Introduction

STATE OF THE MONTANA University System WATER CENTER 2000-2001

The Montana University System Water Center continues to grow into the 21st century. The most exciting news of this past year being the appointment of Gretchen Rupp, P.E. as the new Center Director. Gretchen brings several years of experience as an environmental engineer to our programs.

As in the past, our energies are divided among our missions of water research, outreach to water professionals and other adults, and education of future water professionals. In the research arena, the Center administers three principal programs. Through the Water Resources Research Act, both state (104-B) and regional (104-G) programs are managed. Dollars are awarded through the state program to Montana researchers by a competitive grant review process that calls on the expertise of water professionals from varied positions. These small allotments, described in detail elsewhere in this report, serve as seed grants as well as support for several graduate students throughout the state university system. Our success rate at the regional 104G competition generally averages 2 proposals per cycle. As the research lists indicate, several successful grants from previous years are now winding up. The Whirling Disease research program has completed five years of intensive investigations into the disease that is devastating populations of salmonids throughout the West. Federally funded, and directed by a Steering Committee of fisheries experts from around the nation, the program has to date expended more than $2 million in basic laboratory and field science to come to an understanding of the biology of this complex pathogen. Investigators from more than 10 universities and agencies have been involved in this work. Besides administering all the research funds, the Water Center operates a Wild Trout Research Laboratory specialized for the investigation of whirling disease. The third research effort of the Center is the demonstration of promising treatment technologies for small public water systems. Funded by EPA for the last six years, this year’s program involved pilot- and full-scale testing at water systems in several states. Outreach programs took several forms this year. As always, the Center co-sponsored Montanas week-long Water School - intensive professional training for water and wastewater system operators. We also continued our commitment to co-sponsor an annual Montana water research conference, along with our state section of the American Water Resources Association. We continue to consult with the chief water organizations and agencies in the state to maintain a central website -- MONTANA WATER (http://water.montana.edu). Our Media Team continued development of interactive computer-based training materials for water system operators. Both undergraduate and graduate education benefitted from the Centers programs this year. Three interns were placed with the USGS district offices of Montana and Wyoming for summer fieldwork. Several graduate students were supported by our grant-funded research, and the Center itself made use of student interns in our day-to-day operations.

Our collaborators during this year were nearly too numerous to mention. We benefitted from both contractual and informal ties with entities ranging from university science departments in several states, to local county government, to conservation districts, to a number of state and federal departments, as well as watershed groups. Establishing and maintaining fruitful collaborations can be
exhaustive work, and the Center does not undertake it lightly. But the complex nature and high public profile of many modern water problems render it essential to include diverse viewpoints, funding sources and areas of expertise in crafting solutions. MONTANA WATER PROBLEMS 2000 - 2001 Montana contains the headwaters for two of the largest river systems in the United States. The Missouri and Yellowstone rivers and their tributaries drain the eastern three-quarters of the state while the Clark Fork flows west to the Columbia River. Montana exports an average of over forty million acre feet of water per year to downstream states. While the headwaters of these rivers appear to have a super-abundance of water, the spatial and temporal distribution of water is such that many places in Montana experience water shortages. In the year 2000-01 the cumulative effects of the ongoing drought continue. Water quality problems of both natural and human origin, affecting both surface and groundwater, also present scientific and water management challenges. An example is the extensive fires of 2000. Increasing demands, conflicting interests, and intense competition for water resources are a large part of Montana’s history and will undoubtedly frame much of its future. Because water is critical to every part of Montana’s economic and natural well-being, a multitude of water-related problems and issues concern Montanans. These include: Providing sufficient opportunities for water education. Identification of pollutants in ground and surface waters; their source, movement and fate; and their remediation and abatement. Technological innovations to protect and improve water quality. Timely predictions of moisture and surface runoff, and development of drought mitigation options. Long-term monitoring of water levels and water quality. Baseline characterization of ground-water resource. Provision of adequate supplies of water for municipal, commercial, agricultural, and recreational uses; and means for resolution of competing demands. Protection of aquatic habitat. Public access to streams flowing across private lands. Adjudication and quantification of water rights, including Indian water rights. Apportionment and protection of water flowing across interstate and international boundaries. These are complex problems and issues. Many are currently under review by executive, legislative and judicial branches of government. The mission of the Montana Center is to contribute and disseminate useful information to those who need it in ways that help resolve problems and advance policy. Because of limited financial resources, the Center’s focuses are the result of public/government/peer ranking, and they concentrate on the most critical issues in which there is available expertise. The following sections of this report address the priorities and projects of the Fiscal Year 2000 program.

Research Program

The Montana University System Water Center March 1, 2000 - Feb. 28,2001

Basic Information
Title: Development of a GIS for Analyzing and Mapping Ground-Water Elements Related to Coal-Bed Methane Production

Project Number: B01

Start Date: 4/1/2000

End Date: 2/28/2001

Research Category: Ground-water Flow and Transport

Focus Category: Surface Water, Groundwater, Water Use


Lead Institute: Montana Tech of UM

Principal Investigators: Patrick J. Kennelly, Teresa Donato

Publication


Report Summary

Problem and Research Objectives
Commercial methane production from regional coal-bed aquifers has begun and is rapidly expanding. In semi-arid southeastern Montana the coal beds are the regional aquifers for the agricultural community. Development of this energy resource will have significant impacts on the regional hydrologic resources.

Methane production is accomplished through the process of drilling wells and pumping ground water from coal beds to decrease hydrostatic pressures. Methane gas then desorbs from the coal and is captured in the well, and co-produced with water discharged at ground surface. Water co-produced with the methane can exceed tens of thousands of gallons of water per day from each well, and from the thousands of wells that may be drilled could exceed millions of gallons per day. The production of coal-bed methane, the associated declining static water levels and surface discharges of sodic ground water are expected to last for decades.

Landowners have voiced concerns over all the above issues. To understand where concerns are warranted, landowners need access to maps showing potential and actual coal-bed methane development, well status, and other hydrologic features. The GIS products of this study provide the landowners, government agencies, industry officials, and other interested parties with this important geographic data.

The Geographic Information System (GIS) developed in this study provides the basis for addressing the above concerns and serves four main purposes.

1.) To provide a basis for spatial analyses in future research projects related to ground-water withdrawal and methane production.
2.) To produce an easily updated map product on a planimetric base.
3.) To provide a map producible at different scales.
4.) To provide the public with the above information.

Methodology
The GIS product was developed using Environmental Systems Research Institute (ESRI) ARC/INFO and ArcView software. Hardware included resources at the Montana Bureau of Mines and Geology’s (MBMG) GIS Laboratory in Butte and at MBMG’s Billings office. GIS data was compiled from a number of sources. Coal, methane, geologic, and hydrologic data available at MBMG were used to develop key GIS coverages. Well data on water use were incorporated from MBMG’s Ground Water Information Center’s (GWIC) Sybase database. Gas well locations and status were input from the Oil and Gas Conservation Division. MBMG monitoring well locations were retrieved from GWIC, and coal-mine company monitoring well locations were requested from mine companies. Planimetric GIS data were obtained from the Montana Natural Resource Information System (NRIS).
**Principle Findings and Significance**

This study was significant in that it used GIS to capture data that will assist in the visualization, documentation, and analysis of several factors that will influence or be influenced by the production of coal-bed methane in Montana’s Powder River Basin.

One product, a MBMG Geologic Map of the Anderson and Knobloch coal beds, delineates the areas of the Powder River Basin that have potential for coal-bed methane development. This will be an important tool in evaluating the potential extent of development, and the issues that may face these areas.

The second product, an Open-File Report of hydrologic features in the potential development area, provides an important inventory of features that may be influenced by coal-bed methane development. Springs, ground water wells, and coal-bed methane wells will all be important factors in the overall ground water system as the location and extent of development changes. As well as providing an indication of current conditions, the GIS project will provide a framework with which future changes can be monitored and analyzed.
## Basic Information

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## Publication

Progress Report:

Cattle are often implicated in contamination of nearby water supplies with pathogenic bacteria such as *Eschericia coli* O157:H7 in bovine fecal matter. The goal of this study was to test the method of fluorescent amplified fragment length polymorphism (FAFLP), as a means of water analysis for detecting the origin of the contaminating bacteria. In this study, 12 bovine, 5 feline, and 3 water isolates of *E. coli* were analyzed and compared to two lab strains. FAFLP was performed on the genomic DNA isolated from each sample. An unlabeled selective MseI forward primer (MseI + AT), and a FITC labeled nonselective EcoRI primer (EcoRI + 0) were used in the generation of polymorphic fragments by PCR. PCR products from each sample, and an internal lane standard, were analyzed on an ABI 377 sequencer using Genscan software. Genographer software (http://hordeum.msu.montana.edu/genographer/) was then used to identify unique polymorphisms among the samples, with fragments ranging in size from 100-500 bp. Using this approach a unique fragment (138 bp) was identified that was present only in the feline isolates. A 173 bp fragment was absent from bovine *E. coli*, but found to be present in non-bovine types of *E. coli*, (ie. Game range water sample, and feline isolates). Fragments of 194, 327, and 441 bp, unique to only wild *E. coli* isolates, were absent from laboratory strains of *E. coli*. A fragment of 435 bp, was unique to only laboratory strains, while a 443 bp band was unique to bovine *E. coli*. These results demonstrate that FAFLP can be used to detect the presence of polymorphisms that distinguish isolates of *E. coli* from diverse sources. The results also suggest that FAFLP may be useful in determining the host from which the bacteria originated.
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Publication

Problem and Issue Addressed:
Montana has a critical need to address sources of non-point source pollution and develop Total Maximum Daily Loads (TMDLs) and watershed scale plans for improving water quality for the many endangered streams on its 303 d list. In order to accomplish these improvements, it is critical that the public be educated about the nature of TMDLs, the issues related to TMDLs, how water monitoring and land use plans are conducted and the need for watershed based planning and management. Although there are specific educational programs on water, forestry, grazing, and mining, few if any spend much time on the direct connections between the aquatic and terrestrial parts of the watershed.

Project Objectives:
- To organize and conduct an after-school in-service teacher training and a unique one-day student field trip for forest watershed issues on a small creek in the Swan Valley of western Montana
- To use the above to create a model for an in-service training and Forest Watershed Issues field trip that illustrates and investigates the practical implications of watershed management including TMDLs, terrestrial monitoring techniques, and the land use decision making process for a small creek watershed.
- To provide a field trip which enables students to:
  a) describe what a TMDL is, why it is a critical issue in Montana today, why it is one concern for watershed management, and why the cumulative effects of non-point source pollution are a major contributor to TMDLs for Montana streams
  b) describe why in addition to water monitoring efforts, land use planning at a watershed scale is necessary to address the contribution of non-point source pollution to TMDLs
  c) describe at least one economic, social, political and ecological factor that contributed to the complexity of land use decisions made in the watershed's uplands, midlands, and lowlands
  d) explain the positive and negative impacts that land use decisions may have on the forest, range, wildlife, soils, and vegetation resources and non-point source pollution in the watershed.
  e) describe how BMPs might reduce potential non-point source pollution in the watershed.

Project Activities:
April 2000 - pilot tested a Forest Watershed Issues field trip in Missoula's Deer Creek create with the help of the Clark Fork Watershed Education Network and the Montana Natural History Center

Summer and early fall 2000 - worked with Swan Ecosystem Center to plan and implement a September pre-trip teacher in-service training and a revised Forest Watershed Issues field trip in the Swan River Watershed (Appendices A and B.)

October 2000 - shared the field trip format with teachers participating in the five-day Blackfoot Watershed Tour.

Winter 2000-2001
1. wrote a supplemental two-week Forest Management curriculum to broaden students understanding of their experience on the watershed issues trip (Appendix H).
2. developed a model for pre-trip in-service training and the watershed issues field trip that includes links to other programs, field trip planning considerations, schedules, maps,
worksheets, introduction and summary, station activity outlines, pre-trip and follow-up activity ideas (Appendices A-G.)

March 2000 to present - shared model with the Montana Environmental Education Association, the Missoula Curriculum Consortium, Forest Discovery Days, Project Wet, Teller Wildlife Refuge, and the Bitterroot Ecological Awareness Resources Cooperative.

Project Methods:

During the Forest Watershed Issues field trip:
1. Forty middle school students investigated land use issues for a small watershed in the Swan Valley through field investigations on upland road and forestry sites near Smith and Falls creeks, and lowland ranch and recreation sites along the Swan River.
2. Resource specialists told students at each site how a specific resource was used, the income obtained from that resource, the cost of measures that could be taken to reduce impacts to resources during that land use and resource protection laws and guidelines that must be followed for that resource.
3. Resource specialists helped students collect data and do an activity that illustrated and reinforced their points.

At the conclusion of the field trip:
Students reviewed what they had learned about social, political, and economic concerns and were asked to consider how these concerns contributed to the decision whether or not to follow Best Management Practices (BMPs) and how these practices have the potential to help reduce the current total maximum daily load (TMDL.)

After the field trip: -- Teachers helped students internalize what they had learned via one of several activities:
1. creating a poster presentation of land use issues in the watershed,
2. completing a giant puzzle activity which required students to know at least one social, political, economic, and ecologic concern that was addressed by the land use decision and how that decision contributed to or helped reduce the non-point source pollution, and
3. planning for future land use on the ranch they visited.

Details of program links, each field trip station, the schedule, student worksheets, and follow-up activities can be found in appendices A-G.

Principle Findings and Significance:
Teacher, resource specialist, and other educator evaluations of the field trip and student follow-up projects indicated that the field trip successfully met its goals.

Teacher comments:
"Keep up the good work and effective projects" and
"The more people we communicate with, will help us to help others to do the best or right things for the land"

Student comments:
"I learned about how much wood needs to be in the water and how the water level changes"
"I learned how much it takes to crown a road and how much a culvert costs"
"When camping leave no trace and make sure you aren't polluting the water"
"I did not know that you had to cut 50 feet away from the stream"
"Fish have a hard time going up culverts and a lot about logging"
"Trees affect water temperature more than I thought"
"Put a bridge in instead of a culvert"
Conclusions:
This model provided a unique field trip experience for several reasons.

- Resources specialist/educator teams helped resource specialists to:
  a) understand how to share their knowledge at a level the students could understand and in a time frame students could handle
  b) develop an activity that would illustrate and emphasize the importance of the information the resource specialist conveyed in their talk while also meeting school and environmental education standards (Appendix E.)
- Students received specific real-life examples of how both the costs of protection measures and the protection laws and practices affected the land owner's income and in turn their land use decisions that may or may not have followed the Best Management Practices that help reduce non-point source contributions to the TMDL of a watershed (Appendix D.)
- Students visited four different land uses
- The field trip introduction and summary reinforced how collaborative land use decisions and resource protection are part of the complex TMDL aspect of water shed planning that involves determining the contaminating factors, setting a total allocation for each cause, and determining remedies (Appendices C and F.)
**Recommendations:**
The team recommends that this model be distributed and implemented among as many resource specialists, conservation educators, and middle school teachers as possible to help improve the public's understanding of the complex TMDL process. However, in order to implement the model, existing learning centers such as the eleven included in the Montana Ecosystem Management Learning Center Program and other interested agencies will need to seek grants to provide staff time needed to locate suitable field trip locations and gather materials and pay for busses for the field trips. Once this model has been implemented at a site, less staff time will be needed for field trips since there will be less planning time needed.

Our thanks to the Montana University System Water Center for the funds that made the creation of *this Forest Watershed Issues Field Trip* model possible. We will continue to share this model and our field learning sites with other programs throughout Montana.
List of Appendices

Note: Appendices A-G compose The Watershed Issues Field Trip Model and Appendices A-H have been © 2001 University of Montana. All detailed Appendices are available in Report # 1.210 at the Montana University System Water Center.

A. In-Service Training Information
   1. Agenda
   2. Chart - Links Among Programs and Standards
   3. Pre-Trip Classroom Activities
   4. Pre-Test & Answer Key for Field Trip
   5. General Pre-Trip Information For Resource Specialist Presenters

B. Field Trip Information
   1. Planning considerations
   2. Schedule
   3. Maps

C. On-Site Field Trip Introduction

D. Important Factors to Consider During Watershed Planning, Protection, and Management
   - Station Leaders and Teacher Key and Student Worksheet

E. Field Trip Station Descriptions
   1. Forestry/Trees Station
      Alternate Forestry Activities
      Supplemental Forestry Activities
   2. Roads/Soils Station
      Alternate Roads/Soils Activities
      Supplemental Soils Activity
   3. Recreation/Riparian Station
      Alternate Recreation/Riparian Activities
      Supplemental Riparian Activities
   4. Ranching/Other Vegetation Station
      Alternate Grazing/Range Activities
      Supplemental Range Activity

F. On-Site Field Trip Summary
   Alternate On-Site Field Trip Summary Activity

G. Post-Trip Classroom Activities
   Alternate Post-Trip Classroom Activity

H. Draft Forest Management Unit Outlines
Basic Information

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Publication

1. Progress Report
Problems and Research Objectives

Septic systems have been shown to contaminate groundwater at a variety of locations. Nutrients and pathogens have been identified as parameters that limit water quality for some streams in the Gallatin Valley, and residential rural subdivision development has been identified as a potential source of this contamination. The nutrient and microbial load delivered to water-quality impaired streams by shallow groundwater that flows beneath subdivisions has not been directly measured. To date, the traditional approach used to assess contamination from septic systems has been to examine countywide trends in groundwater quality, and trends have been elusive. The Montana Department of Environmental Quality (DEQ) has suggested a reexamination of subdivisions studied by Peavy et al. in 1980 to determine

- if groundwater quality has been degraded below and down flow of subdivisions and
- whether the Bauman-Schafer and phosphorus breakthrough methods (currently used for subdivision review and non-degradation assessment) reasonably predict impacts of development on groundwater quality.

