The Alta Mine:
A Multidisciplinary Analysis of an Acid Mine Drainage Environment

LRES 442 Fall, 2007

Capstone Course Final Report

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Notes and Observations from the Lead Instructor

The LRES 442 capstone course "provides disciplinary and interdisciplinary knowledge, experiences, and skills related to Land Resource and Environmental Sciences" (MSU Course Catalog Description), and include research topic(s) relevant to current land management issues. Successful completion of this course requires the students to participate in sub-disciplinary research groups of their choice, and to become fully engaged in appropriate research activities that address hypotheses that they develop.

This course emphasizes field measurement and analysis techniques related to soils, plants, water, and microclimate. Students are also required to summarize their findings, developing written and oral communication skills. The 2007-2008 Capstone research site was selected so as to provide students with an opportunity to explore many of the core teaching and research strengths of the Department of Land Resources and Environmental Sciences. This year these included hydrology, (re)vegetation analysis, environmental chemistry, and environmental microbiology; all disciplines highly appropriate to the study of acid mine/rock drainage associated with a closed mine that is representative of those found throughout western United States.

As a group, the students worked diligently, using a wide range of analytical techniques and applied measurements in their analyses. Their efforts were strongly supported by excellent faculty participation, all of which was sincerely appreciated.

This was a solid experience for all involved, including myself.

Timothy R. McDermott
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Introduction and Background

In the 1800's a massive gold rush into Montana left a legacy of abandoned mines scattered throughout the state. These mines were abandoned when their productivity declined to unacceptable levels or declining prices of gold and copper compressed profit margins. The now closed Alta Mine is one of these abandoned mines. It is located in the Colorado Mining District, Jefferson County, Montana (latitude 46° 22' 24", longitude 112° 05' 12") approximately 1.5 miles southwest of the town of Corbin. The Alta Mine site is at an elevation between 5,600 and 6,200 feet above mean sea level in Sections 9 and 10, Township 7 North, Range 4 West.

The Alta Mine was established in 1869 and mined until 1956 for gold and silver from a network of underground tunnels. The mine was initially operated by Helena Mine and Reduction Company between the years 1883 and 1896, where 150 to 200 tons of ore were mined per day. In 1896 mining ceased, and in 1909 the mine was purchased by Boston and Alta Mining Company. Since 1909, multiple small companies have owned and operated the Alta site with limited amounts of extraction and small profits (Tetra Tech EM Inc., 1997).

In 1999 the Montana Department of Environmental Quality (MDEQ), the United States Department of the Interior, and the Office of Surface Mining oversaw a remediation project that aimed to remove mine waste that posed a human health hazard and affected the surrounding ecosystem (MDEQ, 1997). The remediation effort encompassed four major goals: 1) removing waste rock from the area by hauling it to a nearby repository and covering the slopes where this material was removed with 12 inches of cover-soil in hopes of establishing plant growth; 2) terracing the adjacent hill slopes to create safe sites for the re-vegetation effort while maintaining slope contour; 3) reconstructing the stream channel that flows out from the mine shaft; and 4) riprap the channel's boundaries and place straw bales in the channel to capture sediment (Tetra Tech EM Inc., 1997). The re-vegetation efforts included fertilization, seeding and mulching.

Since reclamation at the Alta Mine site was completed in 1999; however, problems associated with acid mine drainage (AMD) persist (Schroth, 2001) and the original re-vegetation efforts have failed on a majority of the treated land. AMD is produced when pyrite-rich minerals weather. The iron sulfide in pyrite is exposed to air and water, resulting in the production of sulfuric acid (Bigham et al., 1990). The low pH of AMD increases metal solubility.

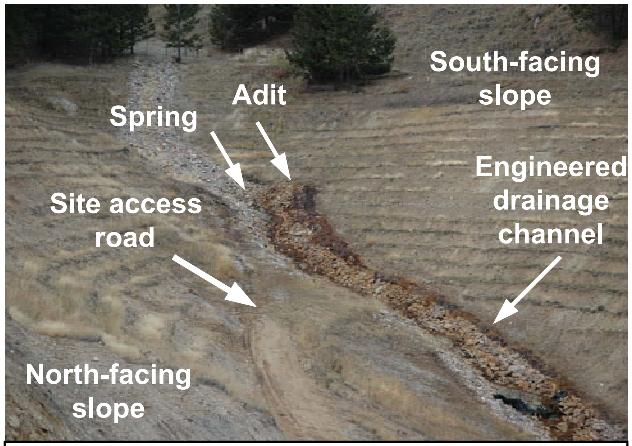

Figure 1. Present day Alta Mine site. Important aspects of the research site are identified.

Capstone Research

The Capstone student research site is shown in Figure 1. Research projects were organized into student groups that focused on: hydrology, re-vegetation analysis, chemistry, and microbiology. Their efforts represented a multidisciplinary analysis of the site with the combined, overarching goal being to assess: i) the relative quality of the water; ii) plant diversity and the correlation of vegetative cover with environmental factors; iii) how the abiotic chemical and biotic biochemical reactions occurring in the drainage channel could influence the quality of the water draining from this site; and iv) whether there was evidence that the microbial community would be impacted by the harshness of an AMD environment. Each group conducted a preliminary assessment of the Alta Mine site and then formulated hypotheses to be tested. Experimental design and procedures were then established to address these hypotheses. In consultation with their faculty advisors, the students were responsible for determining which experiments, analytical procedures, and measurements were appropriate for their work. The students then analyzed their data and prepared a written summary of their findings, which are provided below.

Hydrology

Research Background

The overarching goal of the Hydrology Group was to better understand the dynamics of the water flow within the drainage and in outflow channel. Hydrologists use this flow information as a basis for determining inputs and outputs at the system level. Water movements in and out of a system are not constant, and thus temporal variations were also of great interest, as was metal discharge, which was calculated based on the combination of flow rate information and aqueous chemical analysis. These direct measurements were combined with modeled data derived from instrumented sites to address the following hypotheses.

Hypotheses Tested.

H_1: Total dissolved metal discharge varies seasonally.

H_2: Dissolved metal transport varies during high and low flow events due to groundwater inputs.

H_3: Mine impacted groundwater is the primary source of dissolved metals.

Hydrology: Methods

Hydrologic Site Description and Statistical Modeling Methods. Alta creek watershed is un-gauged for stream discharge and precipitation. Therefore, for modeling purposes, the Tenmile and Prickly Pear watersheds were selected because of their long record of gauged discharge and spatial proximity to the Alta Creek watershed. The drainage areas of Alta, Tenmile, and Prickly Pear are 0.462 square miles, 30.9 square miles, 192 square miles, respectively. Tenmile and Prickly Pear Creeks were chosen for their similarity to the Alta drainage with respect to proximity and elevation. Tenmile Creek is near the town of Rimini and Prickle Pear Creek is near the town of Clancy; the closest town to the Alta watershed is Corbin.

For similar reasons, the Rocker Peak and Frohner Meadow Snotel sites were used for precipitation data. For these types of efforts, proximity again plays a key role in predicting runoff coefficient for un-gauged watersheds (Franchini et. al., 2004). The information from the Snotel sites and the stream gauge data was used to model annual discharge. Alta watershed area was delineated using USGS topographic maps of the area, and area was measured using map scale. United States Geological Survey (USGS) data was compiled using Excel for a twenty-year period, from 1986- 2006. Annual precipitation for both Snotel sites was compiled and averaged, and annual discharge from Prickly Pear creek was also compiled, and area normalized. Using this data, the runoff coefficient was calculated using the equation (Hornberger, 1998):

$$\mathbf{R = (r_s/p)}$$

where: **R**= runoff coefficient; $\mathbf{r_s}$ = annual runoff; and **p**= annual precipitation

R was then used to estimate annual Alta Creek discharge by the equation:

$$\mathbf{D = (p^* R^* A)}$$

where: **D** = discharge; **p** = annual precipitation; **R**= runoff coefficient; and **A** = Alta watershed area, which is equal to 357805.75 sq. miles. Discharge was then converted to liters/minute.

$$\mathbf{Z = \frac{x - \bar{x}}{S_x}}$$

Exceedance probability was calculated using:

where: **Z** = normalized z-value; **x** = maximum value of precipitation; **x(bar)** = the mean of annual precipitation; and $\mathbf{S_x}$ = the standard deviation of annual precipitation (Hornberger, 1998). The probability of Z was extracted from standard normal distribution tables. Exceedance probability was then calculated using:

$$\mathbf{P_T = 1.0- Z}$$

where: $\mathbf{P_T}$ = Total Probability; and **z** = normalized calculated z-value. Return period was calculated by taking the inverse of the exceedance probability.

Field Methods. Hydrologic discharge was measured simplistically with a bucket, a graduated cylinder and a stopwatch. Flow rate was too low to use more precise methods. Discharge was measured at five locations (Table 1) and water samples were collected at the same points as discharge measurement. Water samples for chemical analysis were collected in the field with a sterile syringe and filtered (0.4 µm). After transport to the laboratory, water samples were further filtered (0.2 micron) to remove particulates and acidified with nitric acid (for preservation). Inductively coupled plasma (ICP) spectrometry was then used to measure for the following metals: B, Ca, Mg, Na, K, Cu, Fe, Mn, Zn, P, S, Al, As, Pb, and Si.

Hydrology: Results

Table 1. Hydrology sampling locations

Sampling Site (m)	Feature
0	Adit
1	Spring
59	Waterfall
110	Piezometer
143	Control Dike

Sampling locations are represented in Table 1. Average measured base flow for the site ranged from 18 L/min to 37 L/min (Fig. 2). Seasonal variation was observed, with spring flow rates being consistently greater than in fall. No additional surface water inputs were observed during either sampling time, though the base flow data clearly suggests groundwater or near surface water inputs from stored precipitation (Fig. 2). Groundwater to surface water interactions would be expected in such an environment. Increase in-flow near the adit is due to a groundwater spring, which represents an unpolluted source, indicating pre-disturbance groundwater surface water interactions. Additional sub-surface inputs are evident at 59 m (i.e. Waterfall), where flow increased by 15-17 L/min (Fig. 2), and is indicative of a gaining reach. However, with increasing distance from the adit, decreased in-flow was observed and indicated a losing reach. Complex groundwater interactions are present throughout the area and change seasonally. Possible diurnal changes occur due to whole system inputs (Sullivan and Drever, 2001).

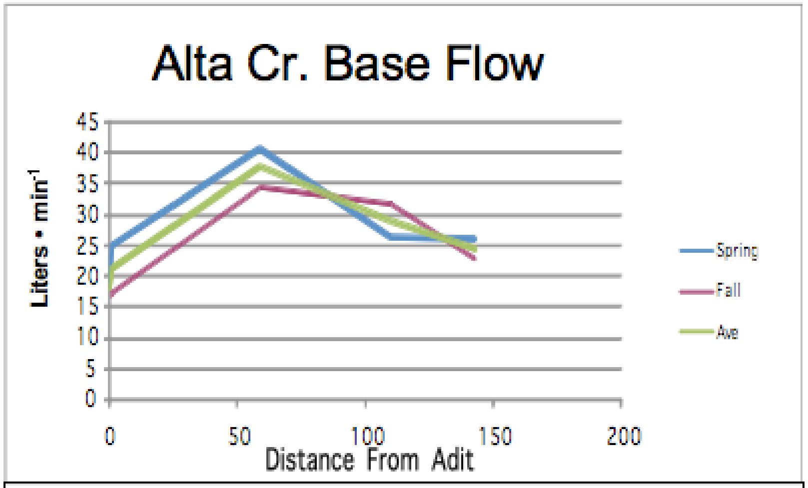

Figure 2. Alta Mine channel base flow measured in Spring and Fall.

Changes in aqueous chemistry along the channel also suggested variable and seasonally-influenced ground water inputs. Metal(loid) export between Spring and Fall varied by a factor of 10-30. Total arsenic discharge decreased as a function of increasing distance from the adit (Fig. 3A) and probably reflects adsorption to the iron solid phase (discussed below). A significant As spike at the piezometer location (110 m) was evident during the Fall sampling, suggesting sub-surface inputs. Such inputs were also indicated by a significant increase in apparent copper discharge (Fig. 3B). Such variability is likely due to fluctuation in water table levels that control seeps of mine-impacted water. Similar variability in metal concentrations has been previously noted in Rocky Mountain ecosystems (Sullivan and Drever, 2001).

A

B

Figure 3. Variable metal and metalloid concentrations with increasing distance from mineadit. **(A)** Arsenic concentrations and **(B)** copper concentrations.

Predicted flow was based on a twenty-year sample, assuming a 1:1 linear relationship between predicted runoff and flow. Annual flow ranged from 24.4 to 29.1 liters per minute (Fig. 4), varying due to annual changes in precipitation. Frequency analysis of high flow events was calculated assuming normal distribution of the data. The exceedance probability that flow would exceed the maximum estimated flow was 5%. The return period for the data was once every twenty years. Metal concentrations for high flow events cannot accurately be predicted due to the uncertain nature of groundwater inputs.

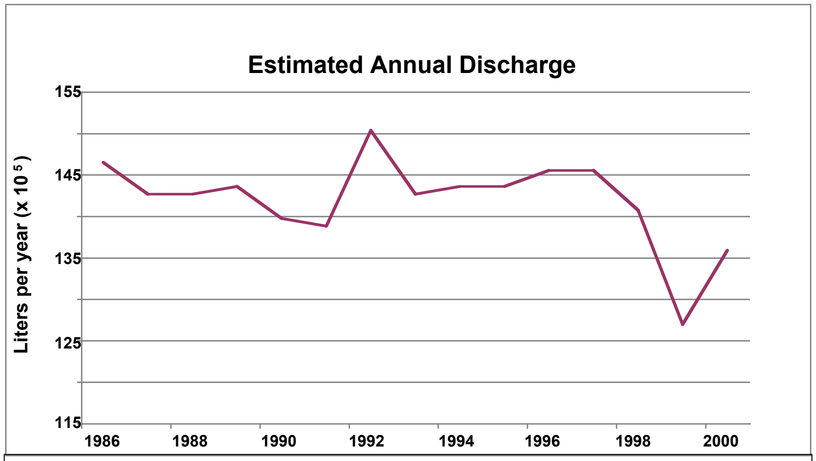

Figure 4. Estimated annual discharge for the Alta mine drainage.

Hydrology: Discussion

General Assessments. The data derived from the hydrologic analyses draw on meteorological and hydrological measurements across space and time and were used to establish a predictive model for similar yet unmeasured phenomena for another geographically related space: the Alta watershed. By adding our predicted watershed inputs to our measured base flow we can estimate discharge. Assuming a linear relationship between export of solutes and discharge, we can then predict export for a specific time point or period. Being able to use this working model to predict, at minimum, the base discharge and export of solutes in potentially hazardous concentrations could prove useful on many levels.

Flow data and aqueous chemical analysis indicate that Alta Creek is both a gaining and a losing stream. That is, water is entering the outflow channel and then exiting to the vadose zone and possibly the phreatic zone. Further research is needed on groundwater flow to determine the control for metal concentrations. Measuring groundwater flow, along with groundwater metal concentrations would allow an accurate prediction of metal discharge from the stream at estimated high and low flow events.

Hypothesis Testing.

***H_1*:** Total dissolved metal discharge varies seasonally. **ACCEPT**

Differences in flow measured in May and August were significant (Fig. 2), and indicated that annual events such as spring snow melt are important to water flow at the system level. Increased moisture inputs due to spring snow melt no doubt greatly influence water movement through the sub-surface, resulting in increased leaching of metals and a complex hydrology at this site as indicated by the increasing and decreasing flows along the reach (Fig. 2) as well as varying metal fluxes (Fig. 3). This variation should be taken into account when calculating annual export and for predicting what is actually happening at the whole drainage level.

***H_2*:** Dissolved metal transport varies during high and low flow events due to groundwater inputs. **ACCEPT**

Acceptance here was based on the measured flow data and metal concentrations. Evidence of groundwater inputs greatly complicate this site. The extent of groundwater flow in the mine works is unknown. Over time, greater levels of dissolution could generate additional sources of pollution, almost certainly increasing metal loads in the stream.

***H_3*:** Mine impacted groundwater is the primary source of dissolved metals. **ACCEPT**

This is based on metal concentrations from the sampled waters as well as the already elevated metal levels found in waters exiting the adit. The latter is viewed to be directly connected to flooded mine shafts. Spring snowmelt in this environment no doubt enhances erosion of metal-laden upslope soils. However, very large increases in copper and arsenic (Fig. 3) during seasonal periods totally lacking measurable precipitation indicates that groundwater inputs, whether from the adit or inputs documented as increased flows along the reach (Fig. 2), are the main drivers of metal concentrations and are evidence of a complicated groundwater – surface water system.

Vegetation Analysis

General Reclamation Background Information

Mine tailings associated with AMD environments are often comprised of acid-producing minerals such as pyrite and have high heavy metal levels due to increased solubility of these metals in acid environments. Tailings are generally removed from a reclamation site and placed in a repository where water will not leach through them. After removal of tailings, cover soil and topsoil are placed on the site before seeding. Cover soil is unpolluted soil that may include subsoil horizons and is generally low in organic matter, and erosion mats are sometimes used for soil stabilization. These mats may incorporate seed mixtures and can be effective at reducing soil loss and sediment delivery to streams where steep slopes and flash precipitation events combine to erode cover soil.

Vegetation can reduce erosion when plant roots bind and stabilize soil particles, preventing surface soils from being detached by the wind (Martinez-Ruis et al. 2005). Moisture, temperature, nutrients, and light are the main factors influencing the success of re-vegetation efforts. South facing slopes tend to dry quickly because they receive more direct sun and hence greater evaporative losses than north-facing slopes. This can result in increased seedling mortality (Grumwald et al. 1988). The presence of heavy metals and low pH levels can also hinder the success of re-vegetation efforts on disturbed sites. High concentrations of heavy metals inhibit root growth, increasing susceptibility to drought (Tordoff et al. 2000). In addition, upward transport of phytotoxic materials by groundwater can cause salts and metals to accumulate to phytotoxic levels in the soil surface, further exacerbating re-vegetation attempts. The factors that influence re-vegetation success in AMD environments were addressed by testing the following hypotheses:

***H_1*:** Vegetation cover and richness will be negatively impacted by soil metals.

***H_2*:** Vegetation cover and richness will be negatively impacted by low pH.

H_3: Species richness will be greater on the north aspect than the south aspect

Vegetation Analysis: Methods

Field Measurements. Three transects of 100 m in length, were established on each aspect (North and South) at three different positions on the slope (low, middle and top). Transects followed terrace treads laterally across the slopes at similar elevations for each aspect. Along each transect at three randomly chosen locations, two 0.5 m^2 quadrat frames were placed, one on the terrace tread, and one on the riser directly adjacent to the tread. A random number generator was used to determine the number of paces between each pair of quadrats for unbiased placement. One person paced out all of the distances for consistency.

For each quadrat, percent plant cover was estimated to the nearest 5% for bare ground, litter, rock, and foliar cover for each species present. In addition, soil cores were taken using tulip borers (4 cm wide) that sampled to an average depth of 10 cm within the quadrat. In each quadrat, three soil cores were combined and labeled for laboratory analysis. A Trimble GeoExplorer 3 (accuracy 1-3 m) was used to mark the location of each pair of quadrat frames.

Chemical Analysis.

Composite soil samples were air-dried and approximately 500 g placed into one-quart ziplock bags and mailed to Agvise Laboratory to be tested for organic matter, pH, NO_3, Olsen P, K, and Mn. Soil tests for Pb and As were performed at Montana State University, using water paste extraction (1:1 soil/distilled H_2O, 72 h), followed by sequential filtration through Whatman # 1 filter paper and 0.2 µm filters, and preservation by acidification with HCl. A total of 36 samples were then analyzed by inductively coupled plasma – atomic emission spectroscopy (ICP-AES) for Pb and As content.

Statistical Analysis. Statistical analysis was completed using SPlus 2000 (Mathsoft Inc.) and Microsoft Excel. Analysis of variance (ANOVA) was used to evaluate the effect of different environmental variables on plant species richness, diversity and percentage cover. In addition, regression was used to compare some of the continuous variables (e.g. pH and species richness).

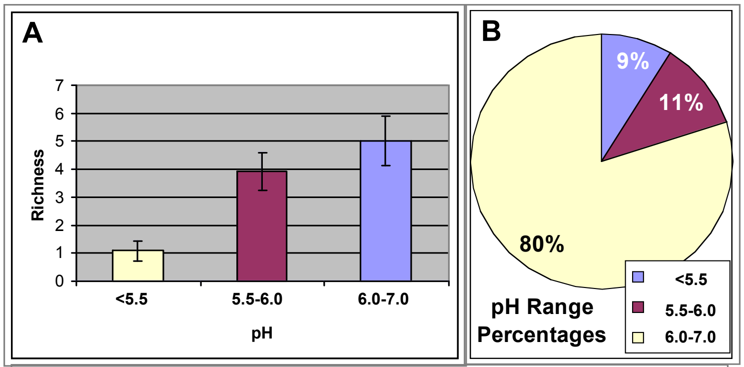

Figure 5. Influence of soil pH on plant species richness. **(A)** Species richness as a function of soil pH. **(B)** Distribution (% of total sampled) of soil pH at the Alta Mine site.

Vegetation: Results and Discussion

ANOVA failed to demonstrate soil metal effects on plant diversity or richness (results not shown). However, soil pH, and slope aspect and tread/rise were found to be important in this regard. A comparison of plant species richness and soil pH values, categorized by reference to plant growth parameters, showed that species richness was significantly higher where soils had a circumneutral pH (6.0 to 7.0) than in more acidic soils (Fig. 5). This relationship was illustrated by regression analysis (Fig. 6), with more variation in richness being explained by pH on the north aspect (R^2 = 0.7902) than the south aspect (R^2 = 0.5072). Plant available water on the north-facing slope is higher than on the south-facing slope where soils become dry earlier in the growing season. The lack of water probably explains more of the variation in richness on the south slope than does pH.

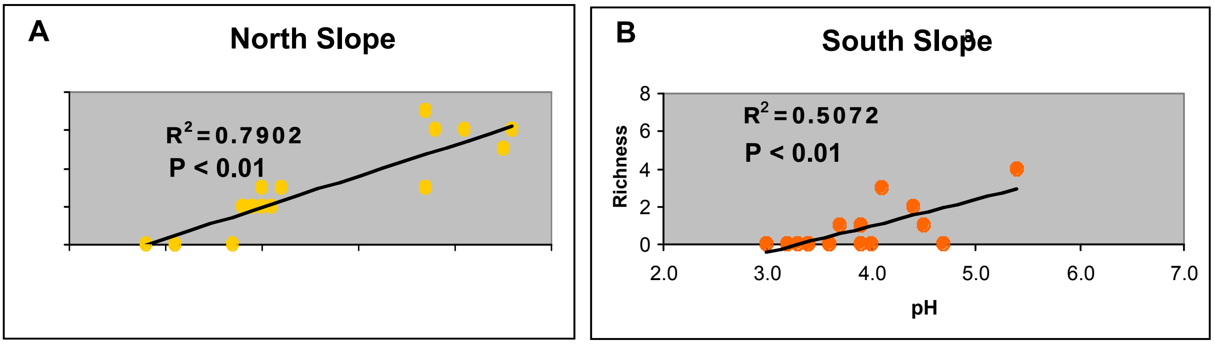

Figure 6. Regression analysis of plant species richness vs soil pH. **(A)** north aspect and **(B**) south aspect of the Alta Mine drainage.

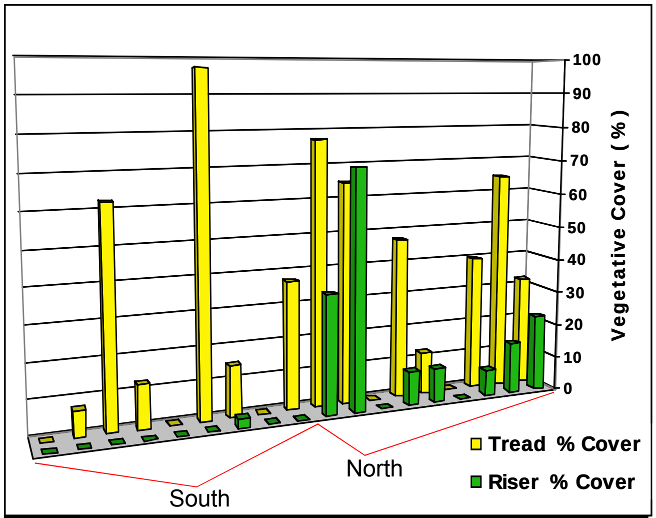

Figure 7. Plant density (as % cover) as a function of drain aspect and of terrace tread/riser.

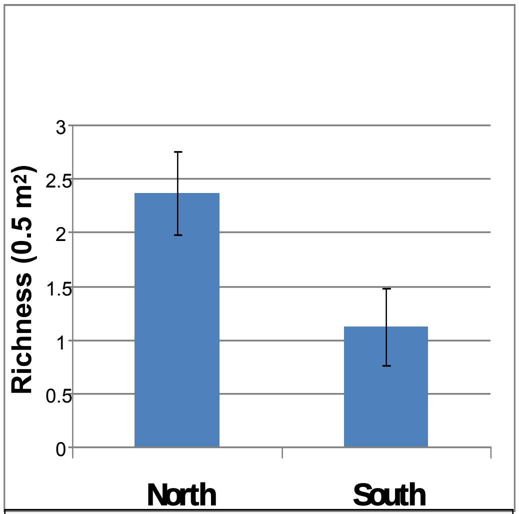

Figure 8. Plant species richness observed on the north and south aspects of the Alta Mine drainage

Comparing aspect and percent cover on tread and riser showed some interesting patterns. Terrace treads had a higher percent of vegetation cover than the risers (Fig. 7). Furthermore, the north aspect tended to have more vegetation than the south as was predicted from initial visual inspection. Again, this is likely due to more intense solar radiation on south slopes, and the resultant drying of soil causing increased plant mortality. Plant species richness was higher on the north aspect for this site (Fig. 8). Mean species richness was 2.4±0.4 for the north, while only 1.1± 0.4 for the south.

Hypothesis Testing.

***H_1*: ** Vegetation cover and richness will be negatively impacted by soil metals. **REJECT**

Results from the soil metal analysis were quite variable, and likely due to the highly variable nature of the topsoil coverage. This was particularly an issue on the south aspect where erosion was considerable.

***H_2*: ** Vegetation cover and richness will be negatively impacted by low pH. **ACCEPT**

Low soil pH strongly inhibited plant establishment as shown by the data reported in Figures 5 and 6. Increasing soil pH (e.g. extensive liming) would increase species richness and percent cover, resulting in soil stabilization and lead to an over all increase in sustainability of the Alta Mine site.

***H_3*: ** Species richness will be greater on the north aspect than the south aspect. **ACCEPT**

Initial visual inspection strongly suggested this hypothesis would be accepted. Measurement of plant number and diversity (Fig. 8) clearly established this to be the case.

Chemistry

General Goals and Hypotheses

Reclamation at the Alta Mine site was completed in 1999. The overarching goal of the chemistry group was to examine the site 8 years post reclamation to assess the quality of the water exiting the mine adit, the lower reaches of the Alta Mine site, and downstream drainage locations. Heavy metal content, description, and redox transformations along the outflow channel were of specific interest. The following hypotheses were tested:

H_1: Water emitted from the mine is influenced by pyrite weathering and is laden with iron and heavy metals.

H_2: Chemical reactions that occur as water equilibrates with the atmosphere change the aqueous chemistry.

H_3: pH decreases down stream due to the formation of iron solid phases.

Chemistry: Methods

Sampling. A total of ten samples were taken at the Alta mine site and surrounding locations. Seven samples came directly out of the acid mind drainage (AMD) starting at the source pool (0m) and moving downstream. Sample locations 2-7 were located 5.2m, 10.3m, 17.0m, 22.7m, 38.8m, and 50.8m down from source pool, respectively

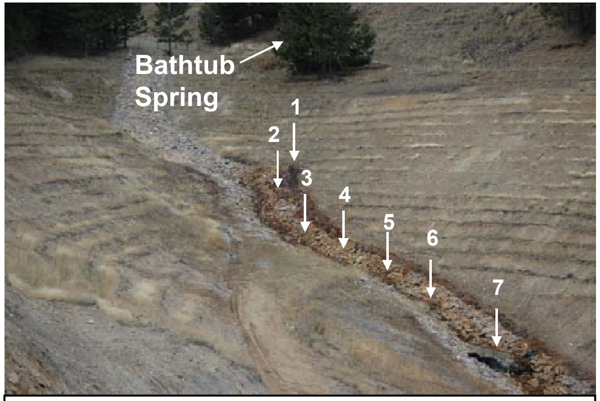

Figure 9. Aqueous and solid phase chemistry sampling sites.

(Fig. 9). Water samples from Bathtub Spring were also taken and represented sub-surface water not connected to the mines shaft(s) creating the AMD. Other sampling sites included the waterfalls (WF) created by the MDEQ during reclamation (150m from source, also corresponds to a site included in the Hydrology component), and a final site considerably further downstream below where the AMD from the Alta creek mixes with Corbin Creek. Corbin Creek runs both on the surface and below ground; a sample was collected where water was on the surface.

pH, Temperature, and Dissolved Oxygen. At each sample site, a pH meter (calibrated in the field with standards of 4, 7, and 10) with a temperature probe (also calibrated that day) was used. Dissolved oxygen (DO) was also measured using a probe and results were converted from percent saturation to mg/L.

Aqueous chemistry. Two samples were taken from each site, filtered (0.22 µm) into two separate 50 mL sterilized Falcon tubes and stored on ice for temporary storage and transport back to Montana State University. One sub-sample was acidified with pure nitric acid (HNO_3) to a 1% acid solution for elemental analysis by ICP-AES and the other sub-sample was used for anion analysis using ion exchange chromatography (IC) (Inskeep et al., 2005). The ferrozine method was used to calculate Fe (III) and Fe (II) concentrations (To et al., 1999), and As(III) and As(V) concentrations were determined using sodium borohydride speciation and hydride generated atomic adsorption spectroscopy (Macur et al., 2007). Samples for inorganic N analysis were prepared similarly to other water samples with no acidification, with (NH_4^+) and nitrate (NO_3^-) determined via a flow injection analyzer (FIA) (Macur, 2007). Total organic carbon (TOC), total inorganic carbon and total nitrogen (TN) were determined with 0.2 µm filtered samples (stored in dark brown glass vials prior to analysis) injected into a Shimadzu TOC V analyzer.

Scanning Electron Microscope (SEM). Solid phase samples were taken in order to analyze the precipitates formed within the stream. Light-colored and dark-colored iron mat solids were collected into small Falcon tubes. Samples were dried, crushed slightly, and then placed on a carbon-mount and cemented in place with graphite liquid binding material.

X-ray Powder Diffraction Spectrometer (XRD). The other method of solid-phase analysis used included the X-ray diffraction (XRD). Samples were analyzed to determine mineral forms within soil matrices, rocks and precipitates.. Samples were dried and crushed to a fine powder with a mortar and pestle, placed in the XRD sample cuvet, and mounted in the XRD machine for analysis.

Table 2. Aqueous pH in the Alta Mine outflow channel.

Sample site	pH
1	4.20
2	4.20
3	3.64
4	3.44
5	3.28
6	3.14
7	3.03
WF	2.70

Chemistry: Results

Water temperatures were fairly constant when measured at the adit in August and in September, but increased slightly with increasing distance; (11.7°C at site 1, 12.8°C at site 7). The pH was 4.2 at site 1 and 2.7 ~150 meters downstream (Table 2). The Bathtub Spring had a pH of 5.5. Dissolved oxygen increased rapidly as the water became oxygenated (Fig. 10).

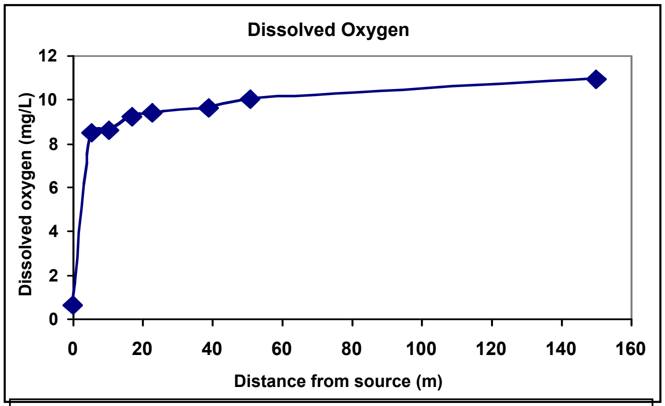

Figure 10. Dissolved oxygen content as a function of distance from adit.

The only anions detected by IC were sulfate and carbonate (Table 3). Additionally, nitrate was detected by FIA. Sulfate levels were variable and did not show increasing or decreasing trends, whereas nitrate concentrations increased slightly along the outflow channel and were opposite of that observed for ammonium. Increasing nitrate and decreasing ammonium suggest the possibility of nitrification in this environment, and is consistent with the phylogenetic analysis of the microbial community (see below). Levels of several metals were found to be higher in the drainage channel than in Bathtub Spring (BS) or in Corbin Creek. Mg, K, Ca, Na, Mn, Zn, and Al all decreased (sample of results shown in Table 3) until about site 6 or 7, and then increased; S and Si behaved similarly (results not shown). Fe and As decreased over the whole Alta Creek stream reach (Fig. 11) while Cu increased (Table 3). Ba, Sb, and Pb (Table 3) remained nearly constant. All of the iron in Alta Creek was basically in the form of Fe(II) (data not shown), whereas most of the As was found as the more oxidized species, As(V) (Fig. 11B). DOC remained relatively constant at ~3 mg C/L, whereas DIC decreased with distance from the adit, suggesting rapidly outgassing of CO_2 (Fig. 12).

	Mn	Zn	Cu	Pb	SO_4^{2-}	NO_3^-	NH_4^+
	(mg / L)					(mg N \ L)	
Bathtub Spring	0	0	0.1	0.4	0	0.03	0.04
1	879	484	0.1	1.1	35	0.01	0.56
2	726	448	0.1	1.1	29	0.01	0.56
3	707	434	0.2	1.1	33	0.01	0.55
5	708	435	0.2	1.1	24	0.05	0.53
6	709	439	0.3	1.1	31	0.04	0.51
7	752	449	0.4	1.0	29	0.04	0.50
Waterfall	768	446	0.5	1.0		0.02	0.44
Corbin	84	27	0.9	0.4	7	0.09	0.07
EPA MCL (mg/L)	0.05	5	1.3	0.015	250	10	

Table 3. Concentrations of specific metals and anions measured along the Alta Mine outflow channel.

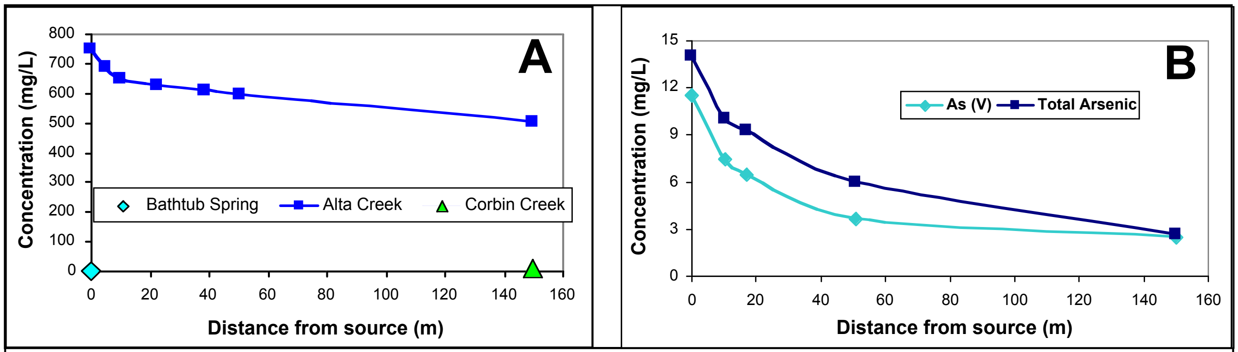

Figure 11. Total Fe and As concentrations in the Alta Mine drainage channel. **(A)** Fe and **(B)** As. Fe data for Bathtub Spring and Corbin Creek are provided for comparison.

SEM analysis of the light and dark colored Fe mat samples indicated the presence of gypsum, iron oxides, and iron sulfides in both types of mat materials (e.g. Fig. 13). None of these compounds seemed to be limited to a certain colored precipitate sample. XRD analysis of these solid phases did not conclusively prove the presence of any pyrite, iron oxides, or iron sulfides. However, it did confirm the presence of silica minerals. There were many peaks that could not conclusively be fit to a known sample, leaving us with a lot of un-quantified, apparently amorphous material. A comparison our XRD data to known low-crystalline ferrihydride and schwertmannite readouts, suggested the presence of these Fe mineral in the Alta mine solid phase samples (Fig. 14).

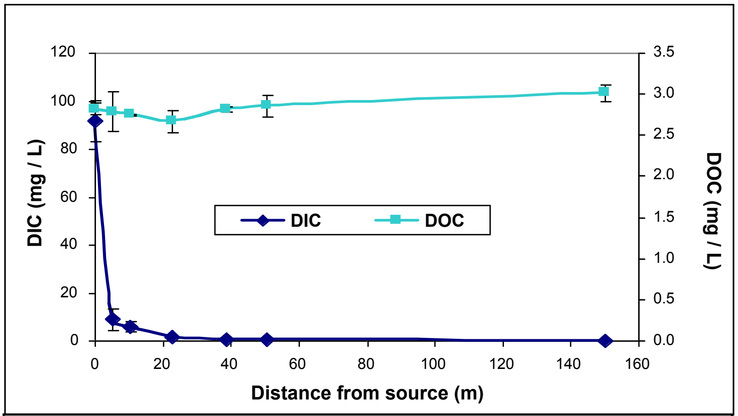

Figure 12. Aqueous and solid phase chemistry sampling sites.

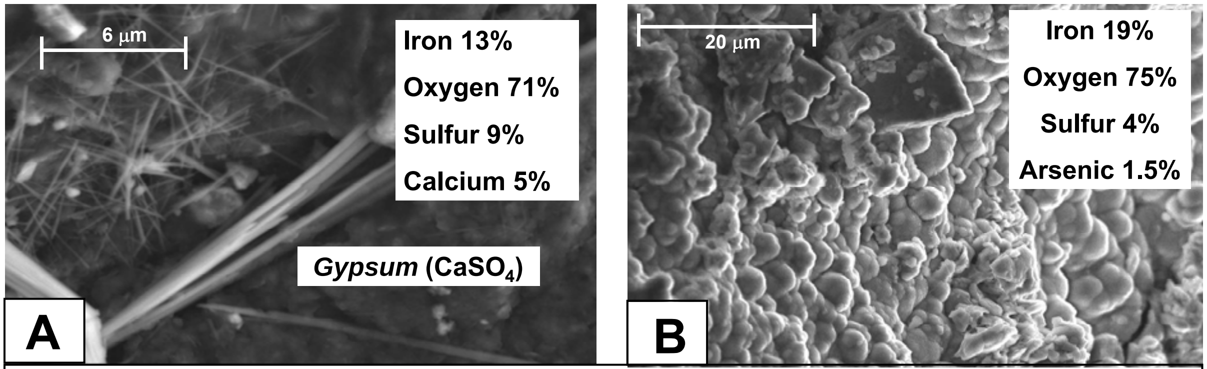

Figure 13. SEM-EDAX analysis of Fe solid phase samples from the Alta Mine outflow channel. (**A**) highly crystalline sample exhibiting elemental content consistent with gypsum; (B) amorphous Fe-oxy-hydroxides containing some sulfur and arsenic.

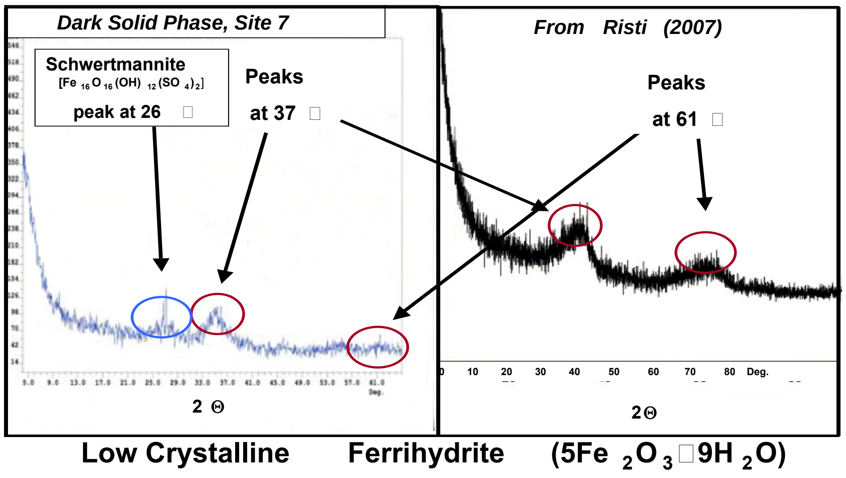

Figure 14. XRD output indicating the presence of amorphous ferrihydrite and Schwertmannite in the Alta Mine solid phase samples. XRD data in right panel is of a pure ferrihydrite and is from Risti (2007).

Chemistry: Discussion and Hypothesis Testing

Concentrations of several metals and metalloids were extremely high, exceeding EPA drinking water standards by several orders of magnitude (Table 3, Fig. 11), and leading us to accept ***H₁*** The decline in pH measured along the outflow channel (Table 2) can be attributed to Fe(III) (from microbial Fe(II) oxidation, see below) complexing with hydroxide molecules from water and forming Fe-oxy-hydroxide solid phases. The protons remaining contribute to acidification (Equation 1) and thus confirms $\boldsymbol{H_3}$.

Eq. 1 $$Fe^{3+}(aq) + 3OH^{-}(aq) + 2H^{+}(aq) \leftrightarrow Fe(OH)_3(s)$$

Total aqueous iron concentrations decreased down the flow channel as a result of the formation of these iron oxides, which also contributed to decreasing arsenic in the outflow channel (Fig. 11B). Chemical speciation determined that the majority of the As in the adit water was in the form of As(V), which strongly sorbs to Fe oxides (Senn and Hemond, 2002). Thus, both Fe and As chemistry changed as a function of the adit water equilibrating with the atmosphere, consistent with accepting $\boldsymbol{H_2}$**.**

Significant increases in Mn, Zn, and Cu between sites 6 and 7 (Table 3) suggests the possibility of ground water inputs and is in agreement with the conclusions drawn by the Hydrology group. We note, however, that these increases are heterogeneous with respect to the various chemical species tracked along the outflow channel; i.e. putative ground water inputs are dissimilar to the chemical profile of the water at site 1. Other changes were due to the mine-impacted water coming into equilibrium with the atmosphere upon surfacing and lead us to accept $\boldsymbol{H_2}$. Specifically, significant and inverse changes in CO_2 and O_2 (Fig. 10, Fig. 12) are consistent with CO_2 outgassing and O_2 ingassing. Organic carbon was fairly constant along the entire channel measured, suggesting that heterotrophic microbial consumption may have been limited. However, decreases in Fe(II) and inverse changes in NO_3 and NH_4 are consistent with significant with the occurrence of chemolithotrophic metabolism. SEM-EDAX analysis showed iron oxides spheres (Fig. 13B) that could represent Fe(III) encrusted microorganisms resulting from enzymatically oxidized Fe accumulating onto cell surfaces.

Finally, we draw attention to the observation that Bathtub Spring water was also acidic, measuring pH 5.5. Water forming this spring is viewed to be of artesian flow and representing ground water unaffected by the mine shafts. Consequently, while sub-surface, mine-impacted water chemistry no doubt contributes some acidity to the Alta Mine outflow channel (adit pH = 4.2; Table 2), it appears that the greatest amount of acidification occurs above ground and is linked to Fe oxidation and thus consistent with the acceptance of $\boldsymbol{H_3}$. Additional work is required to determine how microbial activity is affecting the adit water. A complete understanding of the impacts, good or bad, that microorganisms are having on heavy metal concentrations would require more extensive data collection. The increase in concentrations of certain elements between site six and seven also needs to be studied to recognize how these inputs of heterogeneous groundwater are affecting not only the metal concentrations, but also the microbial life within the stream.

Microbiology

General Reclamation Background Information

The harsh conditions that prevail in AMD environments exert strong selection effects on microorganisms, typically resulting in reduced diversity (Gui-Liang et al.2006). Low pH selects for acidophilic organisms, and high concentrations of heavy metals either select for resistant microbes or indeed organisms capable of using the metals for energy generation. As an example, microbes commonly found in AMD are *Acidithiobacillus, Thiobacillus,* and *Ferrioxidans;* these are all acidophilic Fe(II) chemolithotrophs and is not surprising given the significant concentrations of Fe(II) normally found in AMD waters. Low pH and metal concentrations were key issues with regards to the formulation of hypotheses addressed in this component of the Capstone Course studies.

H_1: Microbial community structure will change with distance from the adit.

H_2: Shifts in the community structure will correlate with chemical changes in the stream

H_3: Iron redox transformations are biologically driven.

Microbiology: Methods

Sampling sites for microbiology analysis were the same as described above by the chemistry group and thus allowed close for The Alta mine adit was sampled on August 28, 2007. Seven sites along the channel were sampled for light mat, dark mat, and green accumulations. At site one, there was no light mat or green accumulations observed and at site six there were no green accumulations present. DNA was extracted from subsamples (≈0.50g) using the Fast Spin DNA Kit for Soils (Q-Bio gene, Bio 101 Systems). The PCR primers, conditions and techniques used for DGGE analysis were the same as described by Norris et. al. (2002). The extracted DNA was then used as template for the amplification of bacterial 16s rRNA genes by PCR using the primers 1070F and 1392R on a Gene Amp® PCR System 9700 (Applied Biosystem) thermocycler for ten cycles. Ten microliters of this product was then run on a 1% agarose gel, stained with ithidium bromide and viewed on a Kodak Image Station 2000R to verify amplification of the target gene. Two microliters of this product was then used as template for another round of PCR (25 cycles) with GC-clamped primer pair 1070F-GC and 1392R-GC. This final PCR product was checked with a 1% agarose gel for verification. Denaturant Grade Gel Electrophoresis (DGGE) was then performed on this final PCR product with a CBS Scientific DGGE apparatus and an 8% polyacrylamide gel containing a 40% to 70% denaturant concentration (where 100% denaturant contains 7 M urea and 40% [vol/vol] formamide). PCR amplicon bands from the DGGE gel were then cut out and DNA was extracted by crushing them in a 1.5 mL microcentrifuge tube and soaking them in an extraction solution (containing 500 mM ammonium acetate, 0.1% [wt/vol] sodium dodecyl sulfate, and 0.1 mM EDTA). This mixture was then incubated at 75°C for 2 hours and centrifuged for ten minutes at 14,000 G. The DNA was then precipitated by adding 2.5 volumes of 95% ethanol and 0.1 volume of 3M sodium acetate. Five microliters of the band extracted DNA was then quantified on a 1% agarose gel with a weighted ladder using the Kodak ID Software and sent to the University of Nevada

Genomics Center for sequencing. Once the sequences were received, they were cleaned up by trimming the ends to remove any extra sequence left from the PCR. The clean sequences were then compared to the National Center for Biotechnology Information (NCBI) nucleotide database using the Basic Local Alignment Search Tool (BLAST).

Microbiology: Results

With the exception of a single sample, total DNA yields were at least adequate for use as PCR templates (Fig. 15). PCRs with these samples yielded amplicons comprising a single band in agarose gels, suggesting the PCRs were accurate, without artifact, and could be used for DGGE analysis (Fig. 16).

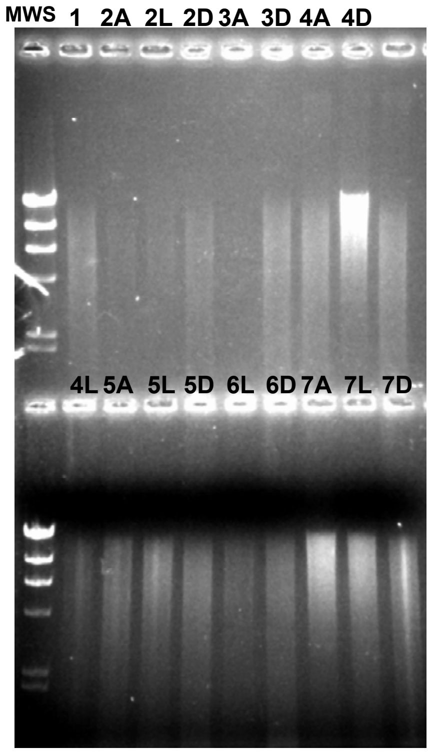

Figure 15. Verification of DNA extraction. Lane designations identify the channel location (number) and the mat type (A=Green; L= Light Brown; D=Dark Brown) of the sample

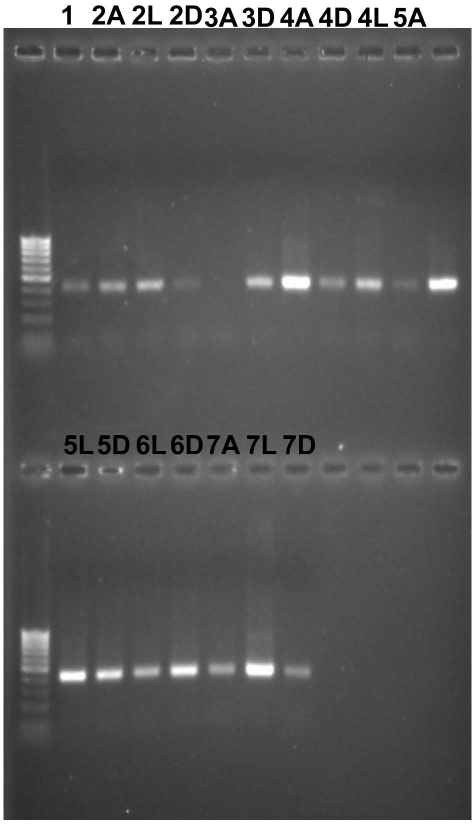

Figure 16. Verification of PCR fidelity. Amplicons were derived from PCRs of DNA extracted from the Alta Mine drainage channel.

DGGE profiles suggested there were changes in the microbial community structure from site to site and among light, dark, and green materials (Fig. 17). After receiving the sequencing the DNA from the bands that we found, we were able to perform a BLAST search to determine near relatives to the organisms that we found (Table 4).

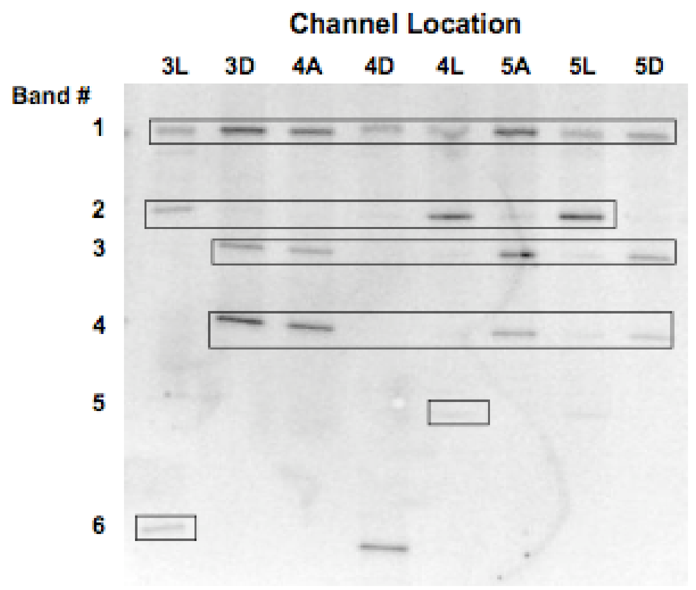

Figure 17. DGGE analysis of microbial community structure along the outflow channel and as a function of mat color type.

The chemistry group, using the ferrozine method for iron species analysis, provided data that was used to determine that there was less total iron and ferrous iron in bottles that contained live mat samples than bottles that contained killed mat samples and bottles that contained filter-sterilized stream water at final time step (Figure 18).

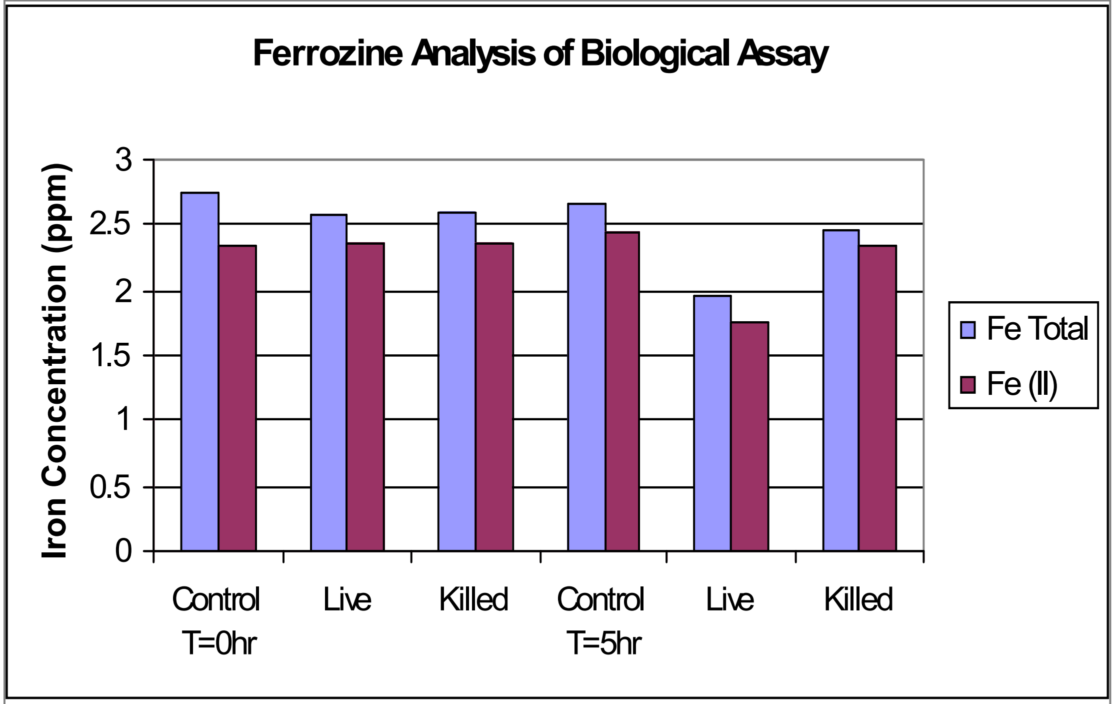

Figure 18. Ex situ assays of biological Fe(II) oxidation. Assays were conducted for 5

Using data from the Chemistry Group, we were able to determine that there were some changes in chemistry with distance from the source (Table 2). Dissolved oxygen increases, total iron and pH decreases with distance from the source.

Microbiology: Discussion and Hypothesis Testing

Some metal(loid)s (e.g. Se, Mn, Zn, and Cu) are important to microorganisms at low concentrations, but at high concentrations can be toxic. Other toxic metals (e.g. Pb, Cd, Sb) have yet to be shown to have a biological function whereas still others (As and Se) are toxic to many microbes but can be used by some to generate cellular energy. Examples of the latter include organisms that can use As(III) as an electron donor or As(V) and Se(VI) as electron acceptors. Some microorganisms can resist high metal concentrations by using inducible mechanisms that reduce the toxicity of the metals. Tolerance means that the organism passively handles the toxicity while resistance indicates an active detoxification mechanism (Gladd, 1992). Microbes can exclude metals, actively exuding them from the cytoplasm at rates greater than uptake or can enzymatically transform the metal into a harmless or less toxic form. Apparent tolerance mechanisms may include the production and release of compounds that influence the mobility of the metals such as polysaccharides that can bind metals. Microbes can also indirectly affect metal mobility and bioavailability by changing the pH of a system (Ledin, 2000). Our studies did not examine mechanisms of metal tolerance, however, it is likely that some or all of the above may occur in the Alta Mine outflow channel.

Our results are similar to those observed by Gui-Liang et al. (2006) who found that AMD waters do not support diverse microbial communities. The DGGE profiles for all sites were relatively simple, suggesting a maximum of six different organism, none of which were *Archaea*. Gui-Lang et al. (2006) also found that most AMD bacteria are poorly represented by cultured organisms; this was the case for some of the organisms encountered in our analysis, although not in all cases (Table 4).

Fe(II)-oxidizing chemolithotrophs are often encountered in these environments due to the high concentrations of dissolved Fe(II). As such, our results are consistent with previous studies in similar environments. The perfect match of band 4 (Figure 17) to *Ferribacter polymyxa*, which has been characterized as an Fe(II) chemolithotroph suggests this organism is present and involved in the biological Fe(II) oxidation found in this AMD environment (Figure 18) that is the basis for Fe(III) mat formation, and the decrease in Fe_{tot} levels as a function of distance from the adit (Fig 11A). Taken together, our data is internally consistent and allows for the acceptance of $\boldsymbol{H_3}$.

Our results are not as conclusive for testing of $\boldsymbol{H_1}$ and $\boldsymbol{H_2}$. While aqueous chemistry measurements clearly showed that the outflow channel waters changed with distance from the adit, DGGE analysis was not conclusive as to microbial community changes. There were differences between the light- and dark-colored Fe mats, however, these differences were fairly consistently found at many locations and not necessarily as a function of distance or chemistry. Furthermore, while major changes in pH, O_2, CO_2, and several chemical constituents were observed, this did not correlate with distinct differences in microbial community structure. Consequently, due to lack of confirming evidence, we reject $\boldsymbol{H_1}$ and ***H2***.

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