

Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review

R.C. Jamieson¹, R.J. Gordon², K.E. Sharples³, G.W. Stratton³ and A. Madani²

¹*School of Engineering, University of Guelph, Guelph, Ontario, Canada N1G 2W1;* ²*Department of Engineering and* ³*Department of Environmental Sciences, Nova Scotia Agricultural College, P.O. Box 550, Truro, Nova Scotia, Canada B2N 5E3*

Jamieson, R.C., Gordon, R.J., Sharples, K.E., Stratton, G.W. and Madani, A. 2002. **Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review.** Canadian Biosystems Engineering/Le génie des biosystèmes au Canada **44**:1.1-1.9. The presence of pathogenic bacteria in public and private water systems has emerged in the past year as a priority water quality issue. Livestock agriculture is considered one of the primary causes of bacterial contamination of surface and ground waters. The application of animal manures to tile drained land, and the subsequent transport of pathogens with subsurface drainage water to surface water systems, has been identified as a major pathogen transport pathway. The objective of this review is to summarize the information that has been produced with respect to the survival of fecal bacteria in soil waste systems and their transport to tile drainage water. Factors influencing fecal bacteria survival include moisture, soil type, temperature, pH, manure application rate, nutrient availability, and competition. Cool, moist environments are considered optimal for bacterial survival. Field scale transport studies have shown significant transport of bacteria to tile drains under common manure management practices. Results from column and field studies suggest that the transport of bacteria through undisturbed soils is primarily controlled by macropore flow phenomena. Manure management strategies intended to reduce bacterial transport to tile drains, such as deep tillage, may conflict with other environmental management concerns. Further research is required to: (i) assess the effects of alternate cultivation practices on bacterial transport, (ii) verify that enteric pathogens behave similar to indicator organisms, and (iii) evaluate the effects of manure pre-application treatment methods, such as long-term storage and composting, on bacterial survival.

La présence de bactéries pathogènes dans les systèmes d'aqueducs privés ou publics est devenue une priorité du débat actuel sur la qualité de l'eau. On considère que la production animale est une des principales causes de contamination bactériologique des eaux de surface et souterraines. On a observé que les pathogènes migraient vers les eaux de surface principalement après l'application de déjection animales sur des sols drainés. L'objectif de cette revue de littérature est de présenter un résumé de l'information qui existe sur la survie des bactéries fécales dans les sols et leur migration vers les drains. Les facteurs qui influencent la survie des bactéries fécales incluent l'humidité, le type de sol, la température, le pH, le taux d'application du fumier, la disponibilité des éléments nutritifs et la compétition. Dans des conditions fraîches et humides, la survie des bactéries est optimales. Lors d'études à l'échelle du champ où des pratiques d'épandage conventionnelles étaient utilisées, on a observé une migration significative des bactéries vers les drains. Les résultats d'études dans des colonnes de sol et au champ suggèrent que dans les sols qui n'ont pas été travaillés, la migration des bactéries est

principalement contrôlée par le mouvement de l'eau dans les macropores. Certaines stratégies de gestion des fumiers comme le labour profond, dont l'objectif est de réduire la migration des bactéries vers les drains, peuvent entrer en conflit avec d'autres préoccupations environnementales. Les besoins de recherche identifiés sont: (i) de déterminer les impacts de pratiques culturales alternatives sur la migration des bactéries; (ii) de vérifier que les pathogènes entériques se comportent de la même façon que les organismes indicateurs; (iii) d'évaluer les impacts de pré-traitements du fumier, comme l'entreposage à long terme ou le compostage, sur la survie des bactéries.

INTRODUCTION

Many Canadians have been negatively affected by lapses in the security of water quality. For example, the Walkerton, Ontario *Escherichia coli* outbreak (O'Connor 2001) questions the safety of potable water sources that have historically been assumed to be secure. The entry of pathogenic bacteria into drinking water sources poses a great risk to human health. Animal manure application to agricultural land is cited as a major source of pathogenic microorganisms in surface and groundwater systems (Reddy et al. 1981). There is an increasing need to develop farm management systems that minimize the risk of water contamination, especially with respect to human pathogens. Pathogenic bacteria associated with agricultural waste that are of concern include *E. coli*, *Salmonella*, *Campylobacter*, and *Shigella*. Pathogenic protozoans of concern include *Cryptosporidium parvum* and *Giardia* (Landry and Wolfe 1999). Difficulties and expenses involved in the testing for specific pathogens, however, have generally led to the use of indicator organisms of enteric origin to estimate the persistence and fate of enteric bacteria in the environment (Crane et al. 1981). Fecal coliforms are the most commonly used indicator organisms. Fecal coliforms (FC) are identified by their ability to produce gas from lactose at 44.5 °C (Geohring et al. 1999). *Escherichia coli* is the most common FC and although most *E. coli* strains are non-pathogenic, some strains, such as *E. coli* O157:H7, pose a serious health risk to humans. Enterohemorrhagic *E. coli* O157:H7 was first identified as a human pathogen in 1982 (Kudva et al. 1998).

Current bacterial enumeration techniques identify only culturable bacteria cells, therefore it is possible that they considerably underestimate actual bacterial populations. Viable,

but non-culturable bacteria are only identified in tests utilizing defined substrate techniques. These tests measure biochemical changes in media rather than colony formation (i.e. the hydrolysis of MUG, 4-methylumbelliferyl b-D- glucuronide, a specific indicator of *E. coli*.) (Zhai et al. 1995).

Numerous studies have revealed the presence of indicator organisms and pathogens in farmed and non-agricultural watersheds. Doran and Linn (1979) monitored surface runoff from grazed and ungrazed pasture land over a three year period in Nebraska. Fecal coliform counts were 5 to 10 times greater in grazed areas than in ungrazed areas, however total coliform (TC) counts differed little. Fecal coliform counts from ungrazed pastures also commonly exceeded water quality standards. Patni et al. (1985) observed that FC bacteria were almost always present in runoff from non-manured cropland, presumably due to non-agricultural sources. Fecal indicators were common in the surface waters of agricultural areas in southern Finland (Niemi and Niemi 1991), generally exceeding 100 counts per 100 mL and occasionally exceeding 1000 counts per 100 mL. Fecal indicators were also found in 50% of water samples from non-agricultural or pristine watersheds, sometimes exceeding 100 counts per 100 mL. Howell et al. (1995) monitored FC and fecal streptococci (FS) in streams, wells, and springs of two agricultural watersheds in Kentucky. All sites that were monitored had samples that exceeded primary contact standards (>200 counts per 100 mL). Streams exceeded primary contact standards between 87 and 100% of the time. Springs and wells exceeded primary contact standards between 28 and 74% of the time. Although fecal indicator bacteria are commonly found in both farmed and non-agricultural watersheds, it is often difficult to ascertain the precise source of contamination in surface water due to the multiple sources of bacteria (Joy et al. 1998).

