## A Seasonal Comparison of the Passive Abandoned Coal Mine Remediation System at Wingfield Pines<sup>1</sup>

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Abandon mine drainage (AMD) in Pennsylvania results in a significant amount of mine pollution entering local watersheds. One solution used to lower the contaminant levels is passive remediation in which contaminants are removed through a series of settling ponds and a wetland. Wingfield Pines, in Bridgeville, PA, contains a passive remediation system that was constructed in 2009. It consists of an aeration pond, four settling ponds and wetlands before water is diverted back into Chartier's Creek, which is part of the Ohio River watershed.

Mixed water and soil samples were taken from the beginning of each pond, the end of the remediation system and just before the water flows back into Chartier's Creek in April 2015, July 2015, October 2015, and January 2016. The samples were centrifuged and separated, and the water was sent to an independent lab for water chemistry analysis of aluminum, barium, copper, lead, zinc, manganese, iron, strontium, nickel, arsenic, cadmium, and selenium levels by ICP-AES and sulfate levels by IC. DNA was extracted from the soil samples and was tagged with a unique 16S rRNA Illumina PCR tag (http://www.earthmicrobiome.org). The amplified 16S *rRNA* fragments of the chromosomal DNA were analyzed by high-throughput sequencing using MiSeq. The data (~2.7 million sequences) was quality filtered and analyzed using python and Qiime programs (Caporaso, 2010). The data show a variety of differences including spikes in different metal levels (1 site in summer, a different site in fall) as well as the type and relative abundance of bacteria communities present. Proteobacteria were most prevalent year round, though prevalence at the Class level varied. Cyanobacteria were only high in the summer. The bacterial communities at the beginning of the remediation change dramatically throughout the year, while the end of the remediation remains fairly constant. The results suggest that the remediation effectiveness and bacterial richness at Wingfield Pines changes, depending on the time of year and contaminate levels.

Additional Key Words: AMD Passive Treatment; Bacterial AMD communities; microbiome analysis; water quality analysis; Wingfield Pines; alkaline mine drainage

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