

Montana Water Resources Research Center

Annual Technical Report

FY 1998

Introduction

Research Program

Basic Project Information

Basic Project Information	
Category	Data
Title	Influence of Organic Mine Reclamation Amendments on Metal Mobility and Water Pollution Potential
Project Number	C-01
Start Date	09/01/1998
End Date	09/30/2000
Research Category	Water Quality
Focus Category #1	Solute Transport
Focus Category #2	Acid Deposition
Focus Category #3	Geomorphological and Geochemical Processes
Lead Institution	University of Montana

Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Tom H. DeLuca	Assistant Professor	University of Montana	01
Donald J. Bedunah	Professor	University of Montana	02
Aimee Vitateau	Student	University of Montana	03

Problem and Research Objectives

PROBLEM - Regional

There are over eight thousand abandoned mines in western Montana alone and thousands more across the North Central Region. Wastes from abandoned sulfide mineral mines have the potential to release significant levels of acidity, heavy metals, and toxic oxyanions (As) to groundwater thereby reducing the quality of ground water resources in this region. Both abandoned and active mines are currently being reclaimed using various surface amendments that enhance the revegetation potential of these sites, but may also increase the loss of metals and oxyanions to subsurface water resources. The wide spread nature of abandoned mines in the West and Midwest creates an insidious, long-term “non-point source” degradation of water resources throughout this region. As more of these inactive mines are reclaimed, it will become increasingly critical that researchers describe the potential effect that organic mine reclamation amendments have on rates of metal transport to ground water via chelate or colloid assisted transport.

Description of Existing Problem

There are currently over 150,000 abandoned mine sites throughout the United States and more than 8,000 in western Montana alone. Many of these abandoned mines involved the mining and processing of sulfide minerals. The formation of fine textured mine tailings in the processing of the ores creates an aerated high surface area that enhances the oxidation of sulfide minerals to sulfate resulting in a highly acidic substrate in which little or no vegetation can be established. Surface runoff and subsurface drainage of metal laden acidic-water results in contamination of ground and surface water resources. The widespread nature of these abandoned mine sites results in sort-of a “point source induced non-point source” degradation of water resources nationwide. The toxicity of several of the metals associated with mine wastes are particularly high to both mammals and fishes, thereby creating a significant health and environmental threat once in ground or surface water (Pierzynski et al, 1994). Treatment of mine waste sites with organic amendments is often considered to be essential for re-vegetation or restoration success on these sites (Sopper, 1991). Common organic amendments used in mine reclamation include sewage sludge or municipal solid waste composts, pulpmill sludge and other wood processing byproducts and various industrial organic byproducts. Such organic amendments are applied to acidic mine wastes (tailings or waste rock) to provide plant nutrients, increase soil buffering capacity and water holding capacity and reduce metal bioavailability. Thus, use of organic amendments in mine reclamation has become a common practice across much of the United States. Unfortunately, we currently have only a limited understanding of how different organic materials influence the mobility of metals at reclamation sites. Low molecular weight organic acids associated with various organic amendments may increase metal mobility via the mechanisms of colloid or chelate assisted transport. Humification of organic matter (via composting) prior to application to mine sites reduces the presence of low molecular weight organic acids and increases the concentration of polymeric organic matter (humus) (Lieta and DeNobili, 1991). The insoluble nature of humic acids coupled with its capacity for the sorption of metals should reduce both the vertical mobility and bioavailability of metals when used in reclamation efforts (Stevenson, 1994; Temminghof et al., 1994). Both humified and non-humified materials are used as organic amendments in acid mine reclamation, however, few studies have been performed to assess how various organic materials influence metal mobility and the potential for metal contamination of neighboring or connected ecosystems. Preliminary research in our laboratory has demonstrated that composted sewage sludge applied to mine tailings greatly reduced levels of soluble and extractable metals. In contrast, log yard waste applied to mine tailings had no effect on levels of

extractable Cu and Fe, and actually increased the levels of water soluble Pb (DeLuca and Lynch, 1997). The composted sewage sludge was shown to have higher levels metal sorption and greater levels of soluble high molecular weight (>10,000 daltons) chelates than the log yard waste (DeLuca and Lynch, 1996). Our studies also showed that composted sewage sludge greatly enhanced revegetation success compared to lime or aged log yard waste. This would also potentially influence net leaching at sites by increasing net evapotranspiration rates and ultimately maintaining higher levels of organic matter in the surface soil. To date there has been inadequate information regarding the nature of organo-metal complexes associated with mine reclamation amendments.

Statement of Results or Benefits

Our research is aimed at providing the much needed information on how organic mine reclamation amendments influence metal mobility and ground water pollution potential. A thorough experimental research program will be designed to investigate the mechanisms by which organic compounds associated with organic mine reclamation amendments sorb or chelate metals and in turn influence the vertical mobility of these contaminants. We will establish both laboratory and field experiments that specifically address the influence of various commonly used organic mine reclamation amendments on metal and oxyanion mobility with particular emphasis on chelate or colloid assisted transport of metals. The results will provide insight into whether certain amendments actually increase metal or oxyanion mobility and thus groundwater pollution potential.