Pathogen fate and transport in soils receiving agricultural waste is a complex issue. Integration of knowledge and skills on this issue, unfortunately has not been forthcoming. Landry and Wolfe (1999) state:

“The range of disciplines conducting fecal bacteria research and the diverse nature of the literature are obstacles to application and synthesis of existing knowledge by animal waste managers and scientists.”

Surface runoff represents the greatest contamination risk for surface water systems. However, under current manure application practices, leachate from manure-amended fields reaching subsurface tile drains also often exceeds drinking water supply and recreational use standards (Warnemuende and Kanwar 2000). Tile drained land represents a large portion of the agricultural landscape in the humid regions of North America. Despite the benefits of drainage systems, they are increasingly being recognized as potential pollution sources. The objective of this review is to specifically assimilate and summarize the information that has been produced with respect to the survival of fecal bacteria in soil waste systems and their transport to, and appearance in, tile drainage water.

SURVIVAL IN SOIL SYSTEMS

The availability of pathogens for transport in runoff and leachate during precipitation events is largely influenced by the die-off rate of enteric bacteria in the soil-waste system (Reddy et al. 1981). A wealth of information has been produced within

the past 30 years on the survival of various enteric bacterial species in soil and groundwater systems. A review presented by Gerba et al. (1975) reported that survival times of enteric bacteria in soil and groundwater ranged from 2 to 4 months. Filip et al. (1988) examined the survivability of several organisms in simulated conditions of saturated soil and observed that most organisms tested for, including *E. coli*, survived for over 100 days at 10°C. Kudva et al. (1998) found that *E. coli* O157:H7 survived for 630 days in sheep manure that was not aerated and stored at air temperatures below 23°C. Entry et al. (2000a, 2000b) monitored concentrations of FC bacteria in soil and runoff water from grassed buffer strips that had received liquid swine waste. After 90 to 120 days, FC levels were not significantly different from strips that had not received waste. Reddy et al. (1981) conducted a review of bacterial survival and attempted to develop first order rate constants to describe the die-off of several indicator organisms and pathogens in soil systems. Average first order die-off rate constants were 1.14 d⁻¹ for FC and 0.41 d⁻¹ for FS. Average rate constants for specific pathogens were 1.33 d⁻¹ for *Salmonella* and 0.68 d⁻¹ for *Shigella*. Sjogren (1994) assessed the survival of *E. coli* and used exponential regression to estimate survival times in soil. They estimated survival times by extrapolating the die-off curve to zero counts of bacteria. Probable survival times ranged from 20.7 to 23.3 months.

Several researchers also have noted the regrowth of indicator organisms in soil systems (Van Donsell et al. 1967; Howell et al. 1996). Continued land application of organic wastes may modify the soil environment to the point where it is a more hospitable environment for pathogenic organisms (Dazzo et al. 1973). This phenomenon, in conjunction with the extended survival times of indicator organisms observed under certain conditions, means that the detection of FC in soil and agricultural drainage water may not represent recent contamination. This may obscure the source and extent of fecal contamination (Howell et al. 1996). The need to quantify the contributions of FC from natural or background sources also has been recognized. Entry et al. (2000a, 2000b) observed that fecal bacteria in soil and surface runoff collected from grass buffer strips not receiving swine waste were as high as 2330 colonies/g soil and 583 colonies per 100 mL runoff water.

There are numerous factors that may influence the survival of enteric bacteria in soil (Gerba et al. 1975). However, attempts to link survival rates of specific enteric pathogens with soil physio-chemical and environmental variables (Reddy et al. 1981) are limited. Additionally, few studies have attempted to verify the assumption that mortality rates of indicator organisms accurately reflect those of pathogenic bacterial species (Crane et al. 1981; Filip et al. 1988). The few studies that have been conducted have found that indicator organisms appear to survive longer than pathogens (Mubiru et al. 2000).

FACTORS AFFECTING SURVIVAL

Moisture

Numerous researchers have suggested that the principal factor affecting the survival of enteric bacteria in soil systems is the moisture status (Gerba et al. 1975; Tate 1978; Kibbey et al. 1978; Crane et al. 1981; Reddy et al. 1981; Faust 1982; Mubiru et al. 2000; Entry et al. 2000b). Tate (1978) found *E. coli*

survival to be greatest in organic soils under flooded conditions. Hagedorn et al. (1978) found *E. coli* populations highest after a rise in the watertable following major rainfall events. *Streptococcus faecalis* died more rapidly under low soil moisture conditions (Kibbey et al. 1978). Mubiru et al. (2000) linked lower mortality rates of *E. coli* O157:H7 to higher unsaturated soil matric potentials and Entry et al. (2000b) correlated the increased survival of FC with increased soil moisture in grass buffer strips receiving swine waste. It is apparent that limited moisture availability in the soil reduces the survival rates of enteric bacteria in manure amended soil systems, although quantitative information on the issue is still lacking.

Soil type

The single soil property that appears to have the greatest impact on bacterial survival is moisture retention, which is linked to particle size distribution and organic matter content. Tate (1978) observed that the survival of *E. coli* in an organic soil over an 8-day period after manure application was threefold greater than in a sandy soil. This was attributed in part to the organic soil's increased ability to retain moisture. Hagedorn et al. (1978) studied the subsurface flow of bacteria over a 32-day period. Fecal bacteria moved faster in coarser soil materials. Chandler and Craven (1980) investigated the survival of *E. coli* and *S. typhimurium* in relation to soil moisture retention and soil type. In dry loam soil, *E. coli* cells were able to survive and proliferate once moisture was restored. Chandler et al. (1981) examined the survival of bacteria on pasture, in topsoil and in subsoil and found that topsoil was the most favorable environment for bacteria. Times required for a 90% reduction in initial bacteria concentrations ranged from 7 to 20 days in topsoil. Zhai et al. (1995) also reported greater survival rates of fecal bacteria in topsoil as compared to subsoil. Mubiru et al. (2000) compared the mortality of *E. coli* O157:H7 in two different soil types. Reduced mortality was primarily influenced by soil type, with soils exhibiting a higher matric potential showing lower mortality rates. They also stated that as well as enhancing moisture retention, fine soil particles could increase bacterial survival because of an increased ability to retain nutrients.

