A PROPOSED BIOLOGICAL SURVEY OF THE BERKELEY PIT LAKE SYSTEM

G.G. Mitman
Department of Biological Sciences, Montana Tech of the University of Montana, Butte, MT

ABSTRACT

Very little is known about the organisms that are impacted by mine waste in the Berkeley Pit Lake System. However, it is known that if heterotrophic and autotrophic organisms are properly nutrified, they can bioremediate mine waste-influenced areas as a benefit of their physiological processes. However, before any type of bioremediation of an ecosystem can begin, it is essential to gain a fundamental understanding of the components of the microbial community. Defining the baseline community structure is the first step toward understanding the interaction of the different biota and toward assessing any improvement in biodiversity within the biotic community. This research will undertake this first step.

The specific research goals are primarily to help to set up a laboratory capable of the identification, isolation, and culture of mine waste organisms; to maintain the isolates in culture; to produce a photographic and written record of these organisms; to determine numerical abundance and species diversity of the site; to determine the organisms' ability to sequester metals; to determine the organisms' capability to raise pH as a result of nutrification; and to collect useful information that may be applied to other mine waste areas. All of this information together will begin to provide an understanding of the interactions among acid mine environments and the organisms that dwell there. Moreover, Butte is an ideal start-up location for these research activities because of its 100-year tradition of mining. In fact, Butte is at the headwaters of the largest Superfund cleanup project in America. Funding of this proposal request may well be a significant opportunity to invest in the future of bioremediation.

Key words: *shoot-root partitioning, zinc, lead, cadmium, phytoremediation*

INTRODUCTION

Very little is known about the organisms that are found in the Berkeley Pit Lake System. Extensive reviews have been written regarding the effects of inorganic stress on algae (Genter, 1996; Rai et al., 1981; and Whitton, 1984), with ever increasing information becoming available about the diversity of protistans, fungi and bacteria that inhabit mine waste sites. Neighboring states are also undertaking research in this area. Consequently, this area of research is quite intriguing because numerous organisms are clearly growing in water as low as pH 2 loaded with high accumulations of metals. What is most significant is that these extremophiles may be potential organisms to bioremediate contaminated sites. Therefore, this proposal requests funds to investigate the species diversity, numerical importance, and potential role of the Berkeley Pit Lake System organisms as possible solutions in bioremediation.

BACKGROUND

The Berkeley Pit Lake System is one of the largest contaminated sites in North America and is located near the headwaters of the largest Superfund site in the U.S. The pit lake is 542 m deep with a lateral extent of approximately 1.8 km by 1.4 km across the rim. The only larger pit mine in the U.S. is the Bingham Pit in Salt Lake City, Utah (Davis, 1989). The chief difference between the two pits is that the Berkeley Pit has a water depth of approximately 266 m, which is rising at a rate of about 8 m/year. Consequently, this represents roughly 1140 billion liters of pH 2.7, metalladen, contaminated water and has been designated a Superfund project for cleanup. This emphasizes how important this research may be to the state of Montana, and to the U.S., but there are even more contaminated, larger sites throughout the world. It is the goal of this seed grant research to begin to gain an understanding of the microbial ecology of the Berkeley Pit Lake System, which will ultimately provide necessary data for bioremediation studies and may apply to other contaminated locales worldwide.

Except for a single sample obtained in June of 1996 (Robins et al., 1996), virtually nothing is known about the microbial ecology of the Berkeley Pit Lake System. The site of this research is one of the few laboratories studying the algae in these acid mine waste areas in Montana. According to Kelly and Whitton (1989): "There have been few studies to relate metal accumulation and toxicity in aquatic plants." However, one extensive study by Sheath et al., (1982) provides information gathered from a three-year study of acidic and metal-influenced tundra ponds in the Northwest Territories. Two species of algae, Euglena mutabilis Schmitz and Chlamydomonas acidiophila Negoro, were the only ones to have been identified at the research laboratory from Berkeley Pit samples so far. However, many other algae, protozoans, fungi, and bacterial species were also observed and remain to be identified. When the Berkeley Pit water was examined, it was literally teeming with life.

This phenomenon is hardly surprising, given the opportunistic nature of microbes. Although conditions within the Pit Lake System are toxic for normal aquatic biota, these same conditions represent an ideal environment for extremophiles which can benefit from lack of competition in this unique habitat. Furthermore, this hostile environment may accomodate new species never before observed. Thus, the research potential of this site is tremendous, primarily because it is located in a geographic area characterized by years of mining, milling, and smelting waste.

