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Evaluation and use of a biotracer to study ground water contamination by leaching bed systems

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Abstract

There is an increasing concern about dangerous levels of bacterial contamination of rural ground water resources in Ontario and throughout the world. Recent studies in rural parts of Canada have identified leaching bed systems as one of the major sources of this contamination. Field studies were undertaken to evaluate bacterial contamination from three different types of leaching bed designs, using nalidixic acid-resistant *Escherichia coli* (*E. coli* NAR) as a biotracer. This biotracer was used rather than passive ground water sampling to clearly identify the source of the contamination and also to allow the determination of travel times and distances more clearly. While this biotracer has been used for other studies its use in actual working septic systems has not yet been reported.

This work has also shown that *E. coli* NAR is an excellent biotracer and can be used to give an accurate assessment of a leaching bed's performance provided it is introduced into the system over a reasonable period of time. Results also show that bacteria are not necessarily removed before the effluent reaches the ground water. The speed, distance of travel and attenuation of biotracer concentrations was found to be highly related to precipitation events, age of system and depth of unsaturated zone below the bed. © 1997 Elsevier Science B.V.

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1. Introduction

Ground water is an important, and limited, resource in most parts of Ontario, Canada and indeed most parts of the world. This is particularly true in the many rural areas primarily dependent on ground water for their potable water sources. Evidence coming to light in recent years has led to a growing awareness of the problem facing this resource due to contamination from a variety of sources. For example, recent studies in Ontario have found that over 25% of rural wells tested have levels of bacterial or nitrate contamination above the drinking water limits (Rudolph and Goss, 1993).

More than one-half of the waterborne disease outbreaks in the United States are due to contaminated ground water (Yates, 1987). In recent years, attention has been focussed mostly on ground water pollution by organic chemicals such as petroleum even though chemicals are responsible for a relatively small percentage of the reported ground water related health problems. In contrast, bacteria, viruses and protozoa present in domestic sewage which are known to cause the majority of waterborne diseases have been given comparatively small attention.

Domestic sewage may contain different types of pathogenic microorganisms and many of these are not removed even if the septic tank is operating properly. These microorganisms can be released into the subsurface from leaching beds where they may travel through the soil and reach the ground water to contaminate wells that may be used for potable water.

Early designs of leaching bed systems relied on results of empirical studies to ensure human health was not threatened by their operation. This included provision of an adequate unsaturated zone beneath the bed, suitable horizontal spacing from water supply wells and ensuring no effluent reached the ground surface due to hydraulic overloading. However, increasing occurrences of contaminated ground water in rural environments, have led to an increase in the concern about the design and siting of septic systems. In some areas septic systems have been identified as major potential contributors of contamination to ground water supplies. In Ontario, a recent public inquiry into land use planning and development identified the septic systems as a "sleeping giant" in terms of their impact on ground water and public health (Commission on Planning and Development Reform in Ontario, 1991).

Satisfactory investigation of septic systems as the source of bacterial contamination can be difficult to accomplish. Few, if any, field studies have been able to conclusively identify levels of bacterial contamination solely due to septic systems. One problem when studying bacterial contamination from individual sources, such as septic systems under field conditions, is that naturally occurring background levels often make it difficult to distinguish between bacteria from the source of interest (i.e. the septic system) and those from other sources.

The use of biotracers that behave similarly to the bacteria of interest, and yet, can be clearly identified in field samples due to their unique characteristics is a solution to this problem. One such biotracer used is a naturally occurring strain of *Escherichia coli* which is resistant to the antibiotic nalidixic acid (*E. coli* NAR). *E. coli* NAR behaves similarly to other strains of *E. coli*, but can be selectively enumerated, since, while it occurs in nature, such occurrences are rare. Hence, its presence or absence in and around

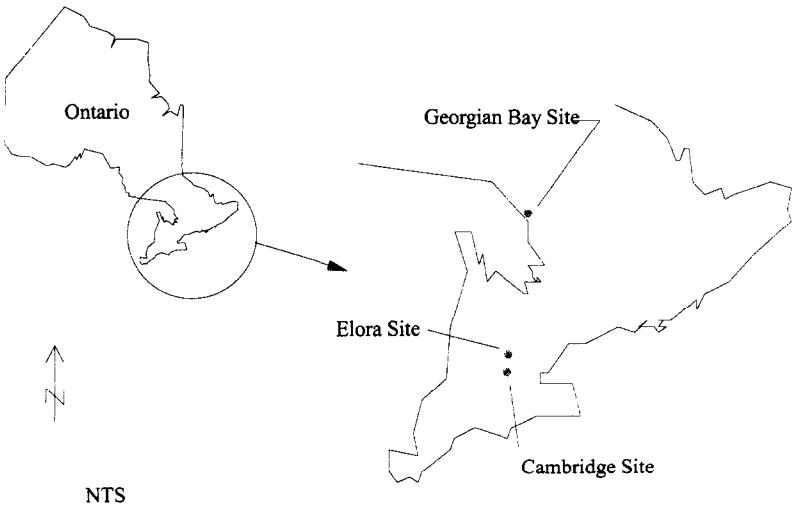

Fig. 1. Locations of instrumented septic systems.

a leaching bed which has been inoculated with the biotracer gives a good indication of the extent and direction of transport of bacteria from the bed. This microorganism has been successfully used in previous studies by Dean and Foran (1991), Fleming et al. (1990) and Palmateer et al. (1989) to monitor bacterial contamination of receiving waters.

The present study was undertaken to study the performance of septic systems installed in different subsurface conditions and to gain an increased understanding of the transport of bacteria through septic systems. This was accomplished by conducting experiments, using *E. coli* NAR as a biotracer, on three instrumented septic systems in Ontario. Locations of the three experimental sites are shown in Fig. 1. The first of these sites is a mounded system located at the Elora Research Station operated by the University of Guelph. The second is a new filter bed system servicing a seasonal dwelling in the shield region of the province, on Georgian Bay near Parry Sound. The third is a conventional septic system located at the Cambridge Research Station, also operated by the University of Guelph.

2. Background studies

Septic systems have been in used for over 100 years in North America. An early reference by Shutt (1904) to septic systems described their construction and operation. Although they have been studied extensively since that time, the basic design described by Shutt nearly 100 years ago is basically unchanged from that described in current regulations (e.g. Ontario Ministry of Environment and Energy, 1982).

