

# **Low Phospholipid Fatty Acid Abundances in Cobourg Formation Rock Core and Water Samples: Implications for Long-term Stability of Canada's Deep Geological Repository for Used Nuclear Fuel**

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Construction of a multi-barrier deep geological repository (DGR) has been proposed for disposal of used nuclear fuel for hundreds of thousands of years. Information on the presence and characteristics of microorganisms living within the deep geological systems is an important consideration for predicting long-term DGR stability. For instance, some microbial metabolisms, such as sulfate reduction, can pose a corrosion threat to the copper coating on fuel containers. Herein, we used phospholipid fatty acid (PLFA) based analysis to assess microbial abundances and distributions in deep surface rock core and groundwater samples from Cobourg Formation in southern Ontario, which is in proximity to one of the locations under consideration for a DGR. Only trace amounts of PLFA were detected in all samples. The abundances were near or below the quantification limits of 0.08 - 0.48 pmol PLFA g<sup>-1</sup> core or mL<sup>-1</sup> water (corresponding to 10<sup>3</sup> - 10<sup>4</sup> cells g<sup>-1</sup> or mL<sup>-1</sup>), and were similar to those in the field blanks in many cases. The total PLFA abundances in the rock cores were less than 1.5 pmol g<sup>-1</sup>, corresponding to cellular abundances of ~10<sup>4</sup> cells g<sup>-1</sup>. PLFAs in the water samples were often below field blanks, indicating minimal microorganisms. The PLFA profiles were dominated by general microbial markers, primarily saturated PLFAs like C16:0 and C18:0 (50-90% in water and 35-60% in core samples). These PLFAs are very commonly detected and are likely the most long lived in low biomass conditions where biotic PLFA turnover rate may be slow. Concurrent microscopic cell counts also showed very low abundances with 10<sup>2</sup> to 10<sup>4</sup> cells mL<sup>-1</sup> groundwater and 0-10<sup>3</sup> cells mL<sup>-1</sup> in field blanks, values similar to the PLFA quantification limits. Parallel 16S rRNA gene quantification by digital PCR showed less than 10 copies mL<sup>-1</sup> in most groundwater samples as well as field blanks. Only six near-surface groundwater samples showed abundances of 10<sup>2</sup> to 10<sup>3</sup> copies mL<sup>-1</sup>. These initial results enhance our understanding of deep surface microbial communities and suggest very low microbial abundances in the Cobourg Formation subsurface, supporting a future safety case for a proposed DGR in such a host rock system.