This research will:

- Assist in our understanding of the effect of various organic amendments on metal and oxyanion mobility in mine reclamation efforts.
- Provide operational guidelines for the use of organic amendments in acid mine reclamation.
- Enhance the potential for successful mine reclamation and minimize the potential for metal loss to groundwater.
- Serve as a source of base line information for the development of models that predict metal loss to groundwater with and without surface organic amendments.

Purpose and Objectives

The purpose of this proposed work is to assess the mobility and bioavailability of toxic mine waste contaminants including As, Cd, Cr, Cu, Fe, and Pb, following treatment with various organic mine reclamation amendments. The specific objectives are as follows:

1. Assess the characteristics of organo-metal and organo-oxyanion (As) complexes associated with different organic amendments with or without liming.
2. Assess the vertical mobility of the complexed or sorbed metals and oxyanions under both laboratory and field conditions.
3. Assess the potential for groundwater and surface water contamination by the complexed contaminants.
4. Assess the short and long term fate of organo-metal and organo-oxyanion complexes in tailing piles following treatment with different organic amendments with or without liming.

Methodology

Methods, Procedures and Facilities

Methods and Procedures

We will investigate the effect of several organic materials commonly used in the reclamation of abandoned mines including composted sewage sludge, composted or raw logging slash, paper mill pulp sludge, log yard waste. To assess the influence of these materials on metal mobility and groundwater contamination potential, both greenhouse and field studies will be employed. A single uniform field site will be chosen with the assistance of the Montana Bureau of Mines and Geology, the Montana Department of Environmental Quality, and possibly the US Forest Service or BLM.

We would identify a site that is streamside, is known to have high concentrations of soluble or exchangeable Pb, Cu, Cd, Cr, and As, and is large enough, yet uniform enough to allow for application of blocks of all 6 treatments to similar materials. We would avoid any sites that are currently under any form of state or federal action. Tailings would be sampled and analyzed for pH, particle size distribution, total C, and total and exchangeable Cu, Pb, Cd, Cr, and As. These sites would later serve as monitoring sites for the long-term analysis of the influence of organic amendments on metal mobility.

Characterization of Organo-Metal Complexes Associated with Organic Treatments

Tailings from the selected mine site will be air dried and sieved to 2 mm. Tailings samples of 100 g would be amended with 10 g of: (1) sewage sludge composted with wood waste (Eko Kompost, Missoula, MT), (2) raw log yard waste, (3) primary pulp mill sludge (Stone Container, Frenchtown, MT), (4) straw, (5) raw chipped logging slash, (6) a no treatment control. Five replicates will be treated with lime as required to bring the final pH to 6.5 (Adams and Evans, 1962) and five will receive no lime (creating a 2 x 6 factorial experiment).

The treated tailings will be brought to 60% water holding capacity, mixed thoroughly, placed in 750 ml containers, and allowed to incubate for 12 weeks. At the end of this period tailings will be fractionated using the procedure outlined by Sposito et al. (1980) and modified by McGrath and Cegarra (1992) with extracts analyzed for Cu, Cd, Cr, Pb, and As. Briefly, this fractionation method includes a water soluble fraction (extraction of the tailings with 0.01 M CaCl₂), exchangeable fraction (extracted with 1 M EDTA), organic bound fraction (extracted with 1 M NaOH), and aqua regia digestible fraction (digested in nitric and sulfuric acid). Each fraction will be analyzed for total Cd, Cr, Cu, Pb, and As on AAS (Amacher, 1996; Bartlett and Bruce, 1982; Reed and Martens, 1996; Huang and Fujii, 1996).

The water-soluble and NaOH-soluble fractions will then be subject to further fractionation by ultrafiltration to assess the percent of soluble metals that exist in an organic complex. Samples will be fractionated into two classes of molecular weights as described by Temminghoff and others (1994) using molecular weight cutoff ranges of 3,000, 10,000 and 30,000 daltons. The filtrate will be split into two samples with one analyzed for total soluble Pb, Cd, Cu, Cr, and As by AAS. An aliquot of 3 ml will be removed, filtered through 10,000 and 30,000 molecular weight cutoff filters (MSI, Westboro, MA) and the filtrate analyzed for total Cd, Cr, Cu, Pb and As by AAS, and the humic acid complexed metals will be determined by difference (Humic acid complexed metals = total metals - fulvic acid (< 10,000 dalton) complexed metals).

Field Research

During the Fall of 1996 (or Spring of 1997), field plots will be established at all five sites in a randomized complete block design. Plots will be amended with the treatments described above, (1)

composted sewage sludge, (2) raw log yard waste, (3) primary pulp mill sludge, (4) straw; (5) raw chipped logging slash, (6) a no treatment control. Each material will be amended with a N source to bring each to a total C:N of 20:1. Lime will be studied as a separate factor (to create a 2 x 6 factorial experiment) to allow us to investigate the mobility of metals under acidic and near neutral conditions (pH adjusted to approximately 6.5 using the method of Adams and Evans, 1962). Lime will be incorporated to a depth of 15 cm and organic amendments incorporated to a depth of 7.5 cm. Plots will be seeded with 20 kg/ha of slender wheatgrass and thick spike wheatgrass, raked to a depth of 3 cm, and watered weekly for the first 6 weeks.