The literature is replete with studies of the performance of septic systems under a variety of conditions and the reader is referred to several extensive review articles

(Hagedorn and McCoy, 1979; Minear and Patterson, 1973; Yates, 1987). Recent studies have tended to focus on the performance of alternative systems such as those utilizing peat as a leached bed material (Brooks and McKee, 1992) or some specific contaminant such as nitrogen (Robertson et al., 1991). The concern of the present work is the movement of bacteria from septic systems and thus the focus is on studies involving bacterial movement near septic systems.

Viraraghavan (1978) studied the movement of coliforms near a leaching bed in a shallow, sandy clay system. Microorganisms were found to travel at least 15 m from the tile during the study period. Significant attenuation in the concentration of the microorganism was seen with increasing distance from the tile line.

Seventeen septic systems were studied by Chen (1988) to determine pollution from faecal coliforms near lakeshore developments in New York. Eleven of these had measurable faecal coliform counts at distances up to 80 m from the discharge points. Evidence in the study suggested that the larger travel distances were due to large cracks in the soil, intensive rainfall, steep slopes and/or an impermeable soil layer near the surface.

Total and faecal coliform bacterial movement from three systems in Virginia were determined in a study by Reneau and Pettry (1975). Large reductions in both total and faecal coliform levels were detected within a 13.5 m distance.

Anderson et al. (1994) focussed on the effectiveness of the vadose zone on the treatment of septic tank effluent in sandy soils. An extensive set of pan lysimeters installed under a tile line of a bed showed basically no movement of faecal coliform from the bed.

A difficulty with the studies mentioned above are that since there are other sources of bacteria such as coliforms, the travel times and pathways of the microorganisms could not be clearly identified. Indirect techniques, such as considering the ratios of faecal to total coliforms (Bouma et al., 1972) are only partially successful at establishing the links between detection location and source. A solution to this is to use a biotracer, such as *E. coli* NAR, and introduce it at the potential source. This approach has been used by Hagedorn and McCoy (1979) in which they describe the testing of three types of antibiotic resistant strains of *E. coli*. They conclude that strains tested were suitable for use as ground water tracers since they have the four essential characteristics necessary for use. This includes:

1. recovery on a strain specific media;
2. extremely small chance of finding indigenous organisms with similar antibiotic resistance profiles;
3. stability of the markers under stress conditions, and
4. the low probability that the antibiotic resistance will be passed on to other bacteria.

In spite of the obvious advantages to using a biotracer to study the performance of septic systems few studies are known. Rahe et al. (1978) used marked strains of *E. coli* in a hillslope area of western Oregon, USA under conditions similar to a leaching bed but without the use of septage. Their objective was to evaluate the various strains of *E. coli* as biotracers and to determine the degree of movement in saturated soils. Soil conditions were saturated via the use of a sprinkler system and the movement of the bacteria monitored using piezometers for sampling. Due to the soil conditions and high

gradient, significant movement was found. Biotracers were found to be superior to dye tracers for the assessment of connectivity between source and destination.

Sinton (1986) has also used a marked strain of *E. coli* to assess the contamination by septic tank effluents. This research examined disposal systems other than leaching beds (i.e. a soakage pit and a deep injection well) and thus is not directly related. However the use of the biotracer very clearly identified the pathways of microbial contamination as well as travel times from the disposal point to monitoring point.

3. Site descriptions

3.1. Elora Site

The Elora Site (ES) is located near Guelph, Ontario, Canada. Soils in the area are primarily silt loam and loam. Fig. 2 illustrates the layout of the leaching bed at the Elora site. It is a raised bed, necessitated by a water table only 0.9 m below the soil surface. The leaching bed services the sink and toilet facilities of a dairy barn. It does not handle waste water from the general cleaning of the dairy operation such as the milk house or

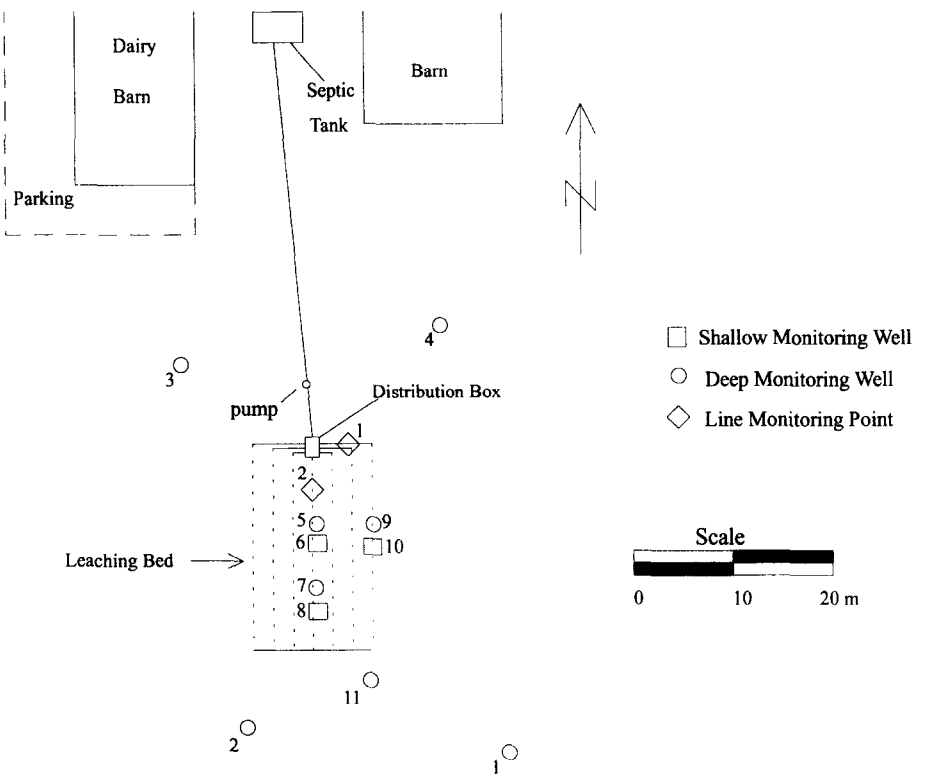

Fig. 2. Leaching bed layout at Elora Site.

animal enclosures. This system has been in operation for approximately 25 years and consists of a 9000 L septic tank, distribution box and the raised leaching bed with seven 20 m long, lines of ceramic tile. Discharge to the bed is approximately 3.0 m^3/day^{-1} and is consistent from day to day due to the regularity of the barn-use schedule.

