THE IMPACTS OF ACID MINE DRAINAGE ON THE BLACK CREEK WATERSHED, WISE COUNTY, VIRGINIA

by

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(ABSTRACT)

Black Creek is a small watershed located in Wise County, Virginia, west of the town of Norton. At the time of this survey, the watershed encompassed approximately 929 hectares of mine and forest lands with a small recreational area. Black Creek proper is a third-order stream approximately 6.7 km in length from its headwaters to its confluence with the Powell River in Kent Junction. Black Creek and several of the tributaries within the watershed were previously identified as areas impacted by acid mine drainage. The watershed was used in a study to identify sources of acid mine drainage and the best methods for its evaluation.

The acid mine drainage sources were first identified using visual inspection and field chemistry. Additional stream segments were then included in the assessment process using metal (aluminum, copper, iron, magnesium, manganese, and zinc) analyses of both overlying water column and sediments.

Using an upstream reach of Black Creek as a reference, short-term toxicity testing was employed, as well as a long-term purge study. The pH at sampling locations ranged from 2.75 to 7.87 SU, and conductivity ranged from 196 umhos/cm to 2040 umhos/cm. All metals were elevated when compared to the reference. Water column samples collected from locations with low pH were acutely toxic to *Daphnia magna* and *Pimephales promelas*. Mortality was high in the elutriant test at locations where pH was low, conductivity was elevated, metals were high, or a combination of these.

In the initial sediment tests, all sampling locations were significantly different than the reference for survival of *Chironomus tentans* and reproduction of D. *magna*. One location was significantly different than the reference for survival of *D. magna*. In the sediment tests completed after two months, survival of *C. tentans* was only different from the control in three locations but was significantly different for growth at all locations. Reproduction by *D. magna* was again significantly less than the reference at all locations. At eight months, only two locations were significant for survival of *C. tentans* and after 15 months, no significant differences occurred between any stations. The study indicates that stream segments that are severely impaired by acid mine drainage are easy to identify using visual inspection and field water chemistry. Those that are moderately impaired require more investigation and may not be responsive to short-term toxicity tests.

Benthic macroinvertebrates, leaf packs, and periphyton were evaluated in the field. Benthic macroinvertebrate communities and leaf-pack breakdown were evaluated at nine locations, while periphyton was evaluated at the mouth of Black Creek, as well as five sites in the Powell River receiving system. While leaf-pack information and benthic macroinvertebrate samples yielded similar information, benthic sampling was much simpler and less time consuming.

Additionally, benthic macroinvertebrate sampling, particularly over several sampling events, was more sensitive at the most severely impacted AMD stations.

The stations were broken down into five different categories in order to better determine which evaluation techniques were most sensitive and cost-effective. The five categories were Non-Impaired, Slightly Impaired, Moderately Impaired, Severely Impaired, and Severely pH impaired. Once the locations were categorized, each method used to evaluate toxicity was examined to determine which methods best identified acid mine drainage impairment in the Black Creek watershed. The methods utilized include the following: basic water chemistry; metals analysis of sediments and water column; acute toxicity testing using both *D. magna* and *P. promelas*; short-term elutriant and sediment tests; chronic sediment test using *C. tentans* and *D. magna*; a purge study; benthic macroinvertebrate sampling; leaf-pack and algal-tile studies. After evaluating these methods, it was determined that using basic water chemistry and benthic macroinvertebrate sampling were the best methods for evaluating acid mine drainage impairment in this watershed.

The reference station was identified as Non-Impaired. Two stations located in the lower portions of Black Creek (L11 and L1) were also Non-Impaired or only Slightly Impaired with the benthic macroinvertebrate results indicating little impairment. Stations U2, U6, U7, and BBM were also found to be Slightly Impaired. The station on the margin of the wetland, U5, was Moderately Impaired. Two previously identified areas of impairment, U9 and U10, (Cherry et al. 1995) were identified as Severely pH Impaired and Severely Impaired, respectively.

To

My Grandmother – Clara Ann Border Sykes~Stromberg

You taught me to seek things wondrous. You taught me that there are no limits.
You taught me to believe.

&

My Father – L.O. Yeager, Jr.

Your strength inspired.

REST IN PEACE

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1.0 CHAPTER ONE

1.1 GENERAL DESCRIPTION

Coal mining has had a long and tumultuous history in the Appalachian region. Discovered in the New World by Father Louis Hennipin in 1649, coal was first mined in the 1750's using picks and shovels (Skousen and Ziemkiewicz, 1995). Technological advances ,mirrored by increased demand, steadily boosted coal output so that by the 1840's, coal exceeded wood consumption in the region. The advent of electricity and the use of rail opened up the coal mining industry in the late 1800's and early 1900's. Mechanization of the coal industry allowed continuous production with more tons mined per man, while the railroad helped supply the increasing demand. This development also left a marked impact on the aquatic environment.

By the 1800's, it became apparent to naturalists that the alteration of the landscape would have a dramatic effect on the aquatic environment (Clarke, 1969). In 1918, Ortmann recorded the slow, steady deterioration of mussel fauna (Unionidae) due to stream pollution. While the coal mining industry is not specifically mentioned in the early literature, it has been implicated in the decline of water quality (Clarke, 1969). In 1939, the first legislation relating to coal mining and land reclamation was passed in West Virginia (Slousen and Ziemkiewicz, 1995). This legislation created the Department of Mines, and bonds of $150 per acre of disturbed area were implemented.

Unfortunately, these and other regulations afforded little protection for the environment. By 1973, an estimated 1.8 million acres of land, most of it forest in Appalachian states, had been disturbed by mining activities (Curtis, 1977). Much of this land was inadequately reclaimed, resulting in impacted streams and rivers (Herlihy et al. 1990; Skousen and Ziemkiewicz, 1995; Santopietro and Zipper, 1996). In 1973, it was estimated that over 18,000 miles of streams in Appalachia had been destroyed due to mining activities (Neves et al. 1997). Acid Mine Drainage (AMD) and siltation, two of the impacts from mining activities, are often identified as the most widespread and detrimental impacts (Appalachian Regional Commission, 1969).

In 1977, congress passed the federal Surface Mining Control and Reclamation Act (SMCRA). SMCRA and the Clean Water Act (1977) require mines to meet a minimum standard for permitting. These statutes also created uniform reclamation standards (Dickens et al. 1989). In its inception, it was hoped that SMCRA regulation would help reduce the environmental damage caused by the coal mining industry. Recent legislation states the purpose of SMCRA is to "establish a nationwide program to protect society and the environment from adverse effects of surface coal mining operations" and "to assure that surface coal mining operations are so conducted as to protect the environment" (West Virginia Consent Decree, February 17, 2000). Areas mined prior to 1977, which were left inadequately or unreclaimed, are referred to as abandoned mined lands (AML).

1.2 ABANDONED MINED LANDS

Abandoned mined lands are areas that were mined prior to implementation of federal controls over coal-mined land reclamation and were left inadequately reclaimed (Santopietro and Zipper, 1996). Many AMLs have environmental problems, largely due to insufficient regulations associated with them. Title IV of SMCRA established the AML program, which provides for the "restoration of eligible lands and waters mined and abandoned or left inadequately restored." Fees of 35 cents per ton of surface-mined coal, 15 cents per ton of coal mined underground, and 10 cents per ton of lignite mined are collected by the Office of Surface Mining (OSM) on all active mining operations. These fees are deposited in an interest bearing Abandoned Mine Lands Fund, which is used to help pay reclamation costs of AML projects.

AMLs have several impacts associated with them. These impacts are the result of moving large amounts of geologic material (Skousen and Ziemkiewiez, 1995), which alters soil profiles and outslope areas (Curtis, 1973). Additional impacts result from denuding forested lands and building haul roads (Curtis, 1977 and 1979). Impacts on land and soil include the following: increased soil erosion; overburden swelling and subsidence; soil instability and landslides; acid drainage; altered infiltration characteristics from soil inversion; exposure of toxic strata to weathering; and drainage from spoil and coal piles. Potential impacts on water resources include the following: ground water contamination; alteration of aquifer characteristics; surface water contamination from increased sedimentation; mine drainage and toxic substances; and modification of watershed characteristics (Curtis, 1973; 1977; 1979; Dickens et al. 1989; Leary, 1991).

1.3 ACID MINE DRAINAGE

One of the most common environmental effects of inadequate reclamation of deep and surface mines is acid mine drainage (AMD). AMD forms as a result of oxidation of minerals containing reduced forms of sulfur, pyrites, and sulfides in the presence of water. The process may occur naturally but is more often a result of the exposure of mineral deposits by mining activity. Iron pyrite is commonly found associated with the geologic strata above or below eastern coal seams. Upon exposure to air and in the presence of water, the following reactions occur:

$$FeS_2 + 3.5O_2 + H_2O \Rightarrow + 2H^{+} + 2SO_4^{-2} \qquad (a)$$

$$Fe^{+2} + .25O_2 + H^{+} \Rightarrow Fe^{+3} + .5H_2O \qquad (b)$$

$$Fe^{+3} + 3H_2O \Rightarrow Fe(OH)_3 + 3H^{+} \qquad (c)$$

or

$$FeS_2 + 14Fe^{+3} + 8H_2O \Rightarrow 15Fe^{+2} + 2SO_4^{-2} + 16H^{+} \qquad (d)$$

Here (a) is the initial reaction then (b) and (c) are resulting reactions. The oxidation of the iron sulfide releases ferrous iron (Fe^{+2}) and sulfuric acid (a). Upon exposure to water, the unstable ferrous iron is oxidized and forms ferric iron (Fe^{+3}) (c). The ferric iron precipitates as insoluble ferric hydroxide “yellow boy” (c) if the pH is above 3.0, or it reacts with the pyrite to form more sulfuric acid (d). This reaction is known as the ferric shunt, where the ferric iron actually has a greater affinity for the pyrite. The overall process describing pyrite oxidation is commonly given by the following incongruent reaction:

$$FeS_2 + (15/4)\,O_2 + (7/2)\,H_2O \Rightarrow Fe(OH)_3 + 2H_2SO_4 \qquad (e)$$

The rate of pyrite oxidation depends on several factors, including reactive surface area of pyrite, form of pyritic sulfur, oxygen concentrations, solution pH, catalytic agents, flushing frequencies, and the presence of *Thiobacillus* bacteria (Skousen and Ziemkiewicz, 1995). The presence of *T. ferrooxidans* is of particular importance because it will facilitate these reactions and increase the ferrous oxidation rate by five to six orders of magnitude. This increased oxidation rate and the ferric shunt make the iron sulfide oxidation reaction a rapid, self-perpetuating process (Nordstrom, 1982).

