The impact of soil type and soil biology on the survival of *Escherichia coli* O157

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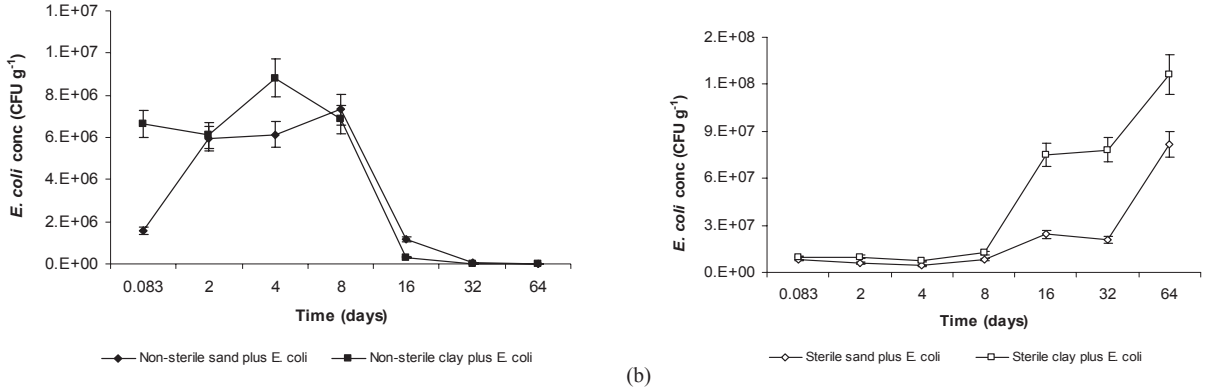
Introduction *E. coli* O157 is a pathogenic micro-organism which is ubiquitous in the agricultural environment as a result of livestock defecation and slurry spreading. This pathogen can cause severe gastroenteritis and haemolytic uraemic syndrome in humans. Thus it is necessary to determine the factors that influence *E. coli* survival in soil to ensure that agricultural practices do not pose a risk to public health. The aim of this work was to determine the impact of soil biota and soil type on the survival of a non-pathogenic strain of *E. coli* O157, which shows similar survival characteristics to pathogenic *E. coli*.

Materials and methods Sandy loam and clay loam soils were collected from Silsoe farm, Cranfield, UK. Soil was sieved to 4mm and moisture content was adjusted to 44 % and 25 % of field capacity for clay and sand soil respectively. 5g sub-samples were transferred to microcosms (sterile glass vials, 30ml). Half of these microcosms were sterilised by autoclaving. Microcosms were stored at 4°C for the first 6 days of the experimental period, and at room temperature (18°C) thereafter. Test microcosms were inoculated with 500µl of culture, containing a known *E. coli* concentration of 2 x 10^7 colony forming units (CFU) per gram of soil. The inoculum culture was prepared by adding 100µl of an overnight culture of *E. coli* O157 to 100ml fresh LB broth and incubating at 37°C for 24 hours on an orbital shaker at 150 rev min^{-1}. This culture was subsequently centrifuged at 10 000 x g and washed three times in sterile ¼ strength Ringers solution. The culture was then serially diluted to a factor of ten, and each dilution was plated onto Sorbitol MacConkey agar amended with Cefixime-Tellurite supplement (CT-SMAC), which is selective for the O157 strain. Plates were incubated at 37°C for 24 hours. Based on these results, it was possible to determine the appropriate dilution to use for microcosm inoculation. Control microcosms were inoculated with 500µl of sterile ¼ strength Ringers solution. Microcosms were mixed gently by hand following inoculation and weighed to establish cumulative initial moisture content. They were sampled destructively immediately after inoculation, and on days 2, 4, 8, 16, 32 and 64 of the experimental period. They were monitored for evaporation by weighing, and sterile water was added when necessary. Sampling consisted of adding 10ml sterile ¼ strength Ringers solution to each microcosm and vortexing, followed by 15 minutes on a reciprocating shaker at 150 rev min^{-1}. Microcosms were then vortexed again, and allowed to stand for 5 minutes for the heavier soil fraction to settle out of suspension. 5-fold dilutions were established for each microcosm in sterile universal bottles. 100µl of each dilution was plated onto CT-SMAC, and incubated at 37°C for 24 hours after which characteristic beige colonies of non-toxigenic *E. coli* O157 were counted and recorded (adapted from Avery *et al*., 2005).

Results *E. coli* concentrations were stable at low temperature (4°C), and there was no significant effect of time, treatment or soil type on *E. coli* survival. Conversely, the three-way interaction between time, treatment and soil type was strongly significant at room temperature (18°C, $p<0.001$). Data also suggest that this interaction differed between sand and clay microcosms. There was a significant effect of the interaction between temperature and treatment on *E. coli* survival ($p<0.001$), but there was no difference in this effect between soil types.



(a) (b)

Figure 1 *E. coli* concentration in (a) sterile and (b) non-sterile sand and clay microcosms over time. Values show mean CFU g^{-1} ± SEM (n=3).

Conclusions These results show that the soil biota has a definite impact on *E. coli* O157 survival, which becomes more pronounced at ambient temperature. The influence of soil type on survival is less clear, due to additional confounding factors such as organic matter, soil spatial structure and microbial diversity.

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References

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