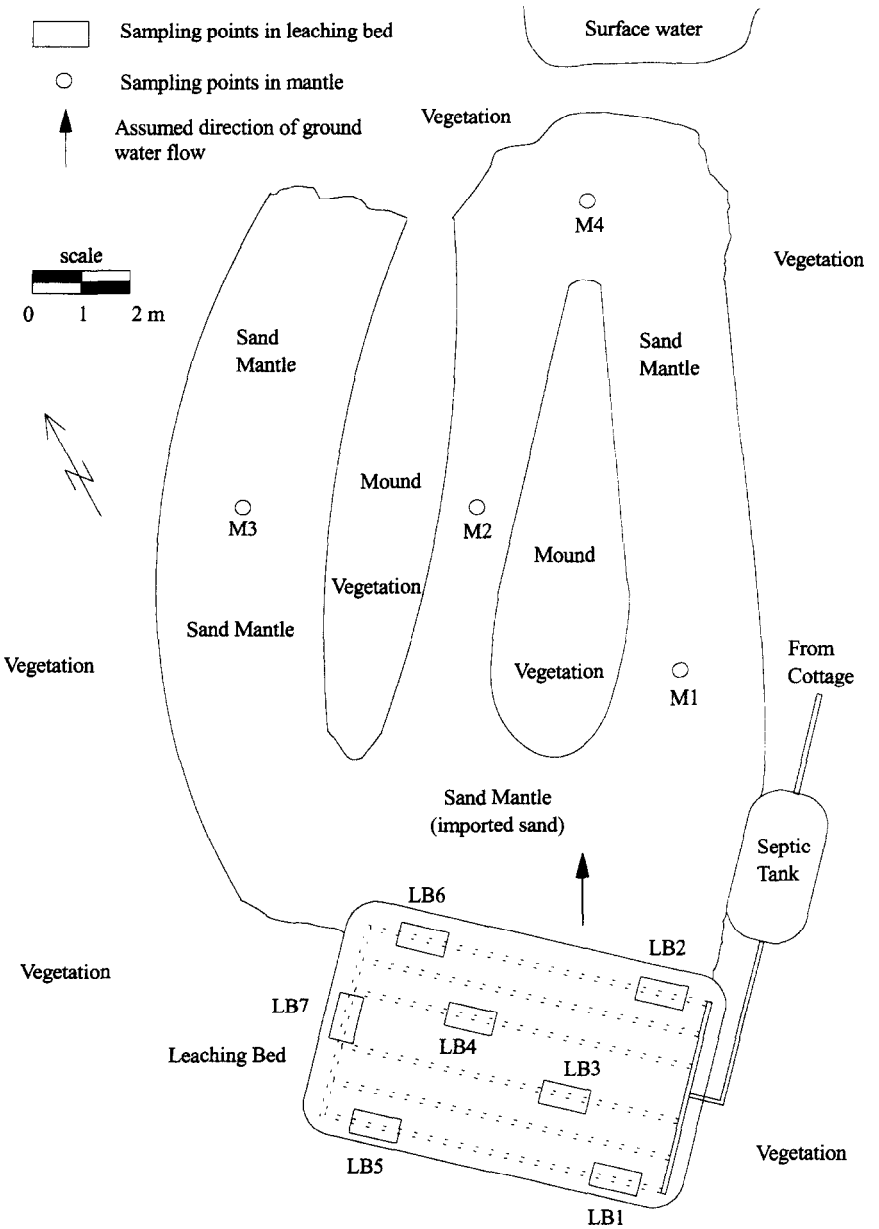

Fig. 3. Leaching bed layout at Georgian Bay Site.

3.2. Georgian Bay Site

The Georgian Bay Site (GBS) is located in the shield region of Ontario, on Georgian Bay near Parry Sound. The tank and bed are situated directly on top of granitic rock with imported fill directly under the bed. Away from the bed, sand fill has been placed on top of mostly complex organic matter in various degrees of decomposition and is acidic in nature.

The filter bed system was constructed entirely of imported material and was installed directly on top of fractured bedrock in a low lying area which could be prone to flooding (Fig. 3). Size of the bed is approximately 8 m by 5 m with 6 lines of perforated PVC drainage pipe. Filter material used for the bed is a well-graded, medium to coarse sand with trace fines. Surface water is located about 17 m horizontally from the bed and is in the direction of subsurface water flow that may come from the bed. This system services a seasonal cottage, which is used extensively during July and August. The estimated flow rate of waste water entering the bed was 0.4 m^3/day^{-1}.

3.3. Cambridge Site

The Cambridge Site (CS) is located near Cambridge, Ontario at a University of Guelph operated research station. It is located above a surficial aquifer comprising

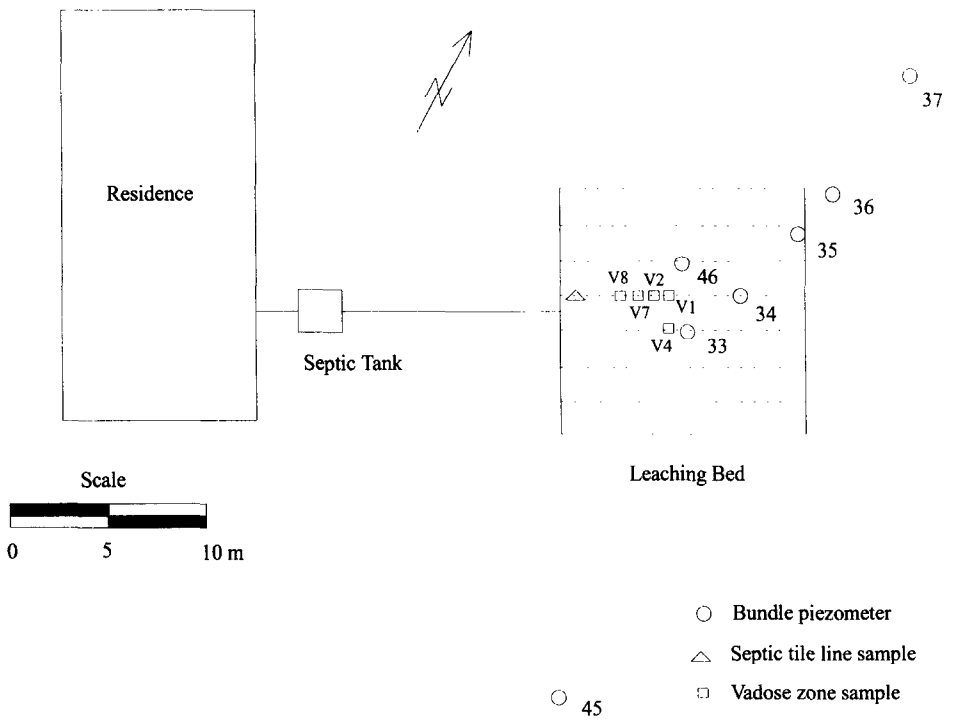

Fig. 4. Leaching bed layout at Cambridge Site.

