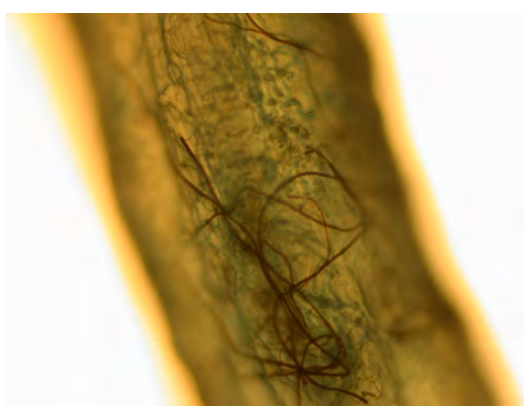
Chapter 7

Biota of the Lehigh Gap Wildlife Refuge – Microorganisms



Mycorrhizae in a Plant Root

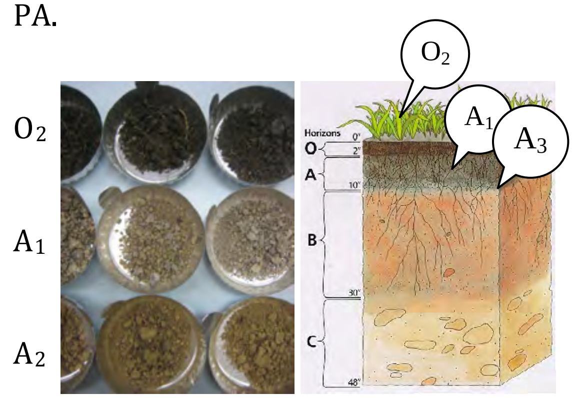
LGWR Biota – Microorganisms

Disturbance of native plant communities, such as what occurred from decades of zinc smelting, is often followed by degradation of both physical and biological soil properties, soil structure, nutrient availability and organic matter. At the LGWR, severe erosion took place leaving behind nothing except mineral soil and bare rock. This damage, along with the loss of vegetation, was obvious. However, the damage to the soil microbial communities is also important to consider. High concentrations of heavy metals have shown adverse effects on soil microbial populations, so it is of interest to study various microorganism communities at the LGWR and throughout the larger Palmerton Superfund site area.

Soil Bacteria

A survey of soil microflora was conducted 32 years ago by Marilyn J. Jordan (formerly known as M.J. Buchauer) and Mary Lechevalier (1975) of Rutgers University's Waksman Institute of Microbiology. They concluded that the heaviest of zinc-contaminated soils experienced the greatest loss in total numbers of bacteria, fungi and actinomycetes. In 2007, Armando Villafañe, Jr. and Dr. Frank Kuserk of Moravian College proposed a study to determine what changes microbial populations and communities have undergone since the 1975 study. A full write-up of this study entitled ***"Current State of the Soil Microflora at the Palmerton, Pa Superfund Site"*** is included as Appendix E-1. This research was accepted for presentation at the 2008 National Conferences on Undergraduate Research held at Salisbury University in Maryland. Villafañe was able to communicate with M. Jordan to determine the sampling sites and methods from the earlier study. Dr. Hank Edenborn of the National Technology Energy Laboratory of the U.S. Department of Energy in Pittsburgh performed the soil metal analyses.

In the previous study Jordan and Lechevalier recorded up to 129,000 ppm of zinc (Zn), 1800 ppm of cadmium (Cd), 2150 ppm of copper (Cu) and 1900 ppm of lead (Pb) in the O_2 soil horizon of the most affected site. Current (2007) metal concentration in the O_2 horizon at this site have decreased significantly with measurements of 4348 ppm of Zn, 68 ppm of Cd, 177 ppm of Cu and 649 ppm of Pb being recorded. Heavy metal concentrations have also significantly declined in the A_1, and A_3 soil horizons within 2 km of the east-plant zinc (Zn) smelter in Palmerton, PA.



Soil Samples Collected from 3 Layers

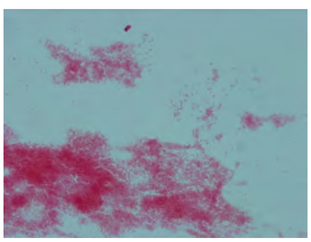
While these metal levels are still considered higher than normal, microbial populations have demonstrated some ability to recover. When compared to the microbial population counts in 1975, total numbers in soil microflora (bacteria and fungi) populations in 2007 were notably higher, particularly in the O_2 horizon of the most affected sites (*S1 and S2*). A strain of *Alcaligens eutrophus*, a bacteria classified by its ability to demonstrate plasmid-bound resistance to Co^{2+}, Ni^{2+}, Zn^{2+} and Cd^{2+} ions, was isolated from *S1* and *S2* soils. The increase in soil microflora (bacteria and fungi) populations at *S1* and *S2* (those sites most severely impacted by smelting) over the last 32 years correlate with reduced metal contamination of these soils and confirms early stages of soil formation at these sites.

As a follow-up to the Villafane, *et al.* study, Vivian Clarke-Ruiz, under the supervision of Dr. Kuserk, conducted a study entitled ***"Evidence for Zinc Tolerance Among Bacteria of the Palmerton, PA Area"***. The entire report for this study is included as Appendix E-2. The project was selected for presentation at the 2009 National Conferences on Undergraduate Research held at the University of Wisconsin, LaCrosse and the poster that was presented is included as Appendix E-3.

This research repeated some of the 2007 field sampling to verify that bacterial numbers at affected sites had indeed recovered from 1975 levels and looked at zinc tolerance among bacteria isolated from soils at the re-sampled sites. Bacteria isolated and identified from soils at all three sites were found to be common soil bacteria, including species from the genera *Staphlococcus, Arhtrobacter, Psudomonas* and *Rahnella.*

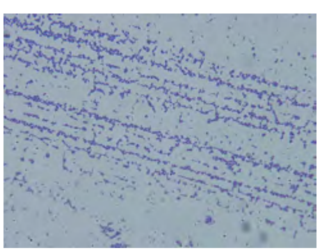
A third study from the Kuserk lab at Moravian College was done by Nicole Sarson to further identify bacteria in the metal contaminated soils. This study entitled ***"Identification of Bacteria found in Metal Contaminated Soils near Palmerton, PA"*** is included as Appendix E-4. In the previous studies, students isolated twenty bacteria samples from contaminated soils near the former smelters. The purpose of this study was to identify as many of the bacteria samples as possible. By utilizing the Biolog® Microbial Identification System, six of the twenty isolates were positively identified. The remaining isolates either were not included in the Biolog® database or represent unidentified species or strains of soil bacteria. The identified bacteria included *Leifsonia aquatic*, *Rahnella aquatilis*, *Corynebacterium*, *Curtobacterium citreum*, and *Pseudomonas fluorescens biotype F*.

Pseudomonas fluorescens biotype F is a gram-negative, rod-shaped, catalase-positive, motile bacterial species. It is found in soil and survives best in a finer textured soil as compared to a coarser soil. *Rahnella aquatilis* (see image below) is also a gram-negative, rod shaped, nitrogen-fixing, motile bacterial species. It utilizes diverse carbon sources for its growth and can be found in both water and soil samples.



Rahnella aquatilis

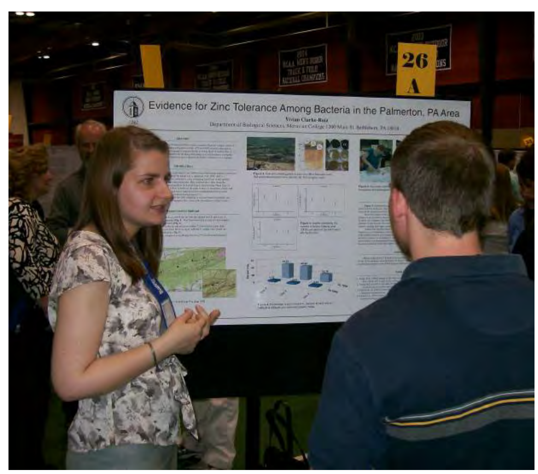
Corynebacterium is the genus within the broader "coryneform bacteria group" for which the most species have been described to date. *Curtobacterium citreum* (see image below) is a gram-positive, coryneform soil bacterial species.



Curtobacterium citreum

Leifsonia aquatica is a gram- positive, rod shaped, non-motile bacterial species found only in aerobic conditions. This species was first identified as *Corynebacterium aquaticum* by Leifson (1962), but the genus name was later changed to *Leifsonia*. He first extracted this species from water samples, but it can also be found in soil.

To better evaluate the microbial communities at the sample sites and hence obtain a better picture of community differences, the use of a community-level physiological profiling (CLPP) technique is recommended for future studies in place of, or in addition to, the MicroLog™ Microbial Identification System. The CLPP method allows for the examination of community metabolism over a week's time, thus creating a profile of the entire bacterial community rather than just the identification of individual members that are randomly isolated from the soil.



