



## **Transport of MS2 phages in the presence of kaolinite in gravel aquifer media**

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**Introduction:** Recent studies show that the transport velocity and concentrations of contaminants (i.e. heavy metals, radionuclides, pesticides) in heterogeneous subsurface media can be significantly increased when they are attached to mobile colloids. Whether this occurs for viruses has yet to be conclusively demonstrated experimentally. Our previous field and column experiments in heterogeneous aquifers (e.g. gravels and fractured rocks) show that MS2 phage, a viral surrogate, could travel not only faster than conservative solute tracers, but also in some cases faster than bacteria. This project tests our hypothesis that viruses hitch a ride on mobile colloids and investigates colloid-virus interactions.

**Methods:** Using kaolinite clay as the model colloid and MS2 bacteriophages as the model virus, we carried out a series of experiments using a 2 m long column packed with alluvial gravel aquifer material under various experimental conditions. Batch experiments without aquifer media were also undertaken to examine the effects of pH, dissolved organic carbon (DOC), ionic strength and the concentrations of clay and phages on virus-colloid interactions, as well as the reaction kinetics. Transmission electron microscopy (TEM), scanning electron microscopy (SEM) and zetasizer were used to visualise and characterise the colloids, phages and their interactions. A particle counter was used to measure particle concentrations. Total and filtered MS2 (through 0.22  $\mu\text{m}$  filter) samples were analyzed using the plaque count method.

**Major findings:** Without kaolinite clay, MS2 phage and Br were transported at the same speed. However, in the presence of the clay, the breakthrough of MS2 phage was significantly earlier than that of Br. The concurrent fronts of phages and clay particles and the significant difference between unfiltered and filtered MS2 phage, suggest that phages were adsorbed onto the clay and co-transported. Phages were mostly transported with the 1  $\mu\text{m}$  particles. The adsorption of the phages onto kaolinite was strong and relatively rapid in the batch tests – up to 95% of the phages can be adsorbed onto the kaolinite within 4 hours. SEM and TEM images showed that MS2 phages were adsorbed to the surfaces and edges of kaolinite particles. Phage adsorption onto clay decreased as pH and DOC increased, but increased with ionic strength and concentrations of clay and phages. Of all these parameters, pH had the greatest impact on phage adsorption onto clay, with a strong negative linear correlation ( $r^2 = 0.94$ ). In column experiments, DOC inhibited the attachment of phages onto the aquifer media, while in the batch tests, DOC prevented the adsorption of phages onto the colloidal particles. An increase in ionic strength had a much greater impact on the attachment of phages to aquifer media (thus resulting in retardation in phage transport) than it had on phage adsorption to colloids. In the batch tests, adding  $\text{CaCl}_2$  salt did not result in cationic bridging between the phages and colloids, but caused phage aggregation. TEM images of phages clearly showed that viruses tended to clump together. The large clumps were associated with the F-pilus surface structure of the host bacteria. An increase in pH or DOC caused the phages to break up, while an increase in ionic strength caused clumping. Field experiments will be carried at a site flood irrigated with sewage effluent. A colloid-facilitated contaminant transport model will be used to simulate the experimental data.