Temperature and pH

Within the majority of the literature an inverse relationship appears to exist between temperature and bacterial mortality (Gerba et al. 1975; Reddy et al. 1981) with higher temperatures decreasing the survival times of fecal bacteria. Van Donsel et al. (1967) found that 90% of coliform bacteria died within 3.3 days of land application in the summer compared to 13.4 days in the winter. The review compiled by Reddy et al. (1981) found that die-off rates approximately doubled with a 10°C increase in temperature. Kibbey et al. (1978) reported optimum survival of FS under cool conditions but noted that freezing and thawing of soils reduced bacterial populations. Zibilske and Weaver (1978) concluded that the only set of conditions that consistently led to the death of *Salmonella* were high temperatures and low moisture. Filip et al. (1988) determined that *E. coli* could survive for over 100 days in water-soil mixtures kept at 10°C. *E. coli* survived longer in sheep and cattle manure at temperatures below 23°C (Kudva et al. 1998). Contrary to this,

Howell et al. (1996) measured FC and FS mortality rates at three different temperatures in feces-amended sediments in the laboratory, noting greater FC survival and sometimes regrowth under warmer conditions. Enteric bacteria have a shorter survival period in soils possessing a low pH (Gerba et al. 1975; Ellis and McCalla 1976) with pH of 6 to 7 being optimum for bacterial survival (Cuthbert et al. 1955; Reddy et al. 1981). Sjogren (1994) found *E. coli* survived longer at a neutral to alkaline pH than at an acidic pH in soils of similar texture and organic matter content.

Manure application rate and characteristics

Concentrations of indicator and pathogenic organisms in animal waste vary widely depending on animal type, waste storage system, and the level of pretreatment prior to land disposal. Detailed summaries of the approximate number of different bacterial species commonly found in wastes for both domestic and wild animals are provided by Reddy et al. (1981) and Crane et al. (1983). Patni et al. (1985) found that long-term storage of manure decreased median counts of FC, TC, and FS by 99%, however, these reductions did not occur when fresh manure was added to old manure. Aeration of sheep manure decreased the survival time of *E. coli* O157:H7 from 21 months to 4 months (Kudva et al. 1998).

In general, manure application rate does not appear to influence bacterial survival. Crane et al. (1981) followed the die-off of indicator organisms in surface applied poultry manure. Rate of manure application had no influence on bacterial survival. Zhai et al. (1995) also observed no influence of manure application rate on the mortality of fecal bacteria. In all experiments, FC was non-detectable within eight weeks of application. Little work has been performed to assess the survival of fecal bacteria in soils that have received excessive applications of manure.

Nutrient availability

The presence of organic matter promotes the survival, and in some instances, the regrowth of enteric bacteria (Gerba et al. 1975). Nutrient availability is a key issue in the survival of microbes in soil. Organic matter increases the retention of nutrients, provides a carbon source for bacterial species, and improves moisture retention properties, as noted earlier. Too much moisture, however, can have a negative effect on *E. coli* survival due to a lack of usable organic carbon in dilute mixtures (Chandler and Craven 1980). A major factor in *E. coli* die-out from soil is its inability to step down its metabolic rate to meet the low availability of usable organic carbon (Klein and Casida 1967) and to adjust to conditions of low nutrient availability (Reddy et al. 1981). Tate (1978) revealed increased bacterial survival in organic soils. Zhai et al. (1995) speculated that higher mortality rates of fecal bacteria in subsoil, compared to topsoil, were partially due to low nitrogen (N) availability.

Competition

Competing microorganisms will limit pathogen survival in soil (Reddy et al. 1981). Enteric bacteria, which have been relocated into the soil-waste environment, must compete with resident soil bacteria for essential nutrients and water. Ellis and McCalla (1976) concluded that indigenous soil organisms were resistant to new microorganisms in their environment. It also has been

found that certain bacteriophage and free-living soil organisms, such as *Bdellovibrio*, can parasitize *E. coli* cells, thus limiting their survival (Klein and Casida 1967). Gerba et al. (1975) and Tate (1978) both reported increased pathogen survival, and sometime regrowth, in sterile soils.

TRANSPORT

The major transport modes of pathogens and indicator organisms in soils receiving manure are through movement with infiltrating water and surface runoff and with the movement of sediment and waste particles (Reddy et al. 1981). Physical filtration is believed to be the primary process which limits bacteria mobility in soil (Gerba and Bitton 1984). Bacteria range in size from 0.2 - 5 μm (Matthess and Pekdeger 1985) and are more subject to straining than smaller microorganisms, such as viruses, which range in size from 300 - 300 nm (Gerba and Bitton 1984). Adsorption is the main process which limits the movement of smaller microorganisms. Removal of bacteria occurs largely at the soil surface by straining and by sedimentation and adsorption (Gerba et al. 1975), however, it is difficult to separate the processes of filtration and adsorption (Reddy et al. 1981). Suspended particles, including bacteria which become deposited at the soil surface, can act as a filter trapping more bacteria (Corapcioglu and Haridas 1984). The physical filtration of bacteria at the soil surface increases the likelihood of losses during runoff (Crane et al. 1981). The capacity of a soil to remove microorganisms is increased at low soil water contents and at greater soil clay contents and cation exchange capacities (Reddy et al. 1981). Bacteria are rarely free in the liquid phase of soil because most cells adhere to clay particles (Reddy et al. 1981). As would be expected, finer grained soil types, such as clays and silts, are more efficient in straining bacteria cells due to the smaller pore sizes (Canter and Knox 1988). Several studies have concluded that the removal of bacteria from percolating liquid within a relatively uniform soil is inversely related to the particle size of the soil (Gerba and Bitton 1984). It also is important to note that bacteria cells tend to aggregate and form flocs and clumps which are more susceptible to filtration (Abu-Ashour et al. 1998). In fact, bacteria are rarely present in solution as single particles (McDowell-Boyer et al. 1986). Individual bacteria particles may also interact to form bridges in pores, preventing further movement in the direction of flow (Gerba and Bitton 1984). Studies have concluded that bridging can occur in soils when the diameter of the suspended particles moving through a medium ranged from more than 0.07 - 0.2 times the diameter of the particles of the medium, depending on how the medium was packed (Sakthivadivel and Irmay 1966).

