

The survival of Faecal Indicator Organisms (FIOs) in soil, following dairy slurry application to land by surface broadcasting and shallow injection

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Abstract

The amended Bathing Water Directive (2006/7/EC) of February 2006 saw the introduction of more stringent microbial parameters for both inland and coastal waters. Two microbial parameters are now required to be examined; intestinal enterococci and *Escherichia coli* (FIOs). Approximately ninety million tonnes of livestock manures are recycled to agricultural land in the UK annually, which is a potential source of FIO export to surface waters. Shallow injection of slurry is a proven method to reduce ammonia emissions, yet there is limited evidence of the survival of FIOs following slurry injection by this technique.

The survival of FIOs within dairy slurry applied via broadcast (splash-plate) and shallow injection techniques was investigated at the plot scale. Soil core samples (2 cm depth) were taken and analysed for FIOs from fifteen randomised 4 m² plots comprising three treatments: 5 broadcast applied and 5 shallow injected, (both receiving fresh slurry at the equivalent rate of 45 m³ per hectare) and 5 control plots (no slurry addition) during distinct periods of the agricultural year. Application method and season affected the survival rate of FIOs. The ramifications of extended FIO survival in soil following slurry application by shallow injection may have to be addressed in the wider context of pollution swapping when considering holistic mitigation options at the farm scale.

Introduction

The Water Framework Directive (WFD) is a significant piece of EC water legislation, designed to protect and improve the quality of water bodies throughout Europe. In the UK, its implementation is being managed by the competent authorities, through government led initiatives. The WFD came into force on 22 December 2000, and was put into UK law in 2003. Member States must aim to reach good chemical and ecological status in inland and coastal waters by 2015. Significant effort has been directed in England at minimising diffuse water pollution from agriculture (DWPA). Historically, DWPA has been synonymous with nutrient and sediment impacts on water quality. The inclusion of daughter directives such as the amended Bathing Waters Directive (2006/7/EC) into the WFD, which introduce stringent microbial parameters for both inland and coastal waters, is an acknowledgement that microbial pollution is a significant contributor to DWPA. Furthermore not only is it recognised that microbial pollution impinges on water quality but also human health (Kay *et al.*, 2007).

Some 90 million tonnes of livestock manures are produced annually in the UK, approximately 73 million tonnes of which is derived from the dairy and beef industries (Chambers *et al.*, 2000). Significant quantities of animal manures collected and stored on dairy farms are done so as slurries, a mixture of faeces, urine and water (Pain and Menzi, 2003). Slurry remains liquid during storage, is rarely batch stored, is continually added to with fresh slurry and does not compost, allowing, potentially, for the prolonged survival of faecal indicators and pathogenic bacteria. Animal slurries contain significant concentrations of nutrients and are routinely applied to agricultural land as fertilisers. The abatement of ammonia (NH₃) emissions from agriculture has been the focus of extensive research across Europe,

resulting in legislation in some European countries, e.g. the Netherlands and Belgium (Misselbrook *et al.*, 2000). An effective method for reducing emissions of NH₃ from the land application of slurries has been to inject slurries into the soil (Misselbrook *et al.*, 1996). Although many European countries have adopted this method of slurry application, few farmers in the UK have. In the UK, the majority of slurries are applied by broadcasting it on the soil surface, often using a splash plate applicator. There is a need to determine if reducing NH₃ emissions through shallow injection increases the risk from another form of pollutant, in this case FIOs, termed 'pollution swapping'.

Slurry applications pose the risk for the spread of pathogenic microorganisms, either directly to streams and rivers through run off from fields or direct spillage from applications made too close to stream boundaries (Oliver *et al.*, 2007). The management and mitigation of such risk is becoming a priority for environmental guardians, seeking practical tools to facilitate effective microbial risk assessments (Oliver *et al.*, 2007, Wilkinson *et al.*, 2006, Sinton *et al.*, 2007). The ability of these risk tools to gauge survival of faecal indicator bacteria from a range of on farm activities, for example the applications of animal slurries for the nutritional benefit of the crops, becomes beneficial to their accuracy.

Faecal indicator organisms (FIOs) are acknowledged as being surrogates for the presence of pathogenic bacteria, they are routinely tested for in the food and drink industry (Jordan *et al.*, 2007) and their concentrations limits in 100 ml volumes of water are embodied in legislation across the World, safeguarding recreational waters for human use, the Water Framework Directive in Europe and the Clean Water Act in the U.S.

The following paper describes the survival of FIOs, with particular focus on *E. coli*, within dairy slurry applied via broadcast (splash-plate) and shallow injection techniques investigated at the plot scale.