While the DEQ’s proposed reexamination is interesting, the original research wells are gone, homeowner cooperation is not assured, and the study areas would require significant travel. An alternative approach is to examine groundwater below mature subdivisions (1970’s-vintage) in the Gallatin County Local Water Quality District (LWQD), which are thought to contribute nutrient and/or coliform to streams. Such a study could be used to answer several important questions of interest both to the state of Montana and to the LWQD.

1) Is microbiological contamination dominantly from poor well seals, septic contamination, or other sources?
2) Are coliform-contaminated or nutrient-contaminated wells on a flow line that contains one or more septic systems?
3) Are wells that are contaminated with nutrients also contaminated microbiologically?
4) Is the contamination level and type (nutrient/coliform) dependent on the texture of the sediments below the subdivision?
5) What nutrient and coliform loads enter and leave a subdivision area via the groundwater, and what are the likely loads being delivered to water-quality impaired streams in the LWQD?

6) Do the Bauman-Schafer and phosphorus breakthrough models correctly predict observed concentrations of nutrients below mature subdivisions?

Methods

Subdivision Selection

Subdivisions were selected as candidates for the project based on the following parameters and ranking criteria: 1) proximity to a water-quality impaired stream, 2) maturity, 3) shallow groundwater depth, 4) proximity to public groundwater supply, 5) previous contamination history, and 6) the absence of other subdivisions on septic systems directly up flow. Once the list of candidate subdivisions was compiled, the subdivisions were grouped according to substrate material, either coarse or fine. Staff from MSU, LWQD, DEQ, and EPA helped in the final selection process. One subdivision was selected as a representative of each group. The remaining subdivisions were rank ordered as back-up research sites if needed.

Obtaining permission from the majority of the homeowners was the final step in the selection process. This involved door-to-door inquiries and form surveys. A brochure with relevant information pertaining to the study, complete with contact numbers, was also developed and hand delivered.

Subdivision Characterization

Well and septic system locations, hydrogeology, and land-use history (mainly determined by visiting with the homeowners and general on-site observation) are the main factors in subdivision characterization. Well and septic system locations were found at the county health department by using septic system permits.

Once the locations of the well and septic systems were determined, either from the septic system permits or by asking the homeowner, GPS locations for each septic system and well, with permission, were mapped. These maps were then used for the hydrogeology characterization.

The hydrogeology characterization was done through well logs, water-level information, and the geomorphology of each subdivision. Water-table elevations were made using the water levels, the GPS-produced ARC View maps, and survey data.
Subdivision Sampling

Two sampling rounds were done, one at low ground water and one at high ground water. Each well, with permission, in the two subdivisions was sampled for pH, specific conductivity, dissolved oxygen, temperature, and pressure using a probe calibrated daily. Also, two sample bottles were filled from each well to be sent to the lab for analysis of total coliform (if present, then also for *E. coli*), chloride, total ammonia as nitrogen, nitrate plus nitrite as nitrogen, and total phosphorus.

The installed well points (up flow, mid flow, and down flow) were also sampled twice. Two different types of wells were installed. Three of the wells were two inch steel well points, and a bailer was used to sample these, removing three well volumes prior to sampling. Nine of the wells were one inch PVC, and these were sampled using a foot-valve to remove three well volumes prior to sampling.

The surface water was sampled at the same time the ground water was sampled. For each stream in the subdivisions, an up flow, a midflow, and a down flow sample were taken along with one discharge measurement per stream.

Analysis

Data is being interpreted in terms of spatial and temporal relationships between well and septic location, hydrogeologic setting, and contamination type and source. The potential effect of travel time on patterns of contamination is being assessed, and expected nutrient loading is being calculated based on available data using the Bauman-Schafer model and a phosphorus breakthrough analysis. Nutrient and microbial loads delivered from subdivisions to water-quality impaired streams via the groundwater flow system are being evaluated.

Progress Report

Subdivision Selection (100% complete)

A meeting was held in January 1999 in Helena with the relevant personnel, and the subdivisions were rank-ordered. For the coarse-grained substrate, Middle Creek Meadows (#2 and #3), located at Four Corners (T2S, R4E, section 13) was selected, and for the fine-grained substrate, Gardner Park and Sourdough Subdivisions, located south of Bozeman, specifically for the area located in between Sourdough Creek and Limestone Creek, were selected (T2S, R6E, section 30).

Subdivision Characterization (100% complete)
All of the wells and septic systems have been mapped by GPS. The water levels have been measured from the sampling round, and the water levels in the monitoring wells are still being monitored. A survey of the wellheads was done to more accurately figure water table elevations.

**Subdivision Sampling** (99% complete)

All sampling has been done except for two of the monitoring wells, which need to be sampled for phosphorus to compare filtered versus unfiltered numbers.

**Analysis** (50% complete)

Analysis is being done on the data to test the hypotheses listed as research objectives. No obvious contamination patterns have been found.
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Publication

ANNUAL PROGRESS REPORT:

1) ABSTRACT
Rapid growth in the west-Billings area has caused an overall change in land use from irrigated cropland to subdivisions. Characterizing surface- and ground-water conditions are needed to evaluate affects of ground water quality and availability as a result of rapid urbanization. Residences in the west-Billings area are not serviced by public utilities and depend solely on ground water from the shallow alluvial aquifer for potable water supplies and septic system/drainfields for sewage disposal.

Distribution of geologic units, and recharge rates are the major factors controlling the flow and quality of ground water in the valley. The Yellowstone River alluvium and terraces form the major aquifer system in the valley. The aquifer system is composed of relatively permeable sand and gravel. Aquifers underlie terrace surfaces stepping up from the modern course of the Yellowstone River. Each progressively higher terrace surface represents an older alluvial floodplain. Former channels have been identified that contain thick potentially high yield aquifers. Terrace scarps are associated with low yield parts of the aquifer and in several areas do not provide enough ground water for domestic uses. In most areas, these scarps form restrictions or even barriers to ground-water flow. Thick plugs of fine-grained materials have been identified near Canyon Creek and Hogan Slough. These areas of thick fine-grained deposits have a significant impact on ground-water flow and quality.

Hydrologic balance data, chloride concentrations, and $^{18}$O and $^2$H analyses indicate that ground-water recharge in the project area is derived primarily from agricultural irrigation. Recharge from precipitation is relatively minor. $^3$He/$^3$H analyses indicate that the age of the sampled ground water ranges from less than one-year to 32 years. The age data correlates with recharge and TDS concentrations.

In areas where applied irrigation water rapidly infiltrates to the ground-water the ground-water quality approached that of the Yellowstone River which is a low TDS bicarbonate-dominated water. This low TDS composition is achieved through dilution from the irrigation water. Where recharge water must infiltrate through unsaturated and saturated fine-grained sediments before reaching the gravel alluvium, its composition evolves to a high TDS sulfate-dominated water. $^{34}$S and $^{13}$C analyses suggest that the evolution is controlled by sulfide oxidation and soil-carbonate dissolution in the fine-grained sediments. The fine-grained sediments range from 0 to over 100 feet thick and are primarily derived from calcareous sulfur-bearing marine shale washed down from the surrounding uplands.

Nitrate concentrations range from <0.5 mg/l to over 20 mg/l. Isotopic and mass balance data suggest that most nitrate in the project area ground-water is likely from fertilizers or soil organic matter from agricultural areas. Septic systems account for a small but growing proportion of the nitrate regionally. On a local scale nitrate impacts can be much more significant.

The primary impact of land use change in the project area is the loss of high quality recharge water from agricultural irrigation. Ground-water under residential subdivisions receive a higher proportion of recharge from lawn irrigation (recycled ground-water) and septic returns. Both sources provide considerably less volume and poorer quality of recharge.
**Basic Information**

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**Publication**

Progress Report

Tritium/helium-3 ($^{3}$H/$^{3}$He) and chlorofluorocarbon (CFC) methods are being used in an attempt to age date ground water in the Missoula Valley aquifer. Sixteen wells along transects of ground-water flow were sampled for tritium and noble gases. The results obtained to date show that much of the ground water has excess terrigenic helium which complicates the $^{3}$H/$^{3}$He dating procedure. Further work will focus on clarifying the source and amount of excess helium with the hope of determining a correction factor to allow $^{3}$H/$^{3}$He dates to be obtained. Twelve of the wells were also sampled for CFCs; ten of those ground-water samples contained CFCs in excess of air-water solubility rendering them unsuitable for age dating. The highest CFC concentrations were detected immediately downgradient from areas of high septic tank density suggesting that septic effluent is a potential source of the excess CFCs.

Problems and Research Objectives

The objective of the proposed research is to evaluate the tritium/helium-3 ($^{3}$H/$^{3}$He) and chlorofluorocarbon (CFC) methods for dating young ground water (0.1–45 years old) with the aim of improving the technical basis for ground-water protection in intermontane alluvial aquifers affected by population increases and land-use changes. The Missoula Valley was chosen because considerable data are available concerning the ground-water flow and ground-water quality for the Missoula Valley aquifer; there are a variety of land uses near Missoula; and land-use patterns have changed over the past 10 years and are likely to continue to change.

Methods

Tritium-Helium and CFCs

Measurement and interpretation of tritium-helium isotopes and CFCs are relatively new techniques that allow direct dating of single ground-water samples. Tritium ($^{3}$H), the radioactive isotope of hydrogen with a half-life of 12.43 yr, is produced naturally in the upper atmosphere and decays to the noble gas helium-3 ($^{3}$He). As water enters the ground-water system and becomes isolated from the atmosphere, $^{3}$He concentrations will increase as ground water becomes older. By determining the amounts of tritium and tritiogenic $^{3}$He in a ground-water sample, an age can be calculated according to the relationship:

$$t = t_{1/2} \ln 2 \times \ln \left(1 + \frac{^{3}\text{He}_{\text{int}}}{^{3}\text{H}}\right)$$

where $t$ is the tritium-helium age, $^{3}\text{He}_{\text{int}}$ is the tritiogenic helium-3 in the sample, $^{3}\text{H}$ is the tritium concentration, and $t_{1/2}$ is the tritium half-life. Ratios of tritium to helium-3 have been used to accurately date shallow ground water with ages ranging from a few months to 50 years.

Chlorofluorocarbons (CFC-11, CFC-12, and CFC-113) are human-made organic compounds first produced in the 1930's. Atmospheric concentrations of CFCs are uniform over large areas and have steadily increased since the 1940's. The atmospheric input of CFCs to ground water can be determined for most localities. Once in ground water, these compounds are soluble and stable. CFC ages are determined by converting CFC concentrations in ground water
to equivalent air concentrations using known solubility relationships and recharge temperature. Limitations to the method include reducing conditions in aquifers that can degrade CFCs in ground water and non-atmospheric sources of CFCs. Under optimal conditions CFCs can be used to estimate ground-water age with about a 1- to 2-year accuracy.

The Missoula Valley aquifer underlies, and is the sole source water supply for the city of Missoula. It is unconfined and composed of 100 to 150 feet of Quaternary sand, gravel, and cobbles. Ground-water generally flows away from the valley margin on the north toward the flood plain in the center of the valley. South of the Clark Fork River ground water flows southwest toward the Bitterroot River and its confluence with the Clark Fork River. The aquifer is recharged primarily by leakage from the Clark Fork River and underflow through Hellgate Canyon as well as precipitation and inflow from deeper units; water leaves the aquifer as base flow to streams, as evapotranspiration, and as pumpage from wells.

During the summer and fall of 1999 and the spring of 2000, sixteen wells were sampled for noble gases and tritium, twelve of the wells were also sampled for CFCs. Most of the wells were monitoring wells, but two of the wells were commercial wells (serving a business) and one well was part of a heating and cooling system on the University of Montana campus. The tritium, noble gas and CFC analyses are being performed by the University of Utah Isotope Geochemistry Laboratory. At this time tritium and noble gas results from about half of the wells have been received; the CFC analyses are complete.

**Principal Findings and Significance (Progress Report)**

The geologic setting and land-use activities in the Missoula Valley are complicating the interpretation of the $\text{³H}/\text{³He}$ and CFC results. Twelve wells located along transects of groundwater flow were sampled for CFCs. All but two of the samples came back with excess CFCs (the CFC concentration was greater than what could be accounted for by equilibrium dissolution from the atmosphere) indicating that the ground water had been contaminated with CFCs. The excess CFCs rendered the samples unsuitable for age dating. The areal distribution of the CFCs in the aquifer suggests that septic effluent may be a potential source. The highest CFC concentrations (CFC-12: 19 to 77.7 pmole/kg) were detected immediately down gradient from areas with high septic tank densities, ground water beneath the sewered areas had much lower concentrations (CFC-12: 2.9 to 7.9 pmole/kg).

Interpretation of the $\text{³H}/\text{³He}$ ages is being complicated by the presence of relatively large amounts of excess helium (helium in excess of air-water solubility) in the ground water. The results to date show that all of the wells sampled in the Missoula Valley aquifer have tritium (8.7 to 13.2 tritium units), however the presence of excess terrigenic helium has hindered successful dating by the $\text{³H}/\text{³He}$ method. The source of excess helium is not clear, potential sources include the mantle or aquifer matrix material.

**Ongoing Work**

In the fall of 2000, samples for noble gas analysis were collected from three wells around
the margin of the valley; two of the wells are completed in deep alluvium and one of the wells is completed in Belt bedrock. Each of these wells had been sampled previously for tritium and the concentrations were below detectable levels. In addition, four wells in the Missoula Valley aquifer were resampled for noble gases using diffusion samplers in the spring of 2001. Total gas pressure was measured in-situ at each well at the time the samplers were retrieved to obtain more precise measurements of gas pressure and therefore, more accurate determinations of gas concentrations in the ground water. The results from the above analyses will provide an indication of background noble gas concentrations in the valley and more accurate measurements of noble gas concentrations in the Missoula Valley aquifer which hopefully can be used to refine the ground-water ages.
## Basic Information

| **Title:** | Development Of Microbial Biobarrers For The Control Of Acid Rock Drainage From Mine Tailings. |
| **Project Number:** | B-04 |
| **Start Date:** | 3/1/2000 |
| **End Date:** | 2/28/2001 |
| **Research Category:** | Water Quality |
| **Focus Category:** | Acid Deposition, Geochemical Processes, Groundwater |
| **Descriptors:** | Acid Mine Drainage, Biobarrers, Biostimulation, Water Chemistry |
| **Lead Institute:** | Montana State University |
| **Principal Investigators:** | Paul Sturman |

## Publication
Progress Report - Abstract

Acid rock drainage from hard rock mine lands is a major environmental problem that impacts both ground- and surface water throughout the Western US. Waste rock and mine tailings often contain high concentrations of metal sulfides which, when contacted by oxygen-containing water, leach metals into solution and radically lower the pH of the receiving water. This research sought to further develop an inexpensive and potentially widely applicable treatment technology to utilize indigenous microorganisms within mine tailings to abate ARD at its source. An innovative method to prevent oxygen transport into tailings is the development and maintenance of a biologically active barrier within the near-surface zone of the tailings. This barrier is made up of naturally occurring aerobic and facultative bacteria which utilize dissolved oxygen in the infiltrating water and therefore maintain the reducing conditions which are necessary for pyrite to remain bound in mineral form. In addition, the generation of anoxic conditions stimulates the activity of sulfate-reducing bacteria (SRB) within the tailings. SRB produce hydrogen sulfide as a metabolic by-product, which may further react with dissolved metals, promoting their removal as metal-sulfide precipitates. This research included laboratory experimentation with columns filled with acid-producing mine tailings. Objectives for the research were to 1) confirm the ability of nutrient dosing to stimulate indigenous aerobic and facultative bacteria in mine tailings, 2) determine the extent to which effluent dissolved metals can be reduced, 3) determine the longevity of treatment effectiveness, and 4) perform initial work necessary to incorporate these research findings into an appropriate mathematical model. Bacterial populations, particularly general heterotrophic bacteria and SRB, were stimulated in molasses- and whey-treated columns compared to control columns. Column effluent pH was increased from approximately 3 to 5 as a result of carbon addition. Effluent aluminum, zinc and copper were reduced in treated columns relative to controls. The effectiveness of whey treatment and molasses treatment were compared. Whey was found to be more effective in reducing effluent pH and stimulating favorable bacterial populations. Molasses treatment enhanced the growth of fungi in some cases, which could have an unfavorable effect on pH and metals in solution. Initial modeling work has identified an appropriate mathematical model, into which the results of this work will be incorporated.

Publications/Citations
This work has not yet been submitted for publication

Student Support
This project constitutes a portion of the PhD thesis work for the principal investigator.
Basic Information

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Publication

   (http://ibscore.dbs.umt.edu/clarkfork)
Abstract:

Since 1985, Clark Fork River basin scientists and citizens have convened every 5 years to discuss ongoing research and management of the Clark Fork basin. In recent years, a number of large scale restoration initiatives have begun to take shape in the basin, so the year 2000 symposium focused on these restoration initiatives as well as many smaller restoration efforts. The goals of the symposium were to:

1) Provide a State of the River assessment.
2) Clarify the goals and objectives of each of the major restoration initiatives with respect to the overall health of the river system;
3) Identify areas of potential conflicts & collaboration between the various efforts;
4) Provide a forum for sharing experiences on river restoration science and projects between government scientists, consultants, watershed groups and other interested citizens.

The Symposium was attended by over 100 participants. Following a State of the River report developed by Vicki Watson of University of Montana and Velda Welch of the Montana State Library, five restoration initiatives were presented (Natural Resource Damage Program on the upper Clark Fork, the Voluntary Nutrient Reduction Program on the upper & middle river; dam mitigation efforts on the lower river; and basin wide fishery & ecosystem restoration programs of the Mt Fish, Wildlife & Parks and the US Forest Service, respectively). These initiatives were critiqued by a panel of basin scientists & citizens. A poster session with over 50 posters on restoration projects and studies was well attended, as was a series of field trips to various restoration projects or businesses. The proceedings of the symposium were published online. In addition, with permission of the Montana Academy of Sciences, the proceedings of the earlier Clark Fork symposia were also published online to increase their availability to the public.