However, the retardation and retention of bacteria in soils is apparently less effective than previously believed, primarily due to preferential flow processes, which can aid in the rapid transport of bacteria from manure application (Smith et al. 1985; Geohring et al. 1999). Quoted lower size limits for macropores have ranged from 30 - 3000 μm (Beven and Germann 1982). The shape of macropores varies from planar slits to cylindrical pipes (White 1985). Beven and Germann (1982) outlined the main processes which contribute to the formation of macropores in natural soils:

- Pores formed by soil fauna such as earthworms, insects, moles, and gophers. They can range in size from 1 - 50 mm, are usually tubular in shape, and found near the soil surface.
- Cracks and fissures formed during the shrinkage of clay soils and freeze/thaw cycles.
- Pores formed by plant roots. These pores are also tubular in shape and found near the soil surface.
- Natural soil pipes that form due to the erosive action of subsurface flows.

Field studies

Several field and watershed scale investigations have specifically looked at enteric bacterial concentrations in agricultural subsurface drainage water. Evans and Owens (1972) monitored *E. coli* and enterococci in tile drainage water over three winters in Scotland on a 0.7 ha sandy clay loam pasture. The pasture was grazed by cattle and sheep during summer. It received no animal manure applications during the first winter, but swine manure was applied three times during the second and third winters. The application of large amounts of manure over short periods of time dramatically increased concentrations of fecal bacteria in drainage water within a few hours of application, but normal levels returned after 2 to 3 days. Bacterial counts were related to the flow rate of the drains and the length of time since manure was applied. Enterococci and *E. coli* also were observed in tile drainage water during the first winter, indicating that these indicator organisms could survive for at least four months. On average, less than 0.05 % of the applied *E. coli* was recovered in tile drainage water. Smith et al. (1972) sampled irrigation and subsurface drainage waters from a southern Idaho irrigation district characterized by silt loam soils underlain by fractured basalt. Irrigation water for the district was obtained from a surface water system, which contained high bacterial counts due to domestic sewage outfalls. The study was intended to assess the ability of soil percolation to remove pathogens because approximately 50% of the applied irrigation water leaves the system as drainage water which is then used to replenish water supply aquifers. The irrigation water consistently had high concentrations of fecal indicators but both FC and FS counts were low in most drainage water samples and below domestic use guidelines.

Rahe et al. (1978) evaluated the movement of *E. coli* that had been injected into the leach lines of septic tank disposal systems that were inundated with groundwater in two different soil types (well drained and poorly drained). Movement rates of 15 m/h were observed at the well drained site. Macropore formation was also identified at this site. *Escherichia coli* exhibited extended survival and appeared to be a satisfactory tracer of saturated groundwater flow. Culley and Philips (1982) focused on survival rates of indicator organisms with winter spread manure. They examined the bacteriological quality of snowmelt subsurface and surface discharge from cropland (corn) receiving different amounts of liquid dairy manure at different times. They evaluated three application rates that were either plowed under prior to seeding, plowed under after harvest, split between fall and spring, or applied to snow covered or frozen ground. They found that spring snowmelt and subsurface runoff from winter applied treatments contained higher bacterial counts than spring and fall applied treatments. They also noticed that surface runoff from June storms on spring

applied plots contained higher bacterial counts than plots that had received manure the previous fall or winter. Dean and Foran (1992) conducted a total of 12 liquid manure spreading trials on five different soil types in Ontario using nalidixic acid resistant (NAR) *E. coli* as a tracer. In nine of the 12 trials bacterial concentrations increased in tile flow within 6 hours of application. In two of the three trials where bacterial concentrations did not increase, the tiles were not flowing at the time of application. In the other trial, the field had been tilled just prior to application. The author speculated that the tillage process had sheared surface macropores.

Cook et al. (1997) studied the influence of manure application methods (broadcast vs inject), timing (fall, spring, late winter), manure vs inorganic fertilization, and N application rate. Trials were performed on 7.6 by 22.7 m plots in corn-soybean rotations. Bacterial densities (FC, *E. coli*, and FS) in drainage water were highly variable with no clear trends. In general, during periods of high drainage flow, higher bacterial densities were observed, but quickly decreased. Warnemuende and Kanwar (2000) reported results from the same experimental set-up as those described above and also reported variable bacterial results. Due to this variability, no conclusions were drawn, but they reported that all plots had FC and FS in drainage water, even those receiving only inorganic fertilizer.

Stoddard et al. (1998) performed a field experiment to measure FC and FS survival and transport through shallow no-tilled and conservation tilled soil amended with dairy manure in the fall and spring. The most rapid mortality of FC occurred after the fall manure application due to the freezing conditions and the freeze-thaw cycles. Neither the timing nor the tillage method significantly affected FC concentrations in the leachate. Fecal coliform concentrations in leachate samples and the potential for groundwater contamination were similar regardless of when manure was applied. This suggests that the mortality rate of FC after manure application has little bearing on the potential for groundwater contamination, but instead contamination depends more on soil structure and water flow.

Joy et al. (1998) used NAR *E. coli* as a biotracer to determine the extent of bacterial movement from manured fields to tile drains under conventional agricultural practices. All experiments were conducted at a single loam soil site over a two year period. Liquid dairy manure was applied in the spring prior to seeding and in the fall after harvest. Manure application rate had no influence on bacterial counts in tile drainage water but there was a strong association between tile bacterial concentrations and rain amount after application. The highest counts occurred when the drains were already flowing when the manure was applied. Soil sampling revealed that the biotracer penetrated the soil profile quickly to a depth of 900 mm in 24 hours in some cases, however, after 20 days the bacteria present in the soil was reduced to less than 1% of the levels found immediately following application, with or without transport. This led to the recommendation that manure applicators should take notice of the flow condition within the tile lines prior to application and the likelihood of precipitation within three weeks after application.

