INACTIVATION OF *E. COLI* IN RIPARIAN AND NON-RIPARIAN SOILS

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ABSTRACT
Intensive farming in New Zealand (NZ) can sometimes mean pastures are grazed to the edges of drains and streams. However farmers using best management practices have retired grazing land to provide riparian protection for streams. Riparian soils perform important functions in attenuating nutrient pollutants and faecal bacteria in overland and subsurface flows. We hypothesize that faecal bacteria entering riparian soils and non-riparian soils via such flows may demonstrate differing rates of inactivation due to differing soil organic matter content and competition from the altered microbial assemblages. Inactivation of *Escherichia coli* from fresh cow manure was measured in 3 replicates each of riparian and non-riparian soils from two NZ dairy farms maintained in a constant temperature room at 15°C. Samples were exposed to artificial sunlight containing both UV-A and UV-B light. Initial inactivation within the first 18 h was very rapid (k=0.932 d^{-1}, base e), although some treatments showed subsequent bacterial re-growth. A much slower inactivation followed (k=0.056 d^{-1}, base e) with *E. coli* still culturable in some of the treatments at 177 d. Statistical analysis showed no significant difference between treatments, and time was the only factor of those tested that influenced inactivation.

Keywords : Riparian protection, faecal indicator bacteria, inactivation rates

INTRODUCTION
Riparian strips can have a variety of benefits for stream water quality within a dairying environment. Riparian plants can stabilise stream banks, which is further improved by preventing stock access to these areas. Riparian plants provide shading of streams to prevent excessive heating, and shading may also lessen the growth of nuisance weeds within streams and drains. Dense swards of grasses in riparian zones can filter particulate matter from overland flow, and build-up organic matter as litter and humus in the soil act as a site of sorption for dissolved nutrients and bacteria from through-flowing water, as well as providing a carbon source for denitrification. As riparian soils develop a physicochemical structure different from adjacent farmland, they are also likely to produce distinct microbiological assemblages that are able to utilize and process nutrients entering from the adjacent pastures.

Runoff from farm land used for grazing can carry high concentrations of faecal bacteria and nutrients (Wilcock et al. 1999) which may enter adjoining waterways as runoff (Doran and Linn 1979; Gary et al. 1983) or drainage water (Teutsch et al. 1991). Collins et al. (2003) found attenuation of faecal bacteria from overland flow in riparian streps, however, information on the rate of inactivation of faecal bacteria within NZ riparian soils, and in pastoral soils under intensive grazing or land application of dairy wastewaters, is limited. Overseas studies have indicated that inactivation depends on a range of factors including sunlight, bacterial competition and soil physical and chemical properties (Crane and Moore 1986; Mubiru et al. 2000). The amount of faecal matter deposited on soils can have a significant effect on soil physical and chemical status (Nguyen and Goh 1992). Since soil moisture, light exposure, resident microbial populations and other bio-physical conditions which prevail in the riparian soils may differ from those in non-riparian (pastoral) soils, the survival of faecal bacteria may differ between these two soil types. Bacterial survival in soils, particularly from manure, is of importance because of the potential pathogen reservoir that they form for contamination of the environment, as well as health implications for farm workers and livestock. The survival of *Escherichia coli*, a useful bacterial indicator of faecal contamination, was tested in two riparian and two non-riparian soils collected from NZ dairy farmlands. The study was conducted for a period of 6 months.