Tailing plots will be instrumented with piezometers to allow for assessment of metal concentration in shallow groundwater. Samples will be collected on a bimonthly basis and analyzed for those metals of interest. Each plot will measure 3 x 3 m with a 2 m buffer zone and have the nested piezometers placed in the plot center to facilitate the collection of metals transported to depth. Tailing samples will also be taken on a regular basis and analyzed for Cd, Cr, Cu, Pb, and As by AAS. We would also subject samples collected at 12 weeks and 12 months to the molecular weight fractionation as described above.

Surface runoff sedimentation traps will also be installed to assess the effect of the amendments on overland flow and loss of free or sorbed As and metals. Treated tailings would be sampled after 12 weeks and 12 months and fractionated as described above and analyzed for Cd, Cr, Cu, Pb, and As by AAS.

Plots will be seeded with two grass species. The grass species will be selected after field plot sites have been identified and will represent species adapted for the climatological conditions of the site. Representative species might include slender wheatgrass or thickspike wheatgrass and these would be broadcast seeded at a rate of 20 kg/ha.

Vegetation establishment will be estimated as density (number/unit area) and aboveground biomass (g/m²). Density will be determined in late spring approximately post-germination. Aboveground biomass will be determined near the end of the growing season clipping and reporting on a dry weight basis. Net evapotranspiration will be estimated using simulation models and site variables measured will be pre-dawn water potential and leaf area index. At this same time tailings samples will be analyzed for microbial biomass by fumigation extraction (DeLuca and Keeney, 1993).

Bioavailability of the metals in mine tailings from several mine sites will be assessed by several means. Tailings will be treated as described above and placed in 20 cm pots seeded with thick spike wheatgrass and placed in a growth chamber or greenhouse for 12 weeks. At the end of this time period plants will be clipped, digested in concentrated nitric acid, and analyzed for Pb, Cd, Cr, and Cu by ICP or AAS. This will provide us with both a measure of metal phytoavailability and revegetation success on tailings treated with the above organic amendments.

Available Facilities

The University of Montana is in an ideal location and is well equipped to conduct the given experiments. In 1995 the University was awarded a large Murdock grant to form a central biogeochemistry facility (Gannon et al., 1995). This facility is home to state-of-the art equipment for the analysis of metals, organic matter and oxyanions. Specifically the laboratory houses an inductively coupled plasma (ICP), ion chromatograph, soluble carbon analyzer, CNSHO analyzer, and an atomic absorption spectrophotometer (AAS). In addition to this facility my laboratory houses vacuum extraction equipment, several drying ovens, incubators, an AAS, and a flow injection analyzer.

Missoula is centrally location in terms of study sites and the availability of tailings. Abandoned mine sites exist on Forest Service lands, Bureau of Land Management Lands, and State Lands, all within 2 hours drive of Missoula.

Principal Findings and Significance

PROGRESS REPORT - YEAR 0.75 OF 2 YEAR GRANT

June 14, 1999 --- We began our search for a field site last fall and continued through this spring. Although we visited several locations last fall and this spring, we determined most to be inappropriate for our field needs due to their small size, presence of standing water, presence of alkaline parent rock, or inaccessibility. Although there are thousands of abandoned mines in Western Montana, there are few that are large and uniform enough to accommodate our proposed field study or they are currently being remediated by state or federal agencies.

We have chosen to perform our studies at two smaller sites rather than the one large site. We have selected a failed reclamation site on privately owned land (Combination Mill Site near Phillipsburg, MT) where lime, log-yard waste, and straw were applied surface applied to a streamside tailing impoundment. Despite the attempted reclamation, there was no successful revegetation and small erosion rills were beginning to form. This site offers some interesting opportunities for our project in that it is an excellent example that amendments such as straw and log yard waste are used to reclaim these sites with no consideration of what effect that these treatments might have on metal availability and mobility. We have attained approval from ASARCO to work on this site and will begin installing plots on June 15, 1999. Because this site was treated with lime, the pH of the tailings are now about 5.5. We will use this as our moderately acidic site and plan on installing plots on a highly acidic site (possibly the Doe Run Mine near Neihart, MT) in July.

Treated tailings will be fractionated to assess metal mobility and bioavailability in late summer and early next spring. Plant biomass will be clipped from plots in early fall, digested and analyzed for metal (Cu, Pb, Zn, Cr, Cd) and As content. Later samples will be collected from lysimeters on a bi-monthly basis and analyzed for the above metals and oxyanion.

During winter months, we located sources for the five organic amendments: straw, pulp mill sludge, log yard waste, cow manure composted with straw, and sewage sludge composted with log yard waste. We obtained samples of these materials and analyzed them for total digestable metals and carbon to nitrogen ratios prior to use in experiments.