Geohring et al. (1999) applied liquid dairy manure at a rate of 47,000 L/ha to a poorly drained silty loam soil which had not received manure for 20 years. They examined the effects of soil

moisture at spreading, method of application, and irrigation timing on the transport of FC to tile drains. Levels of FC were at a maximum before peak discharge indicating preferential flow. The major effect of lower initial soil moisture content was to delay the time it took for the peak concentrations of bacteria in tile outflow to occur. This meant that when rainfall or irrigation occurred on wet plots following manure application, there was a greater cumulative mass transport of bacteria to the tile drains associated with higher tile flow volumes. When the plots were irrigated 6 days after manure spreading, elevated bacterial numbers (although they were 1/3 of day one levels) were still found in the tile outflow. Plow incorporation of applied manure reduced cumulative FC discharge to about 84% of those from disk soil treatments in the same volume of discharge. Geohring et al. (1999) also noted that FC contamination occurred on plots that had not received manure for two years. This contamination was attributed to geese which were frequently observed on the study field. Hunter et al. (2000) monitored FC in drainage water (subsurface and surface) from an upland sheep pasture in England over a 21 month period. Significant fecal contamination was observed with concentrations higher in the summer than in the winter. This contradicts other studies which have found higher concentrations in the winter due to longer survival at lower temperatures and increased wetness. Hunter et al. (2000) believed that artificial land drainage represented an important transport pathway as bacteria were obviously being transported to receiving waters during drier summer months when surface runoff was not occurring.

Column studies

Results obtained from field scale bacterial transport studies are limited with respect to identifying the influences of specific soil physio-chemical properties and manure application methods on bacterial leaching. There is a need to describe bacterial transport in porous media more quantitatively and relate it to known soil properties and processes (Tan et al. 1992). Several column studies have been conducted to examine these processes in further detail with considerable interest directed towards the role of macropore transport of bacteria. Fontes et al. (1991) examined the effects of grain size, soil heterogeneity, and cell size on bacterial transport in column experiments. Results indicated that even in cases with efficient filtration, bacterial transport was significant. The existence of a preferred flow (16 mm diameter of coarser material) path drastically altered the transport behavior with much quicker breakthrough occurring in soils possessing macropores. The transport of an antibiotic resistant *E. coli* strain through 280 mm deep intact and disturbed columns containing varying soil types was examined by Smith et al. (1985). Chloride solutions containing the *E. coli* strain were applied to the soil columns at rates ranging from 5 to 40 mm/h. Results revealed that all soil types became more effective at retaining bacteria when they were sieved and repacked into the columns. For intact columns there was no relationship between soil properties (texture and bulk density) and bacterial transport. The rate of suspension application was the dominant factor affecting transport in intact columns with transport increasing as the application rate increased. Smith et al. (1985) concluded that the transport of bacteria through sieved or mixed soil columns will be negligible when compared to more

structured soils. Tan et al. (1992) attempted to quantify and model the movement of *Pseudomonas fluorescens* strain 2-79 through packed sand columns. The transport of bacteria was retarded relative to the movement of chloride and tritium, probably due to adsorption since the soil pores were not thought to be small enough to strain bacteria. Transport increased in coarser sands and when the soils were treated with acid. Acid treatment would result in a decrease in trace organic material and free iron oxides. Huysman and Verstraete (1992) also examined the influence of soil physio-chemical properties on bacterial transport in disturbed soil columns. They found that increased bulk density and clay content decreased the migration of various *Lactobacillus* strains.

Shrestha et al. (1997) investigated the effect of manure application rate and the presence of macropores on bacterial leaching through 1000 mm soil columns. Both FC and TC transport were greatest in the columns possessing macropores. Coliform transport was not dependent on manure application rate. They concluded that the transport of bacteria will depend on flow rate, type of flow, and the bacterial retention capacity of the soil and that a conventional manure application rate of 168 kg N/ha for corn would be enough to cause bacterial transport through a 1000 mm deep soil profile throughout a corn cropping season. Abu-Ashour et al. (1998) also focused on the movement of bacteria through artificial macropores in soil columns (175 mm soil depth) at different initial water contents using a NAR *E. coli* strain as a biotracer. For all columns without a macropore, no matter what soil type, initial water content or rainfall volume, no biotracer was ever detected. In general, columns with a macropore had detectable concentrations of biotracer in effluent, however, in the columns which had a low initial water content (10%) no effluent was collected, even in columns with a macropore that received rainfall. Abu-Ashour et al. (1998) also discovered that disturbing the top layer of the soil profile in columns that had a macropore appeared to substantially decrease bacterial transport, and noted that this process requires further investigation.

McMurry et al. (1998) studied FC transport through tilled and sod-covered intact soil blocks (325 mm x 325 mm x 325 mm) which received poultry manure and simulated rainfall. An evenly spaced grid collection system was placed beneath the soil blocks to collect percolating water. For all soil blocks, the spatial distribution of outflow was not uniform, indicating preferential flow occurred under all experimental treatments. Fecal coliform levels were highest where the most drainage flowed, consistently exceeding 20,000 counts per 100 mL. The breakthrough of FC in the tilled blocks was delayed as compared to the no-till blocks. They concluded that preferential movement of bacteria in structured soils will occur with rainfall, even under unsaturated flow conditions. Gagliardi and Karns (2000) performed column studies using three soil types in disturbed or intact columns. The transport of *E. coli* O157:H7 strain B6914 was related to soil type, simulated tillage practices, and whether the bacteria was applied with manure or in a clean water solution. Total bacterial counts measured in leachate water exceeded the inoculum's levels for all treatments except intact clay cores. The presence of manure increased B6914 leaching in intact cores but had the opposite effect in disturbed cores. Soluble N levels correlated with bacteria levels in leachate water. They concluded that tillage practices, soil

type, and method of pathogen delivery may influence, but do not prevent, pathogen transport in soil.

Warnemuende and Kanwar (2000) performed column studies (300 mm soil depth) to investigate the effect of timing (spring vs fall) and rate (168 and 336 kg-N/ha) of swine manure application on bacterial leaching. Intact soil cores were collected, swine manure was incorporated, and the columns were placed in growth chambers where spring temperatures were simulated. The fall columns were frozen for several weeks, thawed, and then subjected to the same experiments as the spring columns to simulate overwintering. All columns were subjected to four simulated rainfall events and FC, *E. coli*, and enterococci levels were measured in column drainage water. Spring application of swine manure resulted in higher bacterial densities in leachate than fall applications. Few differences between application rates were detected, but the higher application rate always yielded higher bacterial densities. Also, an interaction between application rate and timing was observed, suggesting that an increase in application rate is more likely to cause greater bacterial contamination in the spring than fall application.