Streams influenced by AMD are acidic and contain sulfates. In coal mining areas, they are usually contaminated by Fe and have a pH value of <6.0 (Skousen and Ziemkiewicz: 1995; ARC, 1969). AMD streams also have high acidity. Acidity differs from pH in that it is a measure of mineral activity that results from dissolved metal ions as well as hydrogen ion concentration. Streams may also be characterized as AMD influenced if they have high concentrations of total iron (1.5 mg/L), aluminum (0.5 mg/L), manganese (1.0 mg/L), and sulfate (SO_4)(250 mg/L) (ARC, 1969). Iron, aluminum, and manganese are mobilized, and aluminum becomes more toxic as a result of low pH (Brezonik et al. 1991; Stiefel and Busch, 1983). Trace metals, such as arsenic (As), cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb), and zinc (Zn) may also be mobilized by acidic waters. Metals in AMD streams may be found in the overlying water column, in the interstitial water of the sediment, or bound to both inorganic and organic materials of which the sediment is comprised. Elevated concentrations of suspended solids are also associated with AMD streams (Curtis, 1979; Dickens et al. 1989).

Acid mine drainage will reach steams via several mechanisms. Streams can be impacted when contaminated groundwater enters them (Leary, 1991). Waters may be intercepted and stored in mining spoils and gradually released as seepage, which eventually enters streams (Dickens et al. 1989). These stored waters may also be released intermittently when the spoil pile or impoundment has exceeded its storage capacity (Dickens et al. 1989), such as during a rain event. Such releases may produce a large portion of headwater streams' base flow (Curtis, 1977).

Acid mine drainage has been studied by both academics and government agencies for several decades. The chemical, physical, and biological aspects of the phenomenon have been studied in both the eastern and western US (Clements, 1991), as well as European and Asian countries (Gray, 1998; Min et al. 1997; Nelson and Roline, 1996). Studies of the biological aspects include but are not limited to microbial activity, wetland delineation, basic toxicity testing, and instream studies monitoring benthic organisms in both lotic and lentic marine and freshwater environments (Demchik and Garbutt, 1999; Gray and O'Neill, 1997, Kim et al. 1999; Lopes et al. 1999).

1.4 STUDY DESCRIPTION

In 1994, researchers at Virginia Tech's Center for Environmental and Hazardous Material Studies and the Biology Department, in cooperation with officials at the Powell River Project, the Department of Mines, Minerals, and Energy's (DMME) Division of Mined Land Reclamation (DMLR) and the Virginia Chapter of the Nature Conservancy, began an investigation of the Powell River in the Tennessee River Basin. The objective of this investigation was to determine the influence of AML on the ability of the Powell River to support aquatic communities (Cherry et al. 1995). The Powell River has historically been an important center of biological diversity for several species of endangered Unionid mussels, and the river in Lee County, Virginia, is Critical Habitat. The Tennessee River drainage also supports the most diverse ichthyofauna in North America (Jenkins and Burkhead, 1994).

These investigations concluded that Black Creek, a third-order tributary of the Powell River in Wise County, Virginia, was a sub-watershed whose AML was "second in environmental severity" as an environmental impact in the watershed. The report indicated that Black Creek contained two active seeps whose "influences of elemental concentrations were apparent at the confluence of the Powell River." This investigation resulted in a subsequent one-year project to gather information necessary to develop a successful and cost-effectivr restoration plan for the Black Creek watershed. This thesis was developed with those objectives in mind, but as a more intensive study.

1.5 OUTLINE OF THESIS

The objectives of this thesis were to determine the impact of AMD on both abiotic and biotic processes within the Black Creek watershed and to suggest the most effective risk assessment strategy. Chapter one discusses the use of visual inspection, basic water chemistry, metal content of water column and sediments, and various forms of toxicity testing to evaluate acid mine drainage. Chapter two uses a more ecological approach with a leaf pack study, benthic macroinvertebrate sampling and algal tiles (used to measure the influence of Black Creek on the Powell River). Chapter three is a summary of the first two chapters, using a weight of evidence approach to best identify AMD impacts in the Black Creek watershed

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2.0 CHAPTER TWO

Water Column and Sediment Toxicity of Acid Mine Drainage at Selected Locations in the Black Creek Watershed, Wise County, Virginia.

(ABSTRACT)

This study was designed to identify sources of acid mine drainage (AMD) in Black Creek, Wise County, Virginia, and then to determine the best method of identifying toxicity using metals analysis and standard toxicity testing. The AMD sources were first identified using visual inspection and field chemistry. Additional areas were then included in the assessment process using metals (aluminum, copper, iron, magnesium, manganese, and zinc) analysis of both overlying water column and sediments. Mock storm events were created to simulate metals in the water column. Short-term toxicity testing was employed as well as a long-term purge study. The short-term toxicity test included water column only test using *Daphnia magna* and *Pimephales promela,* elutriant tests using D*. magna,* and short-term sediment tests using *D. magna*. The long-term purge study flushed stream sediments with clean water for 15 months, and sediment tests were completed using *D. magna* and *Chironomus tentans*. An upstream reach of Black Creek was used as a reference location. The pH at sampling locations ranged from 2.75 to 7.87 SU, with the most severe occurring at a station (U9) below an old highwall. Conductivity ranged from 196 umhos/cm at the reference location to 2040 umhos/cm at U9. Most locations with elevated conductivity also had low pH associated with them. All metals were elevated when compared to the reference, and samples collected from the mock storm event showed a ten-fold increase. Water column samples collected from locations with low pH were acutely toxic to *D. magna* and *P. promelas*. Mortality was high in the elutriant test at locations where pH was low, conductivity was elevated, metals were high, or a combination of these. It took 15 months for toxicity to be flushed from all sediments. In the initial sediment tests, all sampling locations were significantly different than the reference for survival of *C. tentans* and reproduction of D*. magna*. One location, U9, was significantly different from the reference for survival of *D. magna*. In the sediment tests completed after two months, *C. tentans* survival was only different from the control in three locations but significantly different for growth at all locations. Reproduction by *D. magna* was significantly less than the reference at all locations. At eight months, only two locations, one of which was U9, were significant for survival of *C. tentans*. After 15 months, no significant differences occurred between any stations. The study indicates that stream segments that are severely impaired by AMD are easy to identify using visual inspection and field water chemistry. Those that are moderately impaired require more investigation and may not be responsive to short-term toxicity tests. Metals do not constitute the only source of toxicity. Both conductivity and pH may play a role. The issue of AMD toxicity is complex, and this study may indicate that it is a site-specific issue and should be addressed as such in terms of remediation.

2.1 INTRODUCTION

Black Creek, a small watershed located in Wise County, Virginia, has been identified as an area where acid mine drainage (AMD) is the primary source of environmental degradation (Cherry et al. 1995). The next step was to determine which areas were most affected by AMD using classic methodologies, which include acute toxicity testing, water and sediment metals analysis, and sediment toxicity testing to identify impairment. This chapter focuses on standard toxicological testing with emphasis on chemical monitoring.

Black Creek activities commenced with station identification and chemical monitoring, which were followed by laboratory bioassays. Standard field water chemistry was collected at each sampling location, as well as samples for both water and sediment metals analysis. Aside from the water quality standards, there are now several ways to evaluate these data. There is an extensive toxicological database on test organisms, such as the *Daphnia magna* (Cairns, 1990) and their response to the metals of concern in this study. Additionally, there are several databases that contain information regarding sediment and water quality limits (or thresholds) for the metals examined during chemical monitoring. Information from AQUIRE (Biological) and Ecotox/Btag (threshold databases) are used in this evaluation.

Several types of bioassays were employed with the understanding that there are pros and cons to each type of assessment. Acute single species toxicity tests are relatively simple yet provide information about the relative lethality of the test material (Buikema et al. 1982). A 96-hour solid phase test used in conjunction with an elutriate test will aid in determining what dissolved materials are entering the overlying water column (Nebeker et al.1984). Chronic tests are more labor-intensive but measure more sensitive end points, such as growth and reproduction (Buikema et al. 1982; Burton, 1991; Nebeker et al. 1984). Acute toxicity tests have been recognized as a method for evaluation the effects of AMD (Isom, 1993). Several of the toxicity testing methodologies outlined in the following chapter have been used to evaluate AMD (Pereira et al. 1999, Souvek et al. 2000a; 2000b). This work represents a unique combination of test types. There are also other valid and accepted tests for evaluating AMD, such as MicroTox and BOD inhibition, which were not utilized.

Additional focus was directed at the sediments of Black Creek. Sediments are an important component of aquatic habitats, providing food and habitat for several species of biota, which may be affected by the presence of metals (Reynoldson, 1987). Sediment toxicity has become an important issue, and sediment quality criteria are currently being developed by the USEPA. This criteria was deemed necessary because of evidence of environmental degradation in areas where the water quality was not an issue (Chapman, 1989). In AMD streams, sediment may contain elevated levels of metals as compared to those found in the water column. Sediments may serve as both a reservoir and a source of metals to the water column and may integrate metals over time. However, in the absence of continuous metal inputs, the metal levels in the sediment may be depleted over time, which would reduce overall sediment toxicity. This hypothesis was tested by purging seep-influenced, heavy-metal-laden sediments over time through constant exposure to clean stream water in artificial streams.

The first objective of this chapter was to gain site-specific information about several locations within the Black Creek watershed. Visual inspection and basic water chemistry aided in the siting of sampling locations. Water and sediment analyses were utilized to determine, where possible, the extent of metal contaminants. The second objective was to determine toxicity. This objective was met by using a series of different types of toxicity tests that identified locations that were severely impaired and alluded to those that may have been experiencing moderate or minimal toxicity. Chronic toxicity testing indicated that all sampling locations had some level of impairment associated with the sediment fraction. Another objective of this chapter was to determine if clean water flowing over contaminated sediments would cause a reduction in toxicity. This concept is important because if successful, in some cases, it may reduce the need for removal of contaminated sediments, which in itself can contribute to water column toxicity (Bonnet et al. 2000). Conducting a long-term sediment purge study completed this objective. The final objective of this chapter was to determine how well each of these previously stated objectives addressed AMD.

2.2 METHODS

2.2.1 Station Selection

This research focused primarily on nine sampling locations within the Black Creek watershed. These sampling stations were selected by Dr. D. S. Cherry and the Virginia Department of Mines, Minerals, and Energy, Division of Mined Land Reclamation as part of a larger study involving AMD in the Powell River watershed (Cherry et al. 1995). Some information from other sampling locations is included in this study, but the focus is on nine primary stations. These stations are described in Table 2-1 and are represented by their station identification in Figure 2-1.

Table 2-1. Station descriptions of sampling locations in the Black Creek Watershed.

Station Identification	Description
U1	Reference - located upstream of Black Creek Lake in forested region above picnic and recreational area.
U2	Located on large tributary of Black Creek which now flows into the man-made Black Creek Lake. Station located directly upstream of road culvert. Tributary originates in large field, which has been stripped and re-seeded.
U4	Located on a tributary that originated on a hill that had been contour mined and flowed into a small wetland.
U5	Located in the same tributary of Black Creek as U4. Downstream of U4 in a wetland area boarded by pine.
U6	Located in Black Creek upstream of the confluence of the U4/U5 tributary.
U7	Located in Black Creek downstream of the confluence of the U4/U5 tributary.
U8	Located along old highwall (remnant of pre-law contour mining) beside roadway. Station is approximately 50 meters from U9.
U9	Old “Seep 1.” Located approximately 2 km below Black Creek Gate. Seep whose source was unreclaimed highwall.
U10	Old “Seep 2.” Located along roadside below steep slope approximately 150 ft from tributary’s origin. The tributary originated in an abandoned deep mine.
U11	Next tributary below U10 tributary.
L1	Located in Black Creek at the corner of White Oak Gap and Betty “B” Mine Road. A large wetland surrounded the creek at this location.
BBM	Located in Black Creek near the end of White Oak Gap Road over a hillside at the base of the wetland.
L11	Located in Black Creek at the mouth.