Table 1
Summary of conditions at field sites

Site	Type of system	Years of operation	Depth to ground water (m)	Number of sampling points	Dominant soil type
Elora	Raised	25	0.9	15	Loam
Georgian Bay	Filter Bed	< 1	< 1[a]	14	Bedrock
Cambridge	Conventional	17	2	22	Sand

[a]Depth to ground water unknown but assumed to be less than 1 m.

moderately permeable fine sand to very permeable coarse sand with coarser material dominating (Robertson et al., 1991).

The septic system is a conventional design used for permeable soils, consisting of a septic tank and leaching bed approximately 10 m by 10 m (Fig. 4). Leaching bed tiles are perforated PVC pipe, and lie at a depth of 0.6 m at a location where the water table is about 2 m below the ground surface. The system accommodates a family of four and has been in operation since 1977. No flow rate measurements were taken because the septic tank could not be accessed and no feasible method of obtaining average flows could be established.

Table 1 summarizes some of the pertinent details for each of the sites studied.

4. Site monitoring systems

Each site was equipped with a dedicated monitoring system. This included the ability to determine background contamination levels, independent of the system, input concentrations and concentrations within and near the bed. Each of the sites have distinctly different geometries requiring different monitoring systems. These systems, and their installation, are described separately below.

4.1. Elora Site

There are a total of 15 sampling locations in and around the septic system at the Elora Site. Eleven of the sample locations are wells, while the remaining four are in the septic tank, distribution box and 2 of the tile lines. The wells are made of 50 mm diameter PVC pipe, screened at the bottom for a length of 0.8 m. They were installed using a truck-mounted auger to depths of 1 to 4 m. The pipes were placed in the augered holes, packed over their screened lengths with coarse industrial silica sand, and then sealed with bentonite up to the surface. Samples were taken from dedicated 6 mm diameter polyethylene tubing permanently placed in the PVC pipes. Flexible silicon tubing was inserted on the end of the polyethylene tubing so a peristaltic pump could be used for the sampling. Each well had a dedicated silicon tubing to prevent cross contamination between samples. Four 6 mm sampling tubes, one each in two of the leaching bed lines, the septic tank and the distribution box were also permanently installed so samples from these locations could also be taken using a peristaltic pump and dedicated silicon tubing.

4.2. Georgian Bay Site

The monitoring system consists of a total of 14 sampling locations in and around the septic system as shown in Fig. 3. Two of these are surface water locations down gradient from the bed. Seven sampling points are located under the bed at the sand filter/bedrock interface. These samplers were constructed using plastic pans with dimensions of 840 × 420 × 40 mm. Samplers were filled with coarse industrial silica sand to facilitate rapid sampling. Samples were taken via a tube connected to an outlet hole on the bottom using a brass elbow connector and brought to the surface. The samplers were installed during the construction of the filter bed and were placed on top of the bedrock, sloped towards the outlet hole, before the filter material was put in place.

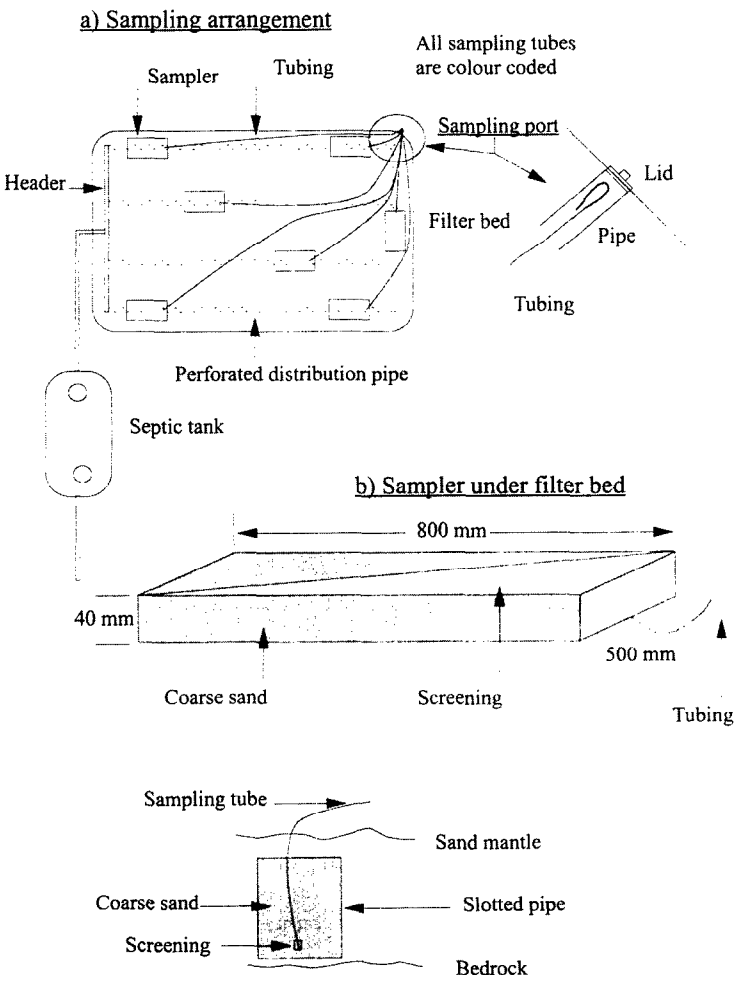

Fig. 5. Samplers at Georgian Bay Site.