MANURE MANAGEMENT STRATEGIES TO MINIMIZE BACTERIA LEACHING

Manure storage and pretreatment

It is apparent that manure management strategies to decrease pathogen concentrations in animal waste before it is land applied are crucial to minimizing the risk of fecal contamination of natural water systems. It has been shown that fecal bacteria densities in manure can be reduced through long-term storage (Elliot and Ellis 1977; Crane et al. 1983; Patni et al. 1985) and composting (Edwards and Daniel 1992; Deluca 1997; Pell 1997). Pathogens are poor competitors outside of host organisms (Elliot and Ellis 1977). Larger manure storage facilities also would provide more flexibility as to when farmers could spread manure (Warnemuende and Kanwar 2000).

Timing and rate

Joy et al. (1998) concluded that the primary factors to be considered when applying liquid manure include the flow conditions of the tile lines prior to application and the possibility of high rainfall after application. When rainfall occurs before or after manure is spread, higher concentrations of bacteria will occur in the drainage discharge (McLellan et al. 1993). The risk of FC transport is greatest when drains are already flowing (Geohring et al. 1999). Warnemuende and Kanwar (2000) stated that animal wastes should not be applied 72 hours prior to a runoff event or to frozen or snow covered ground since bacteria tend to survive longer in cold temperatures. They also found that plots receiving spring manure applications had higher bacterial densities than plots receiving fall applications. However, applying manure in the fall increases the risk of nutrient losses. Although the literature has not shown a clear influence of manure application rate on the survival and transport of bacteria, the impact of excessive or continual application of manure has not been fully investigated.

Manure application method

Subsurface injection of liquid animal manure has been recommended to reduce surface runoff and atmospheric losses of both nutrients and pathogens. Crane et al. (1983) state that

subsurface injection may reduce surface bacteria losses but will decrease the amount of contact between surface soils and the bacteria, thereby increasing transport to groundwater or tile systems. Warnemuende and Kanwar (2000) also speculate that subsurface injection may reduce runoff losses but may increase the likelihood of bacterial movement with drainage water. Incorporation of manure disrupts macropores and creates more soil-bacteria contact. Geohring et al. (1999) observed modest reductions in bacterial transport in plots where the manure was tilled into the soil. McLellan et al. (1993) performed a controlled drainage study which found that blocking the drainage outlets reduced the load of bacteria to surface water bodies by a factor of 1/2 to 1/500 after application of liquid manure to farmland. Also, tillage prior to manure application reduced the amount of bacteria entering drains and broke up the soil macropores at the surface. Fleming and Bradshaw (1992) found that tillage prior to manure application reduced the transport of ammonia-nitrogen to tile drainage systems. However, they did not monitor bacteria transport. Abu-Ashour et al. (1998) found that disturbing the top layer of the soil profile in columns that had a macropore appeared to substantially decrease bacterial transport. McMurry et al. (1998) examined the effects of tillage on intact soil blocks. Although tillage did not produce leachate that could meet water quality standards, the results were encouraging suggesting that more extensive tillage could further retard movement. Culley and Philips (1982) also recommend that manure be applied and plowed under in the fall, prior to freeze up, to minimize bacteria numbers in spring run-off. However this might enhance the survival and transport of bacteria to subsurface drainage.

SUMMARY

Field studies have shown significant transport of enteric bacteria through tile drainage systems under all manure application protocols and environmental conditions. It is difficult to compare studies, however, and attempts to determine specific environmental variables or manure management practices which reduce bacterial transport to tile drainage systems are difficult due to inconsistencies in the organisms used to quantify fecal contamination and procedures used to enumerate them. Additionally, non-agricultural sources of fecal bacteria mask the attempts of many studies which have tried to identify factors which enhance or reduce bacterial survival and transport. Environmental variables that appear to exert the most influence on bacterial transport to subsurface drainage systems are soil moisture content at the time of manure application and precipitation in the 2 to 3 weeks following application. Animal wastes should not be applied to tile drained fields when the tiles are flowing. It has also been shown that enteric microorganisms can survive for extended periods of time, and even grow, under commonly encountered soil conditions. The mortality of enteric microorganisms is greatest during hot dry conditions. These conditions prevail for only a limited period of time in humid climates.

Studies have shown that the transport of fecal bacteria under conditions of ideal matrix flow is inversely related to particle size. Soils consisting of primarily silt and clay particles are very

effective in physically filtering bacterial cells under conditions of ideal matrix flow. However, column and field experiments have indicated that macropore, or non-matrix flow, is the dominant transport pathway for fecal bacteria. Therefore, soils more susceptible to shrinking or cracking, such as clays, could be less effective than sandy soils in terms of limiting bacterial transport. It also has been shown that bacterial survival is greater in finer grained soils, which have an enhanced ability to retain moisture and nutrients.

It appears that management strategies to reduce bacterial transport may conflict with management strategies intended to mitigate other environmental impacts. Studies have shown that tillage can reduce bacterial transport to subsurface drains by disrupting preferential flow paths. However, no-till and conservation tillage are currently being promoted to improve soil quality and reduce other environmental impacts, such as erosion and greenhouse gas emissions. It also has been suggested that manure should be applied during hot, dry conditions to facilitate greater bacterial mortality, but ammonia volatilization is significantly enhanced under these conditions. Also, many producers are hesitant to spread manure during hot, dry weather due to odour problems and subsequent nuisance complaints. Subsurface injection of liquid manure has been recommended to reduce losses of bacteria in surface runoff, as well as decrease odour and ammonia losses, but this may increase survival of pathogens and their transport to subsurface drainage systems. Many researchers have found that filtration and retention of waste applied microorganisms are greatest in the surficial soil layers. Long-term waste storage and/or pretreatment, such as composting, of livestock wastes prior to land application would appear to have the greatest impact on reducing bacterial transport to water systems. However, many producers do not have the resources to implement these types of systems.

This literature review has demonstrated that fecal bacterial transport to surface water systems through subsurface drainage would be expected under current agricultural practices. The location and utilization of receiving water systems receiving agricultural tile drainage outflow should be assessed on a farm by farm basis. The risk of fecal contamination of recreational and drinking water sources should be acknowledged by both farmers and municipal authorities controlling public water systems.