Since we had not yet located a suitable field site, we were initiated laboratory and greenhouse experiments on streamside tailings from Ramsay Flats along the Clark Fork River, just east of Butte, Montana. These experiments will also be conducted on tailings from the Combination Mill Site and the Doe Run site once we establish our field plots. Although this level of duplication was not initially intended, conducting these experiments on tailings from more than one site will only make the experiment more robust and our results more conclusive.

Preliminary data indicate that the organic amendments have a variable influence metal mobility. The

pulp mill sludge greatly reduces Cu solubility, but Cu is readily extracted from the amended tailings with NaOH indicating that the metals are sorbed to humic materials associated with the pulp mill sludge. Both straw and log-yard-waste have little influence on Pb or Cu solubility.

Descriptors

Metals, Oxyanions, Colloid Assisted Transport, Sorption, Humic Acids, Chelation, Organic Matter

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

June 14, 1999 No publications have been prepared based on the research performed under this support. An abstract for the research was submitted to the American Society of Agronomy and Soil Science Society of America to present our findings at the 1999 Annual Meetings in Salt Lake City.

Other Publications

Basic Project Information

Basic Project Information	
Category	Data
Title	Determination of Fuel Oxygenate (MTBE) Biodegradation Potential in Groundwater at Release Sites
Project Number	C-02
Start Date	09/01/1998
End Date	09/30/2001
Research Category	Water Quality
Focus Category #1	Groundwater
Focus Category #2	Toxic Substances
Focus Category #3	Treatment
Lead Institution	Montana State University

Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Al Cunningham	Professor	Montana State University	01
Richard Veeh	Research Associate	Montana State University	02
Paul Sturman	Research Associate	Montana State University	03
Jeff Kuhn	Research Associate	Montana Dept. of Environmental Quality	04
Stephen L. Jester	Consultant	Conoco Inc.	05

Problem and Research Objectives

Alkyl ethers such as methyl-tert-butyl ether (MTBE), ethyl-tert-butyl ether (ETBE), and tert-amyl methyl ether (TAME) have been used as oxygenate additives in gasoline to reduce vehicle emissions such as carbon monoxide and other volatile, toxic organic compounds. MTBE, the most common oxygenate, is currently added at concentrations up to 15% (v/v) to more than 30% of all gasoline sold in the United States. In 1992, more than 1.8 billion gallons of MTBE were used in gasoline, and its use has increased every year since. More than 29 companies now produce MTBE, and it is the second largest organic chemical produced domestically. Due to the widespread use of MTBE in reformulated gasoline, most of which is stored in underground tanks, documented release sites are continually being reported. A recent survey conducted by the USGS identified MTBE as the second most common contaminant of urban aquifers in the nation. Although human health effects are still being evaluated (Leclair, 1997), the EPA has designated MTBE as a probable human carcinogen and issued a draft nonregulatory health advisory of 70 ug/l for nonprimary water sources (Cooney, 1997). Many states have much more stringent maximum contaminant levels for MTBE. The risks to human health posed by MTBE are exacerbated by its high water solubility and mobility in groundwater systems. The relative recalcitrance of MTBE, combined with its high water solubility make it the single largest threat to ground water quality resulting from gasoline releases. Given the widespread use of MTBE and its potential health risk, MTBE and other oxygenate contamination of groundwater is clearly a problem of national scope.

Traditional methods to remove gasoline constituents from groundwater do not work well for MTBE. Air stripping is very difficult because MTBE's low volatility requires large air/water ratios to make the treatment effective. In addition, MTBE's low affinity for organic carbon prevents efficient removal by granular activated carbon. However MTBE is capable of being biodegraded by native soil bacteria, albeit at slower rates than other gasoline constituents. Available research indicates that many naturally occurring microbial populations can biodegrade MTBE as well as tert-butyl alcohol (TBA), an MTBE metabolite of concern, over time. Strategies for enhancing in situ biodegradation (i.e. accelerated natural attenuation) of MTBE are urgently needed.

The proposed research project, which involves a 3-way collaboration between MSU's Center for Biofilm Engineering (CBE), the Montana Department of Environmental Quality (MDEQ) and Conoco Inc., will assess the environmental factors which limit MTBE and other oxygenate metabolite degradation for the purpose of developing improved strategies for enhancing MTBE biodegradation in the field. MSU graduate and undergraduate students from multiple disciplines (i.e. engineering, microbiology, geology, and soil science) will be organized into teams and mentored by state regulatory and industrial officials, together with MSU faculty. The project work plan includes review of all pertinent hydrogeological and geochemical data as well as a full characterization of the microbial population present at an existing MTBE release site located in Ronan MT, and will focus on isolation,

identification, and enrichment of bacterial strains that can biodegrade MTBE metabolites. Strategies for enhancing MTBE biodegradation in situ will be identified and piloted at the Ronan MT study site.

Statement of results or benefits

The proposed study will greatly increase our knowledge of the fate and transport of oxygenate gasoline additives in groundwater systems. The project work plan will determine the physical, chemical and biological processes which limit the rate of MTBE biodegradation. Anticipated results include:

- field and laboratory identification of primary mechanisms of MTBE natural attenuation
- recognition of microbial populations capable of oxygenate degradation
- identification of degradation pathways and metabolite recalcitrance
- optimization of parameters which limit oxygenate biodegradation to maximize degradation rates
- development of biodegradation enhancement strategies and subsequent pilot testing at the Ronan MT field site.

Environmental quality personnel from Montana and many other states have recently begun testing for MTBE at petroleum release sites and are discovering that ground water contamination is far more widespread than originally anticipated. MDEQ is very interested in determining the rate and extent to which MTBE biodegradation can be documented at existing field sites. The results from this study will help states and municipalities decide what level of remediation is necessary to address MTBE concentrations in drinking water supplies and whether specific groundwater remediation efforts can be undertaken to prevent future MTBE impacts (see attached supporting letter from the MDEQ). Development of improved methods for enhancing MTBE biodegradation will clearly also be of use to industrial practitioners responsible for developing remediation strategies for subsurface gasoline release sites (see supporting letter from Conoco Inc.).

Nature, Scope, and Objectives

The **nature** of this project is collaborative in that it involves association between Montana State University's Center for Biofilm Engineering (CBE), the Montana Department of Environmental Quality (MDEQ), and Conoco, Inc. This 3-way collaboration is the major strength of this research project because it will insure that the research results will be of maximum value to practitioners (both regulatory and industrial) concerned with gasoline release site clean-up and related policy development. The research work plan will be executed by graduate and undergraduate students from multiple disciplines organized into project teams and mentored by officials from industry, state government, and academia--thereby providing a unique and valuable learning experience. The role of each of the collaborators is as follows:

CBE. The CBE (P.I's Cunningham, Sturman and Veeh) will direct MSU graduate student laboratory and field activities as outlined in the project work plan. CBE laboratories and equipment will be used and CBE will organize peer-reviewed publication and oral presentation of research results. Graduate students enrolled in ENVE 534, Environmental Engineering Investigations (a graduate level Environmental Engineering course taught by Professor Cunningham) will perform project tasks each year as term projects. In addition CBE will dedicate an NSF supported stipend (\$12,000/yr) for a Ph. D. student to work on the microbiology and molecular biology aspects of this project. CBE will also dedicate \$25,208 in non-federal matching funds.

MDEQ. The MDEQ has provided the major incentive for developing this proposed project by offering the use of an on-going MTBE field site located in Ronan MT (see attached letter). The Ronan MTBE release site is ideal for field study because it lies in a shallow, sandy, well characterized aquifer. These conditions will facilitate extrapolation of results to other field sites. MT DEQ (Mr. Jeff Kuhn) will supply the existing data base for the Ronan site as well as organize the collection of additional field data needed throughout the project. MDEQ officials will work directly with MSU students and researchers to develop and execute the project work plan and review student progress at regular intervals. MT DEQ will contribute \$15,000/yr in non federal support to this project.

Conoco Inc. Conoco Inc. is supportive of the proposed research because of MTBE's relatively high mobility and persistence in groundwater. Because current corporate policy makes it very difficult for Conoco Remediation Technology Division to directly fund remediation related research, the opportunity to participate in a collaborative project with CBE and MDEQ is welcomed by Conoco. Although Conoco does not currently have any remediation sites in Montana where MTBE is a constituent of concern, knowledge gained regarding methods for enhancing MTBE biodegradation will be useful to Conoco (and others in the petroleum industry) at sites outside Montana. Conoco officials (Mr. Stephen Jester) will work with CBE and MDEQ to develop the project work plan, provide review and oversight of the work, attend progress meetings and field trips and mentor graduate students. Conoco will provide \$10,000 of in-kind services and support.

The **scope** of the proposed research will be to assess the environmental factors which limit MTBE, other oxygenate and metabolite degradation with the intent to use this knowledge to develop strategies for enhancing MTBE biodegradation in the field. Accomplishing this broad work scope will require a review of all pertinent hydrogeological and geochemical data as well as a full characterization of the microbial population present the Ronan, MT study site. The project will then focus on enrichment, isolation, and identification of bacterial strains that can biodegrade MTBE, other oxygenates and metabolites. The project scope includes the following **Objectives**:

Objective 1 (Years 1&2) Field Measurement of MTBE Natural Attenuation Parameters.

- Review existing site data from the Ronan, MT site to establish release history, groundwater hydrogeology, geochemistry, availability of potential electron acceptors, free product occurrence, and evidence of natural attenuation. This information will enhance interpretation of laboratory results and will allow site specific recommendations for remedial action.
- Collect groundwater and soil samples from existing or planned monitoring well locations. Establish existing plume dimensions (where necessary). Samples will be indexed and cross-referenced with geochemical conditions present at each sampling location.
- Analyze site groundwater data for evidence of natural attenuation of MTBE, degradation products, and other petroleum compounds.

Objective 2 (Years 1&2) Laboratory assessment of MTBE Biodegradation Parameters.

- Develop and/or substantiate methods for the analysis of MTBE, its known metabolites, etc., in water and soil samples.
- Develop aerobic and anaerobic (e.g. denitrifying, sulfate-reducing, and methanogenic) MTBE-degrading enrichment cultures using microcosms isolated from site samples and selective growth media.
- Identify possible nutrient limitations (e.g. nitrogen and phosphorus) and environmental limitations

- (e.g. dissolved oxygen and temperature) to MTBE biodegradation at the field sites.
- Identify possible hydrocarbon cosubstrates (e.g. propane) that may enhance MTBE biodegradation.
 - Assess optimal rates of MTBE biotransformation using respirometry techniques that involve both aerobic and anaerobic (see above) microbial processes.
 - Isolate and identify specific oxygenate/metabolite-degrading bacteria and enzymes responsible for complete MTBE degradation. Amino acid sequencing of enzymes for location of enzyme-coding gene sequences.

Objective 3 (Years 2&3) Strategies for Enhancing MTBE Degradation in the Field.

- Combine results from objectives 1 and 2 and identify possible strategies for enhancing field rates of MTBE biodegradation at the Ronan, MT site.
- Collect additional field data needed to evaluate feasibility of each alternative.
- Assess engineering feasibility of each alternative strategies and select most promising alternative (s).
- Design and implement a field pilot demonstration project to test MTBE biodegradation enhancement strategies at the Ronan, MT site.

Methodology

Methods, procedures, facilities, related research, and references

Standard **methods** for site characterization, establishing plume dimensions, and aseptic groundwater and soil sampling will be used in all site work. Groundwater and/or soil samples will be analyzed for organic components and geochemical indicators of aerobic and anaerobic biodegradation (O₂, Fe²⁺, Mn²⁺, NO₃⁻, SO₄²⁻, CH₄) following established protocols. Total nutrient concentrations for NH₄-N and PO₄-P will also be assessed. Field analysis methods will be used where possible. Water samples will be analyzed for MTBE, its known metabolites, and other possible oxygenates following the protocol suggested by Steffan et al. (1997) using appropriate standards, gas chromatography, and FID detection.

Aerobic enrichment cultures using batch microcosms will follow the general protocol described by Borden et al. (1997). Anaerobic enrichment cultures using batch microcosms will follow the general protocol described by Yeh and Novak (1994), but will also include modifications of the solution redox potential and added nutrient amendments to test possible oxygenate degradation (i.e. oxidation) coupled to Fe(III) reduction and CO₂ reduction to methane. Nutrient enhancement and biodegradation rate studies will use batch culture and respirometry, respectively, under varying concentrations of added nutrient amendments, especially nitrogen and phosphorus.

Co-substrate investigations will use batch culture techniques. These microcosm enrichments may include carbon substrates such as propane, camphor, propionic acid, ethanol, phenol, t-butanol, butyl formate, isopropanol, pyruvate, and acetone, substrates which have been shown to variably enhance oxygenate degradation or inhibit its degradation, depending on the levels of oxygen and organic matter present (Mo et al., 1997; Steffan et al., 1997).

Isolation of specific oxygenate- and oxygenate metabolite-degrading bacteria will be achieved by repeated transfers through a series of selective liquid enrichment batch cultures followed by single colony isolation on agar plates or in semi-solid agar tubes. Identification of these bacteria will include several independent methods including physiological Biolog tests, phospholipid membrane analysis, and 16S rRNA sequence comparisons to existing sequence databases. Isolation and identification of enzymes responsible for steps in the MTBE degradation pathway will follow protocols similar to those described by Steffan et al. (1997). Because in this study a soluble P-450 monooxygenase has been implicated as one of the enzymes responsible for MTBE degradation, our initial studies will focus there.

Field **procedures** for assessment of MTBE attenuation will utilize methods adapted from the study of natural attenuation of BTEX compounds in groundwater. Existing data from the potential study site in Ronan, MT suggests that MTBE attenuation is occurring, but at a slower rate than benzene, (see in Figure 1). The Figure 1 data, together with information on groundwater velocity, can be used to estimate the in situ degradation rates of each compound of interest using the method of Buscheck et al. (1993). This type of analysis will be utilized to compare apparent field removal rates with laboratory-derived rates for each site. Baseline data for each site will also be compared to data collected over the course of the project to track changes in removal rates and correlate these changes with any site remedial actions.

Figure 1. MTBE and benzene attenuation in the dissolved phase plume, Ronan, MT.

The Center for Biofilm Engineering (CBE) at Montana State University is an NSF-funded Engineering Research Center which specializes in research related to microbial activity on and interactions with surfaces. The CBE **facilities** occupy several thousand square feet of contiguous lab space with hundreds of square feet of bench space. CBE has one laboratory dedicated to all aspects of molecular microbiology including: microcentrifuge, PCR, gel electrophoresis, and light table, camera, and imaging equipment. State-of-the-art DGGE equipment is also available. In addition, a biological respirometer is available for kinetic studies as well as analytical equipment including GC and HPLC instrumentation. Also, several epifluorescence microscopes are in use as well a confocal scanning laser microscope. Adequate room temperature storage space is available as well as cold storage in the form of many refrigerators (4oC) and -40oC and -70oC freezers. An adequate number of laminar flow hoods for microbiological work and chemical hoods are available for use. Laboratory reagent water (18 MÙ) is provided by a Barnstead (4-filter) Nanopure ultrapure water system. Laboratory protocols for handling hazardous waste and other safety-related issues are defined in the CBE Laboratory Manual and enforced by the CBE Laboratory Manager. MSU's Radiation Safety Officer and Chemical Safety Officer enforce university policies toward chemical safety and administer hazardous waste collection and disposal programs.

Related research

The potential for human exposure to MTBE is exacerbated by both its increased use as a gasoline additive and by the physical properties of the compound which minimize soil sorption and make it extremely mobile in groundwater. MTBE is more volatile (i.e. has a higher vapor pressure) than benzene; however, it only has a moderate Henry's Law constant (6×10^{-2} kPa/m³/mol) due to its low octanol-water partition coefficient ($\log K_{ow} = 0.94$ to 1.05) and high aqueous solubility ($\sim 45,000$ mg/L at 25oC) (Davidson and Parsons, 1996; Squillace et al., 1996).

Because MTBE mass at many release sites is predominantly in the aqueous phase, pump-and-treat remediation strategies have been applied, but with limited success. Air stripping to remove MTBE is

very difficult because its high solubility requires large air:water ratios to make the treatment effective (Davidson and Parsons, 1996; Carter et al., 1997). In addition, MTBE's low affinity for organic carbon inhibits removal by granular activated carbon (GAC). In one study, however, the addition of MTBE-degrading bacterial cultures to GAC-containing reactors under aerobic conditions improved initially low MTBE removal from groundwater to greater than 75% (Miller et al., 1997). Although most of the limited data available regarding biodegradation of MTBE suggest that it is degraded only very slowly in the subsurface relative to the BTEX compounds, evidence is mounting that many naturally occurring microbial populations can biodegrade MTBE as well as tert-butyl alcohol (TBA), an MTBE metabolite of concern, both aerobically (Barker et al., 1996; Salanitro et al., 1994; Borden et al., 1997; Steffan et al., 1997) and anaerobically (Yeh and Novak, 1994) over time. Generally, this has been reflected in longer (yet stable) plume lengths associated with MTBE compared to coexistent BTEX plumes. Some studies have indicated that MTBE degrades anaerobically only under slightly acidic conditions and in an environment where more easily degraded organic carbon is absent (Yeh and Novak, 1994).

The research proposed herein will contribute to the literature by providing an extensive characterization of MTBE-degrading isolates at physiological, molecular, and also genetic levels. This will lead to more in-depth knowledge concerning the identification of microbial isolates capable of MTBE degradation, elucidation of the biodegradation process, and optimization of the process at the field scale.

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Principal Findings and Significance

PROGRESS REPORT -- June 14, 1999

The primary goal of this research is the assessment of the environmental factors which limit MTBE, other oxygenate, and metabolite degradation at fuel release sites. In order to meet this goal, work to date has focused on the following objectives in the 4 categories listed:

I. Site Data Review

Existing site data from an MTBE-contaminated site in Ronan, MT has been reviewed to establish release history, groundwater hydrogeology, geochemistry, availability of potential electron acceptors, free product occurrence, and evidence of natural attenuation. This information has greatly enhanced the understanding on contaminant behavior in the subsurface and has provided a framework to assess the performance of the remediation system currently operating at the site.

II. Field Experimentation

- Groundwater and soil samples were collected from 3 locations within the contaminant plume through existing monitoring wells (water samples) and drilling new monitoring wells (soil samples) at selected locations. This sampling assisted in the establishment of plume dimensions, contaminant transport rates, electron acceptor concentrations and biodegradation potential. Samples were indexed and cross-referenced with geochemical conditions present at each sampling location.
- Site groundwater data were analyzed for evidence of natural attenuation of MTBE, degradation products, and other petroleum compounds. Newly collected data and historic data (1994-1998) contributed to our understanding of MTBE (and other fuel constituent) behavior in the subsurface.

III. Laboratory Experimentation

- Methods for the analysis of MTBE and its known metabolites in water and soil samples have been substantiated in CBE laboratories.
- Enrichment cultures from site soil and groundwater samples have led to the isolation of an MTBE-degrading culture using microcosms with selective growth media.
- The potential benefits of possible hydrocarbon cosubstrates (e.g. propane) that may enhance MTBE biodegradation have been investigated in microcosms.

IV. Education and Technology Transfer

The first annual workshop on MTBE fate and transport in groundwater has been delivered to regulators at the Montana Department of Environmental Quality (Helena MT, December 11, 1998). The workshop was organized by the Principal Investigators and delivered by both the PIs and graduate students participating in CE 534 (Environmental Engineering Investigations).

Descriptors

Bacteria, Biodegradation, Bioindicators, Contaminant Transport, Groundwater Quality, Natural Attenuation, Petroleum Additives, Solute Transport, Soil Microbiology, Underground Storage Tanks

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications

Information Transfer Program

Basic Project Information

Basic Project Information	
Category	Data
Title	Montana Watersheds
Description	A WEBSITE to Bring People and Watershed Information Together
Start Date	01/01/1997
End Date	01/01/2003
Type	Publications

Lead Institution	Montana Watershed Coordination Council
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Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Michael McLane	Professional Staff	Montana Watershed Coordination Council	01
Kathy Tohe Stephens	Professional Staff	Montana Water Resources Research Center	02
Gordon Levin	Student	Montana Water Resources Research Center	03
	Student	Montana Water Resources Research Center	03
Ann Marie Gooden	Student	Montana Water Resources Research Center	04

Problem and Research Objectives

These pages have been developed with the guidance of the Montana Watershed Coordination Council (MWCC) for the purpose of encouraging citizen participation in watershed discussions and to make resources available to local groups and committees. Included are 'Events', 'Watershed Groups', 'Funding and Technical Resources', 'Links', and 'All about the MWCC.' All pages are part of an interactive WEB database. Users can access -- add, delete, or edit their information online. Please visit the site at <http://water.montana.edu/WATERNET/watershed/>

This is an ongoing multiyear project.

Methodology

Principal Findings and Significance

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Basic Project Information

Basic Project Information	
Category	Data
Title	The Montana Total Maximum Daily Loads (TMDL) Process
Description	B - 02
Start Date	03/01/1997
End Date	02/28/1998
Type	Library And Database Services
Lead Institution	Montana Water Resources Research Center

Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Kurt Zeiler	Student	Montana Water Resources Research Center	01
Other	Consultant	Montana Dept. of Environmental Quality	02

Problem and Research Objectives

This WEB site - **The Montana TMDL Process**, was created by a senior in Geohydrology with an interest in public policy, and the MT DEQ. Developed for easy access and comprehension, it was also one of the first sites on MONTANA WATER to incorporate data (the 1998 303D List) in a WEB-searchable database format.

Methodology

The WEB site, located at <http://water.montana.edu/docs/tmdl/> contains the following elements:

- Searchable 1998 303(d) List of Waterbodies in Need of TMDL Development.
- What is a TMDL?
- What Problems will TMDLs address?
- TMDL Frequently asked questions.
- Montana TMDL Process Info.
- Montana TMDL Case Studies.
- What is Montana Doing Now?
- Links.
- What happens next?

Principal Findings and Significance

While the state DEQ works at updating the 1999 303(d) list (still unpublished as of 11/09/99), this WEB site has been used consistently by the public, water groups, and water managers seeking basic information on this complex issue.

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Basic Project Information

Basic Project Information	
Category	Data
Title	MONTANA WATER
Description	A WEB switchboard for Montana Water Information
Start Date	03/01/1997
End Date	01/01/2003
Type	Library And Database Services
Lead Institution	Montana Water Resources Research Center

Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Kathy Tohe Stephens	Research Associate	Montana Water Resources Research Center	01
Gordon Levin	Student	Montana Water Resources Research Center	02
	Student	Montana Water Resources Research Center	02
Ann Marie Gooden	Student	Montana Water Resources Research Center	03

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Basic Project Information	
Category	Data
Title	Montana Local Water Quality Districts
Description	WEB Sites
Start Date	03/01/1997
End Date	08/01/1999
Type	Publications
Lead Institution	Montana Water Resources Research Center

Principal Investigators

Principal Investigators			
Name	Title During Project Period	Affiliated Organization	Order
Kathy Tohe Stephens	Professional Staff	Montana Water Resources Research Center	01
Ann Marie Gooden	Student	Montana Water Resources Research Center	02

Problem and Research Objectives

Local Water Quality Districts in Montana

Two of the most important issues of the 90s are water quality and the economy. People who realize the need to protect watersheds, rivers and groundwater are organizing local projects around the country. But at the same time, concerned citizens are facing economic realities and asking, "How can we fund our present activities?" Long range thinkers are asking, "Can we set up a source of continued funding for the future?"

Montanans have found that sound economic development in a vibrant, growing community depends on a clean and healthful supply of water. They convinced the Montana legislature to pass a bill in 1991 allowing counties to form districts with the sole purpose of protecting, maintaining and improving water quality. A "Local Water Quality District" (LWQD) may set fees to fund its water quality program. The district can multiply its resources by using the fees as "matching funds" when applying for grants. Presently, there are four LWQDs in Montana. For more information on LWQDs check MONTNANA WATER at <http://water.montana.edu/docs/lwqdmmain.html>

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USGS Internship Program

Student Support

Student Support					
Category	Section 104 Base Grant	Section 104 RCGP Award	NIWR-USGS Internship	Supplemental Awards	Total
Undergraduate	5	N/A	N/A	N/A	5
Masters	N/A	11	N/A	N/A	11
Ph.D.	N/A	1	N/A	N/A	1
Post-Doc.	N/A	N/A	N/A	N/A	N/A
Total	5	12	N/A	N/A	17

Awards & Achievements

Publications from Prior Projects

Articles in Refereed Scientific Journals

Book Chapters

Dissertations

Water Resources Research Institute Reports

Conference Proceedings

Other Publications