Fig. 5(a) shows the samplers and their arrangement under the bed. Samples were obtained with a peristaltic pump.

To obtain samples from the area down gradient of the bed, four holes were dug to bedrock at depths of less than 0.5 m. Slotted pipe, 100 mm in diameter, was then placed in each of the four holes with 6 mm tubing placed in the middle of the pipe. The pipe was filled with coarse industrial silica sand, so as to surround the tubing, and then covered with a screen to prevent the entry of fine particles. The end of the tubing was also screened to avoid sucking up the sand during sampling. The pipe was then covered and the tubing brought to the surface. Fig. 5(b) and (c) illustrate these samplers.

To get samples from the septic tank, 6 mm tubing was installed through a hole in the septic tank.

4.3. Cambridge Site

The Cambridge Site has undergone field investigations since 1987 by the Waterloo Centre for Groundwater Research (WCGR, Robertson et al., 1991). Multiple piezometer bundles were installed within and near the leaching bed by WCGR and were used in this study. No additional samplers were installed. The system was monitored using a total of 22 sampling points. These included the septic tank, which was accessed indirectly via a sampling tube inserted into the centre weeping tile; four multiple piezometer bundles, sampled at three ground water elevations; two multiple piezometer bundles, sampled at one elevation only; a control sample up gradient of the bed, and six sample points in the unsaturated zone under the tile lines.

The multiple piezometer bundles consisted of 6 mm diameter Teflon sampling tubes attached at 0.6 m depth intervals to a centre stock of 1.6 cm diameter PVC pipe. The bundles were installed into the aquifer with the aid of a 5 cm diameter steel casing and an expendable drive tip. The casing was advanced using a hand held vibrating hammer and was extracted after bundle insertion (Robertson et al., 1991).

The vadose zone samplers, in the tile bed were installed by a local consulting firm for WCGR (Robertson, Pers. Com., 1994). These samplers consist of 200×100 mm pans filled with coarse silica sand. Each pan is attached to a 2 L container, into which any captured water flows. Tubing is connected to this container and goes to the surface so samples can be taken using a peristaltic pump. The samples were installed at various depths around the bed.

5. Biotracer

The biotracer used for the study is a naturally occurring strain of *Escherichia coli* resistant to the antibiotic nalidixic acid (*E. coli* NAR). This was provided by G. Palmateer of the Ontario Ministry of the Environment and Energy (London Regional Laboratory, London, Ontario, Canada.

There are several advantages in using *E. coli* NAR as a biotracer. Firstly, *E. coli* are widely used as indicator organisms in assessing the microbial quality of water. Thus, using an *E. coli* strain as a biotracer more closely resembles the natural passage of this

type of indicator organism. Growth and die-off rates of *E. coli* NAR have been shown to be similar to those of several other naturally occurring *E. coli* strains (Joy et al., 1992). *E. coli* NAR can be selectively isolated from environmental samples and background levels of nalidixic acid resistant *E. coli* strains are extremely low, therefore, the presence or absence of *E. coli* NAR near the source (i.e. leaching beds) is a good indicator of the potential transport of microorganism from the site of interest. The antibiotic marker (nalidixic acid resistance) is chromosomally encoded, and hence more stable. The resistance of the biotracer to other antibiotics, some of which are plasmid-encoded and hence less stable, has not been determined. Finally, *E. coli* NAR has been shown to be safe for introduction into the environment (Palmateer, pers. com.) and has been used in other field studies in Ontario (Dean and Foran, 1991; Fleming et al., 1990, Palmateer et al., 1989). A different nalidixic acid resistant strain of E. coli has been used in field studies elsewhere (Hagedorn and McCoy, 1979). There are no reported studies using the biotracer on existing, operational systems.

5.1. Preparation of inoculum

Preparation of the biotracer, for each inoculation to the septic systems, was done by taking loopfuls of *E. coli* NAR from a Tryptic Soy Agar plate (TSA, Difco Laboratories, Detroit, MI, USA) supplemented with 50 $\mu g\ mL^{-1}$ nalidixic acid (NA), placing it into Tryptic Soy Broth (TSB) and incubating for 40 h at 20°C. For the first test at the Elora Site, cells were washed with 0.1 M phosphate buffer, pH 7.5, before inoculation and a 1 L suspension added each time a system was inoculated. After the first experiment washing was found to be unnecessary. Thus for the second experiment at Elora, and for the other sites, cells were added to the septic systems without washing and a 3 L suspension of cells were added each time a system was inoculated.

5.2. Inoculation of biotracer at experimental sites

In the first experiment at the Elora Site 1 L of a washed cell suspension was transported on ice to the test site where it was added to the septic system by flushing it down the toilet. This was followed by repeated flushing to mix the sample and ensure that it reached the septic tank quickly. The second experiment at the site was conducted in the same manner, except that 3 L of unwashed cell suspension were used and the system was inoculated 6 times (each time with 3 L of unwashed cells) over a 3 week period instead of only once.

At the Cambridge Site the biotracer was also added to the septic tank via the toilet; the system was inoculated twice a week over a 3 week period with 3 L of unwashed cells. Inoculation of the Georgian Bay site involved adding 3 L of biotracer directly to the septic tank and was inoculated 8 times over a 32 day period.

5.3. Sampling and enumeration of biotracer

The sampling and enumeration procedure was essentially the same for all sites. Samples were taken from the desired locations by attaching the installed tubing to a

peristaltic pump and obtaining the required 250 mL sample. To provide background concentrations for both *E. coli* NAR and total *E. coli*, all sites were sampled before the commencement of an experiment. During the inoculation of a site, samples were taken from the sample locations first, followed by addition of the biotracer to the system to avoid cross contamination of the equipment. If possible, sample locations were purged for 1 min to ensure a representative sample from the surrounding area. The sampling period continued until all samples were negative for *E. coli* NAR.

All samples were transported on ice to the laboratory where appropriate filtration and/or dilutions in 0.85% saline were made within 24 h. Gelman sterile cellulose-acetate filters (0.45 μm pore size) were used. After filtration, the filters were placed on mTEC-NA agar in 60 × 15 mm Petri plates, in duplicate, and incubated at 44°C for 24 to 48 h after a recovery period of 1 to 3 h at 20°C. For background *E. coli*, counts on mTEC agar plates were used and incubated for 24 h at 44°C prior to counting. Yellow brown colonies causing a purple to yellow colour change in the agar medium were enumerated as viable colony forming units (CFU) of *E. coli* cells. Values are presented as means of duplicate determinations.