RESEARCH RECOMMENDATIONS

To develop a more complete understanding of the behavior and fate of pathogenic microorganisms in soil and subsurface drainage water, further research within the following areas should be conducted : (i) the effect of soil freeze/thaw cycles on bacterial survival, (ii) the verification that enteric pathogens and indicator organisms behave in a similar manner, (iii) the effects of cultivation practices, such as tillage, on the transport of bacteria through macropores, and (iv) the effects of manure storage/treatment methods, such as long-term storage and composting, on pathogen survival.

REFERENCES

- Abu-Ashour, J., D.M. Joy, H. Lee, H.R. Whiteley and S. Zelin. 1998. Movement of bacteria in unsaturated soil columns with macropores. *Transactions of the ASAE* 41:1043-1050.
- Beven, K. and P. Germann. 1982. Macropores and water flow in soils. *Water Resources Research* 18:1311-1325.
- Canter, L.W. and R.C. Knox. 1988. *Septic Tank System Effects on Ground Water Quality*. Chelsea, MI: Lewis Publishers Inc.
- Chandler, D.S. and J.A. Craven. 1980. Relationship of soil moisture to survival of *Escherichia coli* and *Salmonella typhimurium* in soils. *Australian Journal of Agricultural Research* 31:547-555.
- Chandler, D.S., J. Farran and J.A. Craven. 1981. Persistence and distribution of pollution indicator bacteria on land used for disposal of piggery effluent. *Applied and Environmental Microbiology* 42:453-460.
- Cook, M.J., J.L. Baker, R.S. Kanwar, S.K. Mickelson, J.C. Lorimer and S.W. Melvin. 1997. Bacteria in agricultural drainage water as affected by manure management. ASAE Paper No. 972148. St. Joseph, MI: ASAE.
- Corapcioglu, M.Y. and A. Haridas. 1984. Transport and fate of microorganisms in porous media: A theoretical investigation. *Journal of Hydrology* 72:149-169.
- Crane, S.R., P.W. Westerman and M.R. Overcash. 1981. Die-off of fecal indicator organisms following land application of poultry manure. *Journal of Environmental Quality* 9:531-537.
- Crane, S.R., J.A. Moore, M.E. Grismer and J.R. Miner. 1983. Bacterial pollution from agricultural sources: A review. *Transactions of the ASAE* 26:858-866.
- Culley, J.B. and P.A. Philips. 1982. Bacteriological quality of surface and subsurface runoff from manured sandy clay loam soil. *Journal of Environmental Quality* 11:155-158.
- Cuthbert, W.A., J.J. Pane and E.C. Hill. 1955. Survival of bacterium coli type I and streptococcus faecalis in soil. *Journal of Applied Microbiology* 12:63-70.
- Dazzo, F., P. Smith and D. Hubbell. 1973. The influence of manure slurry irrigation on the survival of fecal organisms in Scranton fine sand. *Journal of Environmental Quality* 2:470-473.
- Dean, D.M. and M.E. Foran. 1992. The effect of farm liquid waste application on tile drainage. *Journal of Soil and Water Conservation* 5:368-369.
- Deluca, T. 1997. Composting for feedlot manure management and soil quality. *Journal of Production Agriculture* 10:235-241.
- Doran, J.W. and D.M. Linn. 1979. Bacteriological quality of runoff water from pastureland. *Applied and Environmental Microbiology* 37:985-991.
- Edwards, D.R. and T.C. Daniel. 1992. Environmental impacts of on-farm poultry waste disposal-A review. *Bioresource Technology* 41:9-33.
- Elliot, L.F. and J.R. Ellis. 1977. Bacterial and viral pathogens associated with land application of organic wastes. *Journal of Environmental Quality* 6:245-251.
- Ellis, J.R. and T.M. McCalla. 1976. Fate of pathogens in soils receiving animal wastes – A review. ASAE Paper No. 762560. St. Joseph, MI: ASAE.
- Entry, J.A., R.K. Hubbard, J.E. Theis and J.J. Fuhrmann. 2000a. The influence of vegetation in riparian filterstrips on coliform bacteria: I. Movement and survival in water. *Journal of Environmental Quality* 29:1206-1214.
- Entry, J.A., R.K. Hubbard, J.E. Theis and J.J. Fuhrmann. 2000b. The influence of vegetation in riparian filterstrips on coliform bacteria: II. Survival in soils. *Journal of Environmental Quality* 29:1215-1224.
- Evans, M.R. and J.D. Owens. 1972. Factors affecting the concentration of faecal bacteria in land-drainage water. *Journal of General Microbiology* 71:477-485.
- Faust, M.A. 1982. Relationship between land-use practices and fecal bacteria in soils. *Journal of Environmental Quality* 11:141-146.
- Filip, Z., D. Kaddu-Mulindwa and G. Milde. 1988. Survival of some pathogenic and facultative pathogenic bacteria in groundwater. *Water Science and Technology* 20:227-231.
- Fleming, R.J. and S.H. Bradshaw. 1992. Contamination of subsurface drainage systems during manure spreading. ASAE Paper No. 922618. St. Joseph, MI: ASAE.
- Fontes, D.E., G.M. Mills, G.M. Hornberger and J.S. Herman. 1991. Physical and chemical factors influencing transport of microorganisms through porous media. *Applied and Environmental Microbiology* 57:2473-2481.
- Gagliardi, J.V. and J.S. Karns. 2000. Leaching of *Escherichia coli* in diverse soils under various agricultural management practices. *Applied and Environmental Microbiology* 66:877-883.
- Geohring, L.D., P.E. Wright, T.S. Steenhuis and M.F. Walter. 1999. Fecal coliforms in tile drainage effluent. ASAE Paper No. 992203. St. Joseph, MI: ASAE.
- Gerba, C.P. and G. Bitton. 1984. Microbial pollutants: Their survival and transport pattern to groundwater. In *Groundwater Pollution Microbiology*, eds. G. Bitton and C.P. Gerba, 65-88. New York, NY: John Wiley and Sons, Inc.
- Gerba, C.P., C. Wallis and J.L. Melnick. 1975. Fate of wastewater bacteria and viruses in soil. *Journal of Irrigation and Drainage Engineering* 101:157-174.
- Hagedorn, C., D.T. Hansen and G.H. Simonson. 1978. Survival and movement of fecal indicator bacteria in soil under conditions of saturated flow. *Journal of Environmental Quality* 7:55-59.
- Howell, J.M., M.S. Coyne and P.L. Cornelius. 1995. Fecal bacteria in agricultural waters of the bluegrass region of Kentucky. *Journal of Environmental Quality* 24:411-419.
- Howell, J.M., M.S. Coyne and P.L. Cornelius. 1996. Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform/fecal streptococci ratio. *Journal Environmental Quality* 25:1216-1220.
- Hunter, C., J. Perkins, J. Tranter and P. Hardwick. 2000. Fecal bacteria in the waters of an upland area in Derbyshire, England: The influence of agricultural land use. *Journal of Environmental Quality* 29:1253-1261.