Citations

See the online conference proceedings (http://ibscore.dbs.umt.edu/clarkfork) which includes abstracts or full papers or power point presentations for over 50 poster papers, 5 restoration initiative papers and the panel report.

Student support

WRC funding was used to pay students who helped put on the symposium and helped put the proceedings on line. Some students also volunteered considerable time in this effort, most notably Ryan Alter who continues to manage the web page even after funding ended.

Over 30 students presented at the symposium or have since posted papers on the web site, including students from several universities and high schools.

Achievements and Awards

The symposium has helped to spark much collaboration on restoration planning & action in the basin. Symposium participants have collaborated on successful proposals for restoration related projects and have continued to communicate concerning restoration vision documents and data & analysis needs for restoration planning. The symposium web page serves as a method of quickly disseminating the results of restoration related studies to the public and scientific community.
Spinoffs of the symposium are too numerous to catalog, so just a few examples will be briefly discussed. The University of Montana is currently funded by the Montana Natural Resource Damage Program to work with the Montana Natural Resource Information System to identify information needs and gaps for watershed restoration planning and action with a view to developing a proposal to build a database to house watershed restoration related data. Several University of Montana students are being funded by the Natural Resource Conservation Service and the Montana Department of Fish, Wildlife and Parks to conduct pre & post restoration assessments of streams that will undergo restoration projects. Symposium participants collaborated with the Governor’s NRDP advisory committee which is in the process of developing a Restoration Work Plan for the upper Clark Fork. These are exactly the sorts of spinoffs hoped for when the Symposium was planned.

The Symposium web page continues to be maintained and expanded.
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Publication

Abstract – Progress Report

MTBE is degraded very slowly in the subsurface relative to other gasoline constituents. Previous research reports indicate that indigenous microbial populations may biodegrade MTBE and TBA at very low rates and after extended acclimation periods. Therefore, dissolved MTBE plumes in groundwater may impact larger areas compared to other gasoline constituents. 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. Research team members collected field data and used chemical and environmental parameters to model degradation of MTBE and plume dynamics at a gasoline release site in Ronan, Montana. All pertinent historical, hydrogeological, and geochemical data were reviewed, and a limited characterization of the site microbial population was performed. This characterization focused on enrichment, isolation, and identification of bacterial strains and consortia that could biodegrade MTBE and its metabolites (e.g. TBA). This study included an educational component, in-depth scientific and engineering components, and a political/management component. Under the auspices of a graduate level class in Environmental Engineering (educational component) and with assistance and guidance from regulatory professionals within the Montana Department of Environmental Quality (political/management component), research advances to date support the following statements:

- A bacterial consortium capable of MTBE biodegradation is present in the subsurface at the Ronan, MT gasoline release site.
- MTBE-degrading isolates degraded MTBE at a higher rate in the presence of a co-substrate (2-propanol), indicating a possible relationship to MTBE-degrading, propane-oxidizing bacteria. MTBE metabolites, TBA and TBF, accumulated as MTBE was biodegraded.
- Genotypic characterization of the MTBE-degrading consortium indicated that at least five bacterial species initially contributed to MTBE degradation. However, over time under the selective pressure of MTBE as the sole carbon source, it appeared that Rhodoferax fermentans became the dominant species. Also, no metabolites were observed.
- Although an MTBE-degrading consortium was obtained from the site, the relatively low MTBE degradation rate observed in the laboratory indicates that microbial biodegradation is of minor importance to overall natural attenuation of MTBE in the field.
- Modeling efforts were somewhat successful in predicting MTBE plume dynamics.

Research advances made through this project have provided a mechanism to assess the biodegradation potential at another MTBE release site near Manhattan, MT. Enrichment cultures for MTBE-degradation from this site were initiated using protocols established with samples from the Ronan site. Experimental data suggest that there is also an MTBE-degrading consortium present at the Manhattan release site.
Research Objectives

The primary goal of this research project was to assess the biodegradation potential of MTBE and to determine what environmental factors may be limiting MTBE degradation at the gasoline release site near Ronan, MT. This work included a review of pertinent hydrogeological and geochemical data and characterization of any MTBE-degrading microbial species that could be identified and/or isolated through enrichment cultures. The objectives of this multi-faceted study included an educational component, in-depth scientific and engineering components, and a political/management component. Educational aspects of the project involved field site assessment, data collection, and transport modeling experience for interdisciplinary students enrolled in an MSU environmental engineering class. The political component of the project was designed to bring students into direct communication with consulting engineers and Montana Dept. of Environmental Quality (MDEQ) personnel both in the field and by presenting their findings in a workshop format of open discussion of logical management options. The MTBE release site was identified through the MDEQ-Underground Storage Tank Program as a threat to a pristine surface water receptor. In order to meet the overall goal, the research was planned to meet the following four general objectives:

I. Site Data Review
- Review of existing 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 was used to enhance interpretation of laboratory results and to allow site-specific recommendations for remedial action.

II. Field Sampling and Data Collection
- Collection of groundwater and/or soil samples from existing or subsequently installed monitoring well locations. Establishment/confirmation of existing plume dimensions. Samples were indexed and cross-referenced with geochemical conditions present at each sampling location.
- Analysis of site groundwater data for evidence of natural attenuation of MTBE, degradation products, and other petroleum compounds.

III. Laboratory Experimentation and Modeling
- Development and/or substantiation of gas chromatographic methods for analysis of MTBE and its known metabolites for monitoring MTBE degradation in enrichment cultures.
- Development of aerobic MTBE-degrading enrichment cultures using microcosms with minimal growth media and composite soil samples as microbial inocula.
- Identification of possible nutrient limitations (e.g. nitrogen, phosphorus, dissolved oxygen) and environmental limitations (e.g. temperature) to MTBE biodegradation at the field site.
- Identification of possible hydrocarbon co-substrates (e.g. propane) that may enhance MTBE biodegradation.
- Isolation and identification of specific MTBE/metabolite-degrading bacteria.
- Application of BIOSCREEN and MODFLOW models to determine predictive capabilities regarding existing plume dimensions, contaminant concentrations, etc.
IV. Education and Technology Transfer

- Organization of components of the laboratory and field research into a graduate level class in Environmental Engineering.
- Development and delivery of annual workshops/discussions on MTBE fate and transport in groundwater.

Methodology

Site Characterization

The release site is located at a fuel station east of U.S. Highway 93, one mile south of Ronan, MT. A 16,000-gallon underground storage tank removed in April, 1994 was estimated to have released approximately 10,000 gallons of gasoline over a one-year period. Historical data were retrieved to estimate initial MTBE concentration in the gasoline to aid in subsequent plume modeling efforts by students involved in the engineering class. The Montana Department of Environmental Quality established 20 monitoring wells at the site to identify plume boundaries, and eight additional wells were installed in 1997 for free product removal. A thorough review of reports containing several years of quarterly monitoring well data was performed. These reports originated from consultants (MSE; Butte, MT) for the Montana Dept. of Environmental Quality and contained complete gasoline contaminant concentrations as well as other pertinent chemical analyses, including dissolved oxygen.

The maximum depth to groundwater is approximately 15 ft. at the point of release. The dominant lithology identified from bore holes was silt and fine sand with scattered clay lenses, typical of lakebed deposits common to the area. Active remediation at the site included a combination of passive recovery skimmers, air sparging systems coupled with soil vapor extraction, and an interceptor trench.

Enrichment Study Inocula

Soil and aquifer materials used as inocula for MTBE-degrading bacterial enrichments were collected from two bore holes drilled to a depth of 1 m below the groundwater surface. One bore hole was near the down-gradient edge of the NAPL-phase gasoline plume at M-12 and the second was adjacent to M-19 near Spring Creek (the impacted surface water receptor). Samples were retrieved from above, at the interface with and below the groundwater surface, and were stored at 4°C for one week prior to inoculation in enrichments. A pristine agricultural soil (Bozeman, MT) was also used as an inoculum in several “control” enrichments.

Enrichment of MTBE-degrading bacteria

Degradation experiments were conducted in closed 125-mL Erlenmeyer flasks under aerobic conditions. The flasks contained 25 mL of a minimal medium (SSE) designed to simulate a “typical” soil solution and contained NH₄NO₃ (1.25 mM), CaSO₄ (2 mM), MgCl₂ (2 mM), KH₂PO₄ (10 uM), KOH (1.25 mM), FeCl₂ (5 uM), supplemented with 100 uL L⁻¹ of micronutrient solution. Subsamples of the composite soil/aquifer material were used as inocula (1% w/v) for the MTBE-degrading microcosms. The slurries were spiked with 10 mg L⁻¹ MTBE and ~ 100,000 dpm flask⁻¹ [¹⁴C]-MTBE. Radiolabeled [¹⁴C]CO₂ evolved from the slurries was captured and used to track MTBE degradation. Solutions of 0.5 M NaOH (0.3 mL) were utilized as [¹⁴C]CO₂-traps and were placed in cups suspended from the stoppers. The enrichment traps were analyzed weekly for [¹⁴C]CO₂ (scintillation analysis), and the aqueous phase was monitored for...
MTBE disappearance using gas chromatography (GC). Treatments were tested in triplicate and compared to autoclaved controls that contained 250 mg L\(^{-1}\) HgCl\(_2\).

Due to headspace losses of MTBE by volatilization during sampling of the base traps, subsequent kinetic degradation experiments with the consortium RS24 were performed in 120-mL serum bottles with sealed teflon-coated septa. MTBE was introduced into 40 mL of SSE medium at concentrations ranging from ~10-70 mg L\(^{-1}\) and 2-mL samples were aseptically taken for GC monitoring of MTBE degradation. At each sampling time, 3 mL of sterile air was also injected into the serum bottle headspace to maintain aerobic conditions.

**Gas chromatography**

Gas chromatographic analysis of 2-propanol, MTBE, TBA, and TBF was conducted using a Hewlett-Packard 5890 Series II gas chromatograph with a FID detector. A Porapak PS 80/100 mesh packed column (2 mm I.D. x 1.22 m glass; Supelco, Bellefonte, PA) was used with a He carrier gas flow rate of 18 mL min\(^{-1}\) at 100ºC and a head pressure of 28 psi. The injector temperature was set at 190ºC and the detector temperature was 250ºC. The initial oven temperature was set at 100ºC for 4 min. and ramped at 10ºC min\(^{-1}\) to a final temperature of 200ºC. 2-propanol, TBA, MTBE, and TBF peaks showed retention times of approximately 2.7, 4.5, 6.3, 8.3 min., respectively.

**Microbial cell counts**

0.5-mL samples of MTBE-degrading cultures were placed above a 0.2-µm, 25 mm-dia. black polycarbonate membrane (Poretics Products; Livermore, CA) clamped in a filter chimney assembly and vacuum manifold apparatus. A 100-µL aliquot of 10 mg L\(^{-1}\) DAPI was added, allowed to stain the cells for 2 min. and rinsed 2-3 times to remove any unbound stain. The direct counts were conducted using a Nikon Plan Apo100x/1.40 oil lens (DIC H, WD 0.13, ∞/0.17) and a Nikon Eclipse E800 epifluorescence microscope with a mercury lamp UV source. The filter block used had an excitation bandwidth of 340-380 nm and an emission bandwidth of 435-495 nm. Total cell counts were based on a calibrated ocular grid, calculated total membrane area, and an average of 20 enumerated grid fields.

**Isolation of MTBE-degrading bacteria**

Soil-derived, MTBE-degrading enrichments were sampled and spread on both R2A and Noble agar (with basal salts) plates. Half of the Noble agar plates also received surface-applied treatments of 50 µL of 20 mg L\(^{-1}\) 2-propanol prior to inoculation with bacteria. All of the Noble agar plates were incubated in closed metal containers and exposed to MTBE vapors by diffusion from MTBE-saturated filter paper. Single colonies from both types of plates were purified through repeated transfer under identical cultivation conditions. Colonies chosen for isolation were based primarily on differences in colony color and size. Isolated colonies were used to inoculate 120-mL serum bottles containing 40 mL of SSE amended with 10 mg L\(^{-1}\) MTBE. 2-propanol (20 mg L\(^{-1}\)) was added as a cosubstrate in selected treatments. Gas chromatography was used to monitor MTBE disappearance and accumulation of degradation products.

**Nucleic acid extraction, polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and sequence analysis**

Cell lysis and DNA extraction protocols were based on established methods. Briefly, cells were lysed by mechanical disruption at 6.5 m s\(^{-1}\) for 45 s using a beadbeater (Savant...
Instruments, Qbiogene; Carlsbad, CA). The crude cell lysates were precipitated with 12M ammonium acetate, and the DNA in the resulting supernatant was precipitated with isopropanol. PCR was performed using primers shown to be highly conserved for numerous bacterial lineages, including the proteobacterial, Gram-positive, cyanobacterial, green nonsulfur, and Cytophagales-Flavobacterium-Bacteriodes lineages; and they amplify a hypervariable-containing rDNA region from nucleotide positions 1070 to 1392, based on the 16S rRNA sequence in *E. coli*. DGGE was performed using a 6-11% concentration gradient of acrylamide in addition to a urea/formamide gradient. DGGE bands were purified for sequence analysis, and band purity was confirmed by comparing the PCR products on a DGGE gel to the parent community. Sequencing of isolate DNA and DGGE bands was performed on an ABI Prism 310 Genetic Analyzer using a 47-cm capillary (PE Applied Biosystems, Foster City, CA) and Prism BigDye terminator cycle sequencing reaction kits (PE Applied Biosystems, Foster City, CA).

**MTBE plume modeling**

MTBE plume modeling efforts by engineering students involved the use of two groundwater models, BIOSCREEN and MODFLOW. Inputs to the models included parameters such as initial MTBE gasoline concentration, partitioning coefficients, groundwater flow rates, contaminant solubility, biodegradation rates, and amounts of free product associated with the site. Although some of these parameter values were based on laboratory experimental data, some of the parameter values were estimated.

**Research Results**

**Soil-derived enrichments**

A 12-treatment matrix of enrichment cultures amended with 10 mg L\(^{-1}\) MTBE (cold) and \(^{14}\)C-MTBE as described was established to assay the presence of MTBE-degrading bacteria at the gasoline-contaminated site. The treatment matrix consisted of three inoculum types (M-12, M-19 and pristine) and two levels of nutrient amendment (1x and 10x SSE) (6 treatments). In addition, 2-propanol was added to an additional set of 10x SSE-treated inocula (3 treatments), and there was one set of killed controls (3 treatments). One flask (RS24) inoculated with soil from M-12 and amended with 2-propanol displayed significant MTBE degradation compared to sterile controls and other treatments. After an initial lag time of approximately 15 days, \(^{14}\)CO\(_2\) analyses indicated a maximum of 33% MTBE degradation after 35 days. Other treatments displayed only minimal degradation over the 35-day trial relative to the sterile controls. Analysis of aqueous phase \(^{14}\)C-MTBE revealed significant losses from all enrichments, apparently due to headspace losses of volatilized MTBE while sampling the base traps. To minimize these losses, all subsequent experiments were performed in sealed serum bottles.

**MTBE degradation by consortium RS24**

During a subsequent 146-day period, the initial MTBE-degrading enrichment was enriched further (10% v/v inoculum transfer) with 14 mg L\(^{-1}\) MTBE and approximately 20 mg L\(^{-1}\) 2-propanol. Interestingly, the 2-propanol had been completely utilized after 7 days and small TBA and TBF peaks were evident. At the end of the second week, the TBA and TBF peaks were still evident; however, by the end of the third week no metabolite peaks were observed. Over this 3-week period, the MTBE concentration had decreased to 12 mg L\(^{-1}\) and continued to decrease steadily at a rate of about 0.1 mg L\(^{-1}\) d\(^{-1}\). After this 146-day trial, another transfer (10%
v/v) was made and amended with 6 mg L\(^{-1}\) MTBE without the addition of 2-propanol. An extended lag time (~18 days) was again observed before significant degradation occurred. However, with subsequent addition of MTBE on days 40, 47 and 53 the onset of degradation became increasingly rapid. As spiked amendments of MTBE increased from 10.3 mg L\(^{-1}\) to 14.7 mg L\(^{-1}\) to 66.4 mg L\(^{-1}\), no significant decrease in degradation rate was observed. Beginning on day 47, MTBE disappearance was monitored daily and MTBE degradation rates of 0.15 mg d\(^{-1}\) and 0.66 mg d\(^{-1}\) were calculated from days 47-53 and days 53-57, respectively. Again, no metabolite peaks were observed in any of the chromatograms. In addition, the culture became noticeably more turbid over this time period suggesting microbial growth was coupled to MTBE degradation. To correlate MTBE disappearance with microbial growth, bacterial cell density was calculated for each sampling time from direct microscopic counts as described above. Increasing bacterial cell density during MTBE degradation in duplicate transfer cultures (10% v/v) of RS24 was observed. A gross estimate of biomass yield based on maximum MTBE utilization rates represented in the graphs was calculated. Assuming an approximate dry mass of 2 x 10\(^{-10}\) mg cell\(^{-1}\), a yield of 0.25 ± 0.02 mg dry biomass mg MTBE\(^{-1}\) was observed.

A significant lag phase was repeatedly observed upon transfer of active MTBE-degrading cultures of RS24 to fresh medium. However, in re-spiking active cultures with MTBE no lag time was observed. To determine if a growth-related factor in the medium may be involved in the degradation of MTBE by RS24, degradation experiments were conducted with spent culture medium and fresh SSE mineral medium. Two actively growing duplicate MTBE-degrading cultures of RS24 were centrifuged at 8,000 rpm for 10 min at 4°C. The supernatant (i.e. spent medium) was decanted and saved and the cell pellets were re-suspended in 3 mL of 1x PBS. Aliquots (1 mL) from each cell suspension were used as inocula for duplicate cultures with 40 mL spent growth medium, and 2-mL aliquots were used as inocula for duplicate cultures with 40 mL of fresh SSE medium. The four new cultures were amended with MTBE to a final concentration of 32 ± 6 mg L\(^{-1}\). MTBE degradation with corresponding increases in cell density for the cultures with spent growth medium was observed. In contrast, the cultures with fresh SSE both show minimal MTBE degradation and much lower cell densities over the 60-day trial period. It should be noted that the average initial bacterial counts for duplicate cultures with fresh SSE (2.85 x 10\(^{6}\) cells mL\(^{-1}\)) were 2-fold greater than for duplicate cultures with spent media (1.39 x 10\(^{6}\) cells mL\(^{-1}\)).

**Isolation of MTBE-degrading bacteria**

Thirteen isolates were cultivated from the original MTBE-degrading enrichment. MTBE degradation kinetics were determined for each isolate in the presence and absence of 2-propanol. Degradation of MTBE was more rapid and complete in the presence of 2-propanol for all isolates tested. Since many isolates had similar colony morphologies and MTBE degradation kinetics, nucleotide sequence analysis of a 340-bp region of the 16S rRNA gene was performed to identify unique isolates. Of 9 isolates characterized, four isolates were 100% identical to *Pseudomonas* sp. Ant9 and five isolates were 100% similar to *Rhodococcus koreensis*. Figure 6 shows degradation kinetics of MTBE in the presence and absence of 2-propanol for two of the isolates identified as *Pseudomonas* sp. Ant9 and *Rhodococcus koreensis*. MTBE degradation by both isolates was minimal in the absence of 2-propanol as a cosubstrate. GC monitoring of isolates grown with 20 mg L\(^{-1}\) 2-propanol revealed an initial decrease in 2-propanol concentration prior to the onset of MTBE disappearance, indicating a probable cometabolic
induction of MTBE degradation. Two metabolites observed during MTBE degradation with both isolates in the presence of 2-propanol were identified by GC/MS analysis as TBA and TBF.

**Molecular characterization of MTBE-degrading consortia**

DGGE profiles of RS24 and two successive subcultures taken from this consortium were generated. Sequence analysis of the DGGE bands revealed a phylogenetically diverse group of bacteria present in the consortium. A decrease in the total number of bands in the profiles was observed with successive subculturing. DGGE bands identified as *Pseudomonas* sp. Ant9 and *Rhodococcus koreensis* showed 100% sequence similarity to the two cultivated MTBE-degrading isolates identified above. Interestingly, the *Rhodococcus koreensis* band disappeared completely by the second subculture, while the band corresponding to *Pseudomonas* sp. Ant9 decreased in intensity with subculturing. However, due to bias inherent in the PCR, differences in DGGE band intensity may not reflect population abundance in the consortium. The band corresponding to *Rhodoferax fermentans* was present in the original consortium and appeared stable in both subcultures. After numerous attempts, bands A and B could not be sufficiently purified for sequence analysis. Bands H1 and H2 are believed to be heteroduplex molecules formed during the PCR reaction, as purification and re-amplification of both bands always yielded H1 and H2 in addition to band A and the band corresponding to *Rhodoferax fermentans*.

**MTBE plume modeling**

MTBE plume modeling using BIOSCREEN and MODFLOW were somewhat successful in describing the plume dynamics observed at the site. Value uncertainty for many of the input parameters to the models no doubt resulted in sub-optimal performance. Nevertheless, this study did show that transport modeling was a valuable tool for describing general plume behavior.

**Annual seminars and discussions**

In December of each year for the duration of the project, meetings were held either in Bozeman, MT or Helena, MT in which students presented the results of their work to site consultants and Montana DEQ personnel in a seminar format. These seminars ranged from topics including hydrogeology, biodegradation, modeling, toxicity, etc. Following the presentations, discussions were held among the students and other official representatives to clarify results and to receive input in guiding further research efforts.

**Discussion**

Enrichments designed to simulate MTBE and nutrient concentrations present in the aquifer revealed a diverse group of bacteria capable of degrading MTBE. Initial screening of gasoline/MTBE-contaminated soils from two monitoring well locations yielded a single enrichment capable of degrading MTBE in the presence of 2-propanol. Maintenance and transfer of this enrichment resulted in a bacterial consortium that could degrade MTBE at relatively high rates in the absence of 2-propanol as a cosubstrate. In this ultimate enrichment, cell growth coupled to MTBE degradation as the sole carbon source and no appearance of MTBE metabolites indicated the bacterial consortium derived energy from the complete catabolism of MTBE. Calculated gross biomass yields for this latter consortium of RS24 (~0.25 mg dry biomass mg MTBE$^{-1}$) were generally similar to the estimated biomass yield of 0.18 mg cells mg MTBE$^{-1}$ and 0.21-0.28 mg dry biomass mg MTBE$^{-1}$ reported by other researchers. A
previously reported bacterial strain capable of MTBE degradation was identified as a member of the Beta subgroup of Proteobacteria by 16S rRNA analysis. In our study DNA sequencing of a gel band of 16S rDNA amplified by PCR and separated by DGGE identified *Rhodoferax fermentans* as the organism probably most responsible for MTBE degradation in consortium RS24. Interestingly, the genus *Rhodoferax* also belongs to the Beta subgroup of Proteobacteria. Although in latter cultural transfers of RS24 without 2-propanol no TBA or TBF metabolites were observed during MTBE degradation, earlier cultures of RS24 displayed both TBA and TBF metabolites in the presence of 2-propanol. Also, accumulation of TBA was observed early in this study when one RS24-transferred culture was grown in the presence of 10 mg L\(^{-1}\) benzene and 20 mg L\(^{-1}\) 2-propanol. Because both TBA and TBF were always observed during MTBE degradation by the isolates, *Pseudomonas* sp. Ant9 and *Rhodococcus koreensis*, in the presence of 2-propanol and because these isolates seemed to be more prominent in DGGE gels of the earlier RS24 consortia, we believe that these two isolates are probably limited to cometabolic MTBE-degradation and eventually became subordinate to *Rhodoferax fermentans* under our experimental conditions. However, others have reported degradation of 28-29% of 200 mg L\(^{-1}\) MTBE as the sole carbon source by three pure culture isolates over a two-week period (degradation rate \(\approx 0.2\) mg d\(^{-1}\)); and one of these isolates was identified as *Rhodococcus* sp. Also, they observed a reduction in MTBE degradation by this bacterium in the presence of butyl formate, \(t\)-butanol, and several other simple organic compounds. These results and results from our study showing an accumulation of TBA and TBF by *Rhodococcus koreensis* may indicate an inhibitory effect caused by accumulation of these metabolites.

The production of TBA and TBF support a previously proposed MTBE degradation pathway in which the enzyme, cytochrome P-450, oxidizes MTBE to tert-butoxymethanol, which then may be converted to TBF via an alcohol dehydrogenase. The TBF may then undergo hydrolysis to TBA. This latter hydrolysis step was also proposed by other researchers; however, they suggested that the initial formation of TBF may simply be a strict chemical oxidation by atmospheric oxygen. We believe our results do not support chemical oxidation as a major MTBE degradation step in that no TBA or TBF peaks were observed in our sterile controls in the presence or absence of 2-propanol.

The fact that no lag phase in MTBE degradation was observed for transferred cultures grown in spent supernatant suggests the presence of a factor in the medium that allowed uninterrupted MTBE degradation to occur. We propose that this mechanism may possibly involve either a repressor-regulated transcriptional system similar to that of the *lac* operon or activator-dependent regulation. In the first case, a constitutively expressed repressor protein would prevent transcription of genes involved in MTBE degradation. Presumably, production or activation of an inducer protein could be initiated by the presence of MTBE or an effector protein. Release of the inducer over time would increase its concentration and effect more binding to and release of the repressor protein and, thus, allow transcription of the MTBE degradation genes. This type of mechanism has already been proposed for other genes involved in contaminant biodegradation. For example, a model has been proposed for dichloromethane (DCM) degradation by *Methylobacterium* sp. in which production of the protein DcmA (responsible for DCM dehalogenase production) is normally repressed by binding of the regulatory protein DcmR to the *dcmA* promoter. De-repression (i.e. activation) of *dcmA* occurs by putative substrate-induced release of DcmR. Also, in another study research showed that the XylR protein has both repressor and activator roles in the control of toluene biodegradation by *Pseudomonas putida*. Undoubtedly, some of these regulatory mechanisms are strictly
intracellular in nature. However, we speculate that extracellular release of regulatory proteins acting as autoinducers such as those already identified in other types of quorum sensing and cell-cell communication would confer a selective advantage. For example, other researchers have suggested that quorum sensing may prevent unnecessary gene induction, allow a rapid sensing of changes in the environment, and serve to coordinate consortial metabolism among bacterial species. At the very least, we believe that this type of concentration-dependent phenomena could result in observations of MTBE degradation such those documented in this study.

Although several MTBE-degrading bacterial species were cultured from the Ronan aquifer, MTBE biodegradation in the aquifer is difficult to assess in relation to other processes of natural attenuation. However, because of the generally low quarterly dissolved oxygen (DO) measurements and low temperatures (4–12°C), we concluded that biodegradation is relatively slow at the site. This conclusion is based on another study in which a low concentration of dissolved oxygen and low temperatures severely slowed MTBE degradation rate and growth rate of the mixed, MTBE-degrading bacterial culture. The low DO levels are likely due to seasonal fluctuations in the groundwater of the region as well as to oxygen utilization coupled to contaminant degradation. For example, DO measurements from the first to the fourth quarters of 1999 were consistently less than 0.5 mg L\(^{-1}\) for MW-10. However, for the first quarter of 2000 a DO measurement of 3.3 mg L\(^{-1}\) was recorded. Over this same time period for the same monitoring well, MTBE measurements first increased over an order of magnitude to 3,580 µg L\(^{-1}\) and then gradually decreased again to < 300 µg L\(^{-1}\). Likewise, TPH measurements increased almost two orders of magnitude to 10,900 µg L\(^{-1}\) and then gradually decreased to < 900 µg L\(^{-1}\). Because background DO levels obtained from MW-11 upgradient of the contaminant plume as well as from MW-6 and MW-16 (adjacent to MW-12 but outside the contaminant plume) were consistently between 4-6 mg L\(^{-1}\), contaminant biodegradation inside the plume is indicated. However, specific evidence of MTBE biodegradation is confounded by the presence of other potential gasoline-related substrates; and no detection of MTBE metabolites has been recorded. Because measured DO levels indicate that oxygen may be limiting biodegradation at this site, we recommend that any applied treatment technology should investigate and/or include oxygen addition, although other important inorganic nutrients such as nitrogen and phosphorus may also be limiting the activity of MTBE-degrading bacteria. One report of enhanced MTBE degradation by injection of pure oxygen into an impacted aquifer supports the above recommendation.

This study has established the presence of bacteria capable of degrading MTBE in the gasoline-contaminated aquifer near Ronan, MT. Other studies targeting the identification of native bacterial populations with the capacity to degrade MTBE in natural environments will help to establish the ubiquity of these organisms and allow better prediction of the efficacy of biodegradation as a means to remediate MTBE-contaminated aquifers. In addition, further laboratory studies designed to elucidate factors that influence MTBE degradation, such as co-substrates or the concentration-dependent factor proposed in this study, may provide insight into more efficient methods of stimulating MTBE degradation by naturally occurring bacteria.

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- Claus Lang, PhD, University of Stuttgart, Germany, July 2000
- Elsa Meiser, MS Environmental Engineering, Montana State University (in progress)
Approximately 20 students were involved in Dr. Al Cunningham’s Environmental Engineering Investigations class (ENVE 534) at Montana State University over a 3-year period. These students received travel reimbursement for visits to the Ronan site for sampling and other data collection. As part of the course requirements, students generated reports on topics related to the site and its contaminants including site characterization, transport modeling, partitioning, biodegradation, natural attenuation, and health risk assessment. These students interacted with consulting engineers from MSE (Butte, MT) and with Montana Dept. of Environmental Quality personnel in the field and also presented their academic reports at annual meetings involving these and other state personnel.

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Basic Information

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Publication

4. Furniss, G., N., 1999, Distinguishing Acid Mine Drainage from Acid Rock Drainage Using Trace Elements in Ferricrete, in abstracts - AWRA-MT Annual Meeting Great Falls MT. (First place paper award.)
10. Furniss, G., 1996, 9,000 Years of Acid Drainage Water Quality Recorded in Ferricrete, New World District, Park County, Montana, Student Poster Session, 1996 Annual Meeting, Northwest Mining Association, December 3-5, Spokane, Washington (won second place award).


19. Sjostrom, D.J., M.A. Poage, C.P. Chamberlain, G. Furniss, 2000, Regional Rocky Mountain Climate Change Recorded By Oxygen Isotopes Of Holocene Ferricretes, Geological Society of America, Rocky Mountain Section Annual Meeting, Missoula, MT.
Abstract

Federal and state regulations require that mining activities be performed in such a way that do not result in degradation of the quality of surface water and groundwater during or after operations. However, because of the nature of host rocks and associated mineral deposits, it is possible that such high water quality standards were never met in many of these streams even prior to mining. Original water quality is debatable as no adequate method exists to determine pre-mining water quality. Herein we report results of a study of modern and ancient mineral compositions as a means of estimating background water chemistry in abandoned mining districts. Based on the observations, no conclusive correlation can be made between solid and solution compositions. Stream, precipitate, and bead chemistry show some correlation but the range of values was not sufficient to establish such a relationship; data from several drainages would need to be combined. However, the continuity of chemical composition as a function of age up to 5,800 years suggests that an empirical relationship could be identified but that with a significant increase in age, such a relationship is subject to diagenetic changes that likely depend on local conditions. Lastly, this study has shown that EPA Method 3051A introduces a smaller uncertainty into the observations and thus is more useful in distinguishing chemical compositions in these stream deposits.
## Basic Information

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## Publication

Influence of Mine Reclamation Amendments on Metal Mobility and Water Pollution Potential

A Final Report to the Montana Water Center

Thomas H. DeLuca and Aimeé Vitateau

The University of Montana

School of Forestry

May 8, 2001

Summary

Waste rock and mine tailings are by products of hard rock mining which contribute large concentrations of soluble and bioavailable heavy metals to water resources. Western Montana alone has some 8,000 no responsible party abandoned mine sites and numerous active mining operations that potentially result in water contamination. Restoration of these acidic, metal rich deposits often involves surface treatment of low priority tailings with lime to neutralize the acidity, and law requires that the materials be treated with an organic matter amendment to simulate natural soil organic matter conditions. It is not clear how different organic matter amendments influence metal mobility or bioavailability. This study was undertaken to determine the influence of raw straw (RS), log yard waste (LYW), composted sewage sludge (CCS), composted cow manure (CCM) and raw primary pulp mill sludge (PMS) on the solubility and bioavailability of Cu, Cd, and Pb and their influence on revegetation success. The research was conducted in laboratory, greenhouse and field settings using both Clark Fork streamside tailings and Combination Mill mine tailings. Both tailings types were enriched in heavy metals, in particular Cu, but the combination Mill site tailings also had relatively high concentrations of Pb. These tailings had also previously been treated with lime resulting in a surface pH of 4.4 where as the Clark Fork tailings had an initial pH of 3.2. The CCM and PMS had the greatest effect on Cu solubility followed by CCS, RS and LYW. Lead solubility most effectively decreased by PMS, but concentrations of organic sorbed Pb appeared to be highest in the CCS. Grass growth on the treated Combination Mill tailings was found to be greatest with CCM followed by PMS in both greenhouse and field studies. There was no revegetation success using CSS. It is not clear why CSS did not perform more effectively as an amendment with the Combination Mill tailings, but may have been a function of the unusually high Pb and Hg concentrations in the Combination Mill tailings.
Introduction

The formation and disposal of fine textured mine tailings during the processing of ores creates deposits of aerated, high-surface, mining wastes that result in the enhanced oxidation of sulfide minerals and the production of acid mine drainage. Mining wastes also contain high levels of heavy metals; geologic factors determine the types and concentrations of the metals. Cadmium (Cd), copper (Cu), and lead (Pb), are examples of metals that are generally expected in high concentrations in tailings from gold mining in western Montana. These metals tend to dissolve readily in the acid water associated with tailings, and once in solution, they are readily available to plants in potentially toxic concentrations. Metals in this form also can be transported from the site to contaminate surface and groundwater. The end result is generally an acidic substrate with little or no vegetation establishment in which soluble metals are readily lost to water resources.

Generally, pH is the most important factor for determining the solubility of metals, and generally most metals tend to be more soluble and therefore more mobile and bioavailable under acidic conditions. For this reason, lime is commonly applied to mine reclamation sites to increase the pH and to reduce the concentration of exchangeable and soluble metals so that vegetation can become established. However, tailings are infertile and on a site where lime alone, or lime and chemical fertilizers, is used vegetation may not survive long enough to benefit the site (Sopper, 1992). Because of this, the use of organic amendments in mine reclamation has become a common practice because of their ability to increase the success of establishing a healthy, self-maintaining vegetative cover by altering both the chemical and physical properties of the site. Organic soil amendments provide organic carbon and plant nutrients (Guisquiani et al, 1992), increase the soil buffering capacity (Hale, 1982), increase the water-holding capacity and increase the aggregation of soils (Sopper, 1992; Khallel et al., 1981). Many different organic soil amendments are being used in mine reclamation, including sewage sludge, straw, wood processing by-products, and other organic, industrial by-products. However, organic amendments can also influence the bioavailability and mobility of metals through several mechanisms. Depending on the type of organic matter added there is potential that the short-term effect can enhance plant uptake and mobilization of certain metals at the site (DeLuca and Lynch, 1997).

There are several forms that metals can assume in the soil environment which effect their potential mobility and bioavailability and which deserve discussion.

Free Ions
Metal ions are generally present in soil solution in cationic form and may sorb to the negative surface charges of the soil constituents, both clay and organic matter particles. The concentration of metals in the free-ion state is maintained by the equilibrium-product of mineral or precipitate forms in the soil.
concentration of the free ion form is greatly pH dependent and organic solute dependent. Although, free ions are considered to move slowly by diffusion through the soil substrate, in soil materials with high metal concentrations the concentrations of metals in the soil solution also rise and become more mobile due to insufficient binding sites (Brummer, 1986). Free ions are the most available form of metals for uptake by plants (Temminghoff et al., 1994; Lieta and DeNobili, 1991).

Complexed/Sorbed Forms
If metal ions are sorbed to colloidal material or involved in a chelate complex then their potential mobility can be greatly altered (McCarthy and Zachara, 1989). Chelates are a class of organic compounds, which are formed by several donor groups of a single organic ligand bonding with a metal ion at different coordinate positions. Chelates are soluble, highly stable, ring-like structures (Stevenson, 1994). Provided that they have the ability to form a ring-like structure when complexed, chelating agents can include the following: fulvic acids, small non-humic organic acids, amines and sugars. Concentration and stability of metal chelates in soil are dependent upon soil pH, concentrations of metal ions, and the ability of metal ions to compete for different chelating ligands in soil (Norvell, 1972).

The most important function of chelates in soil is their ability to increase the solubility of metals. As a result, both the mobility of the metals and their availability for uptake by plants is greatly enhanced (Norvell, 1972). This can be beneficial in agricultural soils with low levels of micronutrients (Lindsay, 1974). But, on contaminated soils this could increase the already potentially toxic levels of metals available to plants and it could contribute to metal contamination of water resources.

Metal ions can also be sorbed to colloids. Colloids are materials that can remain in suspension indefinitely without dissolving. They are insoluble organic and inorganic particles with diameters of less than 10µm. This would include but not be limited to the following: humic acids, non-humic organic acids, clay particles, and mineral precipitates. Because colloids are insoluble by definition, metals associated with colloids are generally unavailable to plants. However, the role of colloids in metal transport is more complicated, and poorly understood. The simple view is that due to the insoluble nature of colloids a sorbed contaminant will be immobilized. Recently, more attention is being given to colloids as a means of contaminant transport rather than immobilization (McCarthy and Zachara, 1989; Amrhein et al., 1992; Amrhein et al., 1993, Ryan et al., 1999).

The colloid concentration in the soil-pore solution is affected by the geological matrix, and precipitation due to changes in pH, ion composition, redox potential, and CO₂ partial pressure (McCarthy and Zachara, 1989). The mobility of colloids at a particular site is influenced by a number of different factors. Water velocity, the colloids ability to sorb to other immobile soil components, electrostatic or
cementing forces (McCarthy and Zachara, 1989), salinity (Amrhein et al., 1992 & 1993), soil pore size, and erosional status of the site all are important in determining if colloids will be immobilized or transported.

We have already briefly mentioned that fulvic acids that are capable of forming soluble ring-like structures can influence metal mobility and bioavailability through chelation, and humic acids can sorb metals and contribute to metal mobility through colloid assisted transport. Humic and fulvic acids are fractions of the soil organic matter. Humic substances are pigmented, heterogeneous, compounds of unknown chemical compositions and in combination with biopolymers of known chemical compositions, called non-humic substances, compose the humus fraction of soil organic matter. Humic substances are further fractionated into humin, humic acids, and fulvic acids based on their solubility in acid or alkali. Humin is the fraction of the soil organic matter that is not dissolved upon the extraction of the soil with either alkali or acid. Humic acids are soluble in alkali and insoluble in acid, while fulvic acids are soluble in both alkali and in acid (Stevenson & Cole, 1999).

The structures of humic and fulvic acids are similar, but they differ in molecular weight, and elemental composition. Both are composed of a mixture aromatic and aliphatic hydrocarbons with a high content of oxygen containing functional groups. Fulvic acids are smaller in size than humic acids and generally range from 500 to 2000-daltons. They also tend to have more acidic functional groups, particularly carboxyl and phenolic groups, per unit area and therefore more sites for metals to bind than humic acids. Humic acids range in size from 50,000 to 500,000 daltons, have higher carbon contents and are less oxidized than the fulvic acids (Paul & Clark, 1996; Stevenson & Cole, 1999). Because of the relatively small size and insolubility of humic acids, they are potentially colloidal in nature. While the small size and solubility of fulvic acids allows them to act as ligands available to form chelate complexes. By acting as chelates and colloids, fulvic acids and humic acids can play an important and very different role in the mobilization of heavy metals. Paré et al. (1999) demonstrated that during composting the fulvic acid fraction of the composted material is decreased and the humic acid concentration is increased during the humification process. At the same time, the easily extractable and exchangeable metals associated with the composted materials decreased, suggesting that they were being converted to non-extractable and non-exchangeable forms, presumably through adsorption or complexation with the humic substances.

In the past, the goal of organic matter additions to a reclamation site has been to aid in the establishment of self-maintaining vegetative cover. However, with increasing attention being given to colloid and chelate-assisted transport of contaminants it is important to determine also if the type of organic matter being added to the soils will have an adverse or favorable influence on metal mobility through chemical changes.
The purpose of our work was to determine how the application of different amendments influences the metal mobility and revegetation success at a mine reclamation site through the implementation of greenhouse and laboratory experiments, combined with a field study. Specifically, we aimed to characterize the presence of cadmium (Cd), copper (Cu), and lead (Pb) in an easily exchangeable fraction, and organically bound fraction in mine tailings. Also, our intent was to further fractionate the organically bound metal fractions into those bound to humic and fulvic acids in an effort to speculate upon the potential mobility of the metals from the site. Finally, we hoped to determine if the amount of biomass produced could be influenced by the different organic amendments.

**Materials and Methods**

**Starting Material Description**

Prior to initiation of the greenhouse and laboratory experiments, and the field study, it was necessary to locate sources for the organic amendments and to analyze these materials for metal content and carbon to nitrogen ratio.

The materials for the preliminary greenhouse and laboratory experiments were gathered in January and February of 1999. Stimson Lumber, of Bonner, Montana donated the log yard waste. The pulp-mill sludge (PMS) is a dewatered by-product of wastewater treatment at Smurffit-Stone Container Corp. of Frenchtown, Montana. Garden City Harvest, and Eko-Compost, both of Missoula, Montana donated the raw straw (RS) and composted sewage sludge (CSS) respectively. The composted cow manure (CCM) was purchased from Lifeline Dairy in Stevensville, Montana. Finally because a suitable field site had not yet been identified, we collected streamside tailings from Ramsey Flats, east of Butte Montana along the Clark Fork River to be used in a preliminary greenhouse and field experiment. In the spring of 1999, the Combination Mill Site was selected as the field site to be used and we collected a composite sample to be analyzed for metal content and to be used in the greenhouse and lab experiments. Prior to applying the treatments to the field plots, we also collected plot samples, which we used for analysis of carbon to nitrogen ratio, and pH.

The tailings were air dried and mixed thoroughly, then sieved to 2.00-mm using a USA standard testing sieve #10. It was also necessary to grind the straw using a Wiley Mill to get it to a manageable size. Next, a small amount of all six of the starting materials was dried at 105° C for 24 hours in order to determine their percent moisture content. Finally, all of these dried samples were ground using a zirconium ball mill for use in the C:N, and metal content analyses.
Metal Analysis

In order to determine the amount of leachable metals in each of the six starting materials I subjected approximately 0.5000 grams of each of the ground samples to EPA Method 3051A (Link et al., 1998) using the MARS-5 microwave. This meant digesting the samples in 3-ml of HCl and 9-ml of HNO₃ while heating the samples to 175° Celsius in 5.5 minutes and maintaining that temperature for 4.5 minutes. Blanks, external standards and preparation duplicates of each of the starting materials were included. After the samples were removed from the microwave, they were allowed to cool then transferred to centrifuge tubes and brought to a volume of 50-ml. Next, the samples were centrifuged and finally decanted so that the samples could be analyzed later on the ICP.

The PMS samples presented two problems that did not occur with the other types of samples. First, when the acid was added, the PMS samples effervesced much more than the other samples did. As a result, a small amount of the sample was deposited on the side of the vessel where it may not have been fully oxidized even by reflux in the microwave. We could have added a strong oxidizer such as H₂O₂ to the samples to alleviate this problem. However, we decided that for our purposes the possibility of a slight negative bias for analytes in the PMS did not warrant changing the method for all of the samples, but that it was important to make a note that one might exist.

Second, after the PMS samples had been centrifuged, a solid pellet did not form so that some particulate matter was transferred to the sample containers. Because of this it was necessary to filter the PMS samples prior to running them through the ICP. This was done using 0.45-micron ion chromatograph acrodiscs and a 10-cc syringe. In order to avoid contamination, we were careful to keep the sample from coming in to contact with the rubber stopper on the syringe plunger.

ICP

We analyzed the samples using a Thermo Jarrell Ash IRIS ICP with a Scott spray chamber and a Crossflow nebulizer. In order to ensure precise and accurate data we used spiked samples, and in-house and external quality control standards. Appendix 1 shows the complete ICP output for the data while Table 1 shows the mean concentrations of the metals of particular interest.

C:N and pH Analysis

In the spring of 1999, we used an EA1110 CHNS-O Elemental Analyzer to determine the carbon to nitrogen ratio of each of the six starting materials. The C:Ns of the five amendments and the Clark Fork River tailings are given in Table 1. The low carbon to nitrogen ratio of the Clark Fork River tailings does not indicate that it is a fertile substrate. On the contrary, the tailings contain only 0.308% carbon, and 0.024% nitrogen. This is a very small amount when compared to a more typical surface soil. Brady and Weil (1999) report the
carbon and nitrogen contents of a Montana mollisol to be 2.06% and 0.74% respectively. Samples of the five organic amendments and the Clark Fork River tailings were analyzed to determine their pH prior to the initiation of the experiments.

Table 1. Starting material summary information is presented below. Total metal concentrations (aqua regia digest) are reported using a 95% confidence interval, except for the Combination Mill Tailings (CMT) because only one sample and a duplicate were analyzed.

<table>
<thead>
<tr>
<th>Source</th>
<th>Cu Conc. (µg/g)</th>
<th>Pb Conc. (µg/g)</th>
<th>Cd Conc. (µg/g)</th>
<th>pH</th>
<th>C:N</th>
<th>% Moisture</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFRT</td>
<td>670±14.0</td>
<td>751±16.4</td>
<td>5.78±0.137</td>
<td>3.2</td>
<td>15:1</td>
<td>1.5</td>
<td>Ramsey Flats</td>
</tr>
<tr>
<td>LYW</td>
<td>12.1±0.100</td>
<td>12.1±2.34</td>
<td>&lt;PQL</td>
<td>4.7</td>
<td>100:1</td>
<td>36</td>
<td>Stimson Lumber</td>
</tr>
<tr>
<td>RS</td>
<td>2.29±0.078</td>
<td>&lt;PQL</td>
<td>&lt;PQL</td>
<td>7.8</td>
<td>85:1</td>
<td>6.2</td>
<td>Garden City</td>
</tr>
<tr>
<td>PMS</td>
<td>40.0±0.266</td>
<td>&lt;PQL</td>
<td>1.20±0.034</td>
<td>8.8</td>
<td>120:1</td>
<td>60</td>
<td>Smurffit-Stone</td>
</tr>
<tr>
<td>CCM</td>
<td>42.8±0.521</td>
<td>&lt;PQL</td>
<td>&lt;PQL</td>
<td>8.2</td>
<td>10:1</td>
<td>67</td>
<td>Container</td>
</tr>
<tr>
<td>CSS</td>
<td>205±1.53</td>
<td>59.7±1.31</td>
<td>1.49±0.031</td>
<td>5.7</td>
<td>20:1</td>
<td>53</td>
<td>Lifeline Dairy</td>
</tr>
<tr>
<td>CMT</td>
<td>3849</td>
<td>4558</td>
<td>20.4</td>
<td>4.4</td>
<td>20:1</td>
<td>2.5</td>
<td>Combinatio n Mill Site</td>
</tr>
</tbody>
</table>

Table 2. CaCl2 and NaOH-extractable Cd, Cu, and Pb concentrations for the reagent blanks and organic amendments.

<table>
<thead>
<tr>
<th></th>
<th>CaCl2</th>
<th>NaOH</th>
<th>CaCl2</th>
<th>NaOH</th>
<th>CaCl2</th>
<th>NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cd</td>
<td>0.086</td>
<td>N/A</td>
<td>0.147</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
</tr>
<tr>
<td>NaOH</td>
<td>N/A</td>
<td>0.019</td>
<td>N/A</td>
<td>0.034</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>LYW</td>
<td>0</td>
<td>0.171</td>
<td>0.514</td>
<td>5.54</td>
<td>0.786</td>
<td>1.06</td>
</tr>
<tr>
<td>RS</td>
<td>0</td>
<td>0.552</td>
<td>2.16</td>
<td>6.68</td>
<td>0.786</td>
<td>0</td>
</tr>
<tr>
<td>PMS</td>
<td>0.171</td>
<td>0</td>
<td>0.749</td>
<td>2.85</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CCM</td>
<td>0.184</td>
<td>0.552</td>
<td>0.514</td>
<td>11.6</td>
<td>1.572</td>
<td>0</td>
</tr>
<tr>
<td>CSS</td>
<td>0.171</td>
<td>0</td>
<td>0.749</td>
<td>43.1</td>
<td>0</td>
<td>1.27</td>
</tr>
</tbody>
</table>

PQL Cd=1.5 Cu= 8.0 Pb= 30
During the laboratory experiment we also analyzed the amendments for CaCl$_2$ and NaOH-extractable Cu, Pb and Cd in order to document if any of the amendments could significantly elevate any of the metals in these extracts. The results of the analysis of each of the starting materials as well as the reagent blanks are presented in Table 2.

**Preliminary Laboratory Experiment with Clark Fork River Tailings**

Prior to conducting the majority of the research, we performed a preliminary laboratory experiment in order to evaluate the effectiveness of the sequential extraction procedure we intended to use. In the fall of 1998 we conducted a preliminary laboratory experiment, because a suitable field site had not been found, and we had prior experience with the Clark Fork River tailings (DeLuca and Lynch, 1997). This preliminary test was a 6 x 2 factorial experiment. Here we tested five organic amendments: 9 tons per acre of raw straw (RS), log yard waste (LYW), pulp mill sludge (PMS), composted sewage sludge (CSS), or composted cow manure (CCM) and a no amendment control with or without the addition of lime. Lime was added to the samples to bring them to a pH of approximately 6.5. Lime rates were determined by using the Evans and Adams buffered lime test extrapolated for acidic mine tailings (see DeLuca and Lynch, 1997). Each treatment was replicated five times. We determined the water content of each of the five amendments and the tailings by oven-drying them at 105ºC for 24 hours. Table1 contains the water content for each of the starting materials. Then we combined the tailings and starting materials in a 10:1 dry-weight ratio in 250-ml French-square bottles. Water holding capacity for each of the treatments was determined using a pressure plate and the samples were then brought to field capacity (60% water holding capacity) and fitted with loose fitting lids to allow air circulation. The samples were placed in a dark place to incubate for 12-weeks. During the 12-week incubation, the samples were weighed biweekly and returned to field capacity.

After 12-weeks, the samples were fractionated using the procedure outlined by Sposito et al. (1980) and modified by McGrath and Cegarra (1992). Briefly, this sequential fractionation includes a water-soluble and exchangeable fraction (extracted with 0.01M CaCl$_2$), an organically bound fraction (digested in 1M NaOH), a carbonate bound fraction (0.05M Na$_2$-EDTA) and a modified aqua regia digestible fraction (digested in HCl and HNO$_3$). Each of these fractions was analyzed for Cu and Pb concentrations using an AAS.

Problems occurred with this method when the data was analyzed and many of the readings were below the detection limit of the instrument. During the course of this study the detection limits of the AAS were determined to be the approximate practical quantification limit calculated as ten times the standard
deviation of a blank analyzed seven times. For the preliminary laboratory experiment this resulted in detection limits for Cu and Pb of 0.9-ppm and 3.0-ppm respectively. In calculating the micro-grams extracted in each of the sequential fractions it is necessary to use the numbers calculated for previous fractions. However, many of the CaCl$_2$ and NaOH extracted samples were below the detection limits for both Cu and Pb but it was necessary to use them in order to develop a complete set of concentrations from the sequential extractions. We replaced concentrations that were reported to be less than zero with zero for calculation purposes. We attempted to replace the values that were less than the detection limit with one half of the PQL; however, it made very little difference when we reported the results of each extract as a percentage of the sum, and so for our final calculations we used the reported concentrations. Also, because of the large number of samples this method generated we decided to not analyze all of the aqua regia samples on the AA for this preliminary experiment. Rather, we analyzed the samples that contained only the amendment, and the limed and un-limed tailings samples that contained no treatment. Then we the averaged the totals and calculated the total amount expected for each of the treatments with the following formula: 0.9(mean total micrograms of metal in U-NT and L-NT samples) + 0.1(total micrograms of metal in amendment). The mean and standard error for each set of replicates for each treatment are reported in Table 3, and Appendix 2 contains more detailed information. Figure 1 depicts the percent contribution of Cu and Pb that each of first three fractions makes to the total for each of the treatments. The remainder to 100% is from the aqua regia fraction.

**Combination Mill Tailings Laboratory Experiment**

Due to the problems experienced and the large number of samples generated during the preliminary experiment we elected to not use a sequential extraction, but rather use only separate, non-sequential, CaCl$_2$ and NaOH extractions. Brummer (1986) reported that the metals in the soluble and exchangeable fraction are frequently extracted using CaCl$_2$, and that it is generally accepted that the organically bound fraction can be extracted using NaOH. We felt that these two extractions were the most important for our interest in metal mobility. Brummer (1986) states that the CaCl$_2$-extractable fraction is representative of the mobile fraction of metals in the soil and is a good estimator of those metals that are available for uptake by plants. However, because of the more recent attention being given to chelate and colloid-assisted transport we obviously felt it was important to consider the NaOH-extractable portion as well. Although we expected that the CaCl$_2$ and NaOH-extracted fractions were the most likely to have concentrations below the detection limits of the AAS, we hoped to avoid compounding the problems by performing the extractions non-sequentially.

A composite sample of tailings was collected from the Combination Mill field study site, air-dried and thoroughly mixed. Then the tailings were sieved to 2.00-
mm using a USA standard testing sieve #10. Because the field study site had been previously treated with lime, lime was not a component of this experiment. In this experiment, there were six treatments that consisted of the tailings combined with the five organic amendments (RS, LYW, PMS, CSS, or CCM) in a 10:1 dry-weight ratio and a control, all replicated five times. The water content of the Combination Mill tailings (CMT) and the starting materials was determined by oven-drying them for 24-hours at 105ºC. The Murdock Laboratory at The University of Montana performed a comprehensive metals-analysis of the Combination Mill tailings using an Environmental Express Hot Block with EPA method 3050B (Table 1). Water holding capacity for each of the treatments was determined using a pressure plate and the samples were then brought to field capacity (60% water holding capacity) and fitted with loose fitting lids to allow air circulation. The samples were placed in a dark place to incubate for 12-weeks to allow microbial reactions to occur. During the 12-week incubation, the samples were weighed biweekly and returned to field capacity.

After 12-weeks, the samples were extracted. We combined 5-grams of each sample with 50-ml of 0.01M CaCl\textsubscript{2} in 50-ml centrifuge tubes. The samples were placed in a shaker on medium for 16 hours, then centrifuged at 30,000-g for 20-minutes. Next, the supernatant was decanted, acidified, and vacuum filtered using a 0.2-micron filter. Finally the samples were refrigerated until they were analyzed for Cu, Pb, and Cd using an AAS. The solution concentration data (ppm) was obtained from the AAS was then converted to soil concentration (µg/g) for statistical analysis. This procedure was repeated using 1M NaOH. In addition to the treatment replicates we also extracted the five organic amendments using the same procedure with both extractants. Because they are less dense than the tailings, it was necessary to use two centrifuge tubes with 3-grams of the amendment and 30-ml of extractant in each and combine them during the decanting process.

After the NaOH-extracted samples had been analyzed for metal content they were then subjected to a simple ultrafiltration technique using centrifuge tubes equipped with a 10,000 molecular weight cut-off membrane. We placed 3-ml of the NaOH-extracted laboratory experiment samples into the centrifuge tubes and centrifuged them at 30,000-g for 20-minutes. Usually this was sufficient to pass the sample through the membrane. In a few instances, it required more time, and we simply re-centrifuged the sample. Temminghoff et al. (1994) explain that ultrafiltration is one of the accepted techniques for fractionating organic matter on a molecular weight basis. Although their technique is more complicated than the one we use here the desired outcome in both cases is the same: to distinguish between the metals in the organically bound fraction that are bound mainly to fulvic acids from those bound mainly to humic acids. Because of the relative size of humic and fulvic acids, we made the assumption that anything that passed through the membrane was either a free metal ion, or a metal bound to the fulvic fraction, and therefore potentially more mobile than the metals bound to the humic fraction that remained in the membrane. The samples were diluted 10:1
and analyzed on the AAS in order to determine the Cu and Pb concentrations bound to the fulvic fraction. We did not analyze for Cd because of the low concentrations found in the unfiltered samples.

**Combination Mill Tailings Greenhouse Experiment**

A greenhouse experiment was executed using the same treatments described above to test the influence of the various treatments on plant growth and metal bioavailability. For each of the treatments, the tailings and soil amendments were combined and mixed thoroughly in a 10:1 dry-weight ratio. Next, the mixture was evenly divided and placed in five, 200-ml containers. The different densities of the different organic amendments created treatments with varying volumes. Cotton balls were placed in the bottom of the containers to ensure that the mixtures filled the containers to approximately the same height. Next, the containers were arranged randomly in a stand and watered heavily for two days. We added seven thick-spike wheat-grass seeds to each container and placed plastic wrap over their tops and allowed the seeds to germinate. After three days, the plastic wrap was removed and the tray was placed in The University of Montana’s School of Forestry Greenhouse. The plants were watered as needed with de-ionized water and the germination and survivorship for each of the replicates was recorded every week. During the third week the containers were fertilized with 10-ml each of a solution containing 3.6-g (NH₄)HPO₄/L. Originally we planned to allow the grasses to grow for 12-weeks at which point we intended to clip, oven-dry and weigh the shoots, and finally digest them in order to determine their metal concentrations. However, after 12-weeks many of the treatments had not generated enough plant material to digest. For this reason, we extended the experiment for 18-weeks in hopes of producing more plant material. However, this was not the case, and many of the replicates actually began losing more material during this time. As a result, we were unable to perform the digest, but we clipped the plant material oven-dried it at 105°C for 24-hours and weighed it in order to perform a between treatment comparison.

**Combination Mill Tailings Field Study**

The site that we chose for the field study was a large area of semi-acidic tailings, mostly devoid of vegetation, that comprises part of the Combination Mill Site in the Black Pine Mining District, approximately ten miles northwest of Philipsburg, Montana. The property is owned by ASARCO and the tailings were deposited across the floodplain of Lower Willow Creek. Lower Willow Creek is a tributary of Flint Creek, which flows into the Clark Fork River and eventually into the Columbia River. Schafer and Associates (1992) of Bozeman, Montana surveyed the site and reported that arsenic, Cd, Cu, iron, Pb, manganese, mercury, silver, and zinc were all present at the site in elevated levels. They also determined through water and sediment samples that the Combination Mill Site serves as a source of these metals to Lower Willow Creek. The Schafer report describes
several reclamation options for the site mainly aimed at reestablishing vegetative cover in order to reduce surface runoff, leaching to groundwater, erosion and sediment transport. Although the benefits of applying lime in conjunction with revegetation are mentioned there is no discussion of organic amendment application. However, RS and LYW appeared to have been applied to the site at some point in the last several years.

The plots were placed in a level, uniform portion of the tailings in proximity to the stream bank using a split plot design with a northern and southern block. In part we chose this design in order to locate the plots on the areas with the least amount of existing vegetation and amendments, and also because much of the LYW and RS that had been applied appeared to have been washed towards the northern end of the site. Each of the blocks contained three replicates of each treatment randomly assigned a position. Due to an error when applying the amendments, the northern block contained an additional plot that was treated with LYW. Each plot was 3x3-m with a one-meter buffer zone. Appendix 3 is a representation of the layout.

Site Characterization

Prior to applying amendments to the site, we removed as much of the previously applied amendments as possible, and staked out the plots. We collected a composite sample from each plot from a depth up to 7.5-cm to assess pre-treatment variability across the site. The organic amendments were incorporated manually to a depth of 7.5-cm. All of the composite samples were oven-dried and sieved to 2.00-mm using a USA standard testing sieve #10. A portion of the composite sample from five randomly selected plots from each of the two blocks was ground using a shatterbox and analyzed by The Murdock Laboratory to determine the carbon to nitrogen ratio. The pH was recorded for each of the 37 plots. We frequently recalibrated the pH meter using a known buffer solution to ensure reliable readings. Finally the pre-treatment aqua-regia extractable Cd, Cu, and Pb concentrations were determined for each of the plots. This was done by digesting five grams of the tailings samples in 50-ml of 4:1 HCl and HNO₃. We allowed the digest to occur for 16-hours then, we vacuum filtered the samples using Whatman 42 filters.

The samples were analyzed for CaCl₂ and NaOH-extractable Cu, Cd, and Pb. We combined 5-grams of each sample with 50-ml of 0.01M CaCl₂ in 50-ml centrifuge tubes. The samples were placed in a shaker on medium for 16 hours, then centrifuged at 30,000-g for 20-minutes. Next, the supernatant was decanted, acidified, and vacuum filtered using a 0.2-micron filter. Finally the samples were refrigerated until they were analyzed for the metal concentrations using an AAS. The solution concentration data (ppm) was obtained from the AAS was then converted to soil concentration (µg/g) for statistical analysis. This procedure was repeated using 1M NaOH.
The pretreatment pH of the field plots in 1999 ranged from 3.7 to 5.8 and averaged 4.4 with a 95% confidence interval of ±0.2. However, an independent samples t-test concluded that there was no significant difference between the pH of the plots in the northern and southern field plot blocks (p=0.203). The carbon to nitrogen ratio ranged from 15:1 to 30:1 also with no significant differences in the amount of carbon (p=0.346) or nitrogen (p=0.579), or in their ratio (p=0.527) between the plots in the northern and southern blocks.

The aqua regia-extractable, soil Cu concentrations for each of the field plots ranged between 1440 and 4438-µg/g with a mean value of 2997-µg/g. The Cd range was 2.41 to 12.9-µg/g with a mean of 8.12-µg/g, and Pb ranged between 260 and 9196 µg/g with a mean of 4168-µg/g. For each metal the minimum concentration was always found in plot 24 and the maximum was always found in plot 1. Independent samples t-tests indicated that there was no significant difference in the aqua regia-extractable Cu concentrations between the northern and southern block (p=0.150), but that the amount of aqua-regia-extractable Cd (p=0.000) and Pb (0.004) was greater in the northern block than in the southern block of plots. However, because we employed the randomized split plot design this variation was not present in the treatment groups. One-way ANOVAs indicated that there was no significant difference in the amount of AR-extractable Cd (p=0.808), Cu (p=0.915), or Pb (p=0.877) in the pre-treatment plots grouped according to which treatment they received. It is interesting to note, that while it is not significant the plots assigned to be treated with LYW had the highest aqua regia-extractable levels of all three metals.

The amount of CaCl$_2$ and NaOH-extractable Cd, Cu, and Pb in the pre-treatment plots was analyzed in the same fashion as the aqua regia-extractable samples to ensure that significant differences did not already exist in metals concentrations between the treatment groups prior to the application of the amendments (Table 6). One-way ANOVAs (1-A) indicated that there was no significant difference in the amount of CaCl$_2$-extractable Cd and Cu, and in the amount of NaOH-extractable Cu and Pb in the pre-treatment plot samples from the different treatment groups. It was necessary to perform Kruskal-Wallis tests (K-W) for the NaOH-extractable Cd and CaCl$_2$-extractable Pb concentrations because the homogeneity of variance assumption was invalid for these data; however, these tests also indicated that there were no significant differences.

Independent samples t-tests indicated that there was no significant difference between the amount of CaCl$_2$ and NaOH-extractable Cd (p=0.101 and p=0.156) and Cu (p=0.819 and p=0.879), and the amount of CaCl$_2$-extractable Pb (p=0.288) in the northern and southern blocks of the Combination Mill field study site. However, the amount of NaOH-extractable Pb was significantly greater in the northern block than in the southern block (p=0.026).
Treatment Application and Post-treatment Metal Analysis

Each plot received either no application (control), or 10 tons per acre of RS, LYW, and PMS, CCS, or CCM. The RS, LYW, and PMS amended plots were treated with fertilizer of N, P, K, and S to create a N and P concentration similar to that of the CCM, and composted sewage sludge treatments. Finally, we seeded the plots with thick spike wheat grass at the equivalent of the recommended rate of twelve pounds per acre.

In June of 2000, we collected a post-treatment set of composite samples from each of the plots and analyzed them for CaCl\(_2\) and NaOH-extractable metal concentrations using the same technique as described above.

At the time we collected the composite samples we also placed resin capsules in each plot in an attempt to measure any differences in the amount of metals leached below the rooting zone. We made our own resin capsules using nude stockings cut into 20x20-cm squares with 15-ml of M614 mixed bed resin (3.9-g oven dried weight) and cotton twine to tie the bags shut. The resin was rinsed 3 times with 0.5M HCl. We buried two resin capsules per plot to a depth 15-20-cm. Two-plots per treatment also received a third resin capsule. The capsules remained buried for eight-weeks. After we removed them they were refrigerated until they could be extracted using 1M HCl.

We encountered several problems using the hand-made resin capsules that might have been avoided had we been able to purchase some of the commercially available capsules. First, the stocking frequently ripped when we removed them from the plots and in some cases they had to be discarded because resin was lost. Second, because of their size and lack of shape the conventional method for extracting the commercially available resin capsules could not be used. In order to extract them, each was placed in an 80-ml beaker with 20-ml of 1M HCl and stirred. We allowed them to sit for five minutes then poured the solution into 200-ml French square bottles. This step was repeated two more times until a total of 60-ml of solution had been added. The wet resin capsules were placed on top of the French-square bottles to allow the remaining extractant to drip out. The third problem with the resin capsules was that the stocking material held sediment that frequently washed off in the extraction and ended up in the solution. This created a problem because the amount of sediment varied greatly from resin capsule to resin capsule, potentially affecting the amount of metal extracted by the HCl wash. Additionally, this made it necessary to vacuum-filter the samples using Whatman 42 filter papers.
Microbial Biomass

The post-treatment composite samples were also analyzed for total microbial biomass. Microbial biomass was determined by CHCl$_3$ fumigation, extraction with 2M KCl, and reaction with ninhydrin as described by Joergenson and Brookes (1990) and modified by DeLuca and Keeney (1993). The resulting solutions were analyzed using a visible light spectrophotometer at 570-nm and the absorbance was converted to soil concentrations of ninhydrin-reactive nitrogen. Microbial biomass C was calculated as the difference between ninhydrin-reactive N in fumigated samples and non-fumigated samples multiplied by a factor of 21 (Joergensen and Brookes, 1990).

Biomass Production

At the end of the growing season, we clipped all of the vegetation from the plots including the seeded thick spike wheat grass as well as other naturally occurring grasses and forbs. The plant material was oven-dried at 105°C for 24-hours and then weighed and the dried weight in grams was converted to biomass (grams/m$^2$) for statistical analysis.

Statistical Procedures

For all of our data, we assumed normal distributions and generally tried to analyze the data using one-way ANOVAs if necessary followed by multiple comparisons using Tukey’s procedure. All p-values are reported, but we used $\alpha=0.05$ to determine significance. In many cases however, the homogeneity of variance assumption was violated. In these cases we used the non-parametric Kruskall-Wallis test instead of the one-way ANOVA, and did no multiple comparisons. However, in these cases the mean and standard error are always included either in the text or in table form.

Detection Limits

As in the preliminary experiment we determined the detection limit for each of the metals on the AAS to be the approximate practical quantification limit (PQL) calculated as ten times the standard deviation of a blank sample analyzed seven times. Averaged over the days that analysis was done the approximate PQL for Cd was approximately 0.15-ppm, which when the samples were converted to soil concentration was equivalent to 1.5-µg/g. The Cu PQL was approximately 0.8-ppm or 8.0-µg/g, and the Pb PQL was roughly 3.0-ppm or the equivalent of 30.0-µg/g. Ideally we would use the he most conservative PQL from all of the days analysis was conducted. However, as we describe later, it became necessary for us to use data that was less than these reported PQLs during statistical analysis in order to depict the overall trend of the data. Therefore, we are reporting the
PQLs here for reference only and to clarify that although we considered them we did not strictly apply them.

Most of the concentrations of Cd, Cu, and Pb from the CaCl$_2$ and NaOH-extracted organic amendments and the reagent blanks were very close to zero and well below the practical quantification limits of the AAS (Table 2). There were two exceptions. The NaOH-extracted CCM and composted sewage sludge both had Cu concentration readings above the PQL. These were 11.6-µg/g and 43.1µg/g respectively. NaOH-extracted Cu also had the highest reading for all of the other amendments as well, but they were all still below the reported detection limits.

Results

Preliminary Laboratory Experiment with Clark Fork River Tailings

Despite the problems encountered with the sequential extraction method (described above), we were able to observe the influence of the different organic amendments on the forms and concentrations of the metals present in the tailings, which in turn influence metal mobility and bioavailability (Figure 1). The PMS and the composted amendments appeared to most effectively reduce the total soluble Cu in the un-limed tailings and the total extractable metals overall. The majority of metals in the tailings treated with PMS were not sorbed to organic material, but rather appeared to be in inorganic precipitates. The addition of LYW appeared to have little influence on the total extractable metals. The addition of lime appeared to increase the proportion of metals found in non-extractable, inorganic, precipitate forms, and none of the amendments appeared to reverse the beneficial effects of the lime. It is interesting to note that the treatment of Clarkfork tailings with PMS resulting in primarily carbonate rather than organic bound Pb.

Combination Mill Tailings Laboratory Experiment

Treatment of tailings with PMS, CCM, and CSS consistently resulted in a significant reduction in soluble (CaCl$_2$ extractable) metals. Most of the insoluble Ca and Pb appeared to be bound to organic materials that were readily solubilized in NaOH. Cadmium concentrations were commonly found to be below the detection limits of the AAS.

Cu Concentrations

Soluble Cu concentrations were significantly reduced by all of the organic treatments (Table 3). However, this effect was extremely modest for the LYW treatment. The CCM and the PMS treatments resulted in the lowest concentrations of of CaCl$_2$-extractable Cu (Table 3, Figure 2). The RS resulted in soluble Cu concentrations similar to that of the CSS treatment.
The NaOH extractions demonstrated that the majority of the reduction in soluble Cu, as a result of the organic amendments, was a result of organic sorption of Cu. The LYW, RS, and PMS treated samples all had significantly less NaOH-extractable Cu than did the CCM and CSS treated samples as well as the control (Table 3, Figure 2). However, the percent of NaOH extractable Cu accounted for by CaCl\textsubscript{2} extractable Cu was 100% for the LYW and Control treatments and 73% for RS. In contrast, soluble Cu accounted for only 37%, 7%, and 5% of the NaOH extractable metals in the CSS, CCM and PMS treated tailings respectively. This means that most of the reduction in metal solubility in these treatments can be accounted for by organic sorption of Cu.

**Pb Concentrations**

The analysis of the CaCl\textsubscript{2}-extractable Pb was identical to that of the CaCl\textsubscript{2}-extractable Cu. Again, the CCM and the PMS treated samples had the least amount of CaCl\textsubscript{2}-extractable Pb, followed by those treated with RS and CSS, then the LYW treated tailings, and finally the control samples contained the most CaCl\textsubscript{2}-extractable Pb (Table 3, Figure 2).

The PMS, CCM, and RS treated samples had significantly less NaOH-extractable Pb than the LYW treated samples or the control did. However, the CSS treated samples were not significantly different from any of the other treatments (Table 3). The NaOH extractions demonstrate that only CaCl\textsubscript{2} extractions accounted for only 10%, 0.6% and 1.2% of the organic bound Pb for CSS, CCM, and PMS respectively. The preliminary study using Clark Fork tailings suggests that much of the Pb bound by PMS likely exist in carbonate forms.
Figure 1. Copper concentrations in sequentially fractionated limed (L) and unlimed (U) Clarkfork tailings treated with no amendment (NA), log yard waste (LYW), raw straw (RS), pulp mill sludge (PMS), composted cow manure (CCM), and composted sewage sludge (CSS) after 16 weeks of laboratory incubation.
Figure 1 (cont). Lead concentrations in sequentially fractionated limed (L) and unlimed (U) Clarkfork tailings treated with no amendment (NA), log yard waste (LYW), raw straw (RS), pulp mill sludge (PMS), composted cow manure (CCM), and composted sewage sludge (CSS) after 16 weeks of laboratory incubation.
Table 3. Probability values (ANOVA) for CaCl₂ and NaOH extractable Cd, Cu, and Pb for combination mill tailings treated with no amendment (NA), log yard waste (LYW), raw straw (RS), pulp mill sludge (PMS), composted cow manure (CCM), and composted sewage sludge (CSS) following 16 weeks of laboratory incubation.

### CaCl₂

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Figure 2. Extractable Cu, Pb, and Cd in Combination Mill tailings treated with no amendment (NA), log yard waste (LYW), raw straw (RS), pulp mill sludge (PMS), composted cow manure (CCM), and composted sewage sludge (CSS) after 16 weeks of laboratory incubation (statistical significance found in Table 3).
**Cd Concentrations**

The CCM and PMS treatments greatly reduced soluble Cd concentrations to levels below detection limits (0.15-ppm) (Table 3, Figure 2). While this is alone is indicative of treatment differences the lack of data points precludes the use of ANOVA. This was a recurrent problem in our data analysis and in order to depict the overall trends in our data we frequently found it necessary to include undesirable numbers. A one-way ANOVA using all of the Cd soil concentration (µg/g) data indicated that there were significant treatment differences (p=0.000) in the mean Cd concentrations extracted from the soil by CaCl₂. In order to create consistency, we did attempt to replace the soil concentrations that were based on solution concentration readings less than the PQL with data points that were based on one-half of the PQL value, in the case of Cd this was 0.75 µg/g. This resulted in the homogeneity of variance assumption being violated, making it impossible to use the ANOVA. Instead, we ran a Kruskal-Wallis test, which concurred with the original ANOVA that there were treatment differences (p=0.000).

The CCM and PMS treatments resulted in significantly lower levels of CaCl₂-extractable Cd than the untreated tailings (p=0.000 for both). The CSS treated tailings contained the next least amount of CaCl₂-extractable Cd although it was not significantly less than the control at the α=0.05 level (p=0.069). The concentrations of Cd in the NaOH extracts were all below the detection limit and therefore no statistical analysis is discussed.

**Ultrafiltration**

Ultrafiltration was used to determine what portion of the metals exist in humic acid complexes. A decrease in concentration with ultrafiltration would imply the presence of high molecular weight organic coordinating compounds (Table 3, Figure 2). A Kruskal-Wallis test confirmed that there were no treatment differences (p=0.078) between the percent difference of the Cu concentrations in the NaOH-extracted samples before and after ultrafiltration. This lack of effect of ultrafiltration on metal concentration suggests that most of the NaOH extractable metals exist as either inorganic complexes, low molecular weight inorganic complexes, or in the free ion form.

The slight increase in some cases was likely the result of inter-day variability in the AAS. There are also two extreme outliers in the data. These likely represent some more severe instrument variability that could be identified with a larger sample size or they could be the result of dilution errors. When they are removed from the data set the Kruskall-Wallis test indicates that treatment differences do exist (p=0.030). In this scenario, the pulp mil sludge and CSS treated samples resulted in the greatest decrease in concentration with 1.8% and 1.5% differences respectively indicating that they have the largest portion of Cu
bound to the humic acid fraction. These samples are followed by the control (0.4%) and the samples treated with RS (0.3%). Finally, the LYW and CCM treated samples both have a no discernable change compared to the control. It is possible that this represents a real trend that could be established with future studies.

There was no notable change in Pb concentrations in the NaOH-extracted samples that were ultrafiltered. Only seven of the 28 samples analyzed showed an increase in NaOH-extracted Pb concentration in the ultrafiltered samples and four of those seven were for PMS amended samples. The tailings samples treated with PMS had the lowest concentration of NaOH-extractable Pb, indicating that it has potentially been precipitated out as insoluble carbonate forms due to the high pH and high carbonate concentration in the PMS.

A Kruskall-Wallis test was used to compare the percent differences between the NaOH-extracted and NaOH-extracted and ultrafiltered samples. The results indicated that there were only significant treatment differences evident in the concentrations of Pb removed using the 10,000-mw cutoff membranes (p=0.020). Interestingly the treatment that had the largest percent difference in Pb concentration was the control (Table 3, Figure 2) followed by the CCM, LYW, RS, composted sewage sludge, and finally the PMS.

**Combination Mill Tailings Greenhouse Experiment**

Thick-spike wheat grass seeds had the greatest percent germination in the tailings treated with the CCM, followed by those treated with the CSS, and PMS. The seeds planted in the LYW treated tailings were next followed by the untreated tailings. The seeds planted in the Combination Mill tailings treated with the RS did not germinate during the entire course of the experiment. This is particularly interesting, because a previous attempt had been made to reclaim the Combination Mill site using lime and straw. A Kruskal-Wallis test did indicate that there were significant treatment differences in the percent of seeds that germinated (p=0.005). Table 4 gives the mean percent germination and standard error for each of the treatments.

Although the seeds planted in the tailings treated with the CSS displayed about a 43% germination rate, during the course of the experiment their physical condition deteriorated. By the end of the 18 weeks, they had 0% survivorship along with the plants in the LYW treated and untreated tailings. Only the grasses growing in the tailings treated with the CCM and the PMS had remaining survivors: 95% and 60% survivorship respectively (Table 4). At the end of the experiment these plant also appeared to be vigorous and healthy. The Kruskal-Wallis test indicated that there were also significant differences in the distribution of the survivorship percentages for the treatments (p=0.000).
Table 4. Percent germination, survivorship and plant dry weight production of thickspike wheatgrass grown in Combination Mill tailings treated with the five organic amendments. Mean and standard error and non-parametric Kruskall-Wallis tests were used to compare the means and the p-values are reported.

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<th>Percent Survivorship (K-W p= 0.000)</th>
<th>Plant Dry Weight (g) (K-W p= 0.000)</th>
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Only the CCM and PMS treated tailings had living plants remaining, thus these were the only pots from which biomass measurements could be made (Table 4). The CCM treatment resulted in significantly (p=0.000) greater biomass production than the PMS in spite of the addition of fertilizer N to the PMS treatments.

**Combination Mill Tailings Field Study**

**Metal Concentrations**

The CaCl$_2$-extractable Cu and Pb concentrations were found to be directly influenced by organic amendments (Table 6). Due to a lack of homogeneity of variance, it was necessary to use the non-parametric Kruskal-Wallis test. Plots treated with the PMS contained dramatically lower CaCl$_2$-exchangeable Cu and Pb than the control. Interestingly, it appears that the plots treated with the CSS actually had somewhat higher concentrations of CaCl$_2$-exchangeable Cu. This may be due to the elevated levels of Cu in the CSS itself. Although, the CSS alone does not have elevated levels of CaCl$_2$-extractable Cu (Table 2) when applied to an acidic soil, the pH of the CSS may have been sufficiently reduced as to experience a slight increase in the solubility of the Cu that was otherwise present as insoluble oxides or carbonates.

The different organic amendments did not appear to have a similar treatment effect on the CaCl$_2$-extractable Cd. The NaOH-extractable Cu and Pb samples exhibited no treatment differences (Table 6). These concentrations are all very close to zero and far below the practical quantification limit of the AAS. We included them for the sake of consistency, but do not believe that they are reliable enough to hold up to accurate statistical analysis.
Figure 3. Field experiment assessment of extractable Cu, Pb, and Cd in Combination Mill tailings treated with no amendment (NA), log yard waste (LYW), raw straw (RS), pulp mill sludge (PMS), composted cow manure (CCM), and composted sewage sludge (CSS) one year following treatment. ** Indicates significantly different than the control at P < 0.01 using a Kruskal-Wallis test.
We attempted to use ionic resins to sorb metals at depth below the treatment plots. Unfortunately, we lost many resin bags to degradation prior to retrieval. The result was that we had only six plots with all three resin bags that were successfully removed and analyzed. For all of our other analysis we used composite samples that would have masked variability, although the between plot variability was still evident. Appendix 4 contains the mean resin capsule Cu and Pb concentration for each plot. Despite the variability, the beginnings of a pattern that generally coincide with the aqua regia-extracted concentrations from the pre-treatment, composite samples are evident in this spatial form. A Kruskall-Wallis test was used to compare the means of each plot to determine if there was any treatment effect. Neither the Cu (p=0.151) nor the Pb (p=0.928) resin capsule concentrations appeared to be affected by the treatments.

**Microbial Biomass**

Only approximately one-quarter of the post-treatment field plot samples contained measurable concentrations of microbial biomass C (mg biomass C/kg soil). There was no significant treatment effect on microbial biomass C as determined by a Kruskal-Wallis test (p=0.239). However, it appears that location of the plots played a more important role in determining if microbial biomass carbon was present as there was greater microbial activity in the northern block than the southern block.

**Plant Biomass Production**

Similar to microbial biomass, plant biomass production was poor on all plots. Plots treated with CCM had the highest plant biomass production of all treatments (Figure 4) followed by PMS, RS, LYW, control, and CSS in that order (Kruskal-Wallis test, significant at P < 0.005). Also, we compared the amount of biomass produced in each plot in the northern and southern blocks using an independent samples t-test. The plots in the northern block produced significantly more biomass than did the plots in the southern plots. (4.07±1.84 g/m²) and (1.26±0.680 g/m²) represent 95% confidence intervals for the biomass production of the plots in the northern and southern blocks respectively. However since each of the blocks contained three of the treatments this inter-block variation is accounted for in the analysis of the treatments variations.
Figure 4. Field plot assessment of plant biomass production (g/m²) 1-year following application of with no amendment (NA), log yard waste (LYW), raw straw (RS), pulp mill sludge (PMS), composted cow manure (CCM), and composted sewage sludge (CSS) to field plots. ** Indicates significantly different than the control at P < 0.01.

**Discussion**

The main objective behind application of organic amendments to acid mine tailings is to ensure revegetation success to stabilize the land surface and reduce overland flow of water, sediment and metals. We set out to determine how various organic amendments influence metal mobility as well as plant establishment. Laboratory experiments with Clark Fork stream side tailings and Combination Mill tailings clearly demonstrate that all five organic amendments, as 10% organic matter addition, reduce the concentration of soluble Cu, Cd, and Pb. In the case of Cu, and Pb all of the organic materials significantly reduce the concentrations compared to the control. However, only the CCM and PMS reduced the concentrations of soluble Cd compared to the control. In field experiments, with treatments applied as a 1% surface soil addition, only PMS effectively reduced soluble Cu concentrations. The limited positive effect of the CSS differs from the findings of DeLuca and Lynch (1997), but might be partially explained by the relatively high concentration of Cu in the CSS and the very high concentrations of Cu at the Combination Mill site (Table 1).

The NaOH extractions verified that the reduction in metal solubility as a result of CCM, PMS, and CSS applications were greatly a function of organic sorption. The high carbonate content of the PMS resulted in the formation of lead carbonate precipitates. Importantly, the PMS and CCM resulted in organic
bound metals that did not appear to be readily soluble (were not extractable with CaCl₂) and therefore should have limited mobility.

The lack of Cu found in high molecular weight Cu complexes in the CSS and CCM goes against the hypothesis that composted materials would have a higher proportion of metals bound to humic acids. Temminghoff et al. (1994) extracted samples from 16 different pH and Cu concentration combinations using 0.0033 M Ca(NO₃)₂ to get an index of the easily extractable Cu species present. Next, they filtered the extract to separate the humic from the fulvic acids, and analyzed the filtrates for Cu concentration using flame and graphite furnace AAS. They found that as the pH decreased the binding of Cu to fulvic acids increased, and that the opposite was true for humic acids. Temminghoff et al. (1994) reported that at pH of approximately 4.4, Cu was predominately bound by fulvic acids, while at pH of approximately 5.7 most of the Cu was bound to humic acids. In our study, the PMS and the CCM elevated the pH of the tailings in the laboratory experiment to 5.82 and 5.36 respectively (Table 9). The resulting pH of the Combination Mill tailings after organic amendment additions was below 4.4. This may explain why the majority of Cu in our samples was associated with fulvic acids and why the PMS showed the greatest overall decrease in Cu concentration between the samples before and after ultrafiltration - corresponding to an increase in Cu associated with humic acids. Although, a 1.8% decrease does not correspond with the “majority” reported by Temminghoff et al. (1994). Also, we did not see similar results with the CCM samples, which show no discernable difference between the Cu concentrations before and after ultrafiltration. The ultrafiltration data we are presenting are likely unreliable because of the inter-day variability of the AAS. But this line of research has important implications for water pollution potential on reclamation sites and deserves further investigation. As we have already stated the ability of fulvic acids to act as chelates could potentially increase water pollution through increased Cu mobility, and according to the research of Temminghoff et al (1994) this would especially be a problem on acid sites associated with mining contamination unless the pH was adequately elevated and maintained during the reclamation process.

Our use of ion exchange resins to capture subsurface flow of metals beneath the field plots was not successful. Interplot variability and the potential lateral movement of metals beneath the plots resulted in inconsistent results.

DeLuca and Lynch (1997) carried out laboratory and field experiments to determine how aged LYW and composted sewage sludge would affect metal mobility in sulfidic mine tailings. They found that the application of the composted material significantly reduced the concentration of exchangeable Cu, Pb, and Fe in the tailings, while application of the uncomposted material only slightly decreased the exchangeable Cu and Fe and increased exchangeable concentrations of Pb. We found no evidence of increased concentrations of exchangeable Pb with the addition of uncomposted material; the addition of all of the organic materials reduced the concentrations of both Cu and Pb compared to
We did observe the same significant difference between the CaCl$_2$-extractable concentrations of Pb and Cu for the laboratory samples treated with composted sewage sludge versus LYW. But we could not corroborate the significant reduction in the exchangeable Cu and Pb concentrations being attributed to the composted and uncomposted materials.

Despite the fact that we used the same source of CSS as DeLuca and Lynch (1997), we found that the PMS and CCM consistently showed the greatest reductions in the CaCl$_2$-extractable concentrations of Cd, Cu and Pb, while the CSS was frequently not significantly different from the uncomposted RS. We believe that this moderate reduction may be due to the elevated levels of Pb and Cu present in the CSS combined with the acid soils acting to increase the solubility of these metals, but it could also be a result of differences in the character of the Combination Mill tailings and those found in Sandbar Creek (DeLuca and Lynch, 1997).

The CCM and PMS treatments resulted in the greatest revegetation success of all of the treatments. By the end of the greenhouse experiment, only tailings treated with these two amendments had surviving plants. In the field study, CCM treated plots had the greatest biomass production (7.77-g/m$^2$) followed by those treated with PMS (3.49-g/m$^2$). The CSS amendments did not increase plant growth or the activity of microorganisms. This again contrasts to the findings of DeLuca and Lynch (1997), but might be partially explained by the high concentrations of Hg and Pb found in the Combination Mill tailings (see Table 1). It is possible that there was a lethal interaction between Hg and the CSS resulting in methylation of mercury thereby creating an environment acutely toxic to microorganisms and plants (Paul and Clark, 1996). Lead may also be methylated by microbes in the presence of luxuriant amounts of organic matter (Paul and Clark, 1996).

The greater variability in the field plot metal concentration data, allows us to speculate only loosely about the results. Because Kruskall-Wallis tests were used to compare the CaCl$_2$-extractable Cu and Pb concentrations among treatments, no multiple comparisons were made. But, it does appear that the PMS treated plots had dramatically reduced concentrations of exchangeable Cu and Pb when compared to the other treatments. Alone this result might not stand up to scrutiny, but when considered in the context of the overall performance of the PMS in the laboratory and greenhouse experiments and the field study, it appears to be accurate.

The size and solubility differences between humic and fulvic acids are important factors for determining their availability to plants. Thornton (1979) reported that the size of organic Cu complexes affects the bioavailability of Cu. He stated that Cu complexes with molecular weights less than 1000 daltons are more available for plant uptake than those with molecular weights of greater than 5000 daltons.
This is in agreement with the behavior that would be expected from metals being chelated by soluble fulvic acid and sorbed to insoluble humic acids.

Only about one quarter of the field plots contained measurable amounts of microbial biomass carbon. As the majority of these plots were adjacent to one another it appears that location is more important than the type of amendment added to the plot in determining how microbial recovery occurs. Visser (1985) also investigated the addition of amendments including NPK fertilizer, sewage sludge and peat in the reclamation of mine spoils. Visser (1985) reported that the addition of the amendments created treatment differences in the amount of microbial biomass carbon after 27 and 39 months respectively on two different sites. We performed our analysis only 12 months after the amendment application and seeding; perhaps our short time frame did not provide ample time for microbial recovery.

Conclusion

The CCM and PMS appeared to consistently out perform the other amendments in decreasing the concentrations of soluble and exchangeable Cu, Cd, and Pb, as well as in vegetation establishment. The composted sewage sludge (CSS) was more effective than RS, LYW or no amendment at lowering the concentrations of exchangeable metals, but had no positive influence on revegetation success. The application of LYW had the least positive impact on the reduction of soluble and exchangeable metals and on revegetation success compared to the control. The results of these experiments suggest that LYW and RS are not suitable organic mine reclamation amendments. The use of PMS in reclamation efforts appears to have significant potential. It is not clear whether the beneficial effect of this amendment is a function of carbonate precipitation of metals, organic sorption of metals, or a combination of both. The high C:N of PMS requires that it be amended with an N source to achieve effective revegetation. The CCM amendments were effective in promoting a reduction in soluble metal concentrations as a result of organic sorption. The high fertility nature of this material also enhanced grass growth in the greenhouse to a greater degree than the other amendments. The differences observed here between CCM and CSS are difficult to explain but could be a result of the high Cu concentration in the CSS or due to the uniquely high concentrations of Pb and Hg found in the Combination Mill tailings making these particular differences somewhat site specific.
Literature Cited


Appendix 1
Throughout its 36 year history, research, education, and information transfer (IT) have been the key components of the Montana University System Water Centers mission - to promote problem-solving partnerships among university, government, and private sector participants. Prior to the advent of the World Wide Web (WWW), small budgets assigned to the IT effort were mainly used up by copying and postage costs, leaving little remaining to support this effort. In 1996, the entire concept of IT at the Water Center was redefined when hosting a WWW site became an affordable reality on the Montana State University campus. As with most fledgling WWW sites, the Centers first attempts at online information were small reports, notices, directories, links, etc. generally highlighting only Center activities. The idea soon evolved however, that the Centers WWW efforts could better be utilized by developing and maintaining an impartial clearinghouse of Montana water information. There was no unified and affordable structure through which scientists, agency personnel, and watershed group participants could access information and resources, and exchange experiences. Through a timely EPA appropriation for watershed development in 1997, the Water Center seized the opportunity to construct a significant Web presentation entitled MONTANA WATER. Concurrently, the Montana Watershed Coordination Council (MWCC) provided guidance (and a few small grants) on watershed group materials, since many isolated watershed projects were moving vigorously forward throughout the Northern Rocky Mountain region.

PURPOSE Create a website to centralize communication and data-sharing efforts among agencies, organizations, and individuals engaged in Montana water issues i.e., watershed modeling and planning, water quality efforts, etc. PARTNERS of MONTANA WATER - (funding) EPA Montana Office, Bureau of Reclamation through the Montana Watershed Coordination Council, U.S. G.S. - Water Institute Program base grant. (others) Natural Resources Information System (NRIS) of the Montana State Library, Montana Bureau of Mines & Geology, MT DEQ, MT DNRC, MT Association of Conservation Districts, USDA Natural Resources & Conservation Service, MT Dept. of Fish, Wildlife & Parks.

OTHER ACTIVITIES

Montana Watershed WEB - a component of MONTANA WATER - this site serves as a bulletin board and resource for a variety of Montana Watershed groups as well as a central switchboard for several ongoing agency activities. AWRA - MT Web - In addition to providing the necessary organizational and logistic support to the annual water conference, the Montana Water Center also designed and maintains the AWRA - MT Web site hosted by AWRA National at http://www.awra.org/state/montana/ Outreach and Information Referral - Using the web sites as tools, the Communications Director of the Center continues to provide information to the general public on a daily basis.

Basic Information
<table>
<thead>
<tr>
<th><strong>Title:</strong></th>
<th>MONTANA WATER - A web information network</th>
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<tr>
<td><strong>Start Date:</strong></td>
<td>3/1/2000</td>
</tr>
<tr>
<td><strong>End Date:</strong></td>
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<td>water information</td>
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<tr>
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<td>Montana Water Resources Research Center</td>
</tr>
<tr>
<td><strong>Principal Investigators:</strong></td>
<td>Kathleen Tohe Stephens</td>
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Publication
MONTANA WATER is an ongoing WEB site designed and maintained as an information switchboard for all of Montana and the region. This is a multi year, ongoing project. Highlights include:

- What’s New - Water News from around the state and beyond - updated continually
- Site updates.
- Development and refinement of several database driven sites - were web users can access, add, and change information online. These include: Events, Water Expertise Directory, Grants and Funding, Technical Resources, Watershed Groups and online library
- the Montana Source Water Protection Site
- Montana Operator Training
## Basic Information

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<tr>
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<tr>
<td><strong>Principal Investigators:</strong></td>
<td>Kathleen Tohe Stephens</td>
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## Publication
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.

Highlights Include:

" upgrades and access to agendas and notes for a variety of Montana Watershed Coordination Council Meetings

" the interactive watershed WEB mapper - hosted by the Montana Natural Resources Information System.

" Under construction - Sponsored through a Montana EPA 319 Non-point Source grant - the Watershed Rehabilitation Projects database.

Montana Watersheds is an ongoing multi year project.
### Basic Information

<table>
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<tr>
<th><strong>Title</strong></th>
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<td>Montana Water Resources Research Center</td>
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<td><strong>Principal Investigators</strong></td>
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### Publication
7th ANNUAL MONTANA WATER CONFERENCE
Holiday Inn
West Yellowstone, Montana
October 5 & 6, 2000

Thursday October 5

**Student Research Project**

 Field Trip to Yellowstone National Park lead by Dr. Stephan Custer Year 2000 Water Legend, plus David Nimick and Phil Farnes. Controlled groundwater ... wetlands ... glaciers ... lava flows ... calderas and geysers. Leaving from the Holiday Inn Parking lot via bus. **Extra fee, so register early. Seating is limited.**

REGISTRATION AT HOLIDAY INN

1:00 - 1:20

Opening Remarks

**Session A. WATER QUALITY**

*Moderator: Jennifer Wintersteen, U.S. EPA Montana*

<table>
<thead>
<tr>
<th>Time</th>
<th>Topic</th>
<th>Presenters</th>
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<tbody>
<tr>
<td>1:20 - 1:40</td>
<td>Diel Variation in Dissolved Metal Concentrations in Streams Draining Abandoned Mine Lands</td>
<td>David A. Nimick, Hydrologist and Thomas E. Cleasby, U.S. Geological Survey</td>
</tr>
<tr>
<td>1:40 - 2:00</td>
<td>Acid Rock Drainage on Butte Hill: A Tale of Two Pits</td>
<td>Pam Newbrough, Graduate Student and Chris Gammons, Geological Engineering - MT Tech of UM</td>
</tr>
<tr>
<td>2:00 - 2:20</td>
<td>Waterline Permeation by Volatile Organic Compounds - A Case Study: Big Sandy, Montana</td>
<td>Mike Trombetta, Chief and Bill Hammer, Environmental Specialist, Hazardous Waste Site Cleanup Bureau - Montana DEQ</td>
</tr>
<tr>
<td>2:40 - 3:00</td>
<td>Enhanced Wet Air Oxidation of Sediments Contaminated with Polychlorinated Biphenyls</td>
<td>James E. Duffy, Assistant Professor, Marc Anderson, Charles G. Hill Jr., and Walter A. Zeltner, Chemical Engineering, Montana State University</td>
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</tbody>
</table>

3:00 - 3:20

**BREAK**
Session B. WETLANDS/WATER TREATMENT  
Moderator: Lynda Saul, Montana DEQ

3:20 - 3:40  
**Geochemical Considerations for the Operation and Closure of Anaerobic Wetlands for the Sequestration of Heavy Metals: The Importance of Metal-Sulfide Complexation and the Importance of Long-Term Sulfide Precipitate Stability**  
Angela K. Fransden, M.S. graduate & USGS Intern - Environmental Engineering - Montana Tech of UM

3:40 - 4:00  
**Constructed Wetlands for Water Quality Enhancement: Research at MSU, 1995-2000**  
Otto R. Stein, Associate Professor  
P.B. Hook, J.C. Cahoon, J.A. Biederman, W.C. Allen, and B.W. Towler  
Civil Engineering - Montana State U.

4:00 - 4:20  
**Cold-region Feasibility of Water Treatment Wetlands: Plant Species Selection is Critical**  
Paul B. Hook, Assistant Professor  
W.C. Allen, O.R. Stein, and J.A. Biederman - Land Resources and Environmental Sciences, Montana State U.

4:20 - 4:40  
**The Fates of Polar Organic Solvents in a Constructed Wetland Treatment System**  
Janet Kowles, M.S. Graduate Student  
O.R. Stein, W.L. Jones, and A.K. Camper  
Civil Engineering - Montana State U.

4:40 - 5:00  
**Effects of Supplemental Organic Carbon Addition on Sulfate Reduction and Zinc Precipitation Using Microcosm Constructed Wetlands in Cold Climate Regions**  
Deborah J. Borden, M.S. Graduate Student  
O.R. Stein, P.B. Hook, and W.L. Jones -- Civil Engineering - Montana State University

Session C. SURFACE WATER  
Moderator: Norm Midtlyng, USGS

3:20 - 3:40  
**Root-Rap or Restoration: The Misapplication of Rosgen-type Structures in Montana**  
Paul Callahan, Senior Hydrologist  
Land & Water Consulting

3:40 - 4:00  
**An Excel Template for Generating Rating Curves for Corrugated-Metal-Pipe Culverts**  
Joel Cahoon, Assistant Professor  
and Dan Baker  
Civil Engineering, Montana State University

4:00 - 4:20  
**Flood Hydrology for Dry Creek, Lake County, Northwestern Montana**  
Charles Parrett, Supervisory Hydrologist  
U.S. Geological Survey

4:20 - 4:40  
**Suspended-sediment Characteristics of the Yellowstone River at the Pine Creek Bridge, near Livingston, MT**  
Kyle W. Griffith, Student Intern  
U.S. Geological Survey

4:40 - 5:00  
**Suspended Sediment in the Ruby River above Ruby River Reservoir**  
Joe Van Mullem, Consulting Engineer

5:30 - 7:00  
**POSTER SESSION AND HAPPY HOUR**  
pop, snacks and partially-hosted bar

7:00  
**DINNER**  
Presentation of 5th Annual Montana Water Legend Award to Dr. Stephan Custer, Montana State University - Earth Science
### Session D. WATER MANAGEMENT - I
**Moderator:** Jane Holzer, MT Salinity Control

<table>
<thead>
<tr>
<th>Time</th>
<th>Title</th>
<th>Presenter(s)</th>
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<tbody>
<tr>
<td>8:00 - 8:20</td>
<td>Instream Flow Requirements for Riparian Vegetation in West-Central Montana</td>
<td>Stephanie K. Mulica, M.S. Graduate Student with Donald Potts, and Ward McCaughey - School of Forestry - UM</td>
</tr>
<tr>
<td>8:20 - 8:40</td>
<td>Watershed Restoration Assessment for Lost Creek -- a tributary of the Upper Clark Fork River</td>
<td>John Lhotak and James Harris M.S. Students Vicki Watson, Professor Environmental Studies, UM</td>
</tr>
<tr>
<td>8:40 - 9:00</td>
<td>Baseline Data and the Methodology for Evaluating Management Changes on a Great Plains River in Montana</td>
<td>O’Brien Hollow, M.S. Student Riparian and Wetland Research Program - UM School of Forestry</td>
</tr>
<tr>
<td>9:00 - 9:20</td>
<td>The Hydrogeologic Conceptual Model for Source Water Protection Planning</td>
<td>James Swierc, Water Quality Specialist Source Water Protection Program - Montana DEQ</td>
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<tr>
<td>9:40 - 10:00</td>
<td>Interactive Access to Comprehensive Watershed Data in Montana over the World Wide Web</td>
<td>Duane Anderson, Water Information System Coordinator Natural Resources Information System (NRIS)</td>
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### Session E. GROUNDWATER
**Moderator:** Kate Miller, MBMG

<table>
<thead>
<tr>
<th>Time</th>
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<tr>
<td>8:00 - 8:20</td>
<td>Hydrogeology of the West Billings Area, Yellowstone County, Montana</td>
<td>Jon Reiten, Assistant Research Hydrologist Montana Bureau of Mines &amp; Geology</td>
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<tr>
<td>8:20 - 8:40</td>
<td>Ground-Water Recharge Characterization Using Isotope and Geochemical Analyses, West Billings Area, Yellowstone County, Montana</td>
<td>John Olson, Asst. Research Hydrologist Montana Bureau of Mines &amp; Geology</td>
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<tr>
<td>8:40 - 9:00</td>
<td>Application of Earth Resistivity to Characterize Groundwater Conditions</td>
<td>Mark Cunnane, Engineer / Hydrogeologist Western Groundwater Services</td>
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<tr>
<td>9:00 - 9:20</td>
<td>Ground-Water Monitoring along Silver Bow Creek, Subarea 2, Silver Bow Crossing to Miles Crossing</td>
<td>Peter M. Norbeck, Hydrogeologist Montana Bureau of Mines and Geology</td>
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<tr>
<td>9:40 - 10:00</td>
<td>Assessment of Groundwater Contamination from Subdivisions</td>
<td>Kerri Fleming, M.S. Student and Steve Custer Dept. of Earth Sciences Montana State University</td>
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Friday Oct. 6 - continued

**Session F. WATER MANAGEMENT - II**  
**Moderator: John Arrigo, MT DEQ**

<table>
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<tr>
<td>10:40</td>
<td>Riparian Timber Harvest Effects on Stream Temperature and Riparian Microclimate</td>
<td>Ron Steiner, Biological Technician and Brian Sugden, Plum Creek Timber Co.</td>
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<tr>
<td>11:00</td>
<td>The Forest Road Sediment Assessment Methodology: A Practical Approach to the Development of Sediment TMDLs</td>
<td>Paul Callahan, Senior Hydrologist, Land &amp; Water Consulting</td>
</tr>
<tr>
<td>11:40</td>
<td>Protecting Montana's Water - An Enforcement Perspective</td>
<td>David Rise, Environmental Enforcement Specialist, MT DEQ</td>
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<tr>
<td>12:00</td>
<td>BUSINESS ANNOUNCEMENTS --- ADJOURN</td>
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**POSTERS** - Authors will be present during the Thursday 5:30 p.m. Poster Session

<table>
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<tr>
<th>Title</th>
<th>Authors</th>
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<tbody>
<tr>
<td>A Rating System for Rural Culvert Crossing Repair and Maintenance</td>
<td>Dan Baker, Joel Cahoon, and Jodi Carson, Western Transportation Institute - MSU</td>
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<tr>
<td>Metal Loading to Basin Creek from Surface and Ground Water near the Buckeye and Enterprise Mines, Jefferson County, Montana</td>
<td>Michael R. Cannon, Thomas E. Cleasby, and David Nimick, U.S. Geological Survey</td>
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<tr>
<td>Characterizing Water, Bed Sediment, and Aquatic Health in a Watershed Affected by Historical Mining, Montana</td>
<td>David A. Nimick, A. M. Farag, and S.E. Church, U.S. Geological Survey</td>
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<tr>
<td>The Montana Ground Water Assessment Program: Progress Report</td>
<td>Tom Patton, Larry Smith, Luke Buckley, John LaFave, and Cam Carstarphen,</td>
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<td></td>
<td>Montana Ground-Water Information Center - MBMG</td>
</tr>
<tr>
<td>Sediment Retention in a SW Montana Riparian Meadow: implications for monitoring and management</td>
<td>Paul B. Hook, J.J. Conner, and P. House, Land Resources and Environmental Sciences - MSU</td>
</tr>
<tr>
<td>Ground-Water Characterization of the Flathead Lake Area</td>
<td>Larry Smith, J. LaFave, T. Patton, C. Carstarphen, D. Mason, and M. Richter, Montana Ground-Water Assessment Program - MBMG</td>
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<tr>
<td>Willow Colonization of Mid-Channel Bars in a Braided Stream Reach, Taylor Fork, Gallatin River</td>
<td>Deb Kanter, P.B. Hook, and W. Locke, Land Resources and Environmental Sciences - MSU</td>
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<tr>
<td>Large Diel Variations in Water Quality in the Big Hole River</td>
<td>Adam Wenz, Chris Gammons, and Rebecca Ridenour, Dept. of Chemistry &amp; Geochemistry, MT Tech of UM</td>
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**THANKS TO ALL WHO MAKE THIS POSSIBLE**

AWRA 2000 Officers: Joanna Thamke, Donna Rise, Larry Dolan

The Water Center - Kathy Stephens and May Mace, Secretary and the Field Trip Leaders!
USGS Summer Intern Program
Student Support

<table>
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<tr>
<th>Category</th>
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Notable Awards and Achievements


The symposium has helped to spark much collaboration on restoration planning & action in the basin. Symposium participants have collaborated on successful proposals for restoration related projects and have continued to communicate concerning restoration vision documents and data & analysis needs for restoration planning. The symposium web page serves as a method of quickly disseminating the results of restoration related studies to the public and scientific community.

Spinoffs of the symposium are too numerous to catalog, so just a few examples will be briefly discussed. The University of Montana is currently funded by the Montana Natural Resource Damage Program to work with the Montana Natural Resource Information System to identify information needs and gaps for watershed restoration planning and action with a view to developing a proposal to build a database to house watershed restoration related data. Several University of Montana students are being funded by the Natural Resource Conservation Service and the Montana Department of Fish, Wildlife and Parks to conduct pre & post restoration assessments of streams that will undergo restoration projects. Symposium participants collaborated with the Governors NRDP advisory committee which is in the process of developing a Restoration Work Plan for the upper Clark Fork. These are exactly the sorts of spinoffs hoped for when the Symposium was planned.

The Symposium web page continues to be maintained and expanded.  

II. Project: Constructed Wetlands for Treatment of Wastewater PI: Otto Stein, Civil Engineering, Montana State University

B-05 FY95-96

In the News -- "Using a Wetland to Clean our Water" MSU News Service 9/2000. See link:

http://www.montana.edu/wwwpb/ag/bullrush.html
Publications from Prior Projects