# Movement of pathogenic bacteria from manure to groundwater resources

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# APPROPRIATE ENVIRONMENTAL AND SOLID WASTE MANAGEMENT AND TECHNOLOGIES FOR DEVELOPING COUNTRIES

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# MOVEMENT OF PATHOGENIC BACTERIA FROM MANURE TO GROUNDWATER RESOURCES

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## ABSTRACT

The objective of our study was to evaluate the potential for bacteria to contaminate ground water after field application of animal manure.
The movement of faecal coliforms through the vadose zone was investigated following the application of animal manure. Two soils were studied at contrasting initial water contents, and two manure types, solid and liquid. Bacteria present in the soil solution were collected using calibrated ceramic-porous-cup samplers. Estimated bacterial migration velocities in the soil profile were consistent with the hypothesis that bacterial transport occurs mostly through soil macropores, faster than the average pore-water velocity. Macropore transport was more likely to occur in wet soils, but it was not necessarily restricted to soils with high initial soil water content. The soil clay content, lower total porosity, and lower saturated hydraulic conductivity resulted in a higher likelihood for suspended bacteria to be funnelled through pores with greater velocity, increasing the potential depth of bacterial contamination. The continuity of macropores was more important for deep transport of faecal bacteria than was total porosity. The potential for deep contamination with faecal bacteria was also correlated with the water content of the manure. We concluded that application of animal manure to soil can readily lead to groundwater contamination with faecal bacteria especially under wet conditions, and that macropores are important in the transport.

## KEYWORDS

Faecal bacteria, manure, soil, transport, vadose zone

## BACKGROUND

Contamination of the water resources with manure-originated bacteria depends on a number of mitigating factors. Survival and transport of bacterial cells, after field application of manure, depend on the physico-chemical properties of both soil and manure as well as the resultants of their interaction. Transport of bacteria from the source to the water bodies is mediated by rain and irrigation water. Therefore the direction this water takes after reaching soil surface is essential in separating the potential for groundwater or surface water contamination. As the rate of precipitation exceeds the rate of water infiltration into the soil more of it can enter through the larger voids within the soil profile or runoff onto adjacent land surfaces or into surface waters. Formation of a soil crust can enhance macropore flow and surface runoff even when relatively

small volumes of water are added to soil (Unc, 1999, McMurry, 1998, Beven and German, 1982), while the underlying soil matrix can still remain unsaturated (Morin *et al.*, 1981).
The strength of a contaminant source depends on the capacity of the manure microbes to survive in the environment. The survival rate of bacteria in soil depends on the animal source, microbial species (Rηprich, 1994), manure application method (Patni *et al.*, 1985), the biological activity of the soil (Aceā *et al.*, 1988), ambient temperature, soil water potential (Gerba and Bitton, 1984, Sjogren, 1981), and nutrient availability (Rattray *et al.*, 1992).
Although some bacteria have cilia and flagella, and are mobile, the range of independent movement is not more than a few mm. Convection of the water (and therefore the advection of suspended particles) is considered to be the main mechanism of transport in surface run-off and in the unsaturated zone. Hence, under field conditions, bacterial movement is limited mainly by the gravitational flow of water in which the cells are dispersed. The relatively large size of bacterial cells allows them to be transported only though the soil macropores. Bacterial movement is therefore limited mainly by the gravitational flow of water in which the cells are dispersed (Stozky, 1985). The presence of a continuous macropore system is one of the main conditions controlling transport of particles through soils (Jacobsen *et al.*, 1997).
The movement of bacterial cells can be retarded by adsorption-adhesion events (Matthess *et al.*, 1988). The significance of these events depends on the physico-chemical interaction between the soil structural and electrochemical properties and the bacterial cell aspect and surface electrochemical properties. Bacteria are adsorbed to surfaces to different degrees as function of the pH, organic matter content, ionic strength and composition of the suspending solution, and characteristics of bacteria cells such as surface charge, hydrophobicity, presence or absence of surface polysaccharide, cilia and flagella (Dickson and Daniels, 1991).
Variability in the soil-water content, soil structure and texture causes the transport and retention mechanisms to be significantly different for vertical and horizontal directions (Stotzky, 1985). While adsorption and adhesion are the major factors controlling retardation, bacteria are believed to be largely removed through straining processes within the manure and at the manure soil interface (Barrington and Jutras, 1983), as well as by filtration within the soil profile due to macropore discontinuities.