Prior to using *E. coli* NAR as a tracer its survival in septic effluent was assessed. This involved inoculating a 333 mL sample of the effluent from the Elora Site with the tracer and sampling over an 11 day period. Although concentrations decreased with time CFU's were still detectable ($> 10^4$ CFU/100 mL) at the end of the 11 day test. Also, comparison of the triplicate results showed excellent consistency between the samples.

6. Results

6.1. Elora Site

Two biotracer tests have been completed on this leaching bed. In the first experiment the septic tank was inoculated with a single dose of *E. coli* NAR, while in the second

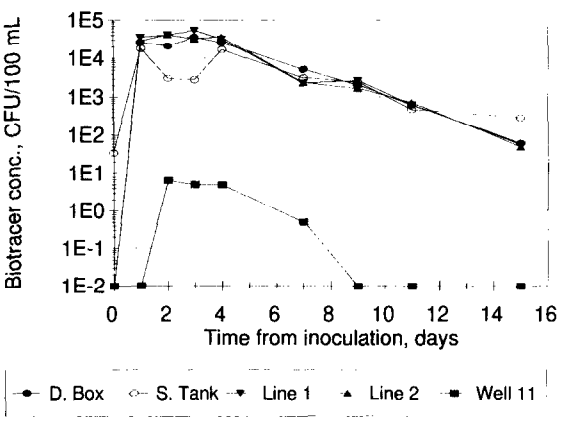

Fig. 6. Biotracer results from single inoculation—Elora Site.

experiment the septic tank was inoculated twice a week for three weeks. The results from these two experiments are described separately below.

The inoculation, in the first experiment, consisted of 2×10^{12} CFU of *E. coli* NAR. All locations where *E. coli* NAR was detected showed expected general trends with zero initial values, rising to a peak and then gradually diminishing back to zero (Fig. 6). Note that concentrations of 0 CFU/100 mL are plotted at 0.01 CFU/100 mL in this and all subsequent figures. *E. coli* NAR was detected in the septic tank, the distribution box, the two monitoring lines and well 11. Of the wells sampled, only well 11 showed evidence of the biotracer in 15 days of sampling, in spite of the fact that this well is outside the leaching bed and 6 of the wells are inside the bed. This was due to the relatively small number of bacterial cell introduced in the single inoculation, which resulted in low overall concentrations. The experiment did, however, confirm the biotracer would remain viable in the septic tank environment and sustain high concentrations for up to 4 days after inoculation. In addition, it showed that the biotracer could be successfully recovered in the monitoring wells, albeit at concentrations 4 orders of magnitude lower than those in the tank and tile lines.

Concentrations determined during the second experiment are shown in Fig. 7. The system was inoculated six times over a 3 week period, averaging 4×10^{12} CFU per inoculation. Results at all locations where the biotracer was detected were similar with zero initial concentrations, rising to a plateau during or shortly after the inoculation period and gradually diminishing after the end of the inoculation period. At the end of the 112 day monitoring period, low levels of the biotracer were still detected in samples from the septic tank, distribution box and some wells. This was attributed to the persistence of the biotracer in the sludge at the bottom of the septic tank. Samples taken 1 year later showed no presence of the biotracer at any location.

Concentrations of biotracers in two of the shallow wells in the bed (6, 8) and a well outside but close to the bed (11) were very similar. Biotracer concentrations at these

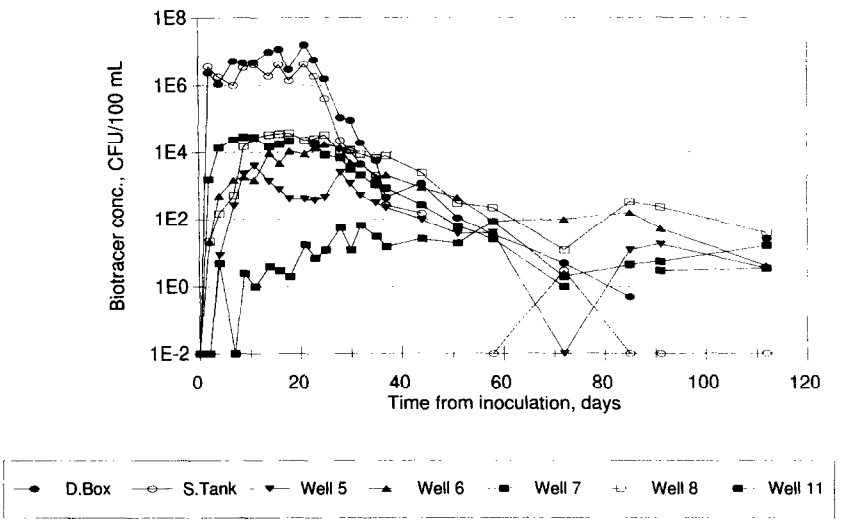

Fig. 7. Biotracer results from continuous inoculation—Elora Site.

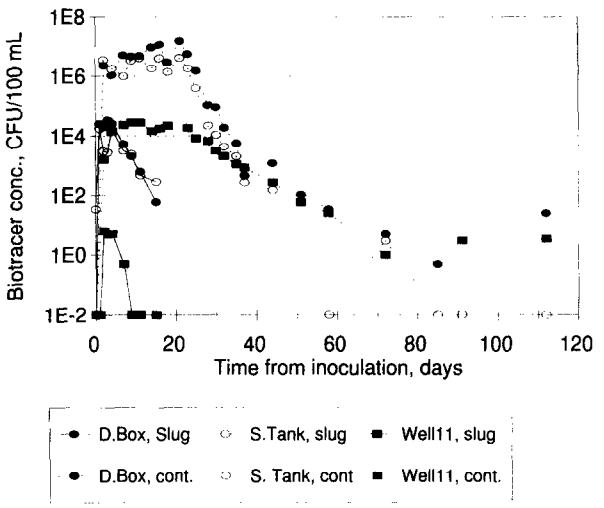

Fig. 8. Comparison of slug and continuous injection—Elorat Site.

locations increased faster than those at two of the deep wells within the bed (5 and 7) and also reached higher levels. This was interpreted as evidence of two system characteristics. First, the formation of a well developed biomat at the base of the gravel trenches significantly slowed the vertical movement of both water and bacteria. This is consistent with the observations of Bitton and Harvey (1992). A subsequent excavation in 1995 of part of the bed showed a well developed mat at the base of the gravel trenches. Secondly, the presence of a till layer, typically less than 2 m below the surface restricts any vertical movement as well, slowing the downward movement both under the bed and in the ground water outside it. This explains not only the lower concentrations but later-to-peak concentrations at the deeper wells (5 and 7) in the bed. Finally, the maximum attenuation of the biotracer from the tank to the nearest well outside the bed was approximately 1000 fold. No biotracer was ever detected at wells 9 and 10 even though these wells were installed adjacent to and below one of the tile lines in the bed. This likely indicates that this portion of the bed was inoperative, either due to clogging of the tile line or the bed in this area.

