

Application of Stable Isotopic and Omics Methods for Assessment of RDX Natural Attenuation in Groundwater

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Background/Objectives. Production and use of explosives has resulted in contamination of soil and groundwater. One of these compounds, RDX, is persistent under most conditions, although natural attenuation mechanisms have been proposed. The goal of this study was to apply traditional and innovative technologies to determine if evidence for natural attenuation of RDX in a large groundwater plume could be demonstrated.

Approach/Activities. Groundwater was collected from multiple wells within transects throughout the RDX plume positioned parallel to the general groundwater flow direction. Samples were analyzed for RDX, known RDX breakdown products, as well as a suite of geochemical parameters. RDX was extracted, concentrated and subjected to compound specific isotope analysis (CSIA) to detect any nitrogen isotope fractionation that could be indicative of biotic or abiotic RDX degradation. Groundwater biomass was extracted for metagenomic analyses to determine if known RDX degrading microbial strains were present, which was followed by proteomic analysis to detect known RDX degradative proteins. Finally, samples of groundwater were used to inoculate enrichments with and without low concentrations of labile carbon to determine the aerobic RDX biodegradation capacity of the microbial community.

Results/Lessons Learned. Groundwater within the plume transects was generally aerobic and oligotrophic. RDX (up to 1700 µg/L) and the RDX degradation product 4-nitro-2,4-diazabutanal (NDAB, up to 150 µg/L) were detected in a large portion of the plume, and lower concentrations of the nitroso-containing metabolites from anaerobic RDX biodegradation were also detected in some areas. Fractionation of nitrogen in RDX measured using CSIA was consistent with RDX degradation via both aerobic and anaerobic biodegradation and alkaline hydrolysis. Combined with previously published isotope enrichment factors for the various degradation process, as well as the available data on groundwater flow velocities, the half-life of RDX due only to natural attenuation was estimated to range from 6 to 55 years depending on the assumed attenuation mechanism. The indigenous groundwater microbial community included bacterial strains known to aerobically degrade RDX (and NDAB), and to degrade RDX under anoxic conditions. Additionally, proteomic analysis of groundwater biomass indicated the presence of both the aerobic RDX degradative enzyme XplA, and the anoxic RDX degradative enzyme XenB. Enrichment cultures derived from groundwater samples exhibited the potential to aerobically degrade RDX and produce NDAB, but only when low concentrations of utilizable carbon were added. These results provide multiple lines of evidence for historic and/or ongoing natural attenuation of RDX, and demonstrate the utility of including of isotopic and –omics technologies when assessing monitored natural attenuation.