## METHODOLOGY

The experiment was conducted at two micro-sites in southern Ontario, Canada, one on a Silty loam soil (ZL) and one on a Sandy loam soil (SL)(Table 1). The size of the individual experimental plot was of 3x1.5m. Two manure types were used, liquid swine manure and solid beef manure (Table 2). Soil solution and any bacteria present were collected using ceramic porous cups samplers (air entry value of 100kPA, average pore size 2.9 μm) inserted at a 45deg angle at three depths, 30, 50 and 75 cm on the SL soil and 30, 75, and 100 cm for the ZL soil (Unc and Goss, 2000). Ten samplers were used on each plot at each depth. Soil water content, before and during the tests, was measured by using time domain reflectrometry (TDR) probes (Topp et al., 1980) inserted to the same depth as the samplers. The insertion depth on the SL soil was limited by the presence of sizeable stones in the soil profile at greater depth. Runoff collectors were placed around the experimental plots. Runoff occurred only at the ZL site.
The experiment was repeated three times at two-three months interval. Every time, for each manure type, there were two soil moisture content levels at the onset of the tests. One soil moisture level was the natural moisture at the time of the experiment while a second plot was irrigated with about 40-50mm water over 2 hours, 4 to 5 hours before the start of the tests. This resulted in six levels for the initial soil moisture for each soil and each manure type during the experimental period. The volumetric soil water moisture varied between 0.18 and 0.37 $m^3$ $m^{-3}$ for the SL soil and 0.17 and 0.42 $m^3$ $m^{-3}$ for the ZL soil.

Table 1. Soils characteristics

| Soil | Depth (cm) | Texture | Sand - Silt - Clay (% weight) | pH ($CaCl_2$) | $CaCO_3$ (%) | OM (%) | $K_{sat}$ [1] (cm $hr^{-1}$) | Porosity ($cm^3$ $cm^{-3}$) |
|---|---|---|---|---|---|---|---|---|
| SL | 0-30 | Loam | 37.7 - 48.3 - 14.0 | 7.5 | 5.5 | 3.7 | 1.7 | 0.43 |
| | 30-75 | Sandy Loam | 48.2 - 42.1 - 9.7 | 7.6 | 17.6 | 2.8 | 2.9 | 0.49 |
| ZL | 0-30 | Loam | 49.2 - 36.8 - 14.0 | 7.35 | 2.87 | 2.02 | 1.69 | 0.41 |
| | 30-75 | Silt Loam | 30.2 - 49.5 - 20.3 | 7.58 | 21.04 | 0.51 | 0.94 | 0.40 |

1) Estimated using the method presented by Saxton (1986)

Table 2. Manure characteristics [1)]

| Manure type | pH | Dry matter (% weight) | Bacteria (log cfu $100g^{-1}$) | |
|---|---|---|---|---|
| | | | Total coliforms | *Escherichia coli* |
| Liquid swine | 8.63 (0.14) | 0.71 (0.35) [2)] | 7.14 (0.67) | 6.38 (0.75) |
| Solid beef | 8.66 (0.09) | 24.69 (3.32) | 8.73 (0.28) | 8.55 (0.28) |

1) Values averaged for all treatments and repeats; 2) Mean and standard deviation

Manure was spread uniformly over soil surface at a rate equivalent to 50 t $ha^{-1}$. Within an hour drip irrigation was started and continued at a rate of 25mm $hr^{-1}$ for a two hours period. Drip irrigation lines were laid on the surface, parallel to each other, uniformly spaced at 12.5 cm. The irrigation system was calibrated before each test. The irrigation area was slightly larger than the actual test area. This was done to minimize the effect of the horizontal soil water moisture gradients under the test plots. Therefore a two-dimensional water flow through the soil could be considered for the drainage calculations. Volumetric soil water content was determined at the onset of the experiment and later when the soil solution was sampled. The soil solution was sampled eight times, once before manure application and six times afterwards up to ten days after application. Soil solution was sampled using the ceramic porous cups to obtain a maximum volume of 7 mL. Solution samples were transported in coolers and preserved at 4° C within 2 hours of collection. Faecal and total coliform counts were obtained within 24 hours of sampling. Drainage at each instrumented depth was calculated using a mass balance approach and considering the changes in the soil water volumetric content, the volume of the irrigation water, the runoff volume, and an estimate of evaporation. The latter was estimated using hourly temperature, dew point and air humidity (Konstantinov, 1971). Average pore water velocity, $PWV_t$ (cm $d^{-1}$) was estimated from the total drainage amount, $q_t$ ($m^3$ $hr^{-1}$), the variation in the soil volumetric water content, $\theta$ ($m^3$ $m^{-3}$), and the drainage cross-sectional area, A ($m^2$) (Eq.1).