The primary goals of this study are both to determine species diversity and numbers for organisms present in these mine waste areas and to determine their potential ecological role in the system for bioremediation. Various beneficial processes occur because of algal and photosynthetic bacterial growth in aquatic habitats. These processes are important because they may affect the chemistry in a number of ways:

- These organisms are primary producers and, as a result of their physiology, naturally produce bicarbonate to raise the pH of acidic solutions in which they are growing.
- As algae grow, they leak excess photosynthates, that in turn promote bacterial growth.
- These microbes play a role in the biological magnification of toxic materials (each trophic level of the food web will increase the concentration of many metals 10x).
- Photoautotrophs oxygenate the water that will promote aerobic activity.
- Photoautotrophs are important in biogeochemical cycling of C, N, P, S, and other elements—most importantly nitrogen fixation and sulfate reduction.

- Algal cells may directly sorb metal ions through several mechanisms that include ion exchange, complexation, and physisorption.
- Accumulation and eventual decomposition of algal biomass will increase the organic carbon component of the mine waste systems which, in turn, will promote heterotrophic growth of bacteria, fungi, and protozoans.
- Algal biotransformation or enzyme-catalyzed conversion of metals will result in less toxic organic compounds.

Heterotrophic bacteria, fungi, and protists are also important because they too play key roles in microbial ecology:

- These organisms naturally raise the pH of the acidic solutions in which they are growing by various physiological processes.
- They are major consumers and decomposers in the food web.
- These microbes play a role in the biological magnification of toxic materials (each trophic level of the food web will increase the concentration of many metals 10x).
- Heterotrophic microbes are important in biogeochemical cycling of C, N, P, S, and other elements—most importantly in nitrogen fixation and sulfate reduction.
- Some microbes have metalothionines—detoxifying enzymes that may have a synergistic effect on the microbial community.
- Biomass accumulation and eventual decomposition will not only increase the organic carbon component of mine waste systems, but will also promote heterotrophic growth of other bacteria, fungi, and protozoans.
- Heterotrophic microbes will release bound nutrients during decomposition–the microbial loop model (Azam et al., 1983).

These combined physiological processes of microbiota have been observed to bioremediate aquatic mine waste environments (Cai et al., 1995). Consequently, if a mine waste site, like the Berkeley Pit Lake System, is properly nutrified with nitrogen, phosphorous, or potassium (eg., aged manure or sewage as inexpensive sources), then this nutrification may cause a successional cascade of increased diversity and biomass that is coupled with an increase in pH. A pH increase, in turn, may lead to a natural restoration process. Thus, if systems are to function correctly and to recover from pollution-induced perturbations, fundamental information both on the autotrophic and heterotrophic components of the microbial community is essential. Defining the baseline community structure is the first step not only toward understanding the interactions of the different groups of organisms, but also toward assessing any improvement in biodiversity within the biotic community. This first step will be taken through this proposed seed grant research.

GOALS AND OBJECTIVES

This project has two principal goals: 1) to determine the baseline community structure of the Berkeley Pit Lake; and 2) to evaluate the isolated species for possible use in bioremdiation. These two goals are necessary both to gain a fundamental understanding of the microbial ecology of acid mine waste areas and to evaluate the potential of these organisms as bioremediators.

The following specific objectives are proposed for this research:

1. to set up a lab capable of identification, isolation, and culture of mine waste organisms;
2. to maintain a culture collection of isolated organisms for possible future use in bioremediation;
3. to produce a photographic atlas and identification manual for these species;
4. to begin a field study of the organisms (numerical abundance and species diversity) present over the course of a year;
5. to determine these organisms' capability to sequester metals by electron microscopy;
6. to determine these organisms' capability to raise pH as a result of nutrification by monitoring cultures;
7. To compile information that may be applied to other mine waste areas in the state or elsewhere;
8. to provide the baseline research that will lead to a larger, nationally competitive project; and
9. to publish results.

EXPERIMENTAL PROCEDURE

Surface water samples will be collected in triplicate for phytoplankton enumeration at randomized sites from the edge to the center of the Berkeley Pit Lake System. A Surface Plankton Net (10µm mesh) and a Thin Layer Water Sampler (TLWS) (Aquatic Research Instruments) will be used. Subsamples will be fixed in Lugol's Solution or 2.5% calcium-carbonate buffered glutaraldehyde and settled for enumeration. These samples will be examined with a Nikon SK-2 inverted microscope according to the method of Utermöhl (1958). Subsurface samples will be collected from the photic and aphotic regions of the pit from the limnetic to the profundal zones by using the TLWS. Samples from as many depths as possible will be examined. Profundal sediment samples will be examined by using a slide hammer coring device. Subsamples at different core depths will also be examined from these cores. Frequencies of sampling will be determined by the accessibility to the Berkeley Pit Lake System. However, sampling is expected to take place at least once per month. As a part of the sampling process, measurements of pH, Eh, of DO, light ($\mu mol \cdot m^{-2} \cdot s^{-1}$), temperature, and the chemical (metal) composition of the water will be made simultaneously with biological sampling.