Material and Methods

Experiments were conducted on an experimental grassland field the soil type described as a poorly drained silty clay loam (Halstow Series Findlay *et al.*, 1984), at the North Wyke Research farm, Devon UK (50°45'N, 3°50'W). Slurry was applied to areas of grassland approximately 2 m x 2 m using two simulated spreading techniques; surface broadcast (splash-plate) and shallow injection. Broadcast spreading was simulated using an adapted watering can with a spoon attachment to provide a suitable splash-plate spread pattern. In order to simulate the shallow injection, 5-6 cm deep slots were cut into the ground with plot scale equipment and a watering can was used to pour the appropriate quantity of slurry into the slots. Dairy slurry was obtained from a slurry lagoon on a nearby dairy farm, after the slurry had been stirred for 1 – 2 hours. Slurry was applied at the equivalent rate of 45 m³ per hectare. The plot scale experiment was designed such that fifteen 4 m² randomised plots comprising three treatments: 5 broadcast applied and 5 shallow injection and 5 control plots were investigated at distinct periods of the agricultural year, May, July and October. The plots had not been grazed or received manure or fertilisers in the previous 20 years, a different set of fifteen randomised plots were used for each application date and there was a 2 m 'race' surrounding each plot, to minimise cross contamination of the plots. Meteorological data was collected in the field using a Skye Minimet 4 meteorological station (Skye Instruments Ltd., UK).

Five soil cores were taken from each plot, bulked and the soil homogenized, samples were taken on day 1, 2, 3 and 4 after slurry application, and then weekly reducing over time until FIO concentrations were undetected or had reached background levels for 2 consecutive samples. For all plots, soil was sampled to a depth of 2 cm. For the control and broad cast applied plots this was achieved using a 2 cm corer. However, for the shallow injection slots a 7.5 cm corer was used, on average the slots were 5.5cm deep

(data not shown) and the 7.5 cm corer effectively retrieved a 2 cm sample from the base of the slot. Soil augers were washed and disinfected in a 1 % solution of Virkon® and rinsed (x 3) with sterile deionised water between plots, to minimise cross plot contamination. Five grams of the mixed soil from each plot was added to 45 ml of Ringers solution, vortex mixed and then shaken for 60 minutes, the remaining soil sample was used to determine the gravimetric water content by drying at 105 °C for 24 h. After standing for 5 minutes the elution was analysed for *Escherichia coli*. Intestinal Enterococci concentrations were also determined, but are not reported here. Appropriate serial ten fold dilutions were made and standard methods of membrane filtration were used to determine bacterial concentrations (Anon part 4, 2002). Samples were washed through the filtration unit with 20 ml of sterile Ringer's solution. Membrane filters of 0.45µm pore size (Pall Gellman Sciences) were aseptically transferred to Membrane Lactose Glucuronide Agar (MLGA) (Oxoid) and incubated inverted, at 44.5 °C (± 0.2 °C) for 18 – 24 hours for *E.coli*. API 20E biochemical kits (bioMérieux) were used as a confirmatory procedure. The API biochemical kits rely on the biochemical profiles exhibited by the bacterial isolates for confirmation of their identity through database comparison.

Results

The numbers of days for *E.coli* concentrations to return to background levels in relation to slurry application method by application date are shown in Table 1. Clearly *E.coli* survived greater in soil samples taken from the shallow injection plots than the broadcast applied plots.

Table 1. Days (averaged of 5 replicates) for *E.coli* concentrations to return to background levels in soil, following the application of slurry, via shallow injection and broadcast

Month of application	Application Method	<i>E. coli</i> Days to control concentrations (days)
May	Shallow Injection	51
	Surface Broadcast	31
July	Shallow Injection	82
	Surface Broadcast	53
October	Shallow Injection	102
	Surface Broadcast	44