$$PWV_t = \frac{q_t}{(\theta_{t1} + \theta_{t2}) \times \frac{1}{2}} \times \frac{1}{A} \times 24 \times 100 \quad \text{Eq. 1}$$

Bacteria migration velocity was estimated from the depth of recovery and the time of sampling. As sampling was not continuous in time, the bacteria migration velocity could only be determined as an approximation that likely underestimated the real value (Unc, 1999). Bacteria were extracted from soil and manure using the standard method for soil bacteria extraction (Klute et al., 1986) and serially diluted before identification and quantification. Soil dilutions as well as soil solution samples were filtered under vacuum through a 0.45 μm pore-size membrane filter that was placed, in a Petri-dish, over a growth substrate of M-FC broth with rosolic acid solution, solidified through addition of granulated agar. After 24 hours ± 2 hours incubation at 44.5° C faecal coliform counts were performed. When it was deemed necessary a confirmatory test was performed. This test used was a lactic fermentation presence-absence test, which is a modification of the standard most probable number - MPN – test, using serial incubations in lauryl-tryptose broth and inositol brilliant green lactose bile broth. Total coliform counts were obtained using M-Endo agar and an incubation temperature of 37 ± 0.5° C (Clesceri et al., 1989). The results of the faecal coliform tests are often considered to represent the incidence of *Escherichia coli* (Charriere et al., 1994).

## RESULTS

Bacterial concentration of the two types of manure used for the experiment was correlated with the amount of dry matter of manure; a greater number of bacteria per unit area being applied with the solid beef manure (Table 2). Preliminary soil and soil solution testing indicated no detectable levels of faecal coliforms. The information on the amount and bacterial concentration of the runoff was used to adjust the initial boundary conditions at the plot surface. Runoff occurred only on the ZL soil and was larger for the liquid manure treatments.

### Contamination Frequency

Although the total porosity was greater on the sandier soil, contamination at depth occurred more frequently on the soil with higher clay content (Table 3) suggesting that more deep continuous macropores are present in the soil with increased clay content.

Table 3. Frequency of detected bacteria transport events (% contaminated sampling locations)

| Soil | Manure | Depth (m) | | | |
|---|---|---|---|---|---|
| | | 0.30 | 0.50 | 0.75 | 1.00 |
| SL | Liquid swine | 15 | 16 | 8 | |
| | Solid beef | 24 | 20 | 8 | |
| ZL | Liquid swine | 12 | | 20 | 16 |
| | Solid beef | 11 | | 27 | 12 |

### Bacteria Migration Velocity

Bacteria migrated through the vadose zone at velocities up to 35 times larger than the average pore water velocity. The difference between the average soil pore water and bacteria migration velocity was more accentuated at increased clay content in the soil (Table 4). The initial volumetric water content of the soil had no significant impact on the rapid macropore transport of bacteria except when liquid manure was applied to the ZL soil ($P_{r=0}$=0.025*). Fast macropore transport was not affected by manure type ($P_{ANOVA}$>0.07ns) but was significantly impacted by the soil characteristics ($P_{ANOVA}$>0.001**) (Table 5). The initial volumetric soil water content had no significant influence on the average pore water velocity within the SL soil for any manure. On the other hand the water movement depended on the initial volumetric ZL soil water content. This indicated that the flow was more uniform within the sandier soil at every water content. The uniformity of the downward movement of the wetting front was also confirmed by the smaller differences in the water movement across the range of the pore size distribution (Table 4 and 5).

