Significance of Mobile Genetic Elements for Microbial Community Adaptation to Pollutant Stress

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The overall goal of this project is to investigate the effect of mobile genetic elements and conjugal gene transfer on subsurface microbial community adaptation and biotransformation of mercury. Special emphasis is given to the contribution of the "nonculturable" fraction of the microbial communities.

Our studies examine the interaction between the fate of these metals in the subsurface and the microbial community structure and activity. Mercury-contaminated soil samples were achieved from the Lower East Fork Poplar Creek Floodplain at the FRC site. The mercury concentration was determined in the contaminated soils. The results showed that the concentrations decreased with depth. These findings were also reflected in the results of a mercury tolerance assay where the soil was exposed to increasing concentrations of mercury. The top soil showed a clear adaptation towards mercury since soil respiration only was reduced slightly even at the highest mercury concentrations (100 ppm), whereas deeper soils (at subsurface depths of 20 in. and 40 in.) only showed minor adaptation. None of the soils showed any adaptation/resistance towards chromate.

The number of CFUs resistant to mercury was determined in the mercury-contaminated and noncontaminated soils. The number of mercury-resistant bacteria was highest in the contaminated soils. Adaptation experiments were also performed, including different microbiological analyses, e.g., plate counts, respiration, and BIOLOG mt2 and EcoMicroPlate readings. In these experiments, it was found that the mercury-contaminated soils were more adapted to mercury than the noncontaminated soils. However, pre-exposure of the noncontaminated soils to mercury demonstrated that these soils also had an indigenous mercury-resistant population, although at a lower level than in the contaminated soils.

The potential detoxification of mercury by bacterial mercuric reductases (*merA* encoded) was determined in a culture-independent manner. *merA* specific PCR reactions were performed, and *merA* amplicons were detected from mercury-contaminated topsoil. We are currently performing quantitative PCR to assess *merA* occurrence in subsurface soil prior to and after $HgCl_2$ stress. We will compare the culture independent qPCR with culture-dependent methods to assess the applicability of qPCR for evaluation of mercuric reductase potential of (subsurface) soils. Finally, methods are being developed for the cultivation of the "nonculturable" mercury-resistant bacteria.