Fig. 8 gives a comparison of the two experiments. In the second experiment, the concentrations of *E. coli* NAR in the septic tank and distribution box were 2 orders of magnitude higher than those of the first experiment as a result of the continuous infusion. The levels in well 11 were four orders of magnitude higher. In addition, *E. coli* NAR were found in wells within the bed (5, 6, 7 and 8) in the second experiment, while in the first no *E. coli* NAR were found.

6.2. Georgian Bay Site

The experiment at the Georgian Bay Site lasted 74 days; the system was inoculated eight times over a 32 day period with an average of 4×10^{11} CFU per inoculation.

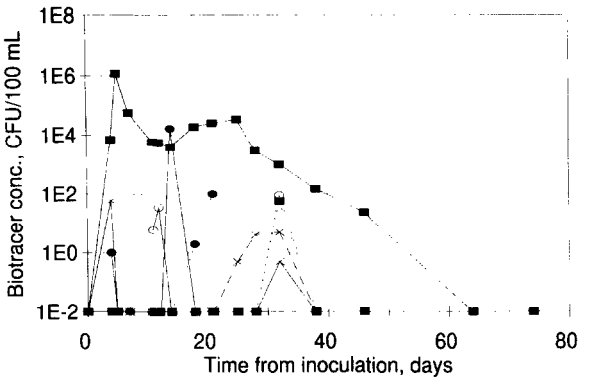

Fig. 9. Biotracer results—Georgian Bay Site.

Lower inoculation concentrations were due to the 5 h travel time to the site. Results are given in Fig. 9 for all locations where positive samples were obtained. Biotracer concentrations in the septic tank did not reach the concentrations found at the Elora Site, in part because inoculation numbers were lower than those used at Elora by an order of magnitude. In addition, a great deal of clothes washing was done during this period; the use of detergents may have adversely affected the viability of the biotracer.

The samplers under the bed were usually dry at the time of sampling. This indicates that little of the bed was being utilized and significant evapotranspiration was taking place. LB3 was the only sampler under the bed from which regular samples could be obtained. Only after periods of significant rainfall were samples obtained from the remaining samplers under the bed. Of the 16 times samples were collected, none were ever obtainable at location LB5; only 1 sample was obtainable at LB6 and LB7; 3 were obtained from LB1 and LB2; 6 were obtained from LB4 and 12 from LB3. In contrast, samples were nearly always obtained from the mantle locations. The number of samples at each of sampling locations clearly shows how the bed is being utilized. Lines closest to the inlet (above LB3 and LB4) are clearly getting the most effluent with decreased amounts at lines away from the inlet (above LB1 and LB2). In addition parts of the bed farthest away from the inlet (LB4-LB7) are receiving little if any of the effluent on a regular basis. Thus the bed is being over loaded in some areas and under loaded in others.

Transport of bacteria below the bed was also found to be related to rainfall events. The only time *E. coli* NAR was detected in significant quantities was after heavy precipitation. High amounts of infiltration of rain would increase the soilwater content in the filter bed, and this would facilitate bacterial movement. In addition rainfall generally lowers the ionic strength of pore fluid and thus promotes bacterial transport through soils (Bitton and Harvey, 1992). Shortly after a significant rainfall of 55 mm on day 12, a sample in LB3 had concentrations of biotracer essentially equal to those in the septic tank.

Samples taken down gradient in both the mantle area and the surface water were generally negative. Only on day 32 were positive samples found at M1 and M2 with

concentrations of 91 and 58 CFU/100 mL, respectively. These indicate travel distances of bacteria of 6 and 7 metres. Of the 28 surface water samples only four samples between day 25 and 32 showed the presence of the biotracer—and then at relatively low concentrations of 5 CFU/100 mL or less.

6.3. Cambridge Site

The experiment at the Cambridge Site lasted 84 days. The system was inoculated twice a week over a 3 week period with an average of 6×10^{12} CFU per inoculation. A total of 255 samples were collected for enumeration, 222 from the multi level piezometers and 33 from the vadose samplers. All but one of the 222 samples from the piezometers came back negative for *E. coli* NAR indicating none of the biotracer reached the saturated zone. *E. coli* NAR was, however, detected in the unsaturated zone at four of the vadose samplers (Fig. 10).

Sampling of the unsaturated zone did not begin until 24 days after the start of the experiment. Two of the locations (V1 and V2) appeared to only have measurable *E. coli* NAR concentrations after the missed 24 day period and not before. V4 and V7 appeared to have significant *E. coli* NAR before sampling of the unsaturated zone started as the first samples obtained at these sites on day 24 had concentrations of nearly 200 CFU/100 mL. However, the results for all indicate that the peak concentrations for the biotracer were not missed. The length of time to reach peak concentration indicates that the transport of bacteria is extremely slow through unsaturated soil. Sampling in the unsaturated zone was irregular due to the fact that at times there was no water in the samplers, with the quantity obtained depending somewhat on infiltration due to rainfall or snowmelt. Samples from V2 and V7 were available nearly every time sampling was attempted, suggesting they were being recharged by the waste water coming from the tile lines whereas samples from V1 and V4 were only obtainable 50% of the time and V8 and V12 could only be sampled 20% of the time.