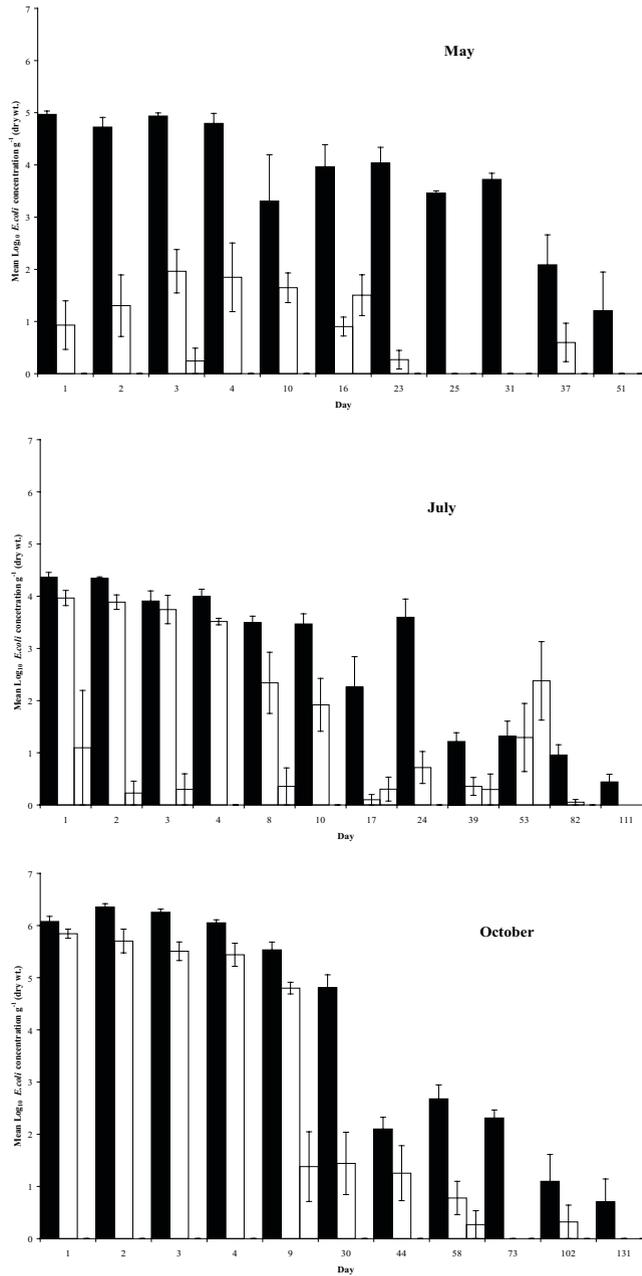
Mean measured levels of UV, air temperature and total rainfall for each slurry application are recorded in Table 2.

Table 2. Mean metrological data, UV, Air temp and total rainfall for each of three slurry application periods

Month of application/ sampling period (days)	Mean UV (wm ²) for sampling period	Mean Air temp (°C) for sampling period	Total rainfall (mm) for sampling period
May 51	236.1	15.1	39
July 111	129.6	14.9	25.4
October 131	60.9	10.7	68.4

Initial *E.coli* concentrations in the fresh dairy slurries varied from; May log₁₀ 5.10 (± 0.04), July log₁₀ 4.86 (± 0.14), October log₁₀ 7.16 (± 0.05) g⁻¹ dry weight. *E.coli* concentrations in the soil cores over time are shown in figure 1. There was a steady decline in viability of *E. coli* concentrations following the three application dates observed in the shallow injection plots, a more rapid decline in viability was observed over the first 10 days in the broadcast plots during July and October. However, in May the concentration of *E.coli*, one day after application, was 4 log below that detected in the slurry initially applied to the plot. Interestingly, an increase of greater than 1 order of magnitude in *E.coli* concentration was observed over the first 4 days, prior to a decline in viability at day 10 during May.

Figure 1. Mean *Escherichia coli* concentrations for spring, summer and autumn, filled bars show data for shallow injection, hollow bars data for surface broadcast application and hatched bars data for the control plots; error bars represent standard errors. N = 5



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Discussion

Based on the results of this plot scale study, it appears that dairy cattle slurry applied to land via shallow injection may remain a substantial source of *E. coli* for at least 30 days after application. Furthermore its survival in the soil at the base of the injection slots, albeit at relatively low concentrations, was detected beyond 100 days for both the July and October applications. Whereas *E. coli* survival in the injection slot, following the May application, was not detected much beyond 50 days, less than half that of the other application dates. In contrast, dairy cattle slurry applied to land via broadcast application did not remain a substantial source of *E. coli* beyond 10 days. Broadcast application of slurry in May, with UV levels recorded at 180 wm^2 and an average ground surface temperature of 15.7 °C on the day of application, a 4 log reduction in viable *E. coli* concentration was recorded within 1 day of application. Interestingly, an increase in *E. coli* population growth of one-order of magnitude was observed during the 4 days after the broadcast applied slurry in May. Kudva *et al.* (1998) observed a similar increase, Kessel *et al.* (2007) investigating *E. coli* survival in cowpats in pasture, observed a 1.5 order of magnitude increase over a similar period, they concurred with a number of other publications that the temperature range between 20 and 35 °C was most favorable for post – deposit growth. Although the mean daily air temperature recorded on day 1 of the May study was 15.7 °C the maximum day time temperature was 26.4 °C. It may well be that even in conditions (elevated temperatures and UV levels) where significant decline in bacterial population may be predicated, post initial land application, a significant growth in population may actually occur, thus underestimating actual FIO concentrations available for surface water contamination in the first few days following manure application or excretion.