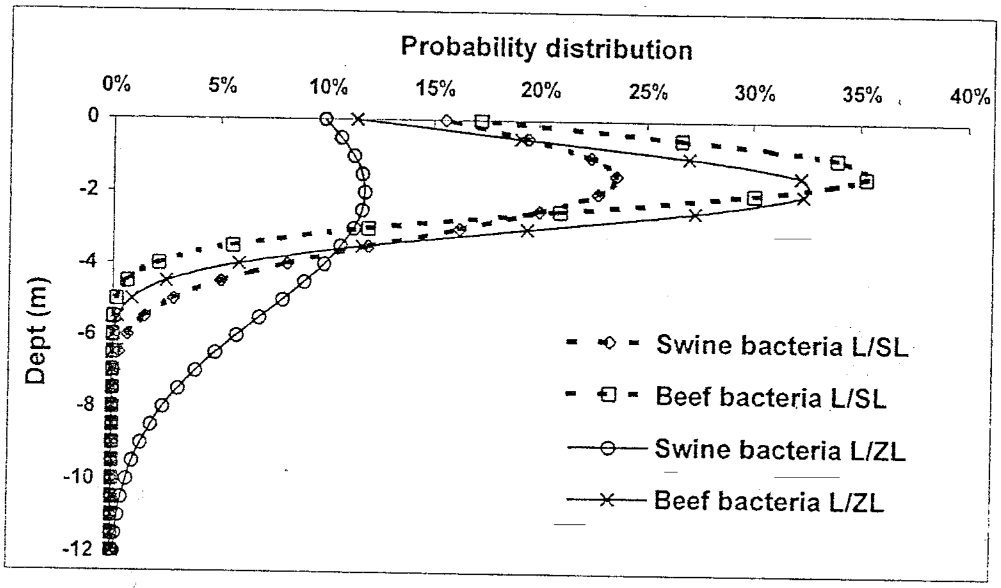


Fig. 2. Estimated potential maximum transport depth of manure bacteria

There was no correlation between the depth of collection and the concentration of E. coli in the soil solution samples ($P_{r=0}$=0.8ns). Similarly there was no correlation between the time bacteria were collected and the depth at which bacteria were found ($P_{r=0}$=0.2ns), or the bacteria loading of the samples ($P_{r=0}$=0.5ns). Deep transport was independent from the initial soil water content ($P_{r=0}$=0.4ns). The only factor that affected the filtration coefficient in a significant manner was the manure type ($P_{ANOVA} < 0.001$***), with most filtration occurring when solid beef manure was applied (Table 6).

## DISCUSSION AND CONCLUSIONS

As the faecal bacteria concentration in manure was correlated to the manure dry matter content application of the solid beef manure resulted in the total amount of bacteria applied per unit surface being 3 orders of magnitude larger than amount applied with the liquid swine manure. Such a simplistic comparison could indicate that solid manure has a greater potential to lead to bacterial contamination of the environment.

Soil-water content at the time of manure application was expected to be an important factor in the velocity of downward migration of bacteria and was also expected to influence the number of bacteria moved to depth (Hegde and Kanwar, 1997). The average wetting front velocity was dependent on the volumetric soil water content status. Bacteria moved up to 35 times faster than the average pore water velocity, the higher speeds being attained in the finer textured soil, in spite of its lower total porosity. This is an indication of the pore size exclusion effect, which results in the relatively large bacterial cells moving through the soil macropores at accelerated speed (Natsch et al., 1996). Bacterial transport velocity was increased on wetter soils, most obviously on the ZL soil. However the amount of bacteria transported at depth was generally independent of the soil moisture, or the presence of continuous macropores. Total soil porosity was found not to be a good measure for the deep transport of bacteria. The uninterrupted length and the structural stability given by higher clay content were more important in facilitating macropore flow and therefore transport of bacteria through the vadose zone (Jacobsen et al., 1997). Manure type proved to be the most important factor in modifying the contaminant flow density. Application of solid beef manure resulted in less deep movement (Fig 2.) despite the greater loading with faecal bacteria. Penetration depth was independent of the bacteria retention rates. Lack of correlation between the sampling time and the amount of bacteria collected poses the question of bacterial survival in the soil. It is not known what the impact of manure characteristics have on the survival of manure bacteria in the field.

The results of this study confirms that macropore flow may be induced by small amounts of added liquids (Beven and German, 1982), and mediate transport of highly concentrated bacterial suspensions independent of the soil moisture status. Increased clay content favoured more pore continuity with depth and hence bacterial transport to greater depths. Lower total soil porosity coupled with a lower hydraulic conductivity of the finer textured soil matrix resulted in more water to be available for macropore flow and surface runoff. The efficiency at which this transport mechanism occurred was most obvious for the treatments with liquid swine manure on drier soils.

Field application of manure represents a potential risk for ground water contamination, by faecal bacteria particularly for shallow water tables.

However all the factors involved in the modifying the transport potential for manure bacteria are not understood. Survival of faecal bacteria in soil can be significant (Cools, 2001). The interaction between the bacterial cells and soil environment is dependent on both cell and soil physicochemical characteristics. Manure is a complex material that can affect both faecal bacteria survival and the interaction of the cell surface with soil mineral and organic surfaces.

More research is needed to explain such interactions if an accurate prediction of the contaminant potential of the manure bacteria is to be achieved.

## REFERENCES


Acea, M. J.,Moore, C. R. and M. Alexander, "Survival and growth of bacteria introduced into soil", *Soil Biology and Biochemistry* Vol.20, No.4, pp. 509-515, 1988.