METHOD
Top soils (loams, 0–10 cm depths) were collected in Spring (1 Oct., 2001) from dairy pastures and adjacent riparian areas from two intensively managed (3-4 cows ha^{-1}) dairy farms in Waikato Region of New Zealand (McBride's Farm, Collie Rd, Te Kowhai, NZ 260 S14 982 813, and MacIntyre's Farm, Settlement Road, Kereone NZ 260 T14 414 863), where riparian strips had been in use for 2 and 8 years respectively. Upon returning to the laboratory, the soil was passed through a 5 mm sieve to remove remaining roots and to create a relatively uniform matrix.
At the same time, fresh cow manure was collected in plastic bags and mixed to a uniform consistency. Soils and the faecal matter were analysed for *E. coli* within 3 hours of collection using the method outlined below. Samples were also taken to determine dry weight content. The manure was then mixed with each soil at a ratio of 1.93 (nominally 2 kg) to 5 kg respectively in large plastic bags and de-ionised water added to achieve final moisture contents of 24% and 35% for riparian soils, and 15% and 35% for non-riparian soils (initial soil moistures were close to 24% and 15% for the riparian and non-riparian soils respectively). Replicates of 250 g of soil:faeces mixture were added to 400 mL plastic containers. The walls of the containers were covered to prevent light entering from the sides, and the tops sealed with translucent plastic film. Containers were kept in a constant temperature room (15°C). Half the samples were additionally covered with aluminium foil to prevent light intrusion. The remainder were left uncovered under a light bank simulating low levels of sunlight. UV-A and UV-B lamps were covered with sheets of acetate to reduce levels of these wavelengths to similar

spectral compositions to sunlight. UV-A and B, and overall irradiance levels (RFF, 400–700 nm, equivalent to PAR) were measured with a Macam 203-3 Radiometer (Macam Photometrics, Livingston, Scotland), and a full spectrum scan was measured with a Licor Li-1800UW Spectroradiometer with a standard cosine receptor. Soil moisture was maintained on a weekly basis by adding deionised water, and any plants that germinated were removed.

During the incubation period of 177 days, one gram subsamples (wet weight) of soil:faeces mixture (or for initial testing, straight soil or faeces) were taken from a depth of 1 cm. Samples collected were extracted with 40 ml of 0.3mM KH_2PO_4 in a sealable tube to remove *E. coli* that were sorbed on soil particles. Large lumps were physically broken up, and the tubes were placed on a shaker-table at 100 rpm for 1 hr. The contents were then allowed to settle briefly (5–10 min.) prior to extracting a portion of the supernatant for analysis for *E. coli* using the Colilert® most probable number method (IDEXX Laboratories Inc, Maine USA, USEPA Method 9223, ONPG-Mug Test). Sampling interval was most frequent at the start of the experiment (daily), reducing to periods in excess of a month during the latter stages of the experiment. Testing continued until more than 50% of the samples were returning values of <10 *E. coli* (MPN) per gram sample (i.e., 177 days or approx. 6 months).

RESULTS

Light levels in the constant temperature room had an overall irradiance level of 24 $W.m^{-2}$ at the surface of the soil/faeces mixture. As lighting was continuous, this equated to around 2.1 $MW.m^{-2}\ d^{-1}$, or 10% of a cloudless day in summer in the Waikato region, or roughly equivalent to an overcast summer day. UV A and B levels were 255.4 and 13.4 $mW.m^{-2}$ respectively. A scan of the full spectrum is shown in Figure 1, along with a sunlight scan on an overcast summer day.

