

## Microbial metabolisms in ancient fracture fluids investigated via MPNs

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Samples were collected from fracture water (FW) from the 2.4 km level at Kidd Creek Mine in Timmins, Canada, where a recent noble gas study discovered fracture waters with mean residence times on the order of 1.1 to 1.6 billion years [1]. In addition, samples were collected from service water in the mine (SW) as a control for contamination. The focus of this study was investigation of possible anaerobic metabolisms in the highly reducing fracture waters using the Most Probable Number (MPN) method. All samples were collected and kept in sterile conditions until (within 48 hours) they were added to a variety of growth media under anaerobic conditions. Once inoculations were complete, samples were monitored for two months and the MPN statistical method used to calculate cells/mL fluid [2].

There was no response for the following metabolisms: nitrogen fixation, heterotrophic methanogenesis, autotrophic methanogenesis, heterotrophic iron-reduction, autotrophic iron-reduction, alkane-oxidizing iron-reduction. In contrast, both FW and SW showed responses to all three sulphate-reducing metabolisms, although those responses were remarkably different for the FW versus the SW. There was a strong positive MPN response in both fluids for alkane-oxidizing sulphate reducers. SW also exhibited a strong MPN response for heterotrophic sulfate reducers. In contrast, in the FW, autotrophic sulfate reducers dominated by the end of the experiment, while heterotrophic sulfate reduction showed only a very small response.

Previous culture-based and non-culture based analyses showed SRBs play a key role in deep subsurface ecosystems [3]. The current study's findings are consistent with this, showing a dominance of sulfate-reducing metabolisms in the FW. Results will be discussed in the context of MPN studies from other sites on the Canadian Shield and from samples at a deeper location (2.7 km below surface) in Kidd Creek mine.

[1] Holland et al. (2013) *Nature* **497**, 357-360. [2] Pedersen (2000) *FEMS Microbio. Lett.* **185**, 9-16. [3] Lin et al. (2006) *Science* **314**, 479-482.