TERATOLOGICAL DIATOM DEFORMITIES IN THE PERIPHYTON OF COLORADO ALPINE STREAMS AS INDICATORS OF ACID MINE DRAINAGE CONTAMINATION

By
Ellie Muhr
University of Colorado at Boulder

A thesis submitted to the
University of Colorado at Boulder
in partial fulfillment
of the requirements to receive
Honors designation in
Environmental Studies
May 2014

Thesis Advisors:
Dale Miller, Environmental Studies
Jason Neff, Environmental Studies
Diane McKnight, Civil, Environmental, and Architectural Engineering, Committee Chair

Abstract

Acid Mine Drainage (AMD) affects stream ecosystems all over the world, and is a prevalent issue concerning water quality in many Colorado alpine streams. This study aims to address this issue by analyzing diatoms in the periphyton of two different alpine streams in Colorado affected by AMD for teratological deformities and comparing them to diatoms observed in Deer Creek; a pristine stream. St. Kevin's Gulch showed evidence of asymmetrical abnormality and bent valves in the genus *Eunotia exigua*. The frequency of these abnormalities was 73.25% of the total species observed at this site. *Eunotia exigua* also dominated the taxonomic richness in this stream. Peru Creek, which is also affected by AMD, did not show evidence of teratological deformities. The results of this study further the need for research to develop an index that analyzes the extent of AMD contamination based on our understanding of conditions that produce these abnormalities in diatoms. Recognizing the specific conditions that produce various deformities could be essential for remediation efforts and early mitigation of AMD to prevent streams from becoming devoid of biota and harmful AMD contamination from draining into valuable water supplies.

Acknowledgements

This project would not have been possible without the aid from an Undergraduate Research Opportunities Program (UROP) grant from the University of Colorado at Boulder. However, funding only stretches so far without the help of an incredible committee and mentors. Thanks to Dale Miller for his constant guidance, positivity, and persistence. I would also like to thank Dr. Diane McKnight for her guidance and understanding throughout this project and several challenges along the way. Thanks also to Jason Neff for assisting me and sticking with me through this long process. Infinite thanks as well to Ian Bishop for mentoring me and sitting with me through countless hours and incessant questions in the lab. Your expertise was of immeasurable value to me during these past few months. In addition, I would like to express my appreciation to Tyler Kohler for helping me with R, the Kiowa labs for processing my samples, and Fred at LEGS laboratory for processing my dissolved metals samples so quickly.

Chapter 2 Introduction

Acid Mine Drainage (AMD) is a pressing threat to freshwater aquatic biomes. It has the potential to alter the chemistry of the stream ecosystem and impact the biological interactions of the biota living within the ecosystem. AMD is caused by chemical weathering of sulfide-rich rocks. Although this process occurs naturally in the form of Acid Rock Drainage (ARD), this study will focus on AMD surrounding abandoned mine sites in Colorado. Understanding the effects AMD has on the stream ecosystems in these areas can provide insight into future remediation efforts. Analyzing diatoms in the periphyton can provide bio-indication of environmental disturbance such as AMD. The objective of this study is to analyze the diatoms present in the periphyton of third order streams in alpine environments in Colorado. Previous studies analyzing diatoms in AMD contaminated streams have found evidence of deformities in these diatoms. In this study, field data and periphyton collection will aim to further investigate, if in fact these Colorado streams show evidence of diatom deformities, and suggest reasoning for these deformities.

Three sampling sites were chosen, two located below abandoned mines and one in the upper reaches of the Snake River watershed in Deer Creek, located near the once booming mining town of Montezuma, Colorado. Deer Creek is designated as a ‘pristine’ stream with a fairly neutral pH and will operate as a control site. The other sites consist of Peru Creek, also part of the Snake River Watershed, and St. Kevin’s Gulch located north of the mining boomtown Leadville, CO. These watersheds are affected by AMD and the contamination is visually and chemically evident.

Mining activity in the late 19th century has increased the surface area of exposed pyrite and sulfide ore. Many of these mines were abandoned once the boom ended providing opportunity for the hydrologic cycle and a series of redox reactions to enable dissolved metals to seep into the surrounding stream segments. These chemical reactions result in a low pH and the identifiable discoloration of the streambeds. AMD can occur from tailing piles, waste rock piles, leach pads, and essentially any aspect of mining practice that can be exposed to the hydrologic cycle and oxygen. Exacerbating this process is the realization that it is nearly impossible to completely prevent the continuation of AMD from an abandoned mine because the hydrologic cycle is so complex and contains many different inputs. Herein lie mitigation issues, which currently do not have devised solutions to end the perpetuity of water treatment plants as the most successful mitigation method.

These particular Colorado stream segments lead into other segments of the water system, which eventually drain into a water source that is utilized by nearby populations of people. In addition to human populations, AMD can also have pronounced affects on the stream biota living in the ecosystem by altering the water's pH, conductivity, and alkalinity (Baker 2003). Stream biota in general is very sensitive to changes in temperature, pH, and nutrient concentrations in their environments. AMD, because of its acidity, lowers the pH and can raise the temperature of the water, which can greatly affect the physiological function of the organisms within the stream ecosystem (Smith 2007). Aquatic habitats in close proximity to AMD generally contain pH values of <5, as well as high concentrations of dissolved metals. In some stream ecosystems such as St. Kevin Gulch in Colorado, the effects of AMD can be clearly seen by the lack of biota and red stream bottom in segments where there are large concentrations of Phosphate due to the photoreduction of iron oxides (Tate 1995). It is important that AMD conditions are recognized and mitigated early on to prevent toxic and acidic conditions from entering the water supplies that support ecosystem life. In some aquatic freshwater environments, the impact of AMD may not be as inherently evident, depending on the intensity of contamination. This is where diatoms are valuable.

Diatoms are a type of single-celled algae and one of the most common phytoplankton. Their cell walls are made of silica and are called frustules. These frustules are reflective of the environments, which they inhabit, so by studying the taxa and morphology of diatoms in an ecosystem it is relatively easy to assess the environmental conditions that are present (Wehr 2003). Diatoms in the periphyton and algal mats have been observed in various extreme environments that a majority of organisms would not be able to withstand. Because of this, they make excellent bio-indicators of environmental disturbance and degradation. By analyzing the types of diatoms in the periphyton of selected streams, environmentally altering conditions can be detected (Smith 2007).

Diatoms can help build healthy stream environments by stabilizing the substrata, and providing food and habitat for other biota within the ecosystem. Without diatoms and benthic algae the stream ecosystem could become dysfunctional and collapse (Blinn 2003). As well, they are easily dispersed manifesting in a variety of ecosystem habitats, and they are easily regenerated, due to a generation time similar to bacteria and macro invertebrates. Diatoms can also tolerate variable environmental conditions, which make them extremely valuable in determining environmental disturbances by comparing pristine ecosystems versus impacted (Blinn 2003).

For research purposes, they are easily collected and have little affect on surrounding biota during the actual collection. As well, their structure consisting of frustules or rigid walls of silicon dioxide, make them easily identifiable and permanently documented in reference slides for future analysis (Blinn 2003). This report aims to analyze morphological and teratological deformities in the diatoms of periphyton mats found in alpine streams in Colorado contaminated by AMD, in order to substantiate them as indicators of high concentrations of dissolved metals.

Chapter 3 Background

Lots of research went into this report before the actual field research was conducted. This undergraduate project was individual in nature and so prior understanding of diatoms and acid mine drainage was necessary for analysis and drawing conclusions from field results. This section provides some of the key studies utilized in the framework and provides context for the subjects discussed in this report.

Anatomy of a Diatom

Photosynthesizing algae are composed of single celled organisms called diatoms, which are found in any reasonably moist environment such as marine, freshwater and even soils. Diatoms are divided into two orders that determine their fundamental shape: Centrales (centric diatoms) have radial symmetry due to their round shape and Pennales (pennate diatoms) which are long and elliptical in shape (Wehr 2003). The skeleton or frustule, is made of silica and is divided into two valves. The epitheca is the older half and a remnant of a previous cell division while the hypotheca is the new half of the shell. These valves contain small pores 0.1~0.6 microns in size, that are essential for obtaining dissolved gases (CO_2 for photosynthesis), solid material and nutrients which pass in and out of these pores or areolae (Blinn 2003). These rows of areolae form striae, which are generally positioned on the transapical axis. Striae are very important in determining the genus and species of a diatom based on their characteristics. These characteristics include: striae density, shape, and orientation. Orientation of striae can be radiate, convergent or parallel. The shape of areolae can be punctate, lineolate, or C-shaped, and the density of striae is determined as the number of striae that appear within 10µm (Spaulding 2010).

Diatoms are responsible for as much as 20% of carbon fixation globally by means of photosynthesis. This process of cycling materials through the areolae is what characterizes diatoms as excellent components for nutrient recycling and uptake. Pennate diatoms have a limited range of mobility and progress short distances by means of a mucus secretion from a ridge or slit running through the center of the diatom called a raphe. Meanwhile centric and some pennate diatom such as, *Fragilariophyceae,* do not contain raphes so they are classified as araphid and are not mobile. These raphes allow the diatoms to move along plants, rocks, and other benthic substrata at varying velocities depending on the genus and formation of the raphe. Diatom cells can occur either solitary or colonial in which the cells are attached by bands or mucous filament into a chain structure or attached by a stalk, which hold them in place against currents and disturbances (Spaulding 2010).

Teratological Deformities

This study is focused on analyzing the morphological deformities in diatoms as a result of AMD and the concurrent dissolved metals. The articles reviewed during research were, therefore, also focused on morphological deformities as a result of environmental modification such as, but not limited to, AMD. A review conducted in 2008 analyzes morphological alterations in diatoms as responses to environmental stresses. In the review, the authors refer to these deformities in response to environmental alterations, as teratological. Classifying whether a deformity is a result of natural evolution, driven by selective pressure, or as a response to environmental alterations, is imperative when analyzing diatoms to ensure accurate evaluation of the deformities as potential indicators of AMD (Falasco 2008). Guidelines for analyzing abnormalities in diatoms evaluates whether the abnormalities are teratological or evolutionary. If the species of interest demonstrates one definite divergence in their morphology, reproducible through several generations and that divergence is consistent through those generations, then the diatom can be considered a variation of the species. If there is significant variability in the morphological alterations under the same ecological conditions, than the deformity can be considered teratological in nature (McLaughlin 1988).

Polymorphism is an adaptive evolutionary response in natural populations, resulting in phenotypic variations attributed to gene pool diversity influenced by natural ecological factors in the environment. Teratological changes occur in immediate response to environmental alterations and the repeated incidence and severity can presumably be correlated with the extent of the stress. These direct morphological responses can be attributed to the lack of genetic underpinning encompassed by this type of deformity and are therefore deemed a response to confined and chronic human and natural environmental disturbances. Characteristics of teratological alterations can be classified by: deformed valve outline, loss of areolae, changes to striation pattern, and potential disruption of the raphe formation. These modifications can potentially cause alterations to the physiological and morphological mechanisms of the diatoms (Falasco 2008).

Current research is limited regarding empirical evidence that teratological diatom deformities in freshwater streams may act as indicators of increased dissolved metal concentrations. A study in the Journal of Freshwater Ecology conducted near Vail, Colorado in the Eagle River, analyzed the relationship between morphologically abnormal valves of the diatom genus *Fragilaria* and elevated concentrations of metals. This study was unique in that the pH of the river was fairly basic (7.7-8.5) but still contained evidence of AMD, allowing the researchers to look at the teratological deformities as a consequence of dissolved metal concentrations independent of pH. The periphyton sample findings identified *Fragilaria*, out of the total 10-17 genera that were identified after collection, as having the highest occurrence of abnormal cells. The study found that the abnormalities found in the genus *Fragilaria* corresponded to the incidence of heavy metal concentrations in the impacted zones. This study used several characteristics to determine teratological deformities. If a diatom contained at least one of these characteristics then it was determined as having morphological deformity. The first stipulation alleged diatoms observed as being asymmetrically abnormal and showing evidence of bent valves were teratologically deformed; secondly, if the diatom exhibited notched or incised valves, and finally if the diatoms contained valves with abnormal striae and patterned axial areas (McFarland 1997). These characteristics will act as guidelines for identifying teratological deformities in this study and will be the basis for determining abnormal morphology.

A more recent study, examining diatom deformities in AMD sites, identifies three genera and six species of diatoms in Friendship Hills National Historic Site in Fayette County, Pennsylvania. The genus *Eunotia* was the most prevalent and was also found to have the most incidence of morphologic deformity. These species, *Eunotia geitlerii* and *E. exigua,* show deformities related to the addition of striae and incised ventrals. As well, the study found the species *Pinnularia subcapitata* showed evidence of punctae that were exceedingly silicified and contained shallow pores (Smith 2007). Smith employs a rubric developed by Miquel in Van Heurck (1896) to determine the types of deformities seen in the frustules of the diatoms. Deformities classified as Type I contained a deformed shape meaning bent or incised valves; Type II had more than one center axes and striations radiating from these multiple axes; and Type III were unsymmetrical and had varied markings as well as added striations. Type I deformities are thought to be caused by external means, while Type II and III are caused by a biological interruption during frustules formation. The most common deformity seen in the diatom samples was Type I, although Type III was also observed with the *Eunotia* genus. The *Pinnularia subcapitata* was more commonly associated with Type III deformities of the punctae (Smith 2007). This rubric for determining teratological deformities is similar to the McFarland and

Smith studies but is not as up-to-date and so will not be depended on as heavily to classify deformities.

A particularly interesting study done in Lac Dufault (Quebec, Canada) describes morphological alterations in diatoms as indicators of pollution throughout history by means of sediment cores. Although this study is looking at lake sediments, the findings regarding AMD are interesting and without any reasonable doubt should hold true when analyzing stream diatoms for AMD. The lake has an extensive past involving mining and as a result, contamination from mining. The study analyzed the sediment cores for diatoms and identified that prior to mining operations the diatom genus *Tabellaria flocculosa* was dominant.

Between the years 1941-1980, high levels of dissolved Cd, Cu, Fe, Pb, and Zn were recorded in association with increased mining production. The once dominant *Tabellaria flocculosa* had a correlated decrease during these years as a new genus, *Fragilaria* cf. *tenera,* gained dominance. Following in abundance in later years after the onset of contamination were *Achnanthes minutissima* and *Brachysira vitrea.* Of more relevant importance than composition were the observed changes in the taxa of the diatoms. Interestingly, almost all of the morphological alterations mentioned were associated with the dissolved metal contamination as a result of the mining activity in the area.

During this period of contamination, the shortest valves were recorded for all taxa. Overall size reduction was most evident in *T. flocculosa* and A. *Formosa*, both of which had higher abundance before contamination. Most crucial to note was that upon contamination onset the valve lengths approximately halved in response for both taxa.

Particularly in the *A. Formosa,* one side of the valve was frequently reported to be rotated 90°. This modification was considered to be an indication of weak silica formation attributed to the AMD. This silica cell wall deformity is a result of silicic acid deficiency. Metals found in streams affected with AMD may weaken typical membrane function of the diatoms, which in turn reduces the amount of silicic uptake and amino acid synthesis. Important to this study is to note the teratological deformities that were observed during the period of contamination. The deformities themselves were generally deformations of the central area and were commonly observed in the *Fragilaria capucina* var. *rumpens* and *Eunotia* spp. Results from the study indicated that both the taxonomical and morphological changes of the taxa observed were correlated with acid mine contamination and an increase in dissolved metals (Cattaneo 2004).

Chemical Composition

In order to understand whether AMD and metal toxicity are to blame for diatom deformities in alpine streams in Colorado, an understanding of the chemical composition of the streams over an extended period of time is critical. As well, understanding how diatoms form and what conditions they favor will be helpful in assessing whether dissolved trace metals from the mining contamination is, in fact, the explanation for the morphological deformities seen in the diatoms. The acidity of AMD comes from exposure of the metals and minerals being mined, to the oxygen in the air and the hydrologic cycle.

$$(1)\quad FeS_2 + \frac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4{}^{2-} + 2H^+$$

Mining increases the probability of rainfall, or snowmelt drainage carrying these reaction products into nearby water sources (Baker 2003). Once these metals are exposed to oxygen they begin oxidizing and can become very acidic. This process is sluggish in a pH range of around 3. Bacteria can exacerbate the effect, acting as a catalyst once the pH is low enough. In particular, the bacteria *Thiobacillus ferrooxidans* have the ability to oxidize pyrite by utilizing ferrous iron to generate a ferric iron catalyst.

$$(2)\quad Fe^{2+} + \frac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \frac{1}{2}H_2O$$

In environments such as AMD contaminated streams, the acidic conditions allow ferric iron, which is a more potent oxidant than oxygen, to oxidize pyrite at a faster rate, forming ferric hydroxide.

$$(3)\quad Fe^{3+} + 3H_2O \rightarrow Fe(OH)_2 + 3H^+$$

If ferric iron is then created with the pyrite, the following reaction occurs and in turn dissolves the pyrite:

$$(4)\quad FeS_2 + 14Fe^{3+} + H_2O \rightarrow 12Fe^{2+} + 2SO_4{}^{2-} + 16H^+$$

This reaction, in addition to the ferrous iron, forms a succession of pyrite dissolution.

The solubility of metal oxide phases increases in these areas and as a result higher concentrations of metals are initiated into the water systems. Metals that are commonly found dissolved in AMD areas that are typically abundant in the sulfide minerals are Cd, Pb, and Mn. Fe and Al are less soluble as pH increases which may be as a result of diurnal photosynthesis by periphyton or inputs from pristine tributaries. When analyzing the results from this experiment it is important to understand the chemical reactions that may cause morphological deformities in diatoms (Todd, McKnight, and Duren 2005).

Based on review of past literature and drawing conclusions from knowledge about chemical reactions in streams affected by AMD, it can be predicted that overall taxa diversity and number will decrease in streams with high concentrations of dissolved metals. In addition, in the observed species from AMD stream samples teratological deformities will be present as a result. These deformities may include bent or incised valves, shortened valves, bent central areas, or abnormal straie density or distribution, based on the similarity of the conditions found in the literature and the conditions present at the 3 sampling sites in this study. If these teratological deformities are present in the diatoms observed in Peru Creek and St. Kevin's Gulch, as well as no deformities of this nature are seen in the control samples taken from Deer Creek, it can be alleged that contamination from acidic mine drainage is the cause of the deformities.

Chapter 4 Research Methods

Information from previous studies as well as advice from Diane McKnight and Ian Bishop was adapted to create the research methods and procedures used in this study.

This methods section includes site descriptions, collection methods for water samples and diatoms, and processing procedures of all samples.

Site Descriptions

Three site locations were sampled in Colorado alpine streams. Two were located in the Snake River basin above Summit County near Keystone. The sites were chosen based on the availability of long-term data to augment the data taken in this experiment, accessibility by vehicle, and allowance to sample (i.e. forest service vs. private property). August and September were chosen as ideal months for sampling as these are typically the driest months in the state of Colorado annually. However, this summer proved to be particularly wet with a torrential precipitation event in the second week in September. As a result sampling was delayed until late September. In Summit County, where two of the sites are located precipitation in August reached 4.22 inches. The annual average for August is 2.26 inches. The historical record on USA.com reported this August is the wettest reported since 1984 for Summit County (USA.com, 2013).

Deer Creek

Figure 1 USGS 2013 CO_Montezuma (Red circle is site location)

This area included the control site at Deer Creek. Deer Creek is a pristine alpine stream that lies on the western slope of the Continental Divide. The area of this watershed is approximately 10.4 km^2 and flows into Snake River at a confluence located in Montezuma, Colorado (Todd, McKnight, & Duren 2005). The Snake River has an area of approximately 11.7km^2 and feeds into Dillon Reservoir, which supports the needs of not only the towns of Frisco, Dillon, and the surrounding ski resorts, but also much of the population on the Front Range where the highest concentration of people in Colorado reside. The sampling site was located less than two miles from the divide itself and approximately two miles from Highway 5 past the town of Montezuma. Montezuma lies about 8 miles from Keystone, Colorado. Montezuma has a rich past, established as a silver mining boomtown in the late 1800's. After the silver bust in 1893, many of the mines were abandoned and the town essentially became a ghost town for many years. The elevation of the site was approximated at 3188.21 m above sea level and the outside temperature was 21.7° C at 9:34am. Approximately 90m above the dirt road there is a slower flowing pool where the samples were taken.

Peru Creek

Figure 2 USGS 2013 CO_Montezuma (Red circle is site location)

The second sample site, Peru Creek, is also part of the Snake River Watershed. The site was located approximately 3 miles east of Montezuma, on frontage road 260, with the Continental Divide located about a mile to the southeast. The elevation of the sampling site was approximately 3096.8 m and the outside temperature was 22.4° C with clear sunny skies at the time of sampling (12:15pm). The Pennsylvania Mine, the major source of AMD contamination, was located less than a mile northeast from the site. The Pennsylvania mine was in operation from the late 1880's until the early 1930's. The main upshot from the mine was millions of dollars in silver, zinc, and lead. Drainages from this mine typically report a pH of between 3 and 5. However, the actual streambed itself typically has a pH above 5. The sampling site was not taken from a drainage area but from the streambed itself (Todd, McKnight & Duren 2005).

St. Kevin's Gulch, Leadville, CO

Figure 3 USGS 2013 CO_Leadville (Red circle is site location)

Finally, the third sampling site was located at St. Kevin's Gulch north of Leadville, Colorado, approximately 2 miles from the turn for County Road 9A. The elevation at the location of sampling was 2932.2 m and the outside temperature was 11.7°

C. Weather at the time of sampling (8:36am) was overcast and by the end of sampling (9:24 am) precipitation occurred, although it did not amount to enough to skew data results. This gulch discharges into the gravel and Quaternary sand aquifer at Tennessee Park in Leadville. St. Kevin's Gulch is the result of heavy iron oxides from the Griffin Mine, which have completely covered the majority of the streambed and left the stream almost void of biota. Photoreduction plays a role during the daytime in this location and as a result, higher concentrations of ferrous iron are present (Paschke, Harrison, & Walton-Day 2001).

Procedure Protocol

To supplement the diatom collection, field measurements were also taken and interpolated into existing field data for the site areas. These field measurements included, pH, temperature, conductivity, and turbidity. Samples for dissolved metals, Total Dissolved Phosphorous (TDP), Total Dissolved Nitrogen (TDN) and Dissolved Organic Carbon (DOC), were taken for analysis back at the lab. All field and lab procedures were executed based on Kiowa Laboratory sample procedures (Seibold, 2013). Procedure protocol, for collecting and analyzing diatoms, is based on the Stream Periphyton Monitoring Manual by Barry J. F. Biggs and Cathy Kilroy (Biggs & Kilroy, 2000).

Field Measurements

Field measurements were taken first using a YSI field meter that was calibrated in solutions of varying pH previous to the sampling. The meter recorded pH, temperature, and conductivity. A Hach turbidimeter was also included in the field to measure turbidity of the streams. Grab samples for dissolved metal analysis were taken in previously bathed reagent-grade nitric acid 500mL and 1L clear plastic bottles and rinsed three times with stream water. Samples for chlorophyll-a analysis were taken in 500mL brown plastic bottles previously washed and rinsed with stream water three times. Samples for dissolved organic carbon (DOC) were taken in precombusted (475 Degrees for 4hrs) glass amber bottles. Four samples for dissolved metals and nutrient analysis, four chlorophyll-a samples, and 2 DOC samples were taken as grab samples from each site. The samples were then filtered back at the accommodation. The samples for dissolved metals and nutrients were filtered through 0.4 (nm) Nucleopore filters while DOC samples were filtered using pre-combusted (475 degrees for 4hrs) glass fiber filters. All samples were filtered using a hand held vacuum/pressure pump and the filtering was carried out as soon as possible after sampling on the same day (within 4 hours of sampling). Periphyton samples were taken in 50mL plastic bottles and were collected from 3 different areas of substrata within the site, using a foil template (2inX2in) for reference and a soft toothbrush. The substrata was removed from the stream, gently brushed in a 2X2 area and brushed into the bottles. A small amount of stream water was used to keep the samples moist until analysis could be conducted back at INSTAAR laboratory.

Water Sample and Diatom Processing Procedures

Water samples being tested for total dissolved phosphorous and nitrogen (TDP &TDN), as well as DOC, were analyzed at the Kiowa Labs at the University of Colorado. The TDP and TDN were digested and then oxidized with potassium persulfate as well as, 3.75 NaOH to release phosphorous from organic matter. The samples were then analyzed using the Lachat Quickchem 8500 Flow Injection Analyzer. DOC was analyzed using a UV-VIS Absorbance Scan with the Agilent 8453 UV-Visible Spectrophotometer. Samples were prepared by adjusting the pH of the samples to obtain an optimal absorbance of 0.1 at 254 nm (Seibold, 2013). Analyses on dissolved metals were conducted at the LEGS Laboratory at the University of Colorado at Boulder using an Inductively Coupled Plasma/Mass Spectrometer (ICP/MS). This machine ionizes the sample with the inductively coupled plasma and then employs the mass spectrometer to separate and measure those ions (LEGS, 2003).

Chlorophyll-a samples were filtered through glass fiber filters in volumes of 250 mL through a hand pump filtering apparatus. The filter was then removed from the pump and placed on aluminum foil and folded in half with the hatched side of the filter facing upwards. The samples were then frozen until analysis at Kiowa Labs back at INSTAAR. Once ready for analysis, the samples were thawed and 20 mL of 90% buffered acetone was added using a pipette to the filters and then wrapped in aluminum foil and frozen. The samples are then warmed to room temperature after 24hrs and then undergo processing in the ISA Jobin Yvon-SPEX Fluoromax-3 Spectrofluorometer and calculations for Chlorophyll-a are made from the data extracted from the system (Seibold, 2013).

Diatom samples were processed using standard protocol (Biggs &Kilroy 2000). Nine samples (3 from each site) were mixed with a solution of 30% hydrogen peroxide and left in a vented chamber to digest all organic material for 24 hours. The samples were then rinsed with distilled water every hour for 8 hours. The cleaned samples were diluted to a cloudy appearance and transferred to cover slips (2 per sample) to dry for 24 hours. Once the samples were dry, permanent slides were created using naphrax as a contrasting agent. Diatoms were then identified and analyzed under a light microscope at 100X magnification with oil immersion using a transect scanning method. 300 valves were identified for Deer Creek, Peru Creek, and St. Kevin's Gulch. The diatoms observed were documented and identified and a percentage of deformities seen were calculated.

Chapter 5 Results

The data resulting from the samples are portrayed exactly as they were when received from which analysis occurred. Errors or outlying data may have resulted from error in the field or filtering after collection. This section aims only to relay the results in an empirical manner by which conclusions are drawn in later sections.

Field Meter Results

Site Name	Temperature (degrees C)	pH	Conductivity (S/m)	Turbidity (NTU)
Deer Creek	3.4	6.39	45.6	1.09
Peru Creek	6.7	5.64	154.3	13.52
St. Kevin Gulch	6.0	4.08	179.8	1.35

Table 1

ICP-MS Results

Sample Location	Al	Ca	Ba	Cd
Peru Creek 1	1066.60	22583.26	118.82	6.82
Peru Creek 2	1003.00	21415.12	123.46	6.63
Peru Creek 3	1034.40	21550.90	110.73	6.68
Deer Creek 1	21.35	10955.46	30.75	DL
Deer Creek 2	17.52	10483.60	26.06	DL
Deer Creek 3	16.58	10478.22	65.63	DL
St Kevin G 1	2259.38	13541.91	84.24	42.89
St Kevin G 2	2486.76	13770.59	119.67	42.84
St Kevin G 3	2285.33	14428.84	129.77	43.03
St Kevin G 3-AD	2233.68	14455.74	125.82	42.89
Machine Detection Limit	1.011	9.608	0.096	0.031
*All values in ppb.				

Sample Location	Ce	Co	Cu	Fe
Peru Creek 1	7.38	5.81	183.96	267.86
Peru Creek 2	8.01	5.52	173.08	244.74
Peru Creek 3	8.13	5.53	176.35	253.00
Deer Creek 1	0.04	0.03	0.63	131.29
Deer Creek 2	0.04	DL	0.32	119.01
Deer Creek 3	0.03	0.03	0.46	114.91
St Kevin G 1	13.02	6.84	79.65	605.09
St Kevin G 2	12.92	6.94	82.69	641.49
St Kevin G 3	12.96	7.01	90.95	634.15
St Kevin G 3-AD	12.43	6.98	89.98	645.19
Machine Detection Limit	0.013	0.027	0.274	13.992
*All values in ppb.				

Sample Location	K	Mg	Mn	Na
Peru Creek 1	601.70	7366.29	2233.60	3469.48
Peru Creek 2	576.94	6991.87	2084.81	3196.81
Peru Creek 3	535.08	7037.47	2118.51	3183.13
Deer Creek 1	449.48	2279.88	9.14	1396.90
Deer Creek 2	390.94	2154.64	8.75	1246.95
Deer Creek 3	210.83	2124.07	8.62	1606.66
St Kevin G 1	815.16	5079.09	4010.20	3179.53
St Kevin G 2	813.28	5053.34	4051.24	3616.49
St Kevin G 3	866.20	5259.17	4170.72	3838.15
St Kevin G 3-AD	845.83	5115.50	4131.12	3723.76
Machine Detection Limit	13.726	0.451	0.233	56.602
*All values in ppb.				

Sample Location	Pb	Si	Sr	Zn
Peru Creek 1	3.59	3606.88	218.29	1828.68
Peru Creek 2	3.34	4203.94	214.65	1746.81
Peru Creek 3	3.27	3947.83	217.77	1761.34
Deer Creek 1	0.16	2674.04	36.66	15.94
Deer Creek 2	DL	2872.46	35.27	12.69
Deer Creek 3	0.07	2511.56	35.02	30.63
St Kevin G 1	5.35	8387.49	36.96	8623.17
St Kevin G 2	5.60	8236.91	37.12	8688.56
St Kevin G 3	5.68	8690.95	38.34	8789.04
St Kevin G 3-AD	5.89	8588.48	37.58	8657.93
Machine Detection Limit	0.062	2.098	0.075	0.491
*All values in ppb.				

Table 2

TDN, TDP & DOC Results

SAMPLE LOCATION	TDN uMOLES/L	TDP mg P/L	TDP uMOLES/L	DOC mg C/L	TDN mg N/L
Deer Creek A	25.55	NP	NP	2.44	0.358
Deer Creek B	15.78	NP	NP	2.24	0.221
Peru Creek A	33.41	NP	NP	0.96	0.468
Peru Creek B	20.53	NP	NP	0.63	0.288
Kevin A	5.01	NP	NP	1.42	0.070
Kevin B	3.47	NP	NP	0.84	0.049
Peru Creek		u	<0.04		
St Kevin Gulch		u	<0.04		
Deer Creek		0.0029	0.09		
u=undetected					
NP=Not Performed					

Table 3

Chlorophyll-a Results

	Chl-a [μg/L]
Deer Creek_01	1.43484021
Deer Creek_02	1.09319104
Deer Creek_03	2.40858708
Peru Creek_01	0.70091653
Peru Creek_02	0.63317637
Peru Creek_03	0.83402379
St. Kevin_01	0.40586569
St. Kevin_02	0.76657127
St. Kevin_03	0.67042626

Table 4

Diatom Results

Teratological Deformities

	Deer Creek	Peru Creek	St. Kevin Gulch	%Abnormal
Achnanthidium gracillimum (Meister) Lange-Bertalot	-	-	-	0
Achnanthidium minutissima (Kutzing) Czarnecki	-	-	-	0
Aulacoseria alpigena (Grunow) Krammer	-	-	-	0
Cocconeis cf. placentula Ehrenberg	-	-	-	0
Cymbella cistula (Ehrenberg) O. Kirchner	-	-	-	0
Cymbella minuta Hilse ex Rabenhorst	-	-	-	0
Diatoma tenuis (Agardh) Van Heurck	-	-	-	0
Diatoma mesodon Kutzing	-	-	-	0
Diatoma moniliformis (Kutzing) D.M. Williams	-	-	-	0
Didymosphenia geminata (Lyngbye) M. Schmidt	-	-	-	0
Encyonema nicafei Spaulding	-	-	-	0
Eunotia areniverma Furey, Lowe, & Johansen	-	-	-	0
Eunotia exigua (Brébisson in Kutzing) Rabenhorst	-	-	+	73.25
Eunotia macroglossa Furey, Lowe, and Johansen	-	-	-	0
Eunotia tenella (Grunow) Hustedt in A Schmidt	-	-	+	7.41
Fragilaria vaucheriae (Kutzing) Petersen	-	-	-	0
Gomphonema angustatum (Kutzing) Rabenhorst	-	-	-	0
Gomphonema brebissonii Kutzing	-	-	-	0
Gomphonema tenellum Kutzing	-	-	-	0
Hannaea arcus (Ehrenberg) Patrick	-	-	-	0
Luticola mutica (Kützing) Mann in Round, Crawford & Mann	-	-	-	0
Meridion anceps (Ehrenberg) D. M. Williams	-	-	-	0
Meridion circulare (Greville) C. Agardh	-	-	-	0
Pinnularia acidophila Hoffman & Kramer	-	-	-	0
Pinnularia divergentissima (Grunow) Cleve	-	-	-	0
Psammothidium sp. (Grunow) Bukhtiyarova and Round	-	-	-	0
Navicula sp. O Muller	-	-	-	0
Nitzschia cf. paleaeformis Hustedt	-	-	-	0
Reimeria sinuata (Gregory) Kociolek and Stoermer	-	-	-	0
Staurosirella leptosteuron (Ehrenberg) Williams and Round	-	-	-	0
Staurosirella pinnata (Ehrenberg) Williams and Round	-	-	-	0
Synedra famelica (Kutzing) Krammer and Lange-Bertalot	-	-	-	0
Synedra ulna (Nitzsch) Ehrenberg	-	-	-	0
Tabellaria floculossa Ehrenberg ex Kutzing	-	-	-	0

Table 4 (+) teratological deformities were found (-) no teratological deformities were found

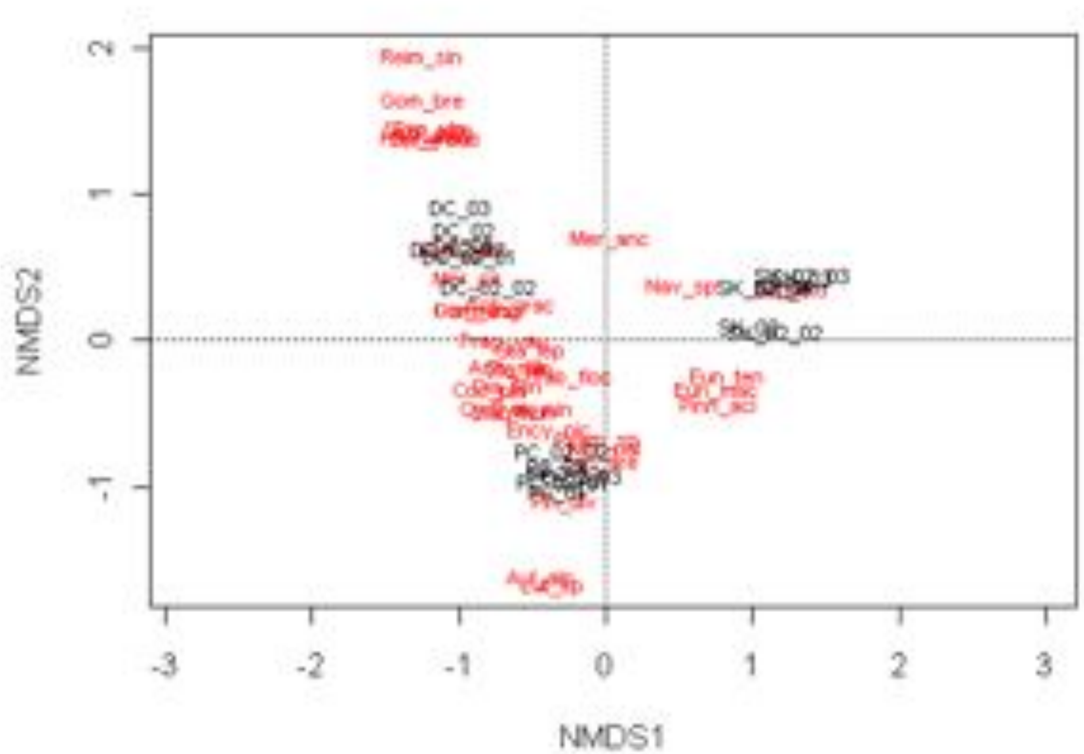

Figure 4 Deer Creek (DC), Peru Creek (PC), St. Kevin (SK); Red (Diatom Species)

Chapter 6 Analysis

Analysis of the results from the field research is a combination of prior review of literature, background knowledge, and direction from advisors and mentors. The analysis is prepared by individual variables and then concludes with an analysis of deformities and evaluation of the data as it relates to the hypothesis of this study. The analysis formulates the conclusions of the study.

Field Meter Analysis

All three sites were measured for turbidity, conductivity, temperature, and pH. Deer Creek was predicted to have the highest pH, and lowest temperatures, turbidity, and conductivity, due to its reputation as a pristine alpine stream. This proved to be accurate with the stream reporting a pH of 6.39, it was only slightly acidic, a turbidity of 1.09 NTU, 45.6 Ms for conductivity and a water temperature of 3.4° C. Peru Creek and St. Kevin's Gulch were expected to have lower pH and higher turbidity, conductivity and temperature as a result of the AMD contamination. Peru Creek resulted in a pH of 5.64, a water temperature of 7° C, and conductivity reading of 154.3 Ms, and a turbidity measurement of 13.52 NTU. St Kevin reported a pH of 4.08, a turbidity reading of 1.35NTU, a conductivity measurement of 179.8Ms and a water temperature of 6°C.

ICP-MS Analysis

Analyses of the ICP-MS results indicate that Peru Creek and St. Kevin's Gulch had higher concentrations of all dissolved metals detected than the site at Deer Creek. This was to be expected considering that both Peru Creek and St. Kevin's Gulch were located in close proximity to abandoned mine sites and therefore subject to AMD. This was also visually evident by the yellow precipitates on the streambed of Peru Creek and the red precipitates on the streambed of St. Kevin's Gulch. St Kevin's Gulch also had higher concentrations of almost all metals excluding, Strontium (Sr), Magnesium (Mg), Copper (Cu), and Calcium (Ca). The higher levels of Strontium and Copper were most likely responsible for the color seen in Peru Creek. Strontium, when exposed to oxygen turns a yellow color and Copper turns an orange-red color. The streambed color seen at St. Kevin's Gulch was most likely attributed to the high concentrations of Iron (Fe) in this area, producing the red tone when exposed to oxygen.

TDN, TDP, & DOC Analysis

In Table 3 the results indicate that TDP concentrations were too low to perform lab analysis. However, TDN produced concentrations large enough for analysis. Nitrogen is an essential nutrient in plant growth and sites with higher observed periphyton abundance would be expected to have higher concentrations of TDP. The values (mg/L) were fairly close for Deer Creek and Peru Creek. This is believable due to similar overlap in diatom species found at both sites. Although observed abundance was higher at Deer Creek these two sites (Peru Creek and Deer Creek) had significantly more diversity and overall taxa abundance than St. Kevin's Gulch. This is reflected in the values given for St. Kevin, as TDN is significantly lower here.

DOC concentrations proved to be representative of the sites as well. Plants uptake carbon during photosynthesis and use it to produce oxygen. In sites with higher periphyton abundance one would expect higher concentrations of DOC available for photosynthesis. In Table 3 DOC is highest in the pristine stream Deer Creek, which also had the highest diversity and abundance of diatoms. A higher value is noted in the sample St. Kevin A. This may have been an error during sample collection or contamination during processing, as one would expect lower concentrations in this site even compared to Peru Creek.

Chlorophyll-a Analysis

Chlorophyll-a is essential in the photosynthesis associated with cyanobacteria, phytoplankton, and eukaryotes. It acts as an electron donor, donating two electrons to an electron acceptor, within the electron transport chain. This process aids organisms in releasing chemical energy (Welschmeyer, 1994). Based on the higher availability of periphyton in Deer Creek, it would be assumed, that higher levels of Chlorophyll-a would exist at that site. This holds true as the results from the samples show Deer Creek on average having higher volumes >1.0µg/L of Chlorophyll-a. The acidic sites, Peru Creek and St. Kevin's Gulch, on average have lower volumes <0.9µg/L.

Diatom Analysis

Figure 4 shows a Nonmetric Multidimensional Scaling (NMDS) program in R run for the diatoms collected and identified at each of the three sites. As described in the methods section, 300 valves from each site were identified and their taxa noted. A ratio was calculated for each species out of the total and inputted into R and run for the NMDS. The six samples for each of the 3 sites resulted in 18 inputs. The black words on the figure are the samples for each site and the red words are the individual diatom species as they relate to each site. It is apparent by this figure that each of the three sites have distinct diatom populations with some overlap. Peru Creek has overlap with Deer Creek containing some diatoms found in more pristine streams such as a couple *Diatoma sp.* and *Achnanthidium sp.* Peru Creek also shares some diatom species with the very acidic St. Kevin's Gulch site. Diatoms in the *Eunotia sp.* and *Pinnularia sp.* are more tolerant of very acidic environments which is why that can often be found in AMD contaminated streams and ponds. The species *Eunotia exigua* however was much more frequent in the St. Kevin's Gulch samples and this species was found to contain the most evidence of teratological deformity.

In the samples collected from St. Kevin's Gulch the only diatom with consistent observed deformities was *Eunotia exiguna* although *Eunotia tenella* showed a few diatoms with teratological deformities. The deformities were consistent in that they all seemed to occur in the same general area and side of the diatom as well as the nature of the deformity. The level of severity was not as consistent and varied greatly, even within the same sample. The deformity observed consisted of a bent central area on the dorsal margin convex.

Figure 2-7 Teratologically deformed *Eunotia exigua* from St. Kevin's Gulch

The deformities seen in the Eunotia exigua are characteristic of the teratological deformities outlined in the McFarland study from 1997. The first stipulation, mentioned in the study for characterizing deformities, is diatoms showing asymmetrical abnormalities and bent valves. These deformities also meet the requirements of being teratological, as defined by McLaughlin, 1988. There is significant variability in the morphological alterations under the same ecological conditions. As well, these deformities meet the characteristic described as being teratological-a deformed valve outline. Deformity classifications in Miquel in Van Heurck rubric also encompass the deformities seen in *Eunotia exigua*. Type I deformities were described as containing a deformed shape; meaning bent or incised valves. With multiple studies reporting deformities analogous to the deformities present in *Eunotia exigua* found in the samples from St. Kevin's Gulch, it can be concluded that these diatoms are teratologically deformed. In Table 4 only two diatoms in total showed evidence of these deformities; *Eunotia exigua* and *Eunotia tenella.* The percent of abnormal valves for *Eunotia exigua* out of the total species observed was 73.25%, *Eunotia tenella* deformities made up only 7.41% of that species observed in total.

Perhaps more important to understanding the conditions that cause teratological deformities in diatoms present in AMD streams, is the correlation between the concentrations of dissolved metals found in St. Kevin's Gulch and Peru Creek and the instance of deformities. Based on the results from the ICP-MS testing, field measurements, and background knowledge, it would appear that the reason for the deformities would be either one of two things or a combination of both; lower pH or the higher concentrations of particular dissolved metals associated with St. Kevin's Gulch. Because certain metals are higher concentrated in Peru Creek than St. Kevin's Gulch, future studies focused on the particular effects of certain metals on diatoms would be useful in understanding what precise factors regarding AMD, cause the teratological deformities observed in this study.

Chapter 7 Discussion

Evaluation and analysis of the results were employed to make final conclusions and summaries. This section discusses the process of determining conclusions regarding acceptance of dismissal of the hypothesis, possible errors found in this study, and future recommendations.

Procedural Deficiencies

Several factors may have influenced the results of this study and should be considered in future research observing diatom deformities. Originally, this project was designed with the three sampling sites consisting of Deer Creek, Peru Creek, and Little James Creek above Jamestown, Colorado. The flooding that occurred throughout Colorado in the second week of September this year isolated Little James Creek and made sampling impossible in this area. Modification to the sampling sites just days before sampling resulted in the third site: St. Kevin's Gulch near Leadville, Colorado. More influential to the outcome of this study perhaps, was the absence of multiple sites along each watershed and need for more seasonally variable sampling times. Although, all three sites comprise the same stream order and are located in similar environments, sampling more locations along the stream and watershed would have been useful in determining more precise explanations for the deformities observed. By sampling in more locations along the watersheds different factors could be reported and more correlation analysis could be conducted. These may include: differing pH levels, concentrations of metals, inputs from AMD drainage, inputs from pristine tributaries, discharge, and precipitation inputs. Another important addition to consider when conducting this study is sampling at multiple times throughout the year to assess whether seasonality has an affect on the types of diatoms seen and the presence or absence of deformities. This study only sampled three locations during a 3-day period in mid September. The sampling period also took place after a particularly wet summer and a torrential precipitation event just a week prior. This influx of discharge may have affected the normal abundance of periphyton and diluted metal concentrations and therefore may have inhibited the overall sample qualities. By sampling throughout several seasons, a better overall representation of diatoms and metal concentrations in the watershed would be recorded.

Additional Research

The results found in this study demonstrate that AMD conditions are responsible for the teratological deformities seen in the St. Kevin's Gulch samples. The original hypothesis, which suggested such results, can therefore be accepted. The aim of this study was to use the deformity findings as a platform for creating proactive remediation efforts. If these types of deformities can be observed in other alpine streams potentially affected by AMD, these diatoms could provide a valuable tool for assessing remediation and prevention of extreme contamination conditions. The streams analyzed in this study showed apparent visual contamination, evident by the colorful metal precipitates on the streambed. Ultimately, with the addition of more studies similar to this one, a potential index for contamination can be established to evaluate different degrees of contamination. In the case of St. Kevin's gulch, where the most frequent abnormalities occurred, the stream is virtually devoid of all living biota. Here more extreme remediation methods would need to be implemented, some of which were already visible below the sampling site. Although it is clear that conditions produced by AMD contamination are responsible for the deformities, there is still uncertainty regarding the specific causes of the teratological valve deformities observed. To truly distinguish whether these deformities are a direct result of the dissolved metals present in a stream or as a result of a pH gradient; more studies like McFarland, 1998 would need to be conducted as well. It is suggested in a few studies that dissolved metals are the reason for these deformities as they weaken silica formation. However, with the addition of pH independent studies new information may give rise to the types of deformities seen and the taxa of diatoms they affect. In the McFarland study the genus *Fragilaria* had the highest abundance of deformities. In this study however, it was evident that the genus *Eunotia* had the highest abundance of deformities and that these deformities were not seen in Peru Creek.

New research opportunities are available to determine whether certain metal concentrations alone are responsible for these teratological alterations or if low pH conditions caused from the metals are also responsible. These questions are important for determining a contamination index as they will help scientists to understand whether these deformities only occur above definite concentrations of certain metals or whether there are other factors coalescing to produce these deformities. In addition, looking at stream conditions in conjunction with the specific deformities themselves can provide more information on the specific inputs that cause different types and severities of the deformities. A new scientific approach is making this possible by utilizing morphometric landmark analysis to further classify and quantify morphological deformities present in streams affected by AMD.

Morphometric Landmark Analysis

Morphometric analysis aims to statistically analyze possible factors that can affect the morphology of an organism. This research would be particularly relevant to identifying and quantifying morphological deformities in diatoms as a result of AMD. Landmark-based geometric morphometrics involve placing a series of coordinates along an organism in 'landmark' areas. These landmark areas are presumably homologous in all organisms of the same taxa and species. For instance in diatoms such as the *Eunotia exiguna,* which was observed as having deformities in this study, landmarks may consist of apices, valves, and even the density of straie. By analyzing these diatoms using landmark-based geometric morphometrics, it provides the ability to understand teratological deformities as a process of a pH gradient or of a dissolved metal concentration gradient. Correlating deformities based on their degree and the environment in which they occur could result in significant information regarding remediation (Dean, Rohlf, & Slice, 2004).

Chapter 8 Conclusion

Evidence of teratological deformities in *Eunotia exigua* can be attributed to AMD, as evidence by this study. With elevated metal concentrations and a low pH, St. Kevin's Gulch provided an acidic environment by which the morphological silica structure of this species was altered. This study, although informational in its observations, leaves uncertainty to precise origins that cause these deformities. A call for future studies done on these or similar streams in Colorado, affected by AMD, may provide more information as to the particular metals or other variables such as pH that change the morphological nature of these diatoms. Understanding these conditions may provide scientists with an opportunity to create a contamination index that would allow them to test for varying levels of contamination by observing deformities in the diatoms of environmentally degraded water systems. This new perspective of water quality has implications for remediation efforts that could limit the need for perpetual water treatment facilities in these areas of AMD. With new species of diatoms being discovered all the time, there is still a great deal to understand about different diatoms and what they reveal about water quality, but more research efforts like this study are stirring progress.

Chapter 9 References

Akcil, A., & Koldas, S. (2006). Acid Mine Drainage (AMD): causes, treatment and case studies. *Journal of Cleaner Production*, *14*(12), 1139-1145.

Aston, R. L. (2001). Water pollution by abandoned mine sites: acid mine drainage; mined land reclamation. *Mineral Resources Engineering*, *10*(04), 467-500.

Baker, B. J., & Banfield, J. F. (2006). Microbial communities in acid mine drainage. *FEMS Microbiology Ecology*, *44*(2), 139-152

Berghorn, G. H., & Hunzeker, G. R. (2001). Passive Treatment Alternatives for Remediating Abandoned-Mine Drainage. *Remediation Journal*, *11*(3), 111-127.

Biggs , B. J. F., & Kilroy , C. The New Zealand Ministry for the Environment, (2000). Stream periphyton monitoring manual (ISBN 0-478-09099-4). Retrieved from NIWA website: http://www.niwa.co.nz/sites/default/files/import/attachments/peri_complete.pdf

Blinn, Dean W., and David B. Herbst. "Use Of Diatoms and Soft Algae as Indicators of Environmental Determinants in the Lahotan Basin, USA." *ANNUAL REPORT FOR CALIFORNIA STATE WATER RESOURCES BOARD CONTRACT AGREEMENT 704558.01.CT766* (2003).

Cattaneo A., Asioli A., Comoli P. and Manca M. 1998. Organisms' response in a chronically polluted lake supports hypothesized link between stress and size. Limnol. Oceanogr. 43: 1938–1943.

Darin, T. F. (2000). Bureau of Land Management's Proposed Surface Management Regulations for Locatable Mineral Operations: Preventing or Allowing Degradation of the Public Lands, The. *Land & Water L. Rev.*, *35*, 309

Davies, H., Weber, P., Lindsay, P., Craw, D., & Pope, J. (2011). Characterization of acid mine drainage in a high rainfall mountain environment, New Zealand. *Science of the Total Environment*, *409*(15), 2971-2980.

Dean, A. C., Rohlf, J. F., & Slice, D. E. (2004). Geometric morphometrics: Ten years of progress following the 'revolution' . Italian Journal of Zoology, (71), 5-16. Retrieved from http://life.bio.sunysb.edu/morph/review/review.html

Drexler, B. (2003). *Icp/ms*. Retrieved from http://www.colorado.edu/geolsci/legs/instrumentation1.html

Duren, S. (2004). *Quantitative hydrologic evaluation of dissolved metal loading and transport in Peru creek, an acid rock drainage stream :Summit county, Colorado*

Falasco, E., Bona, F., Hoffman, G., Hoffman, L., & Ector, L. (2008). Diatom teratological forms and environmental alterations: a review. Hydrobiologia, 623(1), 1-35.

Ford, K. L., United States, & National Science and Technology Center. (2003). *Passive treatment systems for acid mine drainage*. Denver, CO: U.S. Bureau of Land Management, National Science and Technology Center.

Furey, P. (2012). *Eunotia exigua*. In Diatoms of the United States. Retrieved October 22, 2013, from http://westerndiatoms.colorado.edu/taxa/species/eunotia_exigua

Gazea, B., Adam, K., & Kontopoulos, A. (1996). A review of passive systems for the treatment of acid mine drainage. *Minerals engineering*, *9*(1), 23-42.

Gleick, P. H. (1999). *The human right to water* (p. 2). Pacific Institute for Studies in Development, Environment, and Security.

Hamsher E. Sarah, Robert G. Verb & Morgan L. Vis (2004): "Analysis of Acid Mine Drainage Impacted Streams Using a Periphyton Index", *Journal of Freshwater Ecology*, 19:2, 313-324

Hopfenberg, R., & Pimentel, D. (2001). Human population numbers as a function of food supply. *Environment, development and sustainability*, *3*(1), 1-15.

Idaho National Laboratory, United States, & United States. (2006; 1001). *Treatment of acid mine drainage using fishbone apatite iitm*. Washington, D.C: United States. Dept. of Energy.

Johnson, D. B., & Hallberg, K. B. (2005). Acid mine drainage remediation options: a review. *Science of the total environment*, *338*(1), 3-14.

Munk, L., Faure, G., Pride, D. E., & Bigham, J. M. (2002). Sorption of trace metals to an aluminum precipitate in a stream receiving acid rock-drainage; Snake River, Summit County, Colorado. *Applied Geochemistry*, *17*(4), 421-430.

McFarland, B. H., Hill, B. H., & Willingham, W. T. (1997). Abnormal fragilaria spp. (bacillariophyceae) in streams impacted by mine drainage. *Journal of Freshwater Ecology, 12*(1), 141-149. doi:10.1080/02705060.1997.9663517

McLaughlin, R. B., 1988. Teratological forms. The Microscope 36: 261–271

Medley C. Nicolas and William H. Clements (1998): "Responses Of Diatom Communities To Heavy Metals In Streams: The Influence Of Longitudinal Variation." *Ecological Applications*, 8:3, 631-644

Paschke , S. S., Harrison, W. J., & Walton-Day, K. (2001). Effects of acidic recharge on groundwater at the st. kevin gulch site, leadville, colorado. *Geochemistry: Exploration, Environment, Analysis*, *1*(1), 3-14. Retrieved from http://geea.geoscienceworld.org/content/1/1/3.abstract

Patrick, R.M. and Reimer, C.W. (1966**)**. The Diatoms of the United States exclusive of Alaska and Hawaii, V. 1. *Monographs of the Academy of Natural Sciences of Philadelphia 13.*

Patrick, R. & Reimer, C.W. (1975). The diatoms of the United States exclusive of Alaska and Hawaii. Vol. 2, Part 1. Entomoneidaceae, Cymbellaceae, Gomphonemaceae, Epithemiaceae. pp. i-ix, 1-213. Philadelphia: Academy of Natural Sciences.

Round, F.E., Crawford, R.M. and Mann, D.G. (1990**).** The Diatoms. Biology and Morphology of the Genera. *Cambridge University Press, Cambridge, 747 pp.*

Seibold, C. (2013, June 14). *Total phosphorus/total dissolved phosphorus: Lachat quikchem 8500 flow injection analyzer* . Retrieved from http://snobear.colorado.edu/cgi-bin/Kiowa/Kiowa.con.pl?lachtp.doc.html

Seibold, C. (2013, June 14). *Chlorophyll-a and phaeophytin: Isa jobin yvon-spex fluoromax-2 spectrofluorometer* . Retrieved from http://snobear.colorado.edu/cgi-

Smith, Thomas, and Kalina Manoylov. "Diatom Deformities from an Acid Mine Drainage Site at Friendship Hills National Historical Site, Pennsylvania." *Journal of Freshwater Ecology* 22.3 (2007)

Spaulding, S.A., Lubinski, D.J. and Potapova, M. (2010). Diatoms of the United States. http://westerndiatoms.colorado.edu Accessed on 15 October, 2013.

Sullivan, A. B., Drever, J. I., & McKnight, D. M. (1998). Diel variation in element concentrations, Peru Creek, Summit County, Colorado. *Journal of Geochemical Exploration*, *64*(1), 141-145.

Tate, Cathy M., Robert E. Broshears, and Diane M. McKnight. "Phosphate Dynamics in an Acidic Mountain Stream: Interactions Involving Algal Uptake, Sorption by Iron Oxide, and Photoreduction." *Limnology Oceanography* 40.5 (1995): 938-46.

Todd, A. S., McKnight, D. M., & Duren, S. M. (2005). *Water quality characteristics for the Snake River, North Fork of the Snake River, Peru creek, and Deer creek in Summit county, Colorado: 2001 to 2002*. University of Colorado, Institute of Arctic and Alpine Research.

Trumm, D. A., Watts, M., & Gunn, P. (2006, March). AMD treatment in New Zealand–use of small-scale passive systems. In *7th International Conference on Acid Rock Drainage (ICARD)* (pp. 2142-2158).

University of Wyoming Research Corporation, United States, National Energy Technology Laboratory, & United States. (2012). *Treatment and prevention systems for acid mine drainage and halogenated contaminants*. Washington, D.C: United States. Dept. of Energy.

USA.com. (2013). *Summit county weather*. Retrieved from http://www.usa.com/summit-county-co-weather.htm

USGS. (2013, October 27). CO_Leadville North_TM_geo-1 [Print Map]. Retrieved from http://www.usgs.gov/pubprod/maps.html

USGS. (2013, October 27). CO_Montezuma_TM_geo-2 [Print Map]. Retrieved from http://www.usgs.gov/pubprod/maps.html

Wehr, J. and Sheath, R.G. (2003). Freshwater Algae of North America: Ecology and Classification. *Academic Press, 917 pp.*

Welschmeyer, N. A. (1994). Fluorometric analysis of chlorophyll a in the presence of chlorophyll b and pheopigments . Limnology and Oceanography, 39(8), 1985-1992. Retrieved from http://cat.inist.fr/?aModele=afficheN&cpsidt=3523418