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Flood and rainfall mobilisation of *E. coli* and faecal source tracking markers from decomposing cowpats

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The intensification of dairy farming on the agricultural landscape in New Zealand has raised concerns about pollution sources from dairy faecal runoff into waterways. The transport of faecal pollution from farms into waterways is facilitated by overland flow, which can result from rain and flood events, poorly designed irrigation practices and the washing down of milking sheds.

An important step for mitigation of pollution is the identification of the source(s) of faecal contamination. When elevated levels of faecal indicator bacteria (FIB) such as *Escherichia coli* are identified in a waterway, faecal source tracking (FST) tools such as microbial source tracking (MST) using quantitative polymerase chain reaction (qPCR), and faecal steroids (for example, cholesterol) provide information about the sources of faecal contamination. The understanding of the fate (degradation/persistence) and transport of these FST markers in the environment is recognised as an important requirement for the interpretation of water quality monitoring in aquatic environments.

This study investigated the effects of faecal decomposition on bovine faecal indicators (*E. coli* and FST markers: bovine-associated qPCR markers and ten faecal steroids) by monitoring the effect of flood and rainfall events on simulated cowpats over a five and a half month period under field conditions. Two separate spring/summer trials were conducted to evaluate: Trial 1) the mobilisation under simulated flood conditions of the faecal indicators from irrigated versus non-irrigated cowpats, Trial 2) the mobilisation of faecal indicators from non-irrigated cowpat flood runoff versus runoff after simulated rainfall onto non-irrigated cowpats.

The microbial community changes within the decomposing cowpat (as illustrated by amplicon-based metagenomic analysis) were expected to impact on the survival/persistence of the bacterial targets of the MST markers, and also alter the ratio between faecal sterols and their biodegradation products, the stanols. It was hypothesised, therefore, that there would be:

- Changes over time in the concentration of *E. coli* and the bovine-associated MST markers mobilised into the cowpat runoff

- Alterations in the FST ratio signature of the ten measured faecal steroids, resulting in a change from a bovine faecal steroid signature in fresh cowpat runoff to other animal faecal signatures in the runoff from decomposing cowpats
- A difference in the mobilisation decline rates of all FST and microbial markers within a treatment regime and between treatments.

Linear regression analysis was undertaken to establish mobilisation decline rates for each of the analytes in the mobilisable phase from the cowpat runoff treatments, with calculation of the time taken in days for reduction in 90% of the concentration (T_{90}), and statistical comparison of the regression coefficients (slopes) of all analytes. The results will include a discussion of the impacts of the study's observations on the interpretation of faecal indicator assessments for water quality monitoring in waterways influenced by sources of faecal contamination.