between cores, the sampler was cleaned in an alcohol bath and then flamed to remove any residual bacteria. Soil water content was also determined from these cores. Soil samples collected in the field were sealed in plastic sample bags and their soil water content determined by oven-drying in the lab. Flow rates in the tile lines were determined prior to and after manure spreading for up to 40 days. This was accomplished by draining the access chamber and then measuring the volume of tile flow entering the chamber over a timed interval. Soil samples were taken daily immediately after manure spreading and irregularly afterwards until no biotracer was detected.

Concentrations of *E.coli* strains resistant to nalidixic acid in tile water were determined before, during, and after spreading. Individual 200-mL samples were taken from each of the tile lines, at the outlet of the interceptor, and in the drainage ditch up and downstream of the outlet at locations shown in Fig. 1. No biotracer was ever detected at the upstream locations in the ditch. The biotracer was detected at the downstream location and values are reported by Reaume (1994). During each sampling, the volumetric flow rates in the tile lines were also measured.

Manure was spread on the field using a tractor-drawn tanker moving parallel to the tile drains. The volume of the tanker was 9500 L and was drained by gravity. Just before manure application, the inoculum was transported to the manure storage site on ice. At the site 500 mL of inoculum was added to 19.5 L of ultrapure deionized water in a 20-L carboy and shaken vigorously. After taking a 10-mL sample for enumeration, the carboy was emptied into the tanker before the manure was loaded in the tanker. The volume and concentration of inoculum was chosen so as to have a tracer bacteria concentration in the manure similar to that of the indigenous *E. coli*. (~10^6 CFU/g wet basis based on earlier measurements). The action of loading the liquid manure and the approximately 0.5 km travel distance to the site provided ample mixing of the inoculum and the manure. This was later confirmed by the presence of the biotracer in all of the field sample cups.

Prior to spreading the manure on the field, 18 to 20 sterile, 120 mL sample cups were laid out on the field to measure the spreading rate. These were placed flush with the ground surface in areas where surface runoff was not expected to flow into the cup. The layout of the cups was along 3 lines perpendicular to the travel direction of the spreader.

Manure was applied according to the normal practises of the farm. The desired spreading rate of just under 5.6 mm (56,000 L/ha) per application was judged subjectively by the operator. Application direction was primarily parallel to the tile drains. During application the tile effluent was observed. If tile flow existed, periodic 200-mL samples were taken.

Following liquid manure application, the field sample cups were collected and the volumes measured. The samples were then stored on ice prior to bacterial enumeration within 24 hours. Within 48 hours of application soil cores were also collected to determine the immediate depth of penetration into the soil by the biotracer. Tile effluent samples were collected when available and flow rates measured. Daily rain amounts were also measured at a nearby (< 2 km) meteorologic station for the entire duration of the experiments.

Enumeration

All biotracer samples were enumerated using the membrane filtration technique (APHA 1989). Duplicate 100-mL water samples were filtered using Gelman sterile cellulose-acetate filters (0.45 µm). Following filtration, the filters were placed on mTEC-NA agar in 60 x 15 mm Petri plates. Serial dilutions were required depending on the expected concentration in the sample.

Table I. Summary of results.

Exp.	Average depth applied (mm)	Biotracer field concentration in manure, wet basis (CFU/g)	Soil water content (m^3/m^3)	Air temperature (°C)	Maximum detected tile water biotracer concentration (CFU/100 mL) (days since application)		
					Tile drain line		
					1	2	3
F92	4.7	$7.6x10^3$	0.18- 0.22	3	290 (5)	52 (7)	2 (33)
S93	6.9	$1.3x10^5$	0.14- 0.28	25	1 (41)	0	0
F93	5.4	$8.4x10^4$	0.15- 0.25	4	17 (18)	61 (18)	17 (18)
S94	2.9	$4.1x10^4$	0.15- 0.25	20	1400 (2)	1300 (2)	1100 (6)

Note: CFU = Colony forming unit
Water content at saturation = 0.48 m^3/m^3
Water content at field capacity = 0.28 m^3/m^3
Dry soil bulk density = 1.37 Mg/m^3

Soil core samples were first prepared by thoroughly mixing 2-g samples in 199 mL of 0.85% physiological saline. These were then shaken for an hour prior to filtration. Manure samples were also diluted in 9 mL of saline, vortexed, and then spread-plated on mTEC-NA agar after appropriate serial dilutions to obtain countable numbers.