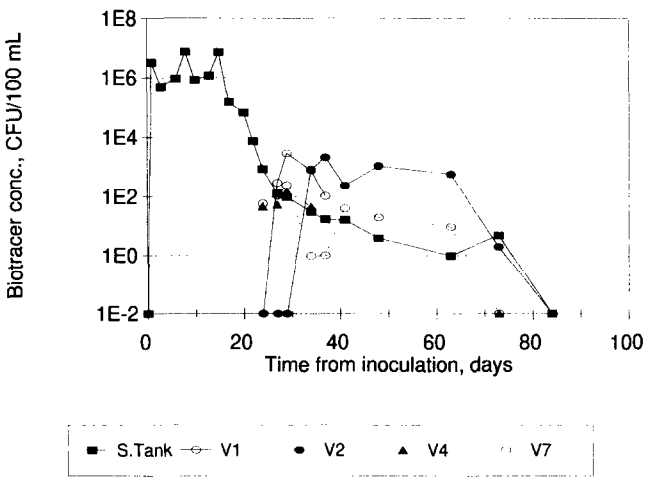

Fig. 10. Biotracer results—Cambridge Site.

Vadose samples began showing significant levels of biotracer 2 days after 32 mm of rain fell over a two day period starting on day 24. Concentrations at V1 and V2 increased from 0 to between 2000 and 3000 CFU/100 mL while at V4 and V7 concentrations increased from approximately 50 to 150 CFU/100 mL.

Overall the results show that this septic system worked well with regard to bacteria. All bacteria were retained in the unsaturated zone and prevented from reaching the ground water. Movement within the unsaturated zone is quite slow and highly affected by precipitation, similar to the Georgian Bay Site.

7. Comparison between sites

A comparison of the results from the three sites is given in Table 2. The comparison is for three measures of system performance using the biotracer results and recognizing one objective of the designs is to restrict the movement of bacteria out of the bed. The first comparison is on the basis of the attenuation of biotracer concentration from the concentrations in the tank (averaged over the surface below the bed). For the Elora Site, this is the lower wells in the bed and for Georgian Bay it is the samplers at the bedrock level. Since no biotracer reached the aquifer at the Cambridge site the attenuation is infinite while at the Georgian Bay Site, after significant precipitation, there was none. At Elora the attenuation was 1000-fold.

The second comparison is on the basis of the reduction of biotracer levels to a point outside the bed where the maximum biotracer concentration was measured. Again at Cambridge this was infinite, while at Georgian Bay the system reduced concentrations 200-fold and at Elora only 100-fold. A final comparison considers the speed at which the biotracer reached the location used for the second comparison. Horizontal travel velocities of biotracer thus determined ranged from 0 at Cambridge, 0.2 m d^{-1} at Georgian Bay and 2 m d^{-1} at Elora. The high velocities determined for Elora are attributed to uneven use of the bed, significant buildup of the biomat and the shallow surficial aquifer. The intermediate velocity at Georgian Bay is probably an underestimation of the velocity since movement probably took place mainly after the significant rainfall on day 12.

These travel distances can be compared to previous results of bacteria movement summarized by Hagedorn and McCoy (1979). They report maximum distances travelled for coliforms of from 0.6 to 830 m and velocities of 0.1 to 30 m d^{-1}. Although these

Table 2
Comparison of biotracer movement in three systems

System	Attenuation of biotracer at Groundwater below Bed	Attenuation of biotracer at Ground water outside bed	Maximum distance travelled (m)	Biotracer velocity (m d^{-1})
Elora	1000	100	4	2
Georgian Bay	1	200	14	0.2
Cambridge	∞	∞	0	0

were all for wastewater none of these were for septic systems and so probably do not accurately reflect conditions for them. Viraraghavan (1978) reported a travel distance of over 15 m from a septic system for coliforms. This was a system with a limited unsaturated zone below the tile (0.15 m) and thus a greater than normal amount of movement would be expected. Attenuation to the location immediately below the bed appeared to be approximately 10. Samples were taken only on two occasions so travel times could not be determined. Alhajjar et al. (1988) reported the results from 17 systems in Wisconsin. Their observation was that indicator bacteria from septic tanks are not transported to ground water and are completely removed by the soil under the bed. The same result was also reported by Anderson et al. (1994) for systems on sandy soils in Florida. The differences between the various studies are most likely due to local sites differences. A clear advantage of the present study is that because the source of the indicator bacteria being sampled for in know a priori, travel distances, time of travel and degree of attenuation are clear.

8. Conclusions

The experiments reported herein show the utility of using a biotracer, *E. coli* NAR, as a means of assessing the performance of various leaching bed designs. This naturally occurring, rare strain of *E. coli* is both convenient to use and allows one to positively distinguish between various potential sources of microbial ground water contamination. In addition, its movement in soil will more closely resemble the transport of microorganisms than would a conservative tracer like chloride or bromide.

Experimental observations of the two experiments at the Elora Site show that to determine the extent of bacterial transport from leaching beds, the biotracer concentration in the septic tank must be maintained at a high level for a prolonged period—3 weeks for these three leaching beds. This prolonged period provides the biotracer extended time to be transported distances that are representative of the system.

Detailed examination of the results at Elora show that an older system, with a well developed biomat, may have significantly retarded vertical infiltration below the tile lines—leading to horizontal transport at levels much higher than the vertical movement.

The filter bed results from the experiment performed at the Georgian Bay Site are encouraging and suggest that this bed worked reasonably well in the first year of use. Results also indicate that only a small portion of the bed ($\sim 35\%$) is being used—probably due to the gravity feed operation. On-going testing is required to determine if this system will continue to operate well in subsequent years and in years in which high precipitation occurs. High-intensity rainfall encouraged biotracer transport through the filter material to the filter bed/bedrock interface and into the surrounding mantel. This gives an indication that at times of high infiltration the efficiency of the filter bed to retain bacteria is reduced. Any infiltration into the bed from above should be discouraged.

Systems with deeper water tables such as those at the Cambridge Site, perform satisfactorily even when constructed in permeable soils. The results at the site indicates that the 2 m of unsaturated soil is more than adequate to ensure that there is no bacterial contamination to the ground water below. Although significant rainfall and thus

infiltration affected the movement of bacteria in the vadose zone from this system, the depth of the vadose zone was effective at attenuating bacterial movement.

Contrary to observations of Alhajjar et al. (1988) and Anderson et al. (1994) bacteria can and do move out of the leaching bed zone for some systems. Use of a biotracer such as *E. coli* NAR has allowed a clear identification of the movement out of the bed, distance moved and speed of travel.

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