- Huysman, F. and W. Verstraete. 1992. Water-facilitated transport of bacteria in unsaturated soil columns: Influence of inoculation and irrigation methods. *Soil Biology and Biochemistry* 25: 91-97.
- Joy, D.M., H. Lee, C.M Reaume, H.R. Whitely and S. Zelin. 1998. Microbial contamination of subsurface tile drainage water from field applications of liquid manure. *Canadian Agricultural Engineering* 40:153-160.
- Kibbey, H.J., C. Hagedorn and E.L. McCoy. 1978. Use of fecal streptococci as indicators of pollution in soil. *Applied and Environmental Microbiology* 35:711-717.
- Klein, D.A. and L.E. Casida. 1967. *Escherichia coli* die-out from normal soil as related to nutrient availability and the indigenous microflora. *Canadian Journal of Microbiology* 13:1461-1470.
- Kudva, I.T., K. Blanch and C.J. Hovde. 1998. Analysis of *Escherichia coli* O157:H7 in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology* 64:3166-3174.
- Landry, M.S. and M.L. Wolfe. 1999. Fecal bacteria contamination of surface waters associated with land application of animal waste. ASAE Paper No. 994024. St. Joseph, MI: ASAE.
- Matthess, G. and A. Pekdeger. 1985. Microbial pollutants. In *Groundwater Quality*, eds. C.H. Ward, W. Giger and P.L. McCarty, 472-482. New York, NY: John Wiley & Sons.
- McDowell-Boyer, L.M, J.R. Hunt and N. Sitar. 1986. Particle transport through porous media. *Water Resources Research* 22:1901-1921.
- McLellan, J.E., R.J. Fleming and S.H. Bradshaw. 1993. Reducing manure output to streams from subsurface drainage system. ASAE Paper No. 932010. St. Joseph, MI: ASAE.
- McMurry, S.W., M.S. Coyne and E. Perfect. 1998. Fecal coliform transport through intact soil blocks amended with poultry manure. *Journal of Environmental Quality* 27: 86-92.
- Mubiru, D.N., M.S. Coyne and J.H. Grove. 2000. Mortality of *Escherichia coli* O157:H7 in two soils with different physical and chemical properties. *Journal of Environmental Quality* 29:1821-1825.
- Niemi, R.M. and J.S. Niemi. 1991. Bacterial pollution of waters in pristine and agricultural lands. *Journal of Environmental Quality* 20:620-627.
- O'Connor, R. 2001. *Report of the Walkerton Inquiry*. Toronto, ON: Publications Ontario.
- Patni, N.K., H.R. Toxopeus and P.Y. Jui. 1985. Bacterial quality of runoff from manured and non-manured cropland. *Transactions of the ASAE* 28:1871-1877.
- Pell, A. 1997. Manure and microbes - Public and animal health problem. *Journal of Dairy Science* 80:2673-2681.
- Rahe, T.M., C. Hagedorn, E.L. McCoy and G.F. Kling. 1978. Transport of antibiotic-resistant *Escherichia coli* through western Oregon hillslope soils under conditions of saturated flow. *Journal of Environmental Quality* 7:487-494.
- Reddy, K.R., R. Khaleel and M.R. Overcash. 1981. Behavior and transport of microbial pathogens and indicator organisms in soils treated with organic wastes. *Journal of Environmental Quality* 10:255-266.
- Sakthivadivel, R. and S. Irmay. 1966. *Quantitative Aspects of Filter Clogging*. Publication Hel-15-4. Hydraulic Engineering Laboratory, University of California, Berkeley, CA.
- Shrestha, S., R.S. Kanwar, C. Cambardella, T.B. Moorman and T.E. Loynachan. 1997. Effect of swine manure application on nitrogen and bacterial leaching through repacked soil columns. ASAE Paper No. 97-2164. St. Joseph, MI: ASAE.
- Sjogren, R.E. 1994. Prolonged survival of an environmental *Escherichia coli* in laboratory soil microcosms. *Water Air and Soil Pollution* 75:389-403.
- Smith J.H., C.L. Douglas and J.A. Bondurant. 1972. Microbiological quality of subsurface drainage water from irrigated agricultural land. *Journal of Environmental Quality* 1:308-311.
- Smith, M.S., G.W. Thomas, R.E. White and D. Ritonga. 1985. Transport of *Escherichia coli* through intact and disturbed soil columns. *Journal of Environmental Quality* 14:87-91.
- Stoddard, C.S., M.S. Coyne and J.H. Grove. 1998. Fecal bacteria survival and infiltration through a shallow agricultural soil: Timing and tillage effects. *Journal of Environmental Quality* 27:1516-1523.
- Tan, Y., W.J. Bond and D.M. Griffin. 1992. Transport of bacteria during unsteady unsaturated soil water flow. *Soil Science Society of America Journal* 56:1331-1340.
- Tate, R.L. 1978. Cultural and environmental factors affecting the longevity of *Escherichia coli* in histosols. *Applied and Environmental Microbiology* 35:925-929.
- Van Donsel, D.J., E.E. Geldreich and N.A. Clarke. 1967. Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution. *Applied Microbiology* 15:1362-1370.
- Warnemuende, E.A. and R.S. Kanwar. 2000. The effect of swine manure application on bacterial quality of leachate from intact soil columns. ASAE Paper No. 00-2053. St. Joseph, MI: ASAE.
- White, R.E. 1985. The influence of macropores on the transport of dissolved and suspended matter through soil. *Advances in Soil Science* 3:94-120.
- Zhai, Q., M.S. Coyne and R.I. Barnhisel. 1995. Mortality rates of fecal bacteria in subsoil amended with poultry manure. *Bioresource Technology* 54:165-169.
- Zibilske, L.M. and R.W. Weaver. 1978 Effect of environmental factors on survival of *Salmonella typhimurium* in soil. *Journal of Environmental Quality* 7:593-597.