Vivian Clarke-Ruiz presenting her research at Moravian College

Arbuscular Mycorrhizal Fungi

Arbuscular Mycorrhizal Fungi (AMF) is a type of mycorrhizae that penetrates the cortical cells of the roots of vascular plants. It is an obligate symbiont that can help plants take up nutrients such as phosphorus and various soil micronutrients. They are of interest at the LGWR because they are frequently found in plants growing on mineral soils (the incidence of their colonization actually is lower in nutrient-rich soils) and they are commonly found in temperate grasslands. An absence of mycorrhizal fungi can also slow plant growth in early succession or on degraded landscapes. Furthermore, the use of AMF in ecological restoration projects has been shown to enable host plant establishment on degraded soil and to improve soil quality and health.[1] In studies in which soil was inoculated with AMF during reintroduction of vegetation, it was demonstrated that a significantly greater long-term improvement in soil quality parameters was attained resulting in increased plant growth and soil nitrogen content and higher soil organic matter content.

Brenda Casper's lab at the University of Pennsylvania is interested in investigating the diversity and function of arbuscular mycorrhizal fungi (AMF) across the metal contamination gradient on Blue Mountain. AMF form mutually beneficial associations with plant roots; in exchange for carbon, they facilitate plant uptake of soil resources, especially phosphorus, which is not very mobile within the soil. However, because of fungi's carbon demand, AMF can also act as parasites under conditions of high soil nutrients. The relationship between AMF and plants in heavy metal contaminated soils is not clear. AMF might prove detrimental if they increase uptake of metals, but on the other hand, they might improve the host's overall wellbeing in a way that enables the plant to better cope with heavy metals.

One study done by Jennifer Doherty involved the examination of the composition of the AMF spore communities across the contamination gradient and the degree that roots of the common cool season grasses are colonized by these fungi. Morphological characteristics of AMF spores, which are produced underground, have been traditionally used to identify species. Abundant spores were found across the contamination gradient but much lower species diversity than is typical of non-polluted grasslands. The relative abundance of the AMF species differs between soils with high and low levels of metal contamination, suggesting that some species are better able to tolerate metals than others. For the cool season grasses *Deschampsia flexuosa* and *Danthonia spicata*, root colonization rates by putative root pathogens (4 %) was greater than colonization rates by AMF (2-3 %), which suggests these grasses are not highly AMF

[1] Jeffries, P.,Gianinazzi, S., Perotto, S., Turnau, K., Barea, J. (2003). The Contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils* **37**: 1–16.

dependent. A greenhouse experiment with *Da. spicata* in the metal-contaminated soils did show that plant growth is slightly improved by the presence of AMF.

Sydney Glassman, a graduate student, focused on the individual species of AMF. She conducted greenhouse experiments to determine if some AMF species are more tolerant of high levels of metal contamination than others, whether some species are more helpful to their plant hosts, and whether ecotypic variation is evident in the fungi. That is, does the same AMF species taken from soils at the high and low ends of the contamination gradient show functional differences? AMF reproduce asexually, so ecotypic variation is not common, but the heavy metal contamination should prove a strong selective force that could generate such differences. Glassman also oversaw a high school student's project with the warm season grasses that were seeded on the mountain. From other research with *Andropogon gerardii*, *Sorghastrum nutans*, and *Schizachyrium scoparium*, it is known these species are highly dependent on AMF, and it is of interest to investigate the role of AMF in the successful establishment of these grasses on Blue Mountain. The plan is to determine the species of AMF associating with these plants in the contaminated soils and percentage of root colonized by AMF fungi and to compare the results with similar data from a serpentine grassland, where these grasses are naturally abundant. A presentation of this research entitled ***"A context-dependent party for three: AMF, non-mycorrhizal soil microbes, and plants in a pollution gradient"*** was given at the August 2010 Ecological Society of America Annual Meeting in Pittsburgh, PA and a summary is included as Appendix E-5.

Other mycorrhizae studies

Researchers from West Virginia University led by Drs. Jonathan Cummings and Dorothy Vesper are interested in a number of questions related to mycorrhizae, metal tolerance and stress responses in poplar trees, and poplar-rhizosphere responses to heavy metals. (The rhizosphere is a narrow region of soil that is directly influenced by root secretions and the associated soil microorganisms.) They have been studying zinc hyper-tolerance in eight hybrid species of popular genotypes and how native fungal species that form symbiotic relationships with poplar root systems can impact metal stress in these trees. They are interested in mycorrhizae from metal-contaminated sites and thus, they isolated soil samples from the LGWR. Their research involves a) analyzing the extractability of zinc and cadmium from the soils; b) how soil-metal interactions are impacted by the presence of mycorrhizae and c) examining a number of zinc-tolerance (biochemical) mechanisms in poplars. Such information could be useful in better understanding the heavy metal stress responses seen in early successional trees at the Refuge and in determining what measures might be important in re-establishing forests in some areas of the Palmerton Superfund Site.