

## Viral Influence on Subsurface Biogeochemical Cycling and Heavy Metal Transport

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While it is well established that microorganisms influence geochemical cycling, the role viruses play in geochemical cycles and metal transport is poorly understood in terrestrial subsurface systems. Here, we investigated production of viruses in relationship to subsurface carbon biogeochemistry and the potential to adsorb heavy metals. Subsurface sediment slurries collected in NE were amended with <sup>13</sup>C labeled organic carbon (OC) as acetate and nitrate. Biostimulation resulted in viral production concurrent with acetate oxidation, <sup>13</sup>CO<sub>2</sub> production, and nitrate reduction. Interestingly, change in viral abundance was positively correlated to OC consumption ( $r^2=0.63$ ) and <sup>13</sup>CO<sub>2</sub> production ( $r^2=0.66$ ) whereas change in cell abundance was not implicating viral lysis of metabolically active cells. These results were not artefacts of bottle experiments. An *in situ* biostimulation experiment (O<sub>2</sub> injection) in a shallow aquifer near Rifle, CO, resulted in an increase in the virus to cell ratio (from 6.4±3.3 to 15.5±4.0) in association with variation in groundwater Eh and OC. This further supports our result that viruses are produced in response to OC or stimulation of metabolic activity. In addition to geochemical cycling, phage have the potential to adsorb and transport heavy metals. Metal adsorption experiments conducted using a model bacteriophage, *Escherichia coli* phage T4, demonstrated Zn<sup>2+</sup> adsorption. Zeta potential analysis further demonstrated the surface of phage T4 is naturally electronegative capable of adsorbing positively charged ions (cations). Interestingly, the presence of Zn<sup>2+</sup> significantly increased infectivity relative to unamended controls (ANOVA  $p<0.05$ ). As such, viruses should also be considered as biological agents influencing biogeochemical cycling and metal transport in the subsurface.