The abatement of ammonia (NH_3) emissions from the livestock sector across Europe has been a focus of research for sometime. Seventy four percent of total estimated ammonia emissions come from animals and 32 % is due to land spreading of slurry (Misselbrook *et al.*, 2000). Emissions of NH_3 can be reduced by an average of 85 % when slurry is applied to land by shallow injection in comparison to conventional splash-plate application. Thus an abatement strategy to reduce NH_3 emissions from slurry is to shallow inject it. However, as this current study shows, injecting dairy slurry significantly increases the survival of *E. coli* compared to broadcast application. So the ramifications of extended FIO survival in soil following slurry application by shallow injection may have to be addressed in the wider context of 'pollution swapping' when considering mitigation options at the farm scale. Although increased survival of FIOs in slurry injection slots has been shown in this study it does not necessarily follow that there is an increased risk of their export to surface waters. There should be a reduction in their mobilisation within slots, where the slots are cut across slopes and not with the slope.

Acknowledgements

This work was funded by the Rural Economy and Land Use (RELU) programme (RES-224-25-0086). North Wyke is sponsored by the Biotechnology and Biological Sciences Research Council (BBSRC).

References

- Chambers, B. J., Smith, K. A., and Pain, B. F., 2000. Strategies to encourage better use of nitrogen in animal manures. *Soil Use and Management* 16, 157-161.
- Jordan, D., Phillips, D., Sumner, J., Morris, S., and Jenson, I., 2007. Relationships between the density of different indicator organisms on sheep and beef carcasses and in frozen beef and sheep meat. *Journal of Applied Microbiology* 102, 1- 57
- Kay, D., Aitken, M., Crowther, J., Dickson, I., Edwards, A.C., Francis, C., Hopkins, M. Jeffrey, W., Kay, C. McDonald, A. T., McDonald, D., Stapleton, C. M., Watkins, J., Wilkinson, J., and Wyer, M. D., 2007. Reducing fluxes of faecal indicator compliance parameters to bathing waters from diffuse agricultural sources: The Brighthouse Bay Study, Scotland. *Environmental Pollution* 147, 138-149.
- Kessel, J.S.v., Pachepsky, Y.A., Shelton, D.R. and Karns, J.S., 2007. Survival of *Escherichia coli* in cowpats in pasture and in laboratory conditions. *Journal of Applied Microbiology* 103, 1122-1127.
- Kudva, I.T., Blanch, K. and Hovde, C.J., 1998. Analysis of *Escherichia coli* O157: H7 survival in ovine or bovine manure and manure slurry. *Applied and Environmental Microbiology* 64, 3166-3174.
- Misselbrook, T. H., Laws, J. A., and Pain, B. F., 1996. Surface application and shallow injection of cattle slurry on grassland: nitrogen losses, herbage yields and nitrogen recoveries. *Grass and Forage Science* 51, 270-277.
- Misselbrook, T. H., Van Der Weerden, T. J., Pain, B. F. Jarvis, S. C., Chambers, B. J., Smith, K. A., Phillips, V. R. and Demmers, T. G. M., 2000. Ammonia emissions factors for UK agriculture. *Atmospheric Environment* 34, 871-880.
- Oliver, D. M., Heathwaite, L. A., Hodgson, C. J., and Chadwick, D. R., 2007. Mitigation and current management attempts to limit pathogen survival and movement within farmed grassland. *Advances in Agronomy* 93, 95-152.
- Pain, B., and Menzi, H., 2003 (Editors). Glossary of terms on livestock manure management. *Recycling Agricultural, Municipal and Industrial Residues in Agriculture Network*.
- Sinton, L.W., Braithwaite, R.R., Hall, C.H. and Mackenzie, M.L., 2007. Survival of indicator and pathogenic bacteria in bovine feces on pasture. *Applied and Environmental Microbiology* 73, 7917-7925.
- Wilkinson, J., Kay, D., Wyer, M., and Jenkins, A., 2006. Processes driving the episodic flux of faecal indicator organisms in streams impacting on recreational and shellfish harvesting waters. *Water Research* 40, 153-161.