The watershed was divided into two segments for this study. The upper watershed was the area above Betty “B” Mine road. This area was accessible via White Oak Gap (Road). The entire study area was void of residences above this point, with the exception of a caretaker’s residence. At the time of the study, there were no road crossings in the upper watershed; however, there were several culverts, and a portion of Black Creek was dammed to accommodate a recreational fishery (stocked lake). A considerable portion of perennial stream existed above this impoundment. The recreational area was gated a few hundred feet below the impoundment.

Figure 2-1. Sampling stations in upper Black Creek watershed.

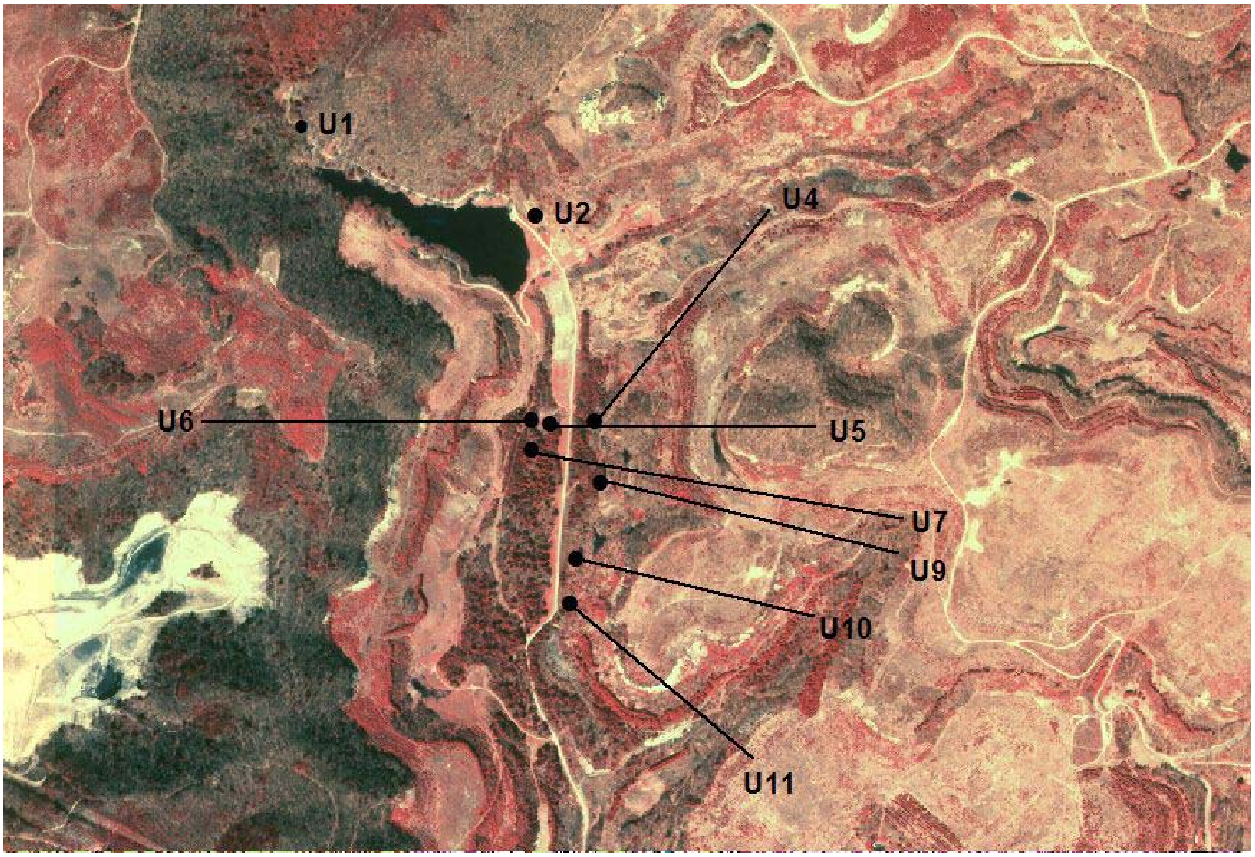
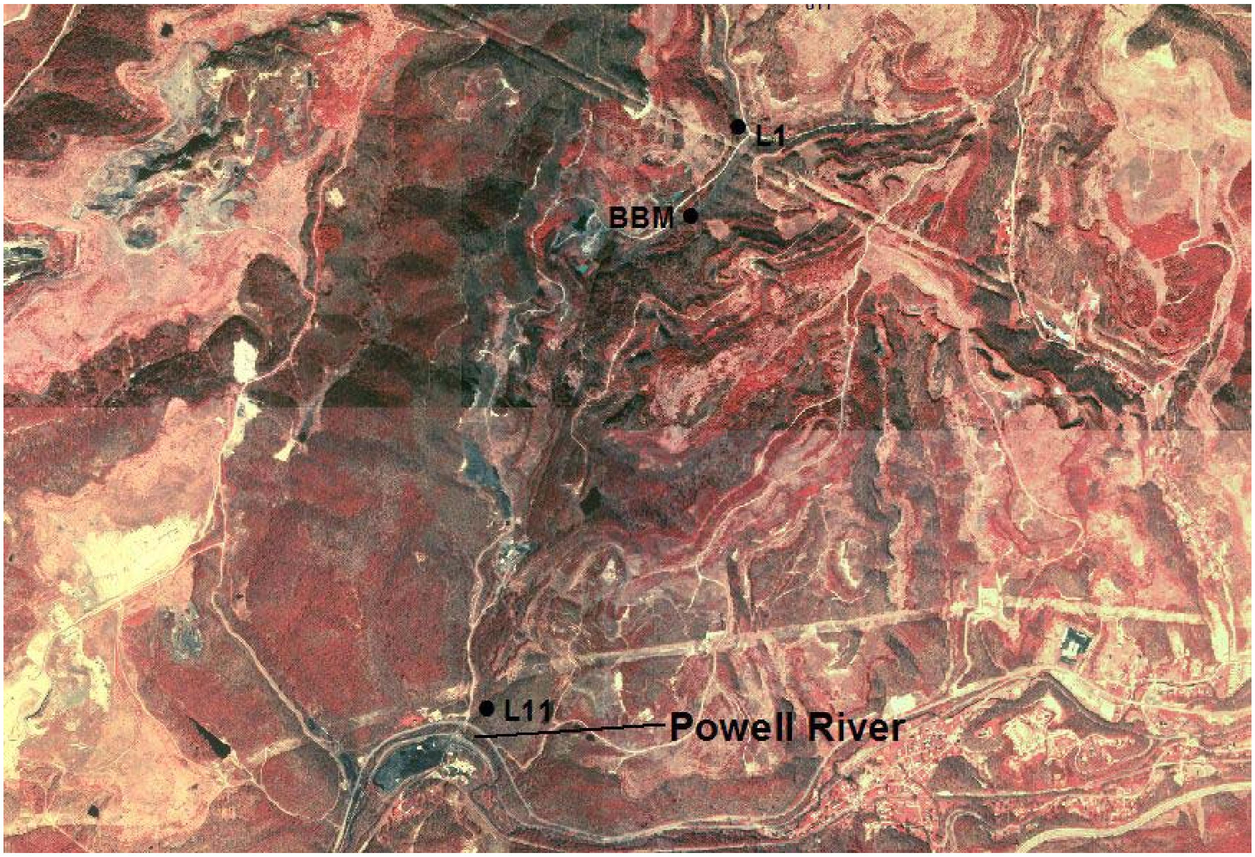


Figure 2-2. ***Sampling stations in lower Black Creek watershed.***



In the Cherry et al. (1995) study, the station referred to as U1 in this study was identified as a reference station. Stations U9 and U10 were identified as “Seep 1” and “Seep 2” in the Cherry et al. (1995) report. These two stations were considered to have serious environmental issues associated with them. Stations U1, U6, U7, L1, and L11 were in Black Creek proper, while the remaining stations were in tributaries. Three stations were located in what is termed the “lower” area of the watershed. Station L1 was located within a wetland area. It was hoped that this station might have some evidence of ecological recovery. BBM was located above an abandoned holding pond near an active deep mine in the lower watershed at the base of the L1 wetland. Station L11 was located at the bottom of the watershed, above the road (Route 58) and railway.

Other stations included in the study were U4, which was located above U5 and closer to the source of AMD; U11, a tributary which was located ~ 25 m below U10; and U8, which was along Black Creek Lake Road, approximately 50 meters from U9. Some stations were monitored solely for water chemistry as part of a larger study (Cherry et al. 1995). These stations are found in Appendix A.

2.2.2 Station Water Chemistry

Station water chemistry completed in the field consisted of temperature, pH, dissolved oxygen (DO), and conductivity. Temperature and pH were measured using a Hanna Model 9024 pH meter. Dissolved oxygen was measured using a YSI Model 57 meter, and conductivity was measured using an Orion Model 122 meter. Water samples were collected in clean Nalgene bottles. Samples collected for metals analysis or hardness were acidified or collected in pre-acidified containers. Samples were placed on ice and returned to the laboratory for analysis. These analyses included acidity, alkalinity, and hardness using methods outlined in Standard Methods (APHA, 1992) and Dr. Cherry's laboratory Standard Operating Procedures found on record at 2006 Derring Hall, Virginia Tech.

Samples for metals analysis were delivered to Environmental Monitoring, Inc. (EMI) of Coeburn, Virginia. These metals included aluminum (Al), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), and zinc (Zn). Chemists at EMI used US EPA method 242.1 to determine Mg and SW method 846 for all other metals.

2.2.3 Station Soil pH and Sediment Metal Analysis

Soil samples were collected from outslope and spoil areas. These samples were collected by plastic shovel, placed in Ziploc bags, stored on ice, and returned to the laboratory. To determine soil pH, 20 grams of air-dried sample was mixed with 20 mls of distilled, de-ionized water for 20 minutes. After mixing, the sample was allowed to settle for an additional 20 minutes, and then pH was measured by placing a pH probe in the aqueous layer. These methods are outlined in the USEPA Handbook of Methods for Acid Deposition Studies (Blume et al. 1990). These results were verified in some samples by measuring pH directly (placing pH probe into moist soil samples).

Sediment metal analyses were completed both on samples collected in the field and those from laboratory testing. Samples in the field were collected by shovel, placed in Ziploc bags, stored on ice, and sent to EMI, where they were analyzed for Al, Cu, Fe, Mg, Mn, and Zn (wet weight in ppm). Methods for handling sediment samples for laboratory testing are described in the test description section.

