Ecological Interactions Between Metals and Microbes That Impact Bioremediation

Allan Konopka,[1] Cindy Nakatsu[2] and Ronald F. Turco[2]
[1]Department of Biological Science and [2]Department of Agronomy, Perdue University, West Lafayette, Ind.

The project's objectives are to:

- develop an ecological understanding of the interactions among heavy metals (lead and chromium), the physico-chemical environment and microbes capable of remediating organic pollutants to supplant the current empirical approach;
- use microbial community diversity and heavy metal tolerance to determine the load of "bioavailable" metal in contaminated sites;
- use experimental microcosms to test ecological conclusions derived from analysis of waste sites. The purpose of these experiments is to optimize organic bioremediation and provide feedback to field site remediators.

Potential limiting factors (heavy metal concentration vs. available organic C) for microbial biomass were tested in soils from three contaminated sites. Soil contamination consisted of either (a) Cr alone (UP site), (b) Pb alone (Avanti site) or (c) Pb, Cr and petroleum (Seymour site). In each case tested, the addition of degradable organic matter caused increases in microbial biomass (phospholipid-P) and microbial respiration rates. We interpret these results to suggest that carbon availability rather than heavy metal toxicity controls microbial activity in these habitats.

The tolerance of microbial communities to heavy metals has been tested in two ways. With the first method, culturable bacteria were recovered and the minimum inhibitory concentrations of metal for growth of individual isolates were determined. A majority of the culturable bacteria in all of the habitats appeared to be sensitive to the toxic metal. However, significant populations of resistant bacteria were also found. In the case of Pb, resistant strains could grow up to 25–70 µM Pb^{++}. In the case of Cr, bacteria resistant up to 50 mM $CrO4^{-2}$ were isolated. The second method involved measuring ^{3}H-leucine incorporation into bacteria extracted directly from soil particles. The functional response of the microbial population was modeled as $Y= 100 X I/(IC_{50} + I)$, where Y is the percentage of microbial activity (relative to the sample with no added metal), and I is the concentration of added metal. The IC_{50} values for lead were in the low (<10) µM range for all soils tested, whereas $Cr_2O_4^{-2}$ concentrations in the low millimolar (< 5) range were necessary to reduce microbial activity by 50%.

We investigated the correlation between the microbial communities and levels of lead, chromium and various organic contaminants present along a 21.3 m transect at a mixed waste contaminated site (Seymour, Ind). Soil chemical analysis showed that total concentrations of xylenes, methylene chloride, toluene, lead and chromium ranged from high to low along the transect. For community analysis soil microbial DNA was extracted in triplicate from a total of 24 locations along the transect. Denaturing gradient gel electrophoresis (DGGE) of PCR amplified 16S rDNA was used to determine bacterial community structure for each sample location. The DGGE patterns indicated that the number of populations at this contaminated site was reduced compared to those typically observed in bulk agricultural soils. Comparison of DGGE community fingerprints using similarity coefficients showed they fell into three groups. The three groups correlated with the concentrations of organic contaminants and not metal concentrations (not totally confirmed, need more organic data). A greater number of high G+C bacteria (indicated by band migration by DGGE) were found in locations with high organic concentrations (e.g., 8,200 ppm methylene chloride, 12,820 ppm toluene, 2,030 ppm xylene). This may indicate populations specifically involved in degradation of high concentrations of these compounds.