E. COLI INTERACTIONS WITH CLAY COLLOIDS: IMPLICATIONS FOR WATER QUALITY

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INTRODUCTION: In some areas of the UK such as South West Scotland problems with compliance with EU microbiological standards for recreational bathing waters persist. The intensive dairy farming region of Ayrshire in SW Scotland has high livestock densities, cool, moist summers and limited effective storage of livestock wastes on farms and steading areas vulnerable to direct loss by runoff of microbially contaminated water to streams. We need to know more about the sources and transport mechanisms. Microbial concentrations in streams are highest at high discharges, and this leads to assumptions that direct runoff is the main source. However it may be that resuspension of sediment retained bacteria is an important mechanism, and we need to know to what extent colloid-bacterial interactions mediate transport processes.

RESEARCH CATCHMENT

Stream samples were collected during a storm event on the Killoch Burn, downstream of Low Holehouse farm (a 55 ha farm with approximately 150 dairy cows and store cattle), within the Cessnock catchment in Ayrshire. The sampling was carried out using ISCO automatic samplers on a 1 hour fixed time interval as part of the Environmental Focus Farm project, led by SAC.

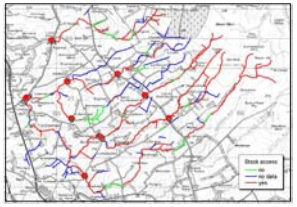

Fig 1. Cessnock catchment

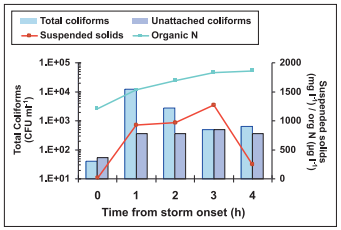

Fig. 2. During a storm event (11-08-08) coliform counts in water decreased, but the proportion of unattached coliforms increased, (see below for methods).

APPROACH/METHODS

We applied laboratory based centrifugation and a novel flow cytometry approach to elucidate the interactions between *E.coli* and pure clay suspensions, as a first step to examining interactions observed in our research catchment.

Flow cytometry uses laser diffraction to separate particles based on particle size (measured as forward scatter); particle complexity (measured as side scatter) and artificial or auto-fluorescence.

Dispersed Montmorillonite and Kaolinite were mixed (individually) with NaCl solutions (0.1- 100 mM) to give a final clay concentration of 0.05g L^{-1}. Washed cells of *E. coli* derived from overnight culture were added to each clay solution at 10^5-10^6 CFU ml^{-1} and shaken on an orbital shaker at 100 rpm for 45 minutes to allow particles and cells to interact. Three replicate runs were performed.

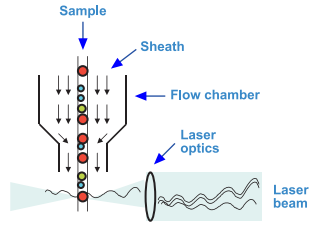

Fig 3. Flow cytometer

Particle bound and unattached cells were separated by centrifugation and culture (Chromocult ES agar) or distinguished by staining with Sytox Green for flow cytometry.

IMPACT OF ELECTROLYTES

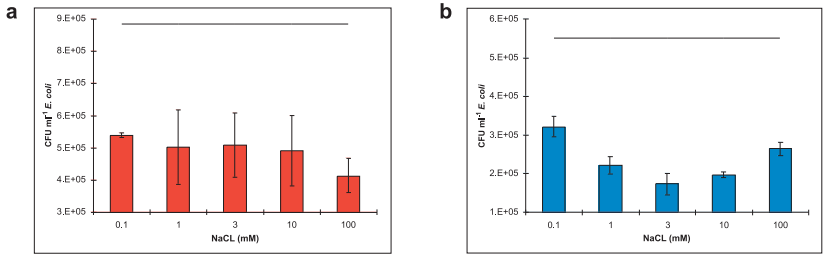

Fig 4: Effect of salt concentration on a) *E. coli* -Montmorillonite interaction and b) *E. coli* - Kaolinite interaction. Error bars show 2 x SD. Solid line represents initial concentration of *E. coli*.

Bars represent "readily transportable" unattached *E. coli* remaining in supernatant after centrifugation.

FLOW CYTOMETRY

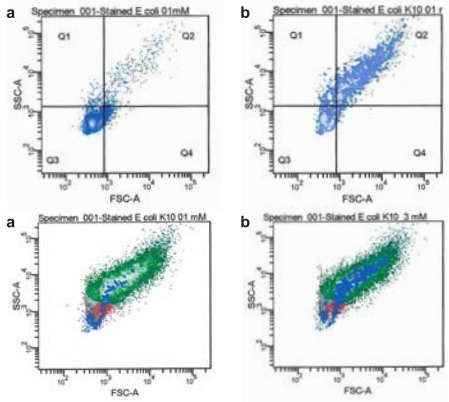

Fig 5. Particle size (forward scatter; FSC-A) and complexity (side scatter – SSC-A) of a) stained *E. coli* cells and b) stained *E. coli* cells plus Kaolinite as measured by flow cytometry.

Fig 6. Particle size (forward scatter; FSC-A) and complexity (side scatter – SSC-A) of stained *E. coli* cells mixed with Kaolinite at a) 0.1mM NaCl and b) 3mM NaCl. Blue areas represent stained cell populations.

SUMMARY

- Flow cytometry shows that stained cell populations shift towards increased particle size and complexity upon addition of Kaolinite indicating aggregation with colloids.
- Shift towards larger more complex particles with increased salt concentration.
- With Montomorillonite – fewer free cells as NaCl concentration increased, with Kaolinite – fewer free cells as NaCl concentration increased from 0.1-3 mM, but less aggregation from 10-100mM

CONCLUSIONS AND FUTURE DIRECTION:

These data demonstrate that changes in electrolyte concentrations can influence binding characteristics of bacteria to environmental colloids. Flow cytometry outputs corroborated the results from centrifugation-derived data and this shows promise as a novel method to differentiate between particle bound and unattached bacterial cells.

Studies are underway to further evaluate the use of flow cytometry for analysis of bacteria-colloid interactions and the technique will be applied to stream water samples from the Cessnock catchment.

These data will be underpinned by further storm event sampling with corresponding hydrological and water chemistry analyses to determine relationships between physico-chemical parameters and microbial binding characteristics.