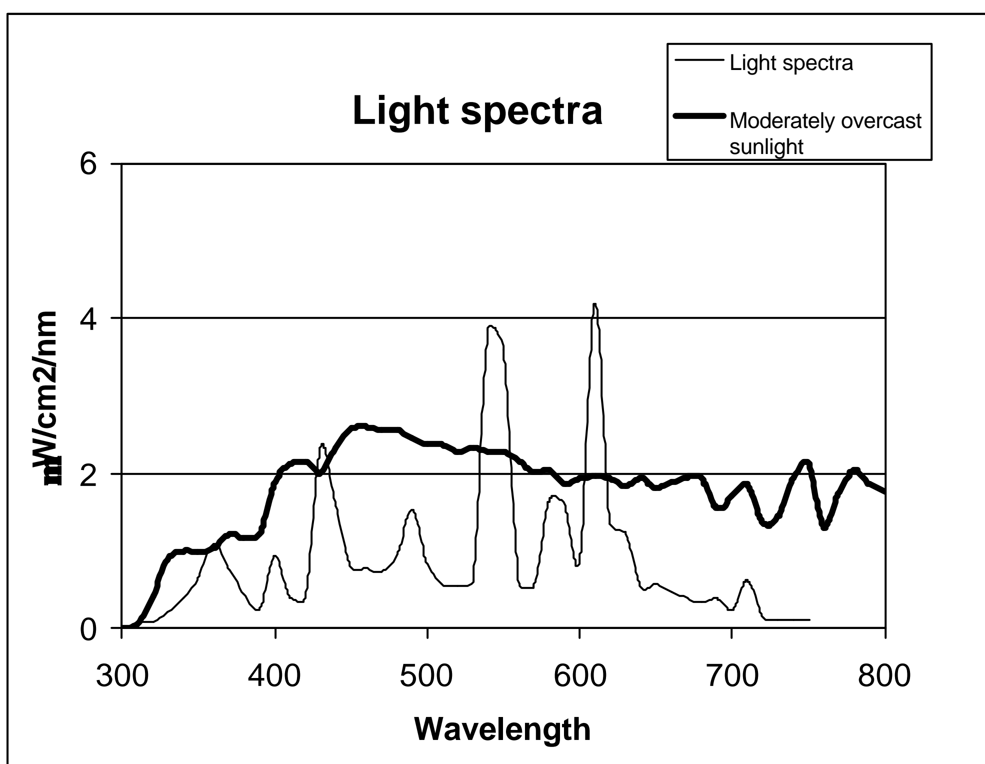

Figure 1. Spectral composition of added light compared with natural light on a moderately overcast day.

Average MPN values for each soil:faeces treatment have been plotted over the approx. 6-month sample period (Figure 2) on a log scale. Initial *E. coli* values for all of the soils were <1 MPN per g, and 2.02 $x10^6$ MPN per g for the faecal matter. Initial mixture concentrations averaged 400,000–800,000 MPN per g. Values for the soil:faeces mixture were variable on the first 3 sampling occasions (0–10 d), with rapid reduction in numbers in most samples, particularly in the first 18 h. Using Chick's Law (Chick 1908) of simple first order reaction kinetics, an inactivation constant k can be calculated according to the following formula,

$$-k = \frac{\ln N_t / N_0}{t_t - t_0} \qquad (1)$$

N_t = number of bacteria at time t
N_0 = number of bacteria at time 0
t = time in days
k = first order or inactivation rate constant (base e)

Using the above formula, the initial constant for the first 18h was k =0.932 d^{-1}. Some treatments showed subsequent increased *E. coli* numbers. The trend in inactivation was much slower after this (combined data k=0.056 d^{-1}), with *E. coli*

numbers falling to background levels in some instances after about 6 months (a 5–6 log reduction). Approximately 17% of samples (25 out of 145) had no culturable *E. coli* on the final sampling date.

Treatment results were compared statistically using ANOVA with interactions (Data Desk®, Data Description Inc, Ithaca NY). Comparisons are shown as box-plots in Fig. 3. There were no statistical differences between replicates, soil type, soil moisture or irradiance (Light) at the 0.05 level. Inactivation of *E. coli* over time (date) was highly significant (≤0.0001). Site was not significant at the 0.05 level (unless 2 extreme values defined as outliers by Data Desk were excluded from the analysis).