2.2.4 Acute Toxicity Testing

Acute toxicity tests were completed using both *Pimephales promelas* and *Daphnia magna*. Both water and sediment samples were collected from each station and returned to the laboratory. Water samples were used within 36 hours and followed the methods outlined in the USEPA Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Webber, 1993). These tests were 48 hours in duration, and the number of surviving organisms was determined in each sample.

The elutriant test and short-term sediment tests were completed using only *D. magna* and were 96 hours in length. Elutriant tests required mixing sediments in a 1:4 ratio with dilution water, vigorously agitating, and settling overnight. The resulting overlying water was removed and centrifuged at 10,000 rpm for 10 minutes (Nebeker et al. 1984). The porewater or elutriant was used as the test solution. Sediment tests were completed in a manner similar to those described below, but for a 96-hour period. *P. promelas* were 13 days old at time of testing, and *D. magna* were less than 24 hours old, except for those used in the long-term sediment test, which were 5 days old.

2.2.5 Sediment Toxicity Test and Sediment Purge Study

A sediment purge study was completed using sediment collected from a series of stations (U1, U5, U6, U7, U9, U10, L1, and L11) in four phases. The first phase was collection of samples and an initial chronic sediment toxicity test. Samples were collected by shovel on November 21, 1995, placed in Ziploc bags, placed on ice, and then returned to the laboratory where they were placed in a refrigerator maintained at 4°C. On December 8, 1995, samples were removed from the refrigerator and placed in baskets (~12in x 6in x 6in) lined with mesh. Each basket contained approximately two liters of sediments; enough to complete at least one toxicity test each. These baskets were then placed into artificial streams (a portion of this sample was also set aside for a sediment toxicity test). Each stream contained sediments from one station only. The streams consisted of a 60-L fiberglass oval that had been fitted with an input and overflow valve. Flow over and around the baskets was maintained by a paddlewheel driven by a 1/4 h.p. continuous-phase electric motor (Figures 2-2 and 2-3). The system was continually fed by tap water, which ran through a carbon filter system. The flow rate was not measured, but the artificial streams all had continuous flow.

As a part of phase one, an initial chronic sediment toxicity test was completed to determine initial levels of toxicity from sediments that were not placed into the artificial streams. This test would indicate baseline toxicity. The methods were a modification (both organisms in the same container) of those outlined for a 10-day, non-renewal test using *Daphnia magna* (Nebeker et al. 1984) and *Chironomus tentans* (US EPA, 1994).

Figure 2-3. Paddlewheel-Driven Artificial Stream System Used in the Laboratory Sediment Purge Studies.

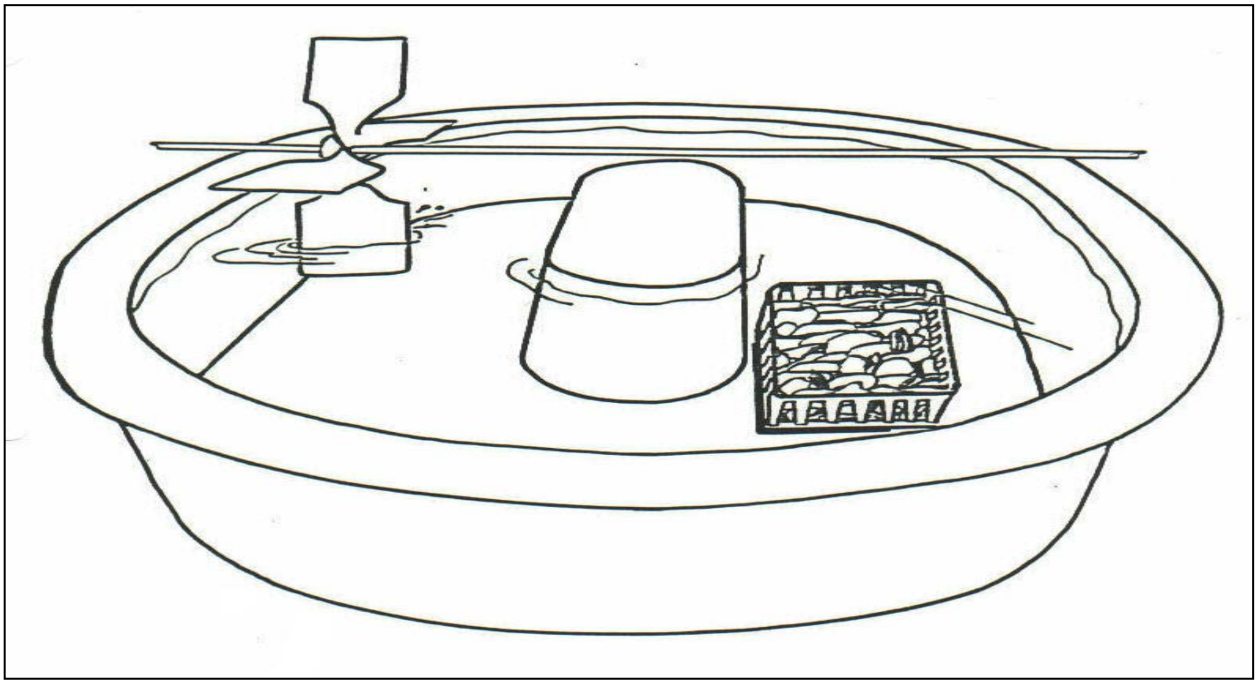
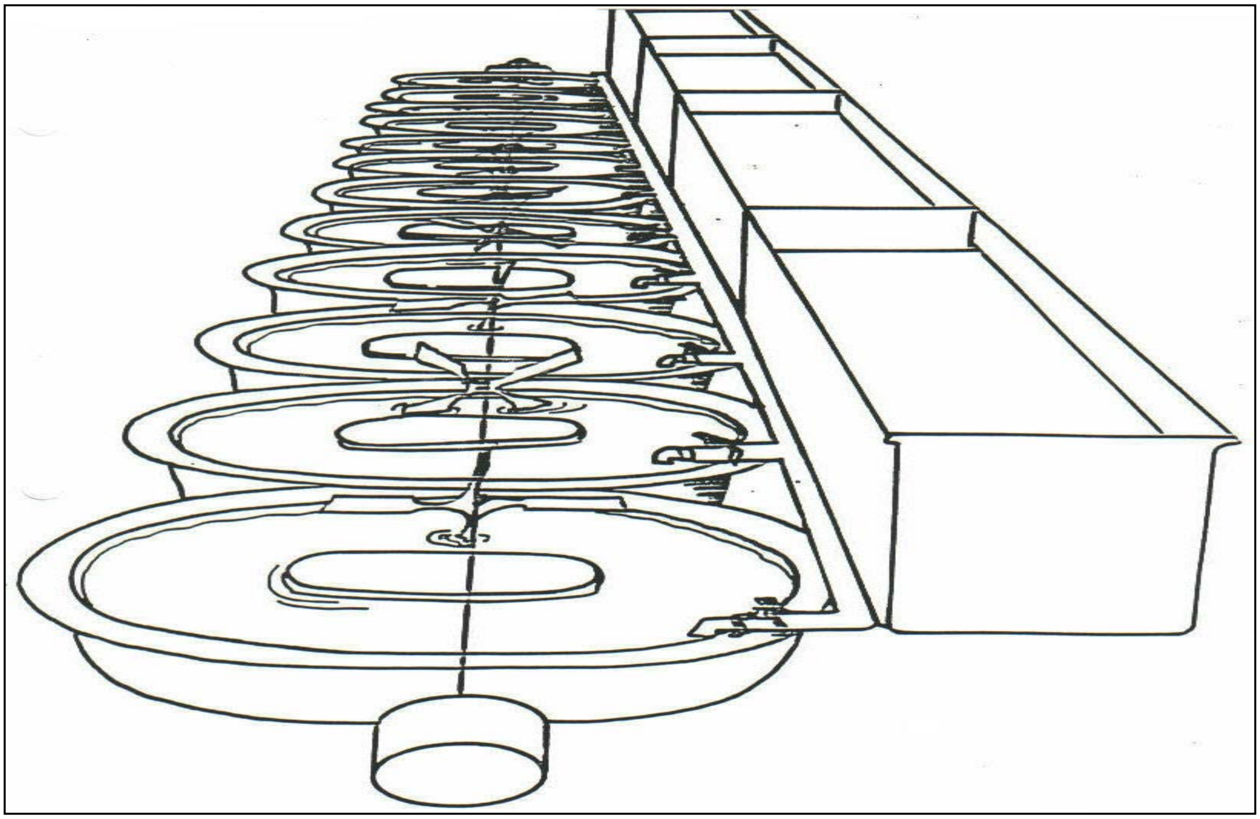


Figure 2-4. Series of Paddlewheel-Driven Artificial Streams.



Sediments were sieved using a 2mm screen to remove indigenous organisms and larger gravel. A portion was removed for dry weight and metals analysis. Samples were then placed in 1-L beakers where a 1:4 sediment to water ratio was achieved. After 24 hours, pH, DO, and conductivity were measured, water samples were removed, and organisms were placed in each container. They were then placed in a waterbath that maintained a temperature of 25° ± 1°C and was aerated constantly at a rate of <100 bubbles/minute. At the end of ten days, water chemistry was completed, a sample was collected for water column metals analysis, and organisms were removed. The water column sample was collected from the center of each test chamber using a clean pipette. These samples were composited so that there was only one sample per station. These tests were then evaluated on the basis of survival, growth (*C. tentans* only), and reproduction (*D. magna* only) to determine if sites were significantly different at the $p<0.05$ level as compared to the reference site. The significance was tested using a One-way ANOVA and Fishers LSD (multiple comparison test). Similar tests were repeated at phase two (two months), phase three (seven months) and phase four (fifteen months).

Sediments from each of the toxicity tests were analyzed for total Al, Cu, Fe, Mg, and Zn. Theses samples were collected prior to material being placed into test chambers. Those sediments collected at the initiation of the study and at two months were analyzed by EMI using methods previously described. Sediments collected from the seven months sampling and the end of the study (fifteen months) were digested using standard methods (APHA, 1992) and analyzed by the Soils Lab at Virginia Tech (US EPA, 1991). The phase three samples were stored at 4°C until analysis, which was eight months after their collection. This exceeds the recommended six months holding time. Samples from phase four were digested within the recommended time frame.

Water column samples were removed from the test containers at the beginning and ending of each test to be analyzed for total Al, Cu, Fe, Mg, Mn, and Zn. As with the sediments, phase one and two samples were sent to EMI, and the final two phases to the laboratory at Virginia Tech. All samples were treated with nitric acid and analyzed within a few weeks of sampling.

Particle size distribution was done on sediments from the initial phase and final testing phase to determine the percent of fine grain sediment (Gee and Bauder,1986). The initial phase sediments were held until the completion of the study (15 months); however, there is no recommended holding time for these types of samples.

2.3 RESULTS

2.3.1 Water Chemistry

Water column chemistry was recorded on at least ten occasions at various locations within the watershed (including during leaf pack study). A summary of the results is described in Table 2-2 (values in Appendix A). Dissolved oxygen was monitored, but it was never below saturation, so it was not included in this table.