Isolated organisms will be examined live, placed in culture, and processed for microscopy as described above. In addition, samples will be fixed for scanning electron microscopy by fixation in 3% glutaraldehyde, dehydration in an ethanol series, and critical point dried and gold-palladium coated (20 nm). Accordingly, material for transmission electron microscopy and light microscopy will be fixed in 3% glutaraldehyde in a 0.1M cacodylate buffer at pH 7.0. The sample will then be split and one half will be stained with 1% OsO_4 in a 0.1M cocodylate buffer. Then both samples will be embedded in Spurr's embedding medium. Both these methods may be modified as necessary and a variety of staining techniques (including immunofluorescence) will be used as appropriate. Finally, it may also be necessary to examine non-embedded material as well, but this should always be available from the culture collection.

Qualitative analysis of samples will use enrichment methods to measure the diversity of the biota in the samples. All isolated organisms will be identified to the most specific taxon possible. Identification will be done by using classical light microscopy (Nikon Eclipse E800-Fluorescence and Normarski). Samples will also be sent to colleagues for verification and/or identification as necessary. (Previously undescribed species or unusual genetic isolates are expected to be found during this study.) Therefore, all isolates will be kept in the permanent culture collection in the department of biological sciences at Montana Tech for further investigations.

As a part of this research, detailed descriptions of all organisms discovered in the Berkeley Pit Lake System will be recorded and will include a photographic atlas and a culture collection. Electron microscopy (scanning and transmission) will also be used to identify species and to determine if any of these isolated species are able to sequester specific metal ions. Furthermore, isolated species will also be evaluated during the culture process to determine both the rate of biomass production and the change in pH of the culture medium (both of enriched field samples and of individual species). Electron microscopy, biomass production, and pH are three factors that will be used to evaluate these microorganisms as potential bioremediators. Collectively, this information will prove to be valuable for future research on acid mine waste areas.

ACKNOWLEDGMENT

This research was funded through the Mine Waste Technology Program at Montana Tech of the University of Montana through EPA/DOE, and implemented by MSE Inc.

REFERENCES

Azam, F, T. Fenchel, J.G. Field, J.S. Gray, L.A. Meyer-Reil, and F. Thingstad, 1983. The ecological role of water-column microbes in the sea. Mar. Ecol. : Prog. Ser. 10, 257-63.

Cai, X.H., S.J. Traina, T.J. Logan, T. Gustafson, and R.T. Sayre, Applications of eukaryoticalgae for the removal of heavy metals from water. Mol. Mar. Biol. Biotechnol. 4(4): 338-344.

Davis, A. and D. Ashenberg, 1989. The Aqueous geochemistry of the Berkeley Pit, Butte, Montana, U.S.A. Applied Geochemistry. 4: 23-26.

Genter, R.B. 1996. In *Algal Ecology Freshwater Benthic Ecosystems* (ed. Stevenson, R.J., Bothwell, M.L., and Lowe, R.L.). Academic Press. New York.

Kelly, M.G. and B.A. Whitton, 1989. Relationship between accumulation and toxicity of zinc in Stigeoclonium (Chaetophorales, Chlorophyta). Phycologia. 28(4): 512-17.

Rai, L.C., J.P. Gaur, and H.N. Kumar, 1981. Phycology and heavy metal pollution. Biol. Rev. Cambridge Philos. Soc. 56: 99-151.

Robins, R.G., R.B. Berg, D.K. Dysinger, T.E. Duaime, J.J. Metesh, F.E. Diebold, L.G. Twidwell, G.G. Mitman, W.H. Chatham, H.H. Huang, and C.A. Young, 1996. Chemical, Physical and Biological interaction at the Berkeley Pit, Butte Montana. Tailings and Mine Waste 97 Conf. Colorado State University, Fort Collins, CO.

Sheath, R.G., M. Havas, J.A. Hellebust, and T.C. Hutchinson, 1982. Effects of long-term natural acidification on the algal communities of Tundra Ponds at the Smoking Hills, N.W.T., Canada. Canadian Journal of Botany. 60: 58-72.

Whitton, B. A. 1984. Algae as monitors ofheavy metals in freshwaters. In *Algae as Ecological Indicators* (L. E. Shubert, ed.). Academic Press. New York.

Utermöhl, H. 1958. Zur Vervollkommung der quantitaven Phytoplankton-Methodik. Int. Ver. Theor. Angew. Limnol. Verh. 9: 1-39.