RESULTS

Distinctly different results were obtained for each of the four experiments despite similar application procedures for each experiment. Table I summarizes some of the key results for each of the four experimental trials.

Application characteristics

The objective of the operator was to spread just under 56,000 L/ha (5.6 mm depth), according to Agricultural Code of Practice Guidelines (Ontario Ministry of Agriculture and Food 1976). The actual rate of application was determined subjectively by the operator and controlled by the speed of the tractor. As would be expected with this level of control, average depths of application among the experiments deviated from this target and ranged from 2.9 to 6.9 mm (Table I). The minimum of 18 samples used to determine these mean depths had coefficients of variation for the four applications that varied between 0.45 and 0.86. This indicates that not only was there significant variation in depth from one application to the next but also within any application.

In addition to the variation in the depth of application spatially and among applications, there was also significant variation in the surface-applied biotracer concentration. The mean values for the four applications ranged from $7.6 x10^3$ to $1.3x10^5$ CFU (colony forming units)/g (wet basis) of manure as determined from the surface samples. There also was significant variation among samples collected for each application. Note that these concentrations represent a reduction by a factor of 100 from the intended concentrations of biotracer and is attributed to die-off of the biotracer when placed in the tanker. For all experiments, some surface ponding of manure was noted after application, but no surface runoff was observed.

Tile drain water characteristics

Tile-drain-water results are only reported here for the measurements taken directly from the tile lines. Their proximity to the surface outlet ensures that any contamination in these three lines will reach the drainage ditch in Fig. 1. However, because the area examined represents only a small fraction of the total area leading into the drainage ditch, only the results for the tile drains in the area examined are presented.

Before and after manure application, flow rates and biotracer concentrations were monitored in the three tile lines (L1, L2, L3). Prior to application no biotracer was detected in

the tile flows. Flow rates and concentrations as a function of time for three of the four experiments are given in Figs. 3 to 5 with time referenced from the time of application. Note that no data are given for the spring 1993 experiment, because only one water sample had *E. coli* NAR (1 CFU/100 mL) detected and flow in the tiles was zero until 40 d after application of manure.

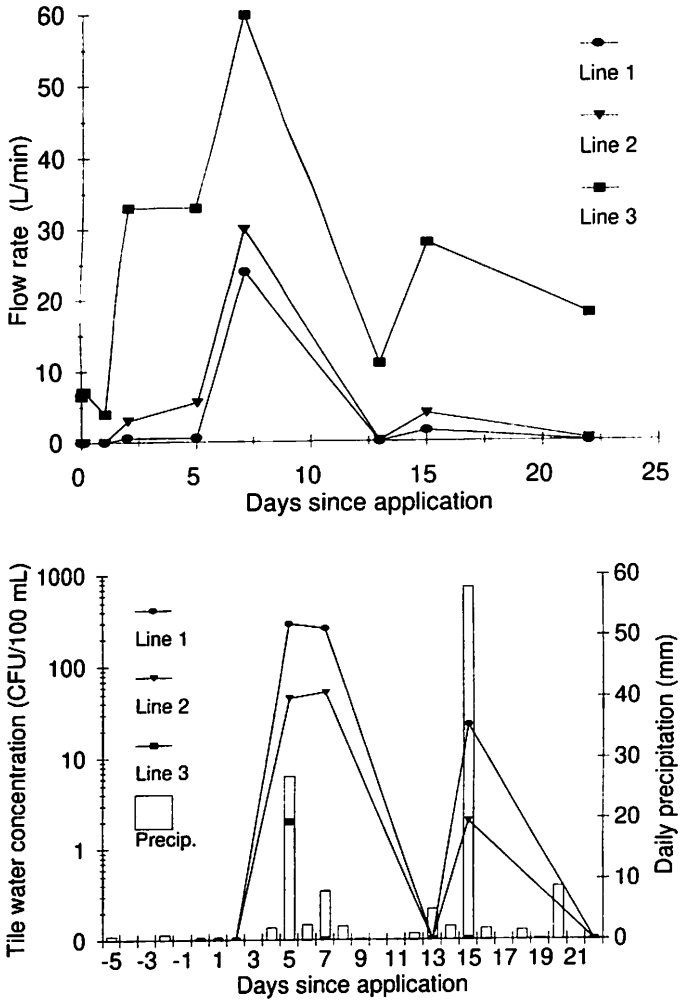

Fig. 3. Tile discharge and biotracer concentrations in the tile-drain-water variations with time in three subsurface tile lines, Elora, ON (Fall 1992).

Peak tile-drain-water biotracer concentrations measured among the four experiments ranged from 1 to 1400 CFU/100 mL (Table I). Within the range of depths applied, depth had no effect on the resulting concentrations. The highest mean depth of application (6.9 mm, S93) had the lowest resulting peak tile drain water concentration (1 CFU/100 mL). In addition, the sampled concentration of biotracer at the field level appeared to have little relationship with the resulting tile-drain-water concentration.

Dean and Foran (1991) have noted a connection between transport of bacteria and the flow rate in the subsurface tiles. This is not evident in the present study. The highest concentrations (S94, 1400 CFU/100 mL) had the lowest tile discharge; whereas, the second highest discharge (S93, 25 L/min) resulted in the lowest measured concentration.

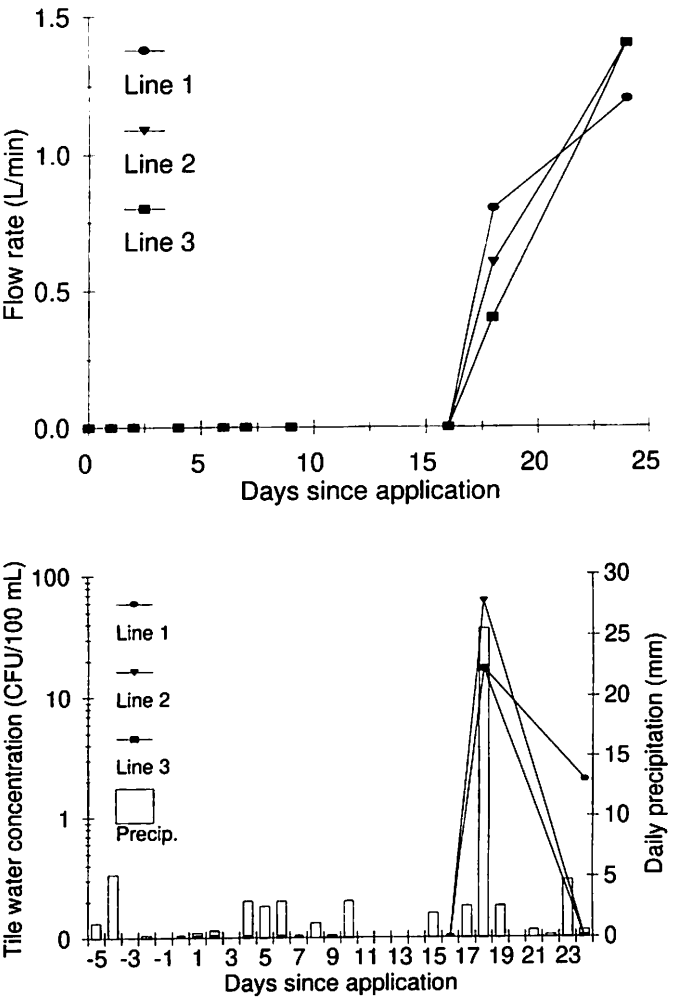

Fig. 4. Tile discharge and biotracer concentrations in the tile-drain-water variations with time in three subsurface tile lines, Elora, ON (Fall 1993).

However, there does appear to be a connection between the presence of flow in tiles prior to application and subsequent detection of biotracer in the tile lines. Tile flow was present before manure application in both the fall 1992 and spring 1994 experiments and both had detectable biotracer concentrations in the tile lines within 5 days of application.

The strongest association with concentrations of the tracer in the file flow was with rain amount after application. Daily precipitation amounts for the experiments are also given in Figs. 3b-5b. The experiments with the two highest concentrations of biotracer in the tile drain water (S94 and F92) both had rain shortly after application. In the case of the spring 1994 experiment, 8.6 mm of rain was recorded within 24 h of manure application. Although this did not result in large flow rates in the tiles (< 0.2 L/min per tile), concentrations of the biotracer quickly reached values over 1000 CFU/ 100 mL. For the experiment in fall 1992, 27 mm of rain on the fifth day after application resulted in tile-drain-water concentrations of over 100 CFU/100 mL. As a result of this storm, flowrates in the tiles increased to between 25 and 60 L/min in each of the tiles.

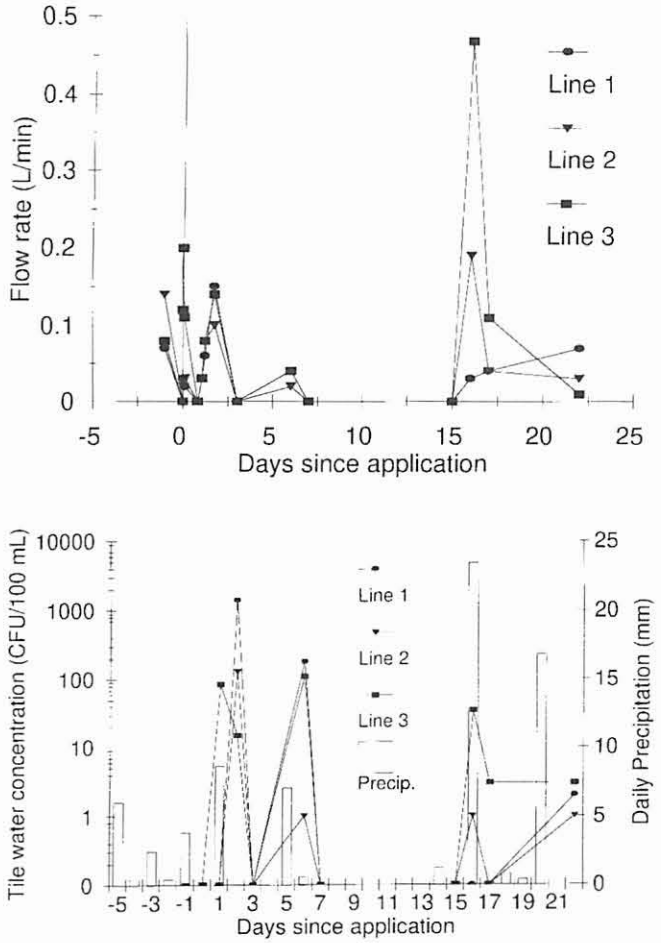

Fig. 5. Tile discharge and biotracer concentrations in the tile-drain-water variations with time in three sub-surface tile lines, Elora, ON (Spring 1994).

For the experiments in fall 1993 and spring 1993, very little rain was recorded for an 18 d period after spreading. In spring 1993, no significant rain fell for 13 days after application followed by 15 mm on day 14. Apparently, this was not enough rain to initiate flow in the tiles. Not until 35 mm fell on day 40 was flow recorded in the tiles and at that time only a marginal (1 CFU/100 mL) biotracer amount was recorded. The experiment in fall 1993 also had no tile flow in the days after application until day 18, just after 25 mm of rain fell. At this time flows increased to over 1.2 L/min in each tile and concentrations peaked at 61 CFU/ 100 mL.

Table II. Application and recovery of biotracer.

Experiment	Number of cells applied at field level (CFU)	Number of cells in effluent (CFU)	Recovered (%)
F92	2.1×10^{11}	2.3×10^{9}	1
S93	6.0×10^{12}	5.9×10^{6}	1×10^{-4}
F93	2.7×10^{12}	2.1×10^{7}	8×10^{-4}
S94	7.3×10^{11}	8.2×10^{7}	1×10^{-2}

As a measure of the overall transport of biotracer to the receiving water, the product of biotracer concentration and flow rate was integrated over the time of measurements for all three tile lines and added together. This was compared to the total amount of biotracer added using the surface-applied concentration and depth measurements to determine the total amount applied (Table II). These results support earlier observations that rainfall shortly after application leads to higher levels of transport. For those experiments with little or no rain after application, the percentage of the biotracer able to move through the soil profile to the surface water was less than 0.001 %; whereas, those instances with large rain amounts within a few days after application had a larger percentage of the biotracer transported to the surface water (between 0.01 and 1% of the original amount applied).

Penetration of bacteria into soil

Soil core samples were collected in the final three experiments before and after (between 24 and 48 h) spreading to determine the degree to which the bacteria had penetrated the soil. Three samples were taken at each of three soil-depth intervals (0-300, 300-600, and 600-900 mm). In two cases, samples were also taken 20 days later to examine if any change had occurred. No biotracer was ever found before spreading, illustrating that, for the first three experiments at least, no residual biotracer was retained six months after prior application.

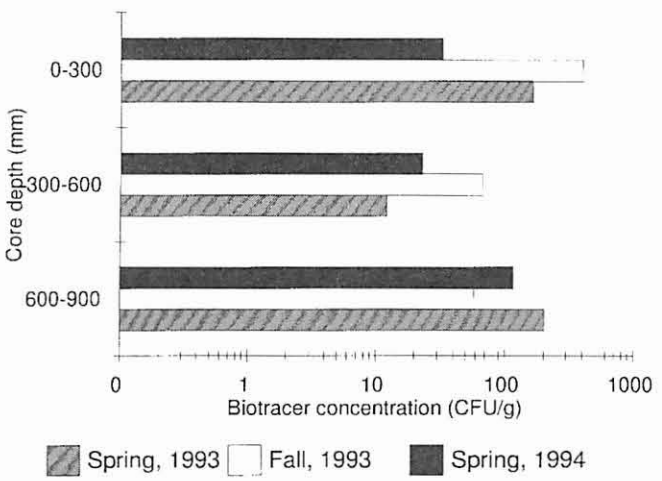

Fig. 6. Biotracer concentration in soil after spreading at Elora, ON.

Figure 6 shows the biotracer concentration as a function of depth immediately after spreading for the last three experiments. The biotracer had clearly penetrated the 900 mm depth and showed a relatively even distribution of concentration with depth. No large differences are evident between the three experiments. At the time of spreading, observations were made of the degree of surface cracking of the soil. In all cases surface cracks in the soil were evident and this in all likelihood led to the rapid distribution of the bacteria down through the soil.

Figures 7 and 8 show the change in biotracer concentrations with depth over time (up to 20 days after

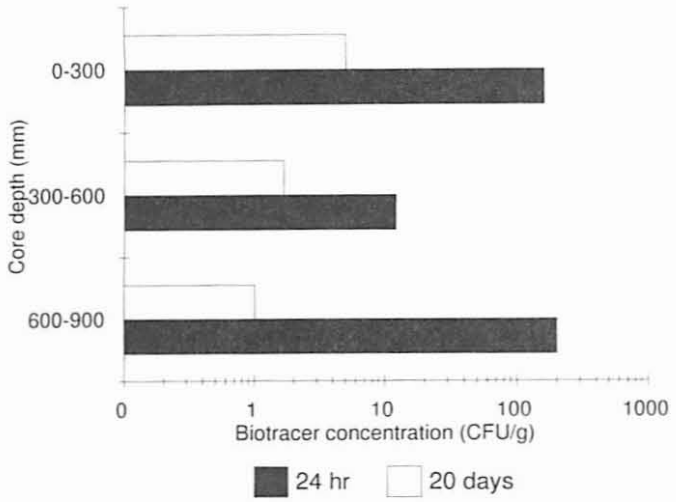

Fig. 7. Change in biotracer concentration in soil with time at Elora, ON (Spring 1993).

application) for two of the experiments. The amounts of bacteria still present after 20 days (a time over which a significant reduction due to die-off alone could be expected) was typically about 1% of the original amount. Reductions in 1994 were somewhat larger than in 1993. The presence of this amount of biotracer after several weeks explains why, for example in the Fall 93 experiment, the biotracer is still detected in the tile flow after a rain event some 18 days after application of manure (Fig. 4b).

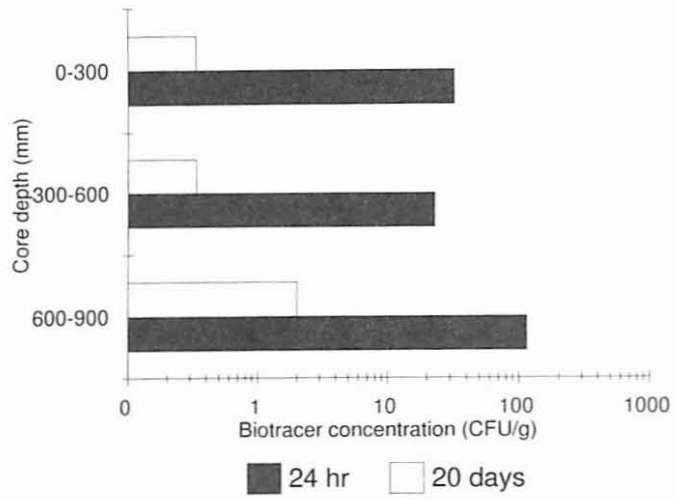

Fig. 8. Change in biotracer concentration in soil with time at Elora, ON (Spring 1994).

CONCLUSIONS

The experiments described are for a single site which received liquid manure four times under normal agricultural practise over the course of two years. Therefore, these conclusions are limited to this location or locations very similar to it.

Under the conditions tested, the application of liquid manure to the field reduced the concentration of bacteria from that applied to that reaching the tile drain water. While the degree of reduction was variable, a reduction of nearly 100% was noted in all experiments. Although this reduction is substantial, biotracer concentrations in the tile-drain-water were frequently measured at concentrations above 100 CFU/100 mL and on one occasion over 1000 CFU/100 mL. With the proximity of these tile drains to the local drainage ditch, contamination of surface water certainly will occur under these conditions.

Depth of application, within the 3 to 7 mm range used here, was not a factor in the degree of surface water contamination. However, flow in the tile drains prior to application and rainfall within two weeks of application led to high levels of bacterial concentration in tile-drain-water.

Biotracer bacteria penetrated the soil column quickly; in most cases a penetration of at least 900 mm was noted within 24 h of application. After 20 days the amount of bacteria in the soil was reduced to less than 1% of amounts immediately after application, with or without transport.

Guidelines for application of liquid manure should take account of flow conditions in the tile prior to application and the likelihood of precipitation creating tile flow within 21 days of application.

The use the biotracer was found to be an efficient method of following the movement of bacteria from liquid manure sources through soils. The method used was relatively simple and reliable. Although the 100-fold reduction in the tracer bacteria concentration from the amount added to the tanker when it was being loaded to that detected at the field level identifies an important factor in using *E. coli* NAR as a quantitative tracer, this could be accounted for in future studies by increasing the amount added.

ACKNOWLEDGEMENTS

Funding for this work was received from the Ontario Ministry of Environment and Energy as well as the Ontario Ministry of Agriculture, Food and Rural Affairs. Laboratory work was carried out by C. Etches and S. Bonte-Gelok and field assistance was received by D. Tiechroeb.

REFERENCES

APHA. 1989. *Standard Methods for the Examination of Water and Wastewater*, 17th edition. Washington, DC: American Public Health Association, American Water Works Association and Water Pollution Control Federation.

Culley, J.L.B. and P.A. Phillips. 1982. Bacteriological quality of surface and subsurface runoff from manured sandy clay loam soil. *Journal of Environmental Quality* 11(1):155-157.

Dean, D.M. and M.E. Foran. 1991. The effect of farm liquid waste application on receiving water quality. Final report submitted by the Ausable-Bayfield Conservation Authority, Exeter, ON. Ontario Ministry of Environment, Toronto, ON.

Fleming, R.J, D.M. Dean and M.E. Foran. 1990. Effect of manure spreading on tile drainage water quality. In *Proceedings Sixth International Symposium on Agriculture and Food Processing Wastes*, 385-392. St. Joseph, MI: ASAE.

Huysman, F., B. Van Renterghem and W. Verstraete. 1993. Antibiotic resistant sulphide-reducing *clostridia* in soil and groundwater as an indicator of manuring practices. *Journal of Water Air and Soil Pollution* 69:243-255.

Joy, D.M., J.L. Abu-Ashour, J.L. Botari, C. Etches, H. Lee, H. Whiteley and S. Zelin. 1992. Microbial transport in soils with and without macropores. In *Proceedings 1992 Technology Transfer Conference.* Ontario Ministry of Environment and Energy, Toronto, ON.

McCoy, E.L. and C. Hagedorn. 1980. Transport of resistance labeled *escherichia coli* strains through a transition between two soils in a topographic sequence. *Journal of Environmental Quality* 9(4):686-691.

Niemi, R.M. and J.S. Niemi. 1991. Bacterial Pollution of waters in pristine and agricultural lands. *Journal of Environmental Quality* 20(3):620-627.

Ontario Ministry of Agriculture and Food. 1976. *Agricultural Code of Practice.* Toronto, ON: Ontario Ministry of Agriculture and Food.

Patni, N.K., H. R. Toxoepeus and P.Y. Jui. 1985. Bacterial quality of runoff from manured and non-manured cropland. *Transactions of the ASAE* 28(6): 1871-1877.

Patni, N.K., H. R. Toxoepeus, A.D. Tennant and F.R. Hore. 1984. Bacterial water quality of tile drainage water from manured and fertilized cropland. *Water Research* 18(2):127-132.

Rahe, T.M., C. Hagedorn, E.L. McCoy and G.G. Kling. 1978. Transport of antibiotic-resistant *escherichia coli* through western Oregon hillslope soils under conditions of saturated flow. *Journal of Environmental Quality* 7(4):487-494.

Reaume, C. 1994. The effect of liquid manure application on the bacteriological water quality of tile-drain effluent. M.Sc. Thesis. School of Engineering, University of Guelph, Guelph, ON.

Rudolph, D. and M. Goss. 1993. Ontario farm ground water quality survey - summer, 1992. Agriculture Canada, Guelph, ON.

Shadford, C.B., D.M. Joy, H. Lee, H.R. Whiteley and S. Zelin. 1997. Evaluation and use of a biotracer to study ground water contamination by leaching bed systems. *Journal of Contaminant Hydrology* 28(3):227-246.