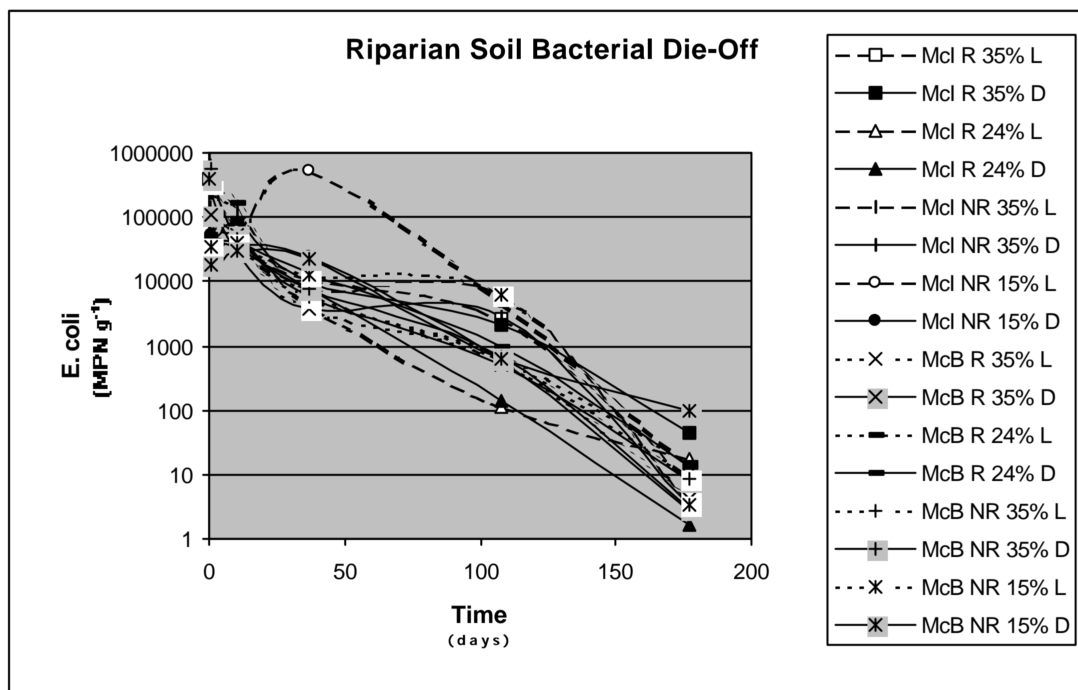

Figure 2. Average E. coli *g^{-1} MPN of 3 replicates for each treatment.*
McI, McIntyre's farm; McB, McBride's farm; R, riparian; NR, non-riparian;
15%, 24%, 35%, % moisture content; L, light; D, dark.

DISCUSSION

Concentrations of *E. coli* in the soil:faeces mixture varied considerably on the first sampling date (day 0), and ranged from 4.0 x 10^4 to 2.8 x 10^6 MPN per g (although averages of the three replicates were more similar, at between 4.0 x 10^5 and 8.0 x 10^5). This variability in individual replicates may reflect difficulty in obtaining a truly uniform distribution of faecal matter in the initial soil:faeces mixture. Each sample demonstrated a rapid decline in viable bacteria over the ensuing 18 hrs, as have been found by other researcher (e.g. Klein and Casida 1967), particularly where initial inoculum size is high. Two treatments showed temporary increases after the initial 3–10 days in culture. This also may have been an artefact of sampling, due to non-uniform distribution of faecal matter within the matrix, however bacterial re-growth has been reported in soil:manure mixtures (Lau and Ingham 2001) and other high-nutrient environments (see Crane and Moore 1986 for a review), and this process cannot be excluded. After this period, the decline in viable bacteria became much slower, declining to levels of $1x10^0$ – $1x10^3$ MPN g^{-1} over a period of six months.

The long survival period for some bacteria in this experiment can be attributed to a combination of a low k value after the first 37 days, and the high number of bacteria in our initial inoculum. The greater the number in our starting material, the longer the time required to return to background concentrations.

Statistical comparison of treatments did not separate out any particular factors as being significant, other than time. There was a possible difference between the two farm sites, but only when two outliers were removed from the calculation.

Various mechanisms are implicated in the inactivation of faecal bacteria, including UV inactivation, bacterial competition and protozoan predation (Crane and Moore 1986; Recorbet et al. 1992). While we provided a source of UV light as a treatment, we anticipated that it would not influence the survival of *E. coli* other than immediately at the surface, and thus we deliberately sampled from within the main body of the soil:faeces matrix. Accordingly, no differences were apparent between the samples exposed to light (including UV) and those kept in the dark. Similarly no differences were apparent between the riparian and non-riparian soils. Nor did the soil moisture levels that we tested appear to provide significant differences in *E. coli* survival, and it may be suggested that potential microbial predators or competitors were not advantaged at the moisture levels tested. Regardless of the microbial inactivation mechanisms which may have operated, the soils from the two farms seemed to have similar overall inactivation rates, both for the riparian and non-riparian soils.

However neither of the riparian soils had been retired from pasture grazing for long periods (2 and 8 years). Differences may become more apparent over time, if the riparian soils alter in physical as well as microbial characteristics.

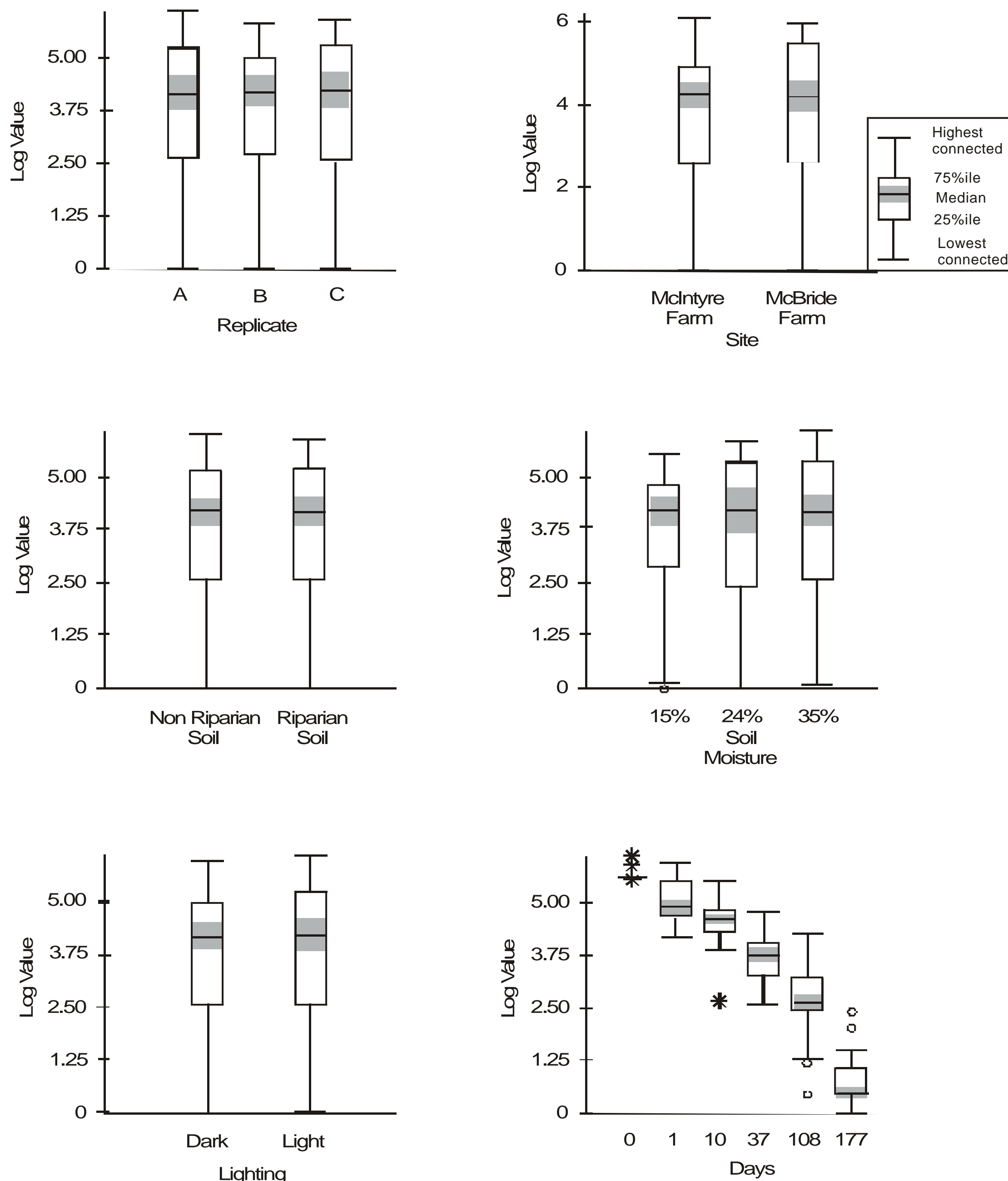

Figure 3. Box plots of log E. coli *numbers for each treatment.*

75%ile and 25%ile are the high and low hinge of each box plot. Shaded areas represent *median ±1.58(high hinge – low hinge)/Ön*. The whiskers extend to the highest data value not above the high hinge + 1.5(high hinge – low hinge), and to the lowest data value not below the low hinge – 1.5(high hinge – low hinge).

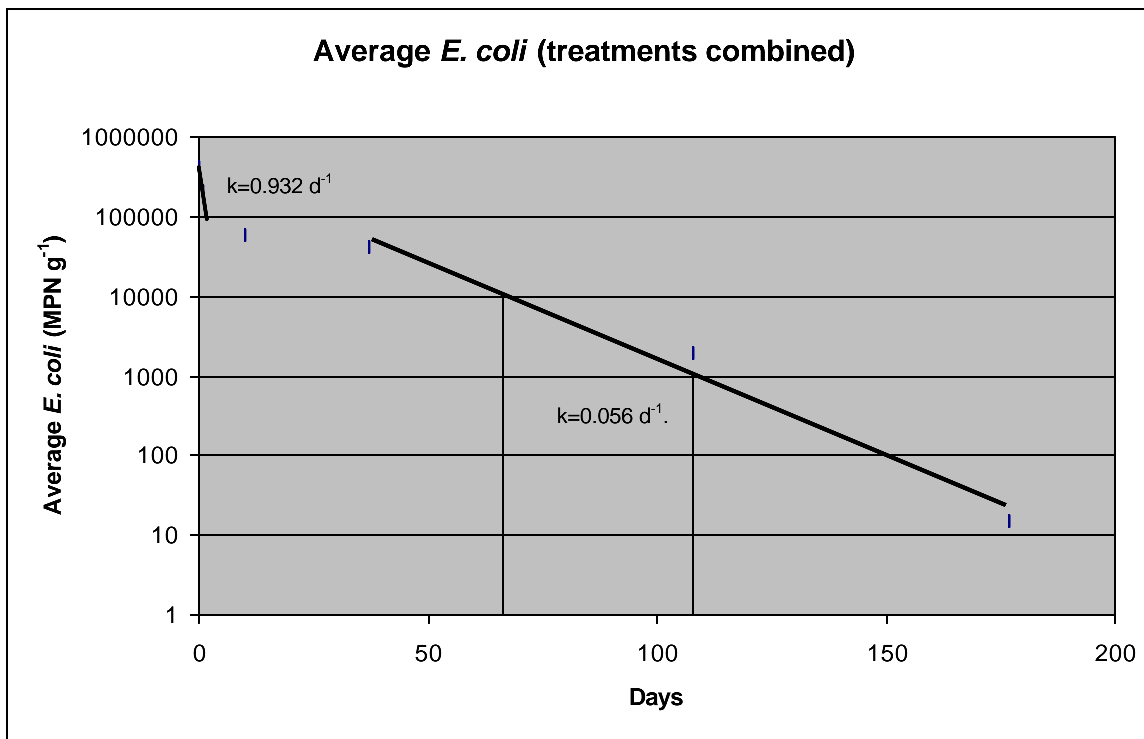

Figure 4. Average inactivation of E. coli *for all treatments combined showing initial* k *and later* k *constants.*

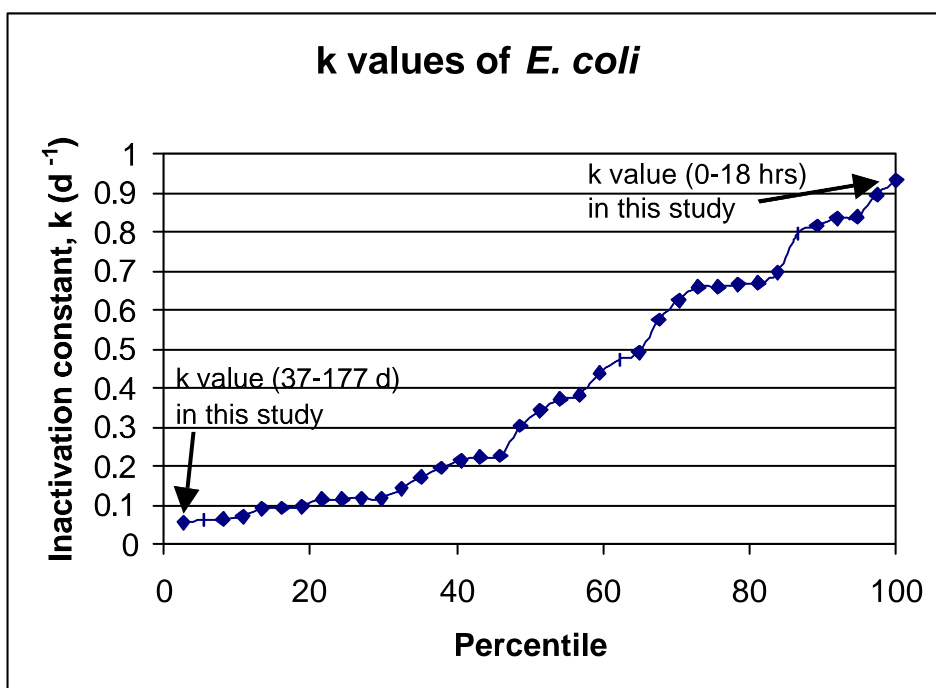

Figure 5. Values of k *for inactivation of* E. coli *in soils as reported by Crane and Moore (1985) and those measured in this study.*

As the ANOVA generally showed no difference between treatments, and just a general inactivation trend in Fig. 2, data from all treatments was combined and averaged in order to determine an initial inactivation constant (base e) of k=0.932 d^{-1} over the first 18 hrs (Fig. 4). A period of re-growth was apparent for some treatment, after which a more gradual inactivation rate was apparent, with a k = 0.056 d^{-1}. This equates to a 1 Log removal period (90% of viable bacteria removed) of 41 days. In comparison with inactivation rates of *E. coli* in soil published in a review article by Crane and Moore (1986), the *k* constants recorded in this study fall at the upper and lower extremes (see Figure 5). This however is consistent with the trend seen in the data from Crane and Moore (1986), with high k values in short term studies (7-14 days), and low k values in longer term studies (>10 weeks). This suggests either the presence of populations of *E. coli* in faecal wastes with differing ability to survive in soil environments (as suggested in Crane and Moore 1986), or different k values due to changing environmental conditions, e.g. competition for available nutrients with time of incubation.

CONCLUSION

Inactivation rates of faecal indicator bacteria, *Echerichia coli*, which had been freshly added to riparian soils retired from grazing for 2 and 8 years respectively, were not significantly different from adjacent un-retired pastures. Moisture content of the soil, and exposure to dark or simulated sunlight also revealed no differences in removal rates between treatments. Initial inactivation rates were higher than recorded in many other studies (k=0.932), but reduced to very low values after 37 days in culture (k=0.056). An average of 41 days was required for a one log reduction in numbers of *E. coli* during the latter period of the study. Some *E. coli* were still cultureable after a period of 177 days (approximately 6 months), although 17% of samples (25 out of 145) had no culturable *E. coli* on the final sampling date. Although our results suggest that the rate of bacterial inactivation in these riparian soils did not differs from that in the non-riparian soils, we cannot rule out the possibility that soil characteristics and microbial fauna unique to riparian soils may take longer to re-establish than the 2–8 years these soils have been retired from intensive dairy grazing.

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