Barrington, S. and P. J. Jutras, *"Soil sealing by manure in various soil types"*, ASAE Winter Meeting, Microfiche collection, fiche no. 83-4571, 1983

Beven, K. and P. Germann, "Macropores and water flow in soils", *Water Resources Research*, Vol.18, pp.1311-1325, 1982

Charierre, G. D., Mossel, A. A., Beaudeau, P., and H. Leclerc, "Assessment of the marker value of various components of the coli-aerogenes group of Enterobacteriaceae and of a selection of Enterococcus spp. for the official monitoring of drinking water supplies", *The Journal of Applied Bacteriology*, Vol.76, No.4, pp.336-344, 1994

Clesceri, L. S., Greenberg, A. E., and R. R. Trussel *"Standard methods for the examination of water and wastewater"* 17th ed., American Public Health Association, Washinghton DC, 1989

Cools, D., Manure-derived antibiotic resistant bacteria: survival in soil and contamination of crop roots, PhD Dissertation, Katholieke Universiteit Leuven, Leuven, Belgium, 2001.

Dickson, J.S. and E.K. Daniels, "Attachment of Salmonella typhimurium and Listeria monocytogenes to glass as affected by surface film thickness, cell density, and bacterial motility", *Journal of industrial microbiology*, Vol.8, No.4, pp.281-284, 1991

Gerba, C. P. and G. Bitton, "Microbial pollutants: their survival and transport pattern to groundwater" In: *Groundwater pollution microbiology*, (G. Bitton and C.P. Gerba, eds.), Wiley, New York, pp.65-88, 1984

Hegde, P. and R. S. Kanwar, *"Impact of manure application on groundwater quality"* ASAE Annual International Meeting, Paper No. 97-2144 AWE, Minneapolis, Minnesota, 1997

Jacobsen, O. H., Moldrup, P., Larsen, C., Konnerup, L., and L. W. Petersen, "Particle transport in macropages of undistributed soil columns", *Journal of Hydrology*, Vol.196, pp.185-203,

Klute, A., Campbell, G. S., Jackson, R. D., Mortland, M. M., and D. R. Nielsen, "*Methods of soil analysis, Part 2 - Chemical and microbiological properties*", ASA-SSSA, Inc., Madison, 1986

Konstantinov A. R., "*Evaporation in nature*", Jerusalem, Israel, 1971

Mathess, G., Pekdegger, A., and J. Schroefer, "Persistence and transport of bacteria and viruses in groundwater-a conceptual evaluation", *Journal of Contaminant Hydrology*, Vol.2, pp.171-188, 1988

McMurry, S. W Coyne M. S. and E. Perfect, "Fecal coliform transport through intact soil blocks amended with poultry manure", *Journal of Environmental Quality*, Vol.27, pp.86-92, 1998

Natsch, A., Keel, C., Troxler, J., Zala, M., Von Albertini, N., and G. Defago, "Importance of preferential flow and soil management in vertical transport of a biocontrol strain of Pseudomonas fluorescens in structured fields" *Applied and Environmental Microbiology*, Vol.62, pp.33-40, 1996

Patni, N. K., Toxopeus, H. R., and P. Y. Jui, "Bacterial quality of runoff from manured and non-manured cropland", *Transactions of the ASAE*, Vol.28, No.6, pp.1871-1877, 1985

Rattray, E. A., Prosser, J. I., Glover, L. A., and K. Killham, "Matric potential in relation to survival and activity of a genetically modified mirobial inoculum in soil", *Soil Biology and Biochemistry*, Vol.24, No.5, pp.421-425, 1992

Rüprich, A., Felduntersuchungen zum Infiltrationsvermögen und zur Lebensfähigkeit von Fäkalkeimen in Boden nach Gülledüngung, PhD Dissertation, University of Hohenheim, Hohenheim, Germany, 1994

Sjogren, R. E. and M. J. Gibson, "Bacterial survival in a dilute environment", *Applied and Environmental Microbiology*, Vol.41, No.6, pp.1331-1336, 1981

Stotzky, G., Mechanisms of adhesion to clays, with reference to soil systems, In: *Bacterial adhesion:Mechanisms and physiological significance* (D.C. Savage and M. Fletcher, eds.), Plenum Press, New York, pp. 195-254, 1985

Topp, G. C., Davis, J. L., and A. P. Annan, "Electromagnetic determination of soil water content: Measurements in coaxial transmision lines", *Water Resources Research*, Vol.16, pp.574-582, 1980

Unc, A., Transport of faecal bacteria from manure through the vadose zone, M.Sc. thesis, University of Guelph, Guelph, Canada, 1999

Unc, A. and M. J. Goss, "Development of a protocol for sampling faecal coliform bacteria originating from manure in the vadose zone", *Water Quality Research Journal of Canada*, Vol.35, No.1, pp.23-38, 2000