Table 2-2. Range and median of pH, conductivity, alkalinity, and acidity values collected in 1995-1997 at select sampling stations in the Black Creek watershed.

Station	PH (SU)	Conductivity(umhos/cm)	Alkalinity *	Acidity *
U1	6.69 – 7.84 (6.98)	196 – 497 (422)	27 – 150 (58)	---
U2	6.15 – 7.58 (7.39)	747 – 923 (816)	56 – 100 (62)	---
U4	3.40 - 4.40 (3.66)	1395 – 1864 (1545)	---	85 – 118 (98)
U5	3.62 - 4.78 (4.06)	1087 – 1795 (1483)	---	67 – 113 (93)
U6	6.43 - 6.98 (6.77)	542 – 1107 (664)	---	15 – 35 (15)
U7	4.70 - 6.89 (3.39)	650 – 1435 (876)	---	10 – 40 (25)
U8	3.10 - 5.72 (3.39)	929 – 1938 (1282)	---	213 – 336 (275)
U9	2.75 - 3.60 (3.14)	1510 – 2040 (1835)	---	270 – 1785 (331)
U10	6.21 - 7.67 (7.14)	800 – 1722 (848)	---	149 – 235 (154)
U11	3.10 - 6.46 (3.92)	745 – 1980 (1632)	---	42 – 200 (135)
L1	6.10 - 7.81 (7.27)	668 – 1376 (1269)	50 – 86 (68)	---
BBM	6.54 - 7.03 (7.85)	802 – 1222 (1012)	56	---
L11	7.12 - 7.87 (7.75)	750 – 997 (780)	43 – 50 (47)	---

* as mg $CaCO_3$/L

Values for pH dropped below 6.0 SU at U4, U5, U7, U8, U9, and U11. Station U7, while in the mainstem of Black Creek, was obviously influenced by the discharge of U5 (note upstream U6 values). Acidity was also elevated at these locations, as well as U6 and U10, which had acceptable pH values (within the 6.0 –9.0 range). Conductivity was elevated throughout the system, with consistent recordings below 500 umhos/cm only found at the reference station. Only five locations had any net alkalinity, indicating that the buffering capacity of the system may be limited. Stations that did have any net alkalinity had acidity ranging from 15 to 1785 mg/l (as $CaCO^3$) (Table 2-2).

Water column samples were collected in October and November 1995 (Table 2-3). Some of these values exceeded Virginia Water Quality Standards (VAWQS) and/or BTag/EcoTox values, which are thresholds (not criteria) used for evaluating Superfund Sites. There is no standard for Mg. Values that exceed the VAWQS, EcoTox and/or BTag are in bold. There are no EcoTox, or BTag values for Mg, and values that are modified by pH or hardness were used at the default value (Hardness = 100 mg/L).

Almost every station examined had a value that exceeded some parameter, with U4, U5, U8, U9, U10, and U11 being the most frequent (Table 2-3). Aluminum values exceeded BTag (0.025 mg/L) at every sampling location. Copper, while often below detection limit, did appear at several stations, including the reference, and exceeded BTag values (0.0065 mg/L) as well. At stations U4, U5, U8, U9, and U11, copper values exceeded the VAWQS (0.018 mg/L), which is hardness dependent (120 mg/L $CaCO_3$ utilized). Iron thresholds were exceeded at almost every location except on either one of the sampling dates. Even the reference location had an elevated value. However, U4, which had elevated Al, Cu, Mn, and Zn, did not have high water column values for iron. Manganese exceeded both EcoTox and VAWQS (0.08 and 0.05 mg/L respectively) at every location except the reference station. However, the reference station had one high value, 0.064 mg/L Mn in October 1995. There were no acute or chronic VAWQS values for this metal, and, in Virginia, as in some neighboring states, it only becomes a concern when near drinking water intakes. Zinc values were low or below detection limits at U1, U2, and U10. The values were higher than the lowest standard (0.11 mg/L BTag) at U4, U5, U7, U8, U9, and U11 on at least one sampling date.

Table 2-3. Water column total metals (mg/L) in October and November 1995 at select sampling stations in the Black Creek watershed.

Station	Al		Cu		Fe		Mg		Mn		Zn	
	Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov
U1	**0.486**	**0.250**	**0.012**	**BDL**	0.237	**0.390**	23.9	16.5	**0.064**	BDL	0.017	BDL
U2	**0.521**	**0.730**	0.002	BDL	**2.76**	**20.2**	51.2	28.5	**2.36**	***2.37***	BDL	BDL
U4	**12.7**	**10.1**	**0.023**	BDL	0.204	0.17	117.0	74.0	**2.38**	***1.87***	**0.285**	**0.23**
U5	**11.2**	**2.31**	**0.019**	---	**2.49**	**0.47**	116.0	49.0	**2.64**	***1.86***	**0.257**	0.08
U6	**2.12**	**---**	BDL	---	**1.31**	**---**	58.9	---	**1.27**	**---**	0.053	---
U7	**6.54**	**---**	0.004	---	**1.87**	**---**	85.9	---	**2.01**	**---**	**0.175**	---
U8	**31.9**	**28.1**	**0.456**	BDL	**22.9**	**11.3**	107	81.0	**5.71**	***5.31***	**0.459**	**0.34**
U9	**31.7**	**22.3**	**0.435**	BDL	**6.75**	**13.6**	104	55.0	**4.26**	***2.01***	**0.435**	**0.30**
U10	**0.556**	**0.94**	BDL	BDL	**14.3**	**8.98**	35.9	23.5	**2.99**	***2.82***	BDL	0.02
U11	**1.08**	**14.7**	**0.018**	BDL	**11.0**	**3.25**	48.1	130	**3.49**	***2.22***	0.018	**0.27**
U11*	**---**	**0.55**	**---**	BDL		**3.59**		33.0		***6.75***		0.05

* Origin of U11 tributary
BDL – Below Detection Limit (0.005 mg/l for Cu, 0.001 mg/l for Mn, 0.01 mg/l for Zn)
--- no sample collected

2.3.2 Station Soil pH and Metal Analysis

Soil samples collected from spoils and outslope areas had pH values ranging from 3.06 to 7.06 (Table 2-4). Values below 4.0 were found in the area from the gate at the entrance to the Black Creek Lake to U9, the abandoned highwall area. There was little difference in the values measured using standard methods and a probe directly.

Table 2-4. Soil pH in Standard Units collected at nine locations in the Black Creek watershed.

Location	**pH Measurement**	
	Standard Method	**Probe**
Highwall Southeast of BC Lake	4.27	4.23
Spoils Pile On Way To Origin Of U11 Tributary	4.71	
Highwall Above U2	5.54	5.55
Spoils Pile Above Seep Which Runs Under Road, 0.5 km From U9	3.73	
Origin of U4 (Seep)	3.54	
Bench Above U4	3.47	
U8	3.06	
U9 - Old "Seep 1"	3.65	
U11	7.06	

Concentrations of metals from sediments removed from the stream in November, 1995 are included in Table 2-5. While sediment quality criteria are currently being developed, there are values for several species developed by NOAA (Gray, 1995; Herr and Gray, 1997; Long et al. 1995), and both EcoTox and BTag have values. All values assume a default for Total Organic Carbon (TOC) of 1.0 mg/kg. Copper values for both EcoTox and BTag are 34 mg/kg. No location in the watershed had a value this high. The NOAA standard for copper is much higher at 390 mg/kg. Zinc values for both EcoTox and BTag are 150 mg/kg, with the NOAA value being slightly higher (270 mg/kg). None of these values were exceeded in the sediment samples. There are no benchmarks for Al, Fe, Mg, and Mn. However, iron, copper, manganese and zinc values were elevated in locations throughout the watershed when compared to values obtained at the reference location (U1).

Table 2-5. Concentrations of total metals from sediments (mg/kg) removed from the stream at select sampling stations in the Black Creek watershed in November and December 1995.

Station	Al	Cu	Fe	Mg	Mn	Zn
U1 (11/95)	2800	2.7	9500	300	450	18.5
U1 (12/95)	3041	18.2	12500	677	995	40.8
U2 (11/96)	1980	12.5	39000	1700	4500	53.1
U5 (12/95)	16520	76.5	20064	3451	417	308
U6 (12/95)	8613	2.80	22832	2416	981	224
U7 (12/95)	9510	46.0	12700	2203	782	351
U8 (11/95)	1000	2.5	176000	150	10.9	20.2
U9 (11/95)	950	5.1	19600	170	7.2	17.6
U10 (11/95)	2600	19.3	77600	360	1520	22.0
U11 (11/95)	2700	7.6	15300	760	110	26.8
L1 (12/95)	4190	28.1	14000	2400	954	79
BBM (12/95)	7860	64.6	69500	1374	3250	142

2.3.3 Acute Toxicity Testing

In an acute toxicity test completed in November at select sampling locations, the most toxic station was U5, where all daphnids and fish died after 24 hrs of exposure (Table2- 6). The six other stations were non-toxic. The pH of the U5 sample was extremely low. Conductivity values in this test were lower than those found in the field. It is believed that this is a result of meter error.

Table 2-6. Survival (%) of Daphnia magna and Pimephales promelas in acute toxicity tests utilizing water column samples collected at seven sampling stations located in the Black Creek watershed.

Station	*D. magna*		**P. promelas**		Water Chemistry	
	24 hrs	48 hrs	24 hrs	48 hrs	pH	Conductivity
U1	100	100	100	100	7.12	100
U5	0	0	0	0	4.16	1100
U6	100	100	100	100	6.95	400
U7	100	100	100	100	6.88	550
L1	100	100	100	100	7.07	600
BBM	100	100	100	100	7.32	600
L11	100	100	100	100	7.52	650

pH is in standard units
Conductivity in umhos/cm

In the elutriant test, the most toxic station was U9, where all daphnids died after 24 hrs of exposure (Table2-7). Two other toxic stations were U10 and L1, where only 15 percent were alive after 96 hrs. Stations U7 and U6 had marginal toxicity, with 55-60 percent survival at the end of the test. Four stations (U1, U5, U10, and L11) had 80 to 95 percent survival and were non-toxic stations. The pH was extremely low at U9 (2.84 SU), and the conductivity was elevated when compared to U1. The conductivity was also elevated at U5, U6, and L1 when compared to U1.

Table 2-7. Elutriant test results at select stations in the Black Creek watershed using D. magna.

Station	Percent Survival At Each Station Per Interval					Water Chemistry	
	0 hr	24 hrs	48 hrs	72 hrs	96 hrs	pH	Conductivity
U1	100	100	100	100	95	6.92	65
U5	100	100	100	90	85	7.57	650
U6	100	100	100	80	60	7.55	800
U7	100	100	100	65	55	6.57	150
U9	100	0	0	0	0	2.84	1300
U10	100	100	100	100	90	7.66	205
L1	100	100	100	70	15	7.75	750
BBM	100	100	90	60	15	7.36	180
L11	100	100	100	85	80	7.24	280

pH is in standard units
Conductivity in umhos/cm

Short-term sediment toxicity tests were run alongside elutriant tests (Table 2-8). Station U9 exhibited the most severe toxicity, with 100 percent mortality occurring within 24 hours. All other stations were non-toxic, with survival ranges from 85 to 100 percent. The pH of station U9 was 2.97 SU with a conductivity of 1050 umhos/cm. All other stations tested had pH values that ranged from 7.21 to 7.98 SU and conductivity below 300 umhos/cm.

The elutriant was more toxic than the corresponding sediments at stations U6, U7, L1, and BBM (Tables 2-7 and 2-8). Values for pH had a greater range within the elutriant test, with the sample from U7 dropping to 6.57. Conductivity was elevated in the elutriant test at U5, U6, and L1 (650, 800, and 750 umhos/cm respectively) when compared to the values in the sediment tests.

Table 2-8. Short-term sediment test results at select stations in the Black Creek watershed using D. magna.

Station	Percent Alive At Each Station Per Interval					Water Chemistry	
	0 hr	24 hrs	48 hrs	72 hrs	96 hrs	pH	Conductivity
U1	100	100	100	100	100	7.66	120
U5	100	100	100	100	85	7.21	210
U6	100	100	100	90	90	7.88	180
U7	100	100	100	90	90	7.98	150
U9	100	0	0	0	0	2.97	1050
U10	100	100	95	85	85	7.53	300
L1	100	100	100	100	100	7.76	150
BBM	100	100	100	90	90	7.82	260
L11	100	100	100	100	100	7.57	250

pH is in standard units
Conductivity in umhos/cm

2.3.4 Sediment Test – Sediment Purge Study

In Test One (phase one), *C. tentans* survival at all sampling stations was significantly lower than that in U1 (Table 2-9). Survival ranged from 0 percent in U9 to 55 percent in U6. Growth measurements for *Chironomus* were not included because survival was significantly lower than the reference at all locations. *Daphnia* survival was only significantly lower in the U9 sample (total mortality). However, all stations were significantly lower in reproduction than U1. Reproduction was lowest at 8.6 neonates per female at station U10 and highest at BBM (16.2 neonates per female). Mean survival and reproduction (with upper and lower 95 percent CL) are listed in Table 2-9. Statistical differences are noted in each table using asterisks. These results served not only as an indication of sediment-associated toxicity but also as a baseline for the purge study.

Table 2-9. Test 1 (initial) survival of C. tentans and survival and reproduction of D. magna in sediment toxicity tests using sediments collected at various sampling stations in the Black Creek watershed, mean and range.

Station	*Chironomus tentans* Survival (%)	*Daphnia magna* Survival (%)	*Daphnia magna* Reproduction (Average # neonates)
p value	0.000221	0.0000001	0.000001
U1	90.0	80.0 (50.9 – 109.1)	33.1 (19.1 – 47.0)
U5	50.0 * (31.6 – 68.4)	80.0 (41.0 – 119.0)	13.3 * (8.8 – 17.8)
U6	55.0 * (21.9 – 88.1)	97.5 (89.5 – 105.5)	13.8 * (0.2 – 27.4)
U7	45.0 * (-4.5 – 94.5)	92.5 (68.6 – 116.4)	15.7 * (2.7 - 28.7)
U9	0 *	0 *	---
U10	20.0 * (4.1 – 37.0)	75.0 (37.1 – 112.9)	8.6 * (2.6 – 14.5)
L1	50.0 * (15.6 – 84.4)	77.5 (50.3 – 104.7)	15.1 * (11.5 – 18.7)
BBM	37.5 * (8.2 – 83.2)	70.0 (57.0 – 83.0)	16.2 * (15.2 – 17.1)
L11	50.0 * (3.2 – 96.8)	85.0 (64.5 – 105.5)	15.2 * (10.1 – 20.3)

Values that are significantly different from U1 are designated by *.
Alpha = 0.05

Test two (phase two at two months) resulted in significantly lower values for survival of *C. tentans* at U6, U9, and U10 (72.5 percent, 70.0 percent and 72.5 percent respectively)(Table 2-10). The remaining stations were significantly lower for *C. tentans* growth than U1 (ranging from 2.3 g at U5 to 4.1 g at U6). All stations had similar survival of *D. magna*; however, all stations were significantly lower for reproduction when compared to U1. Survival at station U1 was 85 percent and ranged from 60 to 100 percent at all other stations. U1 reproduction was 46.0 neonates per female while the remaining stations had values ranging from 13.9 to 31.1 neonates per female.

Table 2-10. Test 2 (two months) survival and growth/reproduction of C. tentans and D. magna in sediment toxicity tests using sediments collected at various sampling stations in the Black Creek watershed, mean and range.

Station	***C. tentans*** **Survival (%)**	***C. tentans*** **Growth (grams)**	***D. magna*** **Survival (%)**	***D. magna*** **Reproduction (Avg. # Neonates)**
p value	0.178610	0.000061	0.321267	0.001195
U1	97.5 (89.5 – 105.5)	5.2 (4.6 – 5.8)	85.0 (69.1 – 100.9)	46.0 (43.8 – 48.1)
U5	77.5 (53.6 – 101.4)	2.3 * (1.2 – 3.4)	85.0 (57.4 – 112.7)	23.3 * (1.8 – 44.8)
U6	72.5 * (52.5 – 92.5)	4.1 * (3.1 – 5.0)	80.0 (67.0 – 93.0)	28.5 * (15.0 – 42.0)
U7	82.5 (55.3 – 109.7)	3.4 * (2.0 – 4.8)	100	31.1 * (17.5 – 44.8)
U9	70.0 * (31.0 – 109.0)	3.6 * (2.4 – 4.9)	60.0 (-12.3 – 132.3)	13.9 * (-3.7 – 31.5)
U10	72.5 * (48.6 – 96.4)	3.8 * (3.2 – 4.6)	90.0 (77.0 – 103.0)	31.0 * (16.6 – 45.5)
L1	95.0 (85.8 – 104.2)	3.7 * (2.9 – 4.5)	85.0 (64.5 – 105.5)	31.1 * (28.9 – 33.3)
BBM	77.5 (42.2 – 112.9)	3.6 * (2.6 – 4.5)	82.5 (58.6 – 106.4)	23.9 * (20.4 – 27.3)
L11	85.0 (64.5 – 105.5)	2.5 * (1.1 – 3.9)	90.0 (58.2 – 121.8)	19.3 * (3.2 – 35.5)

Values that are significantly different from U1 are designated by *.
Alpha = 0.05

In the third test (phase three at seven months), *C. tentans* only had one station significant for survival, which was U9 (Table 2-11). Station U9 had 55.0 percent survival as compared to U1, which was 85.0 percent. Stations L1 and U5 were significantly lower for growth when compared to U1. *C. tentans* growth at station U5 was 3.4 g and growth at L1 was 3.1 g. Growth of *C. tentans* at U1 and the remaining stations ranged from 3.9 g (U7) to 4.8 g (U1). Results for the *D. magna* were not included because this portion of the test was not completed successfully (high mortality throughout test, including controls).

Table 2-11. Test 3 (seven months) survival and growth of C. tentans in sediment toxicity tests using sediments collected at various sampling stations in the Black Creek watershed, range and mean.

Station	***Chironomus tentans*** **Survival (%)**	***Chironomus tentans*** **Growth (grams)**
p value	0.144653	0.302539
U1	85.0 (57.4 – 112.6)	4.8 (2.8 – 6.8)
U5	77.5 (50.3 – 104.5)	3.4 * (2.8 – 4.0)
U6	65.0 (34.5 – 95.5)	4.3 (3.0 – 5.4)
U7	80.0 (41.0 – 119.0)	3.9 (2.8 – 4.9)
U9	55.0 * (17.1 – 92.9)	3.6 (1.7 – 5.4)
U10	57.5 * (42.3 – 72.7)	3.9 (1.8 – 5.9)
L1	82.5 (47.2 – 117.9)	3.1 * (2.1 – 4.0)
BBM	70.0 (57.7 – 83.0)	3.9 (3.1 – 4.6)
L11	85.0 (69.1 – 100.9)	4.0 (2.8 – 5.2)

Values that are significantly different than U1 are designated by *.
Alpha = 0.05

Test 4, which was completed 15 months (phase four) after the purge study was initiated, had no significant differences at any station for any parameter (Table 2-12). Even U9, which exhibited consistent toxicity, was not significantly different from the reference location. Values for survival of *C. tentans* ranged from 47.5 to 75 percent, with the highest survival at station U1. Growth was highest at U6 (3.8 g) and lowest at U9 (3.0 g), neither of which was significantly different from U1 (3.6507 g). *Daphnia magna* survival ranged from 90 percent at U1 to 75 percent at the BBM station. Reproduction ranged from 20.8 to 27.6 neonates per female.

Table 2-12. Test 4 (final - fifteen months) survival and growth/reproduction of C. tentans and D. magna in sediment toxicity tests using sediments collected at various sampling stations in the Black Creek watershed, range and mean.

Station	***C. tentans*** **Survival (%)**	***C. tentans*** **Growth (grams)**	***D. magna*** **Survival (%)**	***D. magna*** **Reproduction (Avg. # Neonates)**
p value	0.480281	0.5916666	0.941760	0.941760
U1	75.0 (47.4 – 102.6)	3.6 (2.7 – 4.6)	90.0 (0.8 – 1.0)	22.5 (14.9 – 30.2)
U5	52.5 (37.3 – 67.7)	3.7 (2.7 – 4.6)	82.5 (0.4 – 1.3)	20.8 (16.5 – 25.1)
U6	47.5 (20.3 – 74.7)	3.8 (2.6 – 5.0)	82.5 (0.6 – 1.1)	22.9 (13.5 – 32.3)
U7	65.0 (34.5 – 95.5)	3.2 (2.9 – 3.6)	87.5 (0.7 – 1.1)	24.0 (16.2 – 31.8)
U9	55.0 (39.1 – 70.9)	2.9 (2.6 – 3.2)	80.0 (0.5 – 1.1)	27.2 (15.3 – 39.0)
U10	55.0 (17.1 – 92.9)	3.0 (2.5 – 3.4)	77.5 (0.6 – 0.9)	22.9 (17.9 – 28.0)
L1	50.0 (-0.3 – 100.3)	3.7 (3.1 – 4.3)	77.5 (0.5 – 1.0)	25.5 (16.9 – 34.0)
BBM	72.5 (32.7 – 12.3)	2.9 (2.7 – 3.2)	75.0 (0.5 – 0.9)	27.6 (21.1 – 34.3)
L11	60.0 (37.5 – 82.5)	3.3 (3.1 – 3.6)	80.0 (0.5 – 1.1)	24.8 (15.8 – 33.9)

Values that are significantly different from U1 are designated by *.
Alpha = 0.05

Water chemistry was completed before and after each sediment test. This information may be found in Appendix A. Table 2-13 represents an example of the data that was collected. During the first test, overlying water had a starting pH of 7.45 and a conductivity of 206 umhos/cm. Twenty-four hours after the overlying water was added to the sediments, the pH dropped below 4.0 in some of the U9 replicates. At the end of the test, the water column pH was below 3.5 in all four replicates each having conductivities above 700 umhos/cm.

Metals samples were taken from the water column before and after each test and a portion of the sediment was also analyzed for metals. Samples collected from the water column and sediments used in each test did not have any trends that would indicate a reduced amount of metals in each progressive test. Table 2-14, contains the water column and sediment metal analysis from Station U9. Some samples had a dramatic decline in amounts of metals present, such as Fe in the sediments (89800 to 10683 mg/kg), while some had an equally substantial increase, for example, Cu in water column samples (0.035 mg/l to 0.271 mg/l). Data from all sampling locations are included in Appendix A. Particle size distributions of sediments used for the initial test were not significantly different from those used in the final sediment test.

Table 2-13. Water column chemistry from the overlying test (reference) water and the beginning and ending measurements from all four replicates at station U9 in the first sediment test.

Rep	Temperature		pH		Conductivity		Dissolved O_2	
	Begin	End	Begin	End	Beg	End	Beg	End
Ref	25.2		7.45		206		7.9	
U9 A	25.1	24.5	5.96	3.33	331	709	7.9	7.4
U9 B	25.1	24.5	3.59	3.11	364	780	7.8	7.3
U9 C	25.1	24.5	3.55	3.16	379	880	7.9	7.5
U9 D	25.1	24.3	3.97	3.16	336	809	7.9	7.3

Table 2-14. Metals analysis of station U9 from the water column and sediments from each sediment toxicity test completed during the purge study.

Metal	Al	Cu	Fe	Mg	Mn	Zn
Water column analysis in mg/L						
Test 1	1.990	0.035	23.000	42.010	5.900	0.450
Test 2	1.780	0.002	3.050	12.000	0.940	0.070
Test 3	1.706	0.002	0.099	3.375	0.018	11.290
Test 4	1.069	0.271	0.268	10.100	0.001	0.031
Sediments analysis in mg/Kg						
Test 1	5219	51.2	89800	1167	168	308
Test 2	3930	40.0	96400	720	118	82
Test 3	5065	54.2	33770	1741	1576	191
Test 4	3889	44.0	10683	1069	283	337

2.4 DISCUSSION

2.4.1 Water Chemistry

Investigations of areas polluted by AMD are aided by visual cues, such as iron floc and aluminum precipitate, and the blackened underside of stones, which may indicate high manganese. Iron, one of the most abundant metals in AMD associated with coal, will range in color from brown to yellow depending on oxidation state or phase, all visible to the human eye (Nagano et al. 1992). These visual cues, as well as barren outslope areas, large filamentous algal mats, or foaming precipitates, may indicate AMD or high metal content in a stream. Lending itself well to (visual) inspection, an index for AMD streams has been developed by Gray (1996a). It is purely descriptive, but it may be calculated rapidly. Other researchers have utilized visual inspection with varying success (Kirby et al. 1999; Shum and Lavkulih, 1999), but it is agreed that color may prove to be an inexpensive and useful tool. In the Black Creek watershed, areas with the most severe impacts (U8, U9, and U10) display dramatic color changes, all of which are associated with AMD. Visual cues and prior reconnaissance were particularly beneficial to this study.

Altered water chemistry is a common result of unchecked mining activities. Throughout the continent, AMD with associated low pH is a common theme in unregulated disturbed areas (Banks et al. 1997; Bell et al. 2001; Gray, 1997; Rahn et al. 1996; Rosner, 1997; Szczepanska and Twardowska, 1998). While pH in some locations may be as low as 2.0 SU, in the Black Creek watershed, values were recorded below 4.0 SU at five sampling locations and below 3.0 SU at one (U9). It has been suggested (Gray, 1996b) that sulfates and conductivity are better indicators of AMD than pH. In the Black Creek watershed, at least one location, U10, had elevated conductivity but did not have low pH. This location was clearly impacted, based on metal analysis and visual inspection, but pH alone as an indicator would have been insufficient to identify this location as a possible AMD impact.

2.4.2 Metal Toxicity

Metal toxicity is a very complex issue, particularly when numerous metal contaminants are present. There is little dispute that water quality is at risk in AML areas, with research on the topic available from several decades (Biesecker and George, 1966; Appalachian Regional Commission, 1969; Minear and Tshcantz, 1976; Beston, 1986; Leary, 1991; Rahn et al. 1997; Soucek et al. 2000a; Souvek et al. 2000b; Cherry et al. 2001). The amount and extent of the pollution varies from area to area, with extensive changes in hydrology making each situation different (Curtis, 1973; 1977; 1979). However, a reoccurring theme in areas that have been disturbed by historical mining, including various types of mineral removal other than coal (Rose and Ghazi, 1998), is that high levels of metals may result from exposed pyritic rock. In the Black Creek watershed, both visual inspection and thorough metal analysis provide evidence of metal contamination.

Visual inspection of U2, U8, U9, and U10 indicate that the associated soils, rocks, and sediments are laden with both iron and manganese. Aluminum precipitate is often present at U7 where the acidic tributary of U4 and U5 meet Black Creek proper. These visual observations were confirmed with metals analysis. Metals concentrations were extremely high in the water column in several sampling locations as well as in the sediments. While it is evident that the severity of metals contamination varies throughout the watershed, water column concentrations for copper, iron, manganese, and zinc exceeded the VAWQS at least once at every sampling location. For example, copper was found above 0.016 mg/L at U4, U5, U7, U8, and U11 in October 1995. This indicates these stream segments are justly placed on the states 1998 impaired waters list (VADEQ, 1998).

The number of metal contaminants as well as their concentration complicates water column toxicity in Black Creek. As a secondary problem, several locations have extremely low pH. Change in pH can result in different metal species being present in the water column fraction. The metal may also be present in the total or dissolved form. Knowing its relative concentration is just the first step in determining its possible biological effect. Using aluminum as an example for all metals present in the system, it is evident that this metal, with water column concentrations above the EcoTox threshold at every sampling location, should be of some concern. However, what is bioavailable to organisms in this system is more directly related to factors such as pH and the resulting elemental species (Witters, 1998; Brenzonik, et al. 1991). It is unclear whether toxicity associated with low pH in AMD streams is the result of acidification or high concentration of associated heavy metals (Lopes et al. 1999), but it is well known that low pH waters carrying high concentrations of acid-soluble metals are toxic to a variety of aquatic organisms.

Acute toxicity was evident in samples collected from U9. However, this was a one-time sampling event, which may not have been extensive enough. Monthly or quarterly sampling may have yielded better information particularly when dealing with stations that do not exhibit acute water column toxicity during a single sampling event. Samples from U9 were also toxic (100 percent mortality) in both the elutriate (porewater) and short-term sediment tests. While there is some dispute as to how sensitive these tests are (Pereira et al. 1999), they clearly indicate severe toxicity at this location. Results were not quite as dramatic in areas that may have been experiencing slight or moderate toxicity (U5, U6, and U7). The short-term sediment test and the porewater test did not yield results that were similar, but they need not be conflicting. For example, L1 and BBM had extremely low survival in the elutriant test while the short-term sediment test survival was comparable to those found in the U1 sediments (100 percent and 90 percent survival). The two tests yield information about two separate exposure routes. These results may indicate that there may be confounding factors associated with the toxicity (Adam et al. 2001). Both L1 and BBM are associated with a wetland. Wetlands are well-known sinks for environmental contaminants, which may complicate toxicity issues.

2.4.3 Sediment Toxicity

Sediments are the dominant sinks for metals in most aquatic environments (Morse, 1991). Despite considerable effort, however, finding a single inexpensive test of sediment toxicity has proven elusive. Samples taken only inches apart from one another, for example, may contain dramatically different levels of contaminants (Herr and Gray, 1997). This introduces a high degree of variability in the data collected from any one particular location. Sediment sampling may introduce variability as well, where researchers must take care so that minute particles or the fine fraction of sediments are not lost (OEPA, 2001). Researchers can introduce additional variability into the sampling regimen by not sampling in consistent habitats, i.e., sampling from pools at one location and riffles in another. This factor is important because if fine material is likely to be the most toxic, stream hydraulics will dictate where this material deposits, and during most flow conditions, these particles are likely found in depositional areas, such as the non-degrading stream bank in river bends and pools. Sampling plans that address the multiple sources of variability in the field, such as composite sampling, may aid in interpretation of laboratory data.

Some investigators suggest a several-tiered approach when investigating sediment concentrations so that all contamination may be accounted for (Herr and Gray, 1997). This includes sediment sampling, Fe hydroxide floc sampling, chemical analysis, interstitial (pore) water collection, sediment elutriates, sediment fractionation, and physical analysis. While extremely costly and time-consuming, this type of strategy allows for repeatability and reproducibility not found in single-method assessment.

Sediments also have several routes of exposure that may present risks to benthic organisms. In this study, and in AMD streams, benthics are already exposed to metals via the water column. They are receiving the metals via inhalation (movement across the gill surfaces) via adsorption, and/or via direct ingestion. Exposure to sediment metals occurs through a similar mechanism. Ingestion may occur through direct ingestion of sediment particles, particularly with burrowing species. Adsorption may occur through direct dermal contact with sediments, particularly in species with exposed gills. Additionally, metal exposure may occur through ingestion of other macroinvertabrates or other benthos that have bioaccumulated the material. Considering the differences in concentration of metal contamination in the sediments versus the water column, it is evident that there may be a much greater exposure risk via the sediments. For example, the highest water column concentration of iron was found at U8, 22.9 mg/L, which equates to 0.0023 percent of the sample being composed of total aluminum. The total aluminum in the sediment sample at this location was 176,000 mg/kg. This equates to 1.7 percent of one kg of the sample. Since the effect the metal has on the organism is related to how much of the material is available, it is a logical conclusion that direct contact with the sediments may result in much higher exposure.

Recommended thresholds exist for sediments (Long et al. 1995), but they are limited to very few metals species, none of which appear to be a potential problem in this watershed. This is not to say that metals like iron, for which no threshold exist, at 176,000 mg/kg lacks potential toxicity (see Soucek et al. 2000a,b). At a minimum, the thick coats of iron deposited on the streambed at stations U2, U9, and U10 are creating a loss of available habitat for benthic organisms on the streambed surfaces and in the interstitial spaces. The presence of multiple species and the various pH levels throughout the watershed may be causing changes in metal speciation from location to location further confounding sediment toxicity at the sampling locations in this watershed.

Transport of metals from the sediments into the water column during storm events may also be an issue in an AMD. Recent studies using resuspended sediments and biological endpoints yielded similar information: the overlying water column can be affected by the resuspension of bottom material (Bonnet et al. 2000).

Sediment toxicity tests were important in this study for two reasons. First, they provided general information on the sediment toxicity associated with the system, and, second, they were part of a long-term purging study. Sediment toxicity tests have been used by various researchers (Cherry et al. 2001; Soucek et al. 2000b). Samples collected at the U9 sampling station had toxicity associated with them until the 15-month point. In initial tests, mortality may have been related to the low pH that occurred in test chambers a few days after test initiation in this station's replicates; however, later tests did not have the dramatic reduction in pH but still had associated toxicity. U9 and U10 remained toxic until the final (fourth) sediment test while U5, U6, and L1 had toxicity that lingered into the second or third sediment tests. Because of the variability in the metals data, it is difficult to determine what factor contributed to the change in toxicity over time at the locations and why some locations improved more rapidly. What is clear is that the sediments, while exposed to a continuous supply of clean water, experienced a reduction in toxicity over time.

The importance of this information is two-fold. The study indicates that if upstream sources of AMD are remediated, downstream segments may recover. Further investigations may be warranted investigating the sources of sediment toxicity so that the mechanism of recovery is better understood. This research may be important is based on the extent to which AMD exists and some resent research regarding AMD discharges. In 2000, the West Virginia Department of Environmental Protection did a study, which indicated that of the 541 potential sources related to 314 permits, 460 of the sources would impact receiving streams if untreated (Faulkner, 2000). This means that if the bonds on these active permits are broken, acid mine drainage will result. This would be in addition to the thousands of miles of stream already polluted by AMD. With such an abundance of contaminant sources, it is not likely that every stream impacted by AMD will be a candidate for restoration. Remediation is costly, and it can be difficult determining which streams are the best candidates. Recently, researchers in both West Virginia and the United Kingdom have made some important discoveries regarding the longevity of acid mine drainage (Demchak et al. 2000; 2002). The research indicates that the life of acid mine drainage from surface mines, which remove the majority of the coal, is short-lived on a geological time scale (10-20 years). Deep mines may exhibit AMD for 10 to 100+ years. Deep mines, which are located below the water table, have a shorter term of AMD than those above the water column. This is related to flushing in deep mines below the water column and cyclic exposure of water and air to pyretic material in mines that are above the water table. Essentially, over-time, clean water is gradually introduced to the downstream sediments, these streams may eventually recover. While this should not be viewed as a mechanism to allow more AMD pollution, it is certainly encouraging to learn that some streams that will never be candidates for remediation and restoration will possibly, in future generations, have some hope of at least partial recovery.

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3.0 CHAPTER THREE

Effects of Acid Mine Drainage on Biotic Indices at Selected Locations in the Black Creek Watershed, Wise County, Virginia

(*ABSTRACT*)

Black Creek watershed in Wise County, Virginia, has historically received inputs from abandoned mined lands in the form of acid mine drainage (AMD). Impacts from AMD in this watershed include low pH (< 3.0 SU), high trace metals, and high conductivity (>1000 umhos/cm) from several seeps (Cherry et al. 1997). This study was developed to determine if biological impairment was occurring within the watershed as a result of AMD. The objectives of the study were to determine impairment using benthic macroinvertebrates, leaf packs, and periphyton sampling. Benthic macroinvertebrate communities and leaf pack breakdown were evaluated at nine locations, while periphyton was evaluated at the mouth of Black Creek as well as four sites in the Powell River receiving system. The results of the benthic macroinvertebrate study and the leaf pack study were then compared to determine if the two methods yielded similar results.

Benthic macroinvertebrate sampling indicated that of the nine stations sampled, at least six of them had some level of impairment as compared to the reference station. The most dramatic differences were seen in the stream segments below an old deep mine shaft that had a discharge flowing from its mouth, (U9), and from the base of an old highwall, (U10). Both of these locations were void of benthic macroinvertebrates at the time of sampling. The most sensitive metrics were those associated with EPT (Ephemeroptera, Plecoptera and Trichoptera genus), a trend similar to that in the literature. Leaf pack breakdown results were similar to benthic macroinvertebrate sampling. Of the nine stations, six of these had leaf penetrance that was significantly lower than the reference. The trend for % loss and k values (exponential breakdown rates) was the same. Sampling stations U9 and U10 again were more different from the reference than any other sampling station. Periphyton samples were not collected at the same locations as the benthics or leaf packs but indicated that Black Creek was having an impact on the Powell River mainstem. All three sampling techniques successfully identified impairment as a result of AMD in the Black Creek watershed. As expected, trends from benthic macroinvertebrate sampling and leaf pack breakdown were similar.

3.1 INTRODUCTION

Black Creek is located in the Powell River watershed in Wise County, Virginia. The creek is approximately 6.7 km long, is bordered on the western side by Black Creek Ridge, and discharges into the Powell River downstream of Josephine at Blackwood and Kent Junction. The watershed has been extensively mined, evidence of which appears on the Norton Quad United States Geological Survey Topographical sheet. It has been determined that the upper reaches of the watershed are severely impacted by acid mine drainage (AMD) from abandoned mined lands (AML) (Cherry et al. 1995; 1997). These impacts include high heavy-metal concentrations in the sediments and water column, high conductivity, and low pH (Cherry et al. 1997). Impacts from AMD in the lower reaches of the watershed include waters laden with heavy metals as well as areas where metals are leaching from sediments.

Acid mine drainage is the consequence of exposure of pyretic rock to water and air. The end result of exposure may be acidic waters as well as waters contaminated with heavy metals (Skousen and Ziemkiewiez, 1995). AMD has affected the water quality of tens of thousands of miles of stream throughout the United States. In the late 1980's, the US EPA completed a study (National Stream Survey) to provide estimates of the number of acid streams in the mid-Atlantic and southeastern US (Messer et al. 1986). This study estimated that 4590 km (+/-1670) of stream were acidic due to AMD, and another 5780 km (+/-2090) were impacted by AMD but not acidic (Herlihy et al. 1990). In a study completed in 1996 by the United States Geological Survey (USGS), 270 abandoned coal mine discharges were located and sampled. A total of 193 of the discharges exceeded standards for pH, 141 for manganese, and 122 for iron (USGS, 1996). This magnitude of water quality degradations will likely result in a decline in general stream health.

In small woodland streams, as much as 99% of the total energy input is from allochthonous material (Paul et al. 1983: Suberkropp and Klug, 1976). The primary component of this material is leaves (Paul et al. 1983), which serve as an important nutrient source for various species of aquatic benthos (Cummins and Klug, 1979), as well as a major source of organic carbon (D'Angelo and Webster, 1992). However, leaf material must be broken down in order for the material to have any nutritional value (Kaushik and Hynes, 1971). Initially, leaf breakdown occurs by leaching of soluble components, which leads to large mass loss, followed by colonization by microorganisms (conditioning) and then degradation by invertebrate feeders and physical abrasion (Lynde, 1994; Oberndorfer et al. 1984). Several studies have indicated that in the presence of acidic waters, leaf decomposition rates are much slower than those in unperturbed streams (Baker and Christensen, 1991; Carpenter et al. 1983; Francis et al. 1984; Hildrew et al. 1984; Mackay and Kersey, 1985; Jenkins and Suberkropp, 1995). Decomposition rates may be measured as the amount of mass loss over a period of time (Benfield et al. 1979; Paul et al. 1978,1983; Peterson and Cummins, 1974; Superklug et al. 1976; Webster and Benfield, 1985). Penetrance or "toughness" of leaves may also be determined. It is another measure of leaf breakdown that compares how much mass is required to penetrate the leaf surface (Feeny, 1970). As the leaf material breaks down, less and less mass will be required to penetrate the leaf.

Nutrient breakdown is continuous when resident benthic aquatic organisms, often identified as shredders, begin to feed on the leaf material (Webster et al. 1999). These organisms reside in first, second, and third-order streams where they grind, digest, and then defecate the leaf material. The organisms take the leaf material, which is commonly referred to as course particulate organic material (CPOM), and turn it into fine particulate organic material (< 1mm)(FPOM). This process makes the detrital material an energy source for other aquatic organisms (Benfield, 1981), which is carried downstream and through the food chain. In order for this energy transfer to be accomplished, these shredding organisms must be present in the water column and residing on leaf material that has already been broken down by microbial processes.

In recent years, there has been increased emphasis on using benthic macroinvertebrates to measure stream health (Karr and Chu, 1999). Many states have developed water quality programs that include monitoring benthic macroinvertebrates. There are several advantages to using benthic macroinvertebrate biosurveys. Benthic macroinvertebrates are abundant and good indicators of local conditions (US EPA, 1999). Surveys are easy to complete, and benthics are relatively easy to identify with a variety of keys available to family and genus levels. Sensitivity of various families has been well documented, including genus level information that may be found in studies, such as the Mid-Atlantic Coastal Stream Workgroups publications. Invertebrate community structure has been widely used to evaluate conditions of lotic ecosystems and as an indicator of sediment quality (Ankley et al. 1994; Karr, 1991; Yeager, 1994). Bioassessment using benthic macroinvertebrates is not a new concept, with some research dating back to the 1950's (Cairns and Dickson, 1971).

Bioassessment has also been used for a wide variety of applications, including impacts associated with effluent discharges, logging, silviculture, and other point and non-point pollution sources (Bradt et al. 1999; Brown et al. 1997: Cairns and Dickson, 1971; Felder et al. 1998; Fore et al. 1996; Garrit et al. 1990; Gurtz and Wallace, 1984; 1986; Macky and Kersey, 1985; Specht et al.1984;Trayler and Davis, 1998). Benthic macroinvertebrates have also been used for AMD studies, with research spanning several decades (Cherry et al. 2001; Cole et al. 2001; Dill and Rodgers, 1974; Griffith et al. 1995; Kimmel et al. 1996; Robeck and Richardson, 1969; Scheiring, 1992;Vinyard, 1996; Warner, 1971; Whipple and Dunson, 1993). However, because of the potential changes in hydrology associated with AML, Bradfield (1986) has warned that special care has to be taken when evaluating data. Bradfield found that simple comparisons between benthic metrics did not always indicate impairment that may be associated with the relationship between stream flow and water quality in mined areas.

Periphyton is the attached "algae" that forms on the surfaces of rocks or other objects in aquatic systems. According to Wetzel (1975), periphyton is the microflora community composed of slime, bacteria, algae, micro- and macrofauna, and detritus (Benfield, 1981; Otten and Willemse, 1988). The entire developmental cycle of periphyton is aquatic (some benthic macroinvertebrates have adult stages that are terrestrial), and therefore is believed to be a good indicator of water quality (Nielsen et al. 1984). Several studies have used periphyton as an indicator of changes in water quality (Clements, 1991; McCormick 1994; Steinman et al. 1992) and different methodologies are well documented (Biggs, 1988; Cooke, 1956; Losee and Wetzel, 1983; Lamberti and Resh, 1985; Nielsen et al. 1984). The US EPA indicates that periphyton is a useful water quality monitor because, as primary producers, algae are directly affected by chemical and physical changes; sampling is easy and inexpensive; and standard methods for their evaluation exist (US EPA, 1999). Decline in water quality may be measured in a number of ways, including loss of mass (measured as Ash-Free Dry Weight - AFDW), chlorophyll *a*, species diversity, and cell counts. There is also evidence that several species of algae are tolerant of AMD environments (Warner, 1971**).**

Based on earlier reports (Cherry et al. 1995; 1997), these methodologies were reviewed in an effort to develop a sampling plan that would identify AMD stream segments using biotic methodologies. This research had a two-fold objective. The first objective was to determine which reaches of stream in the Black Creek watershed were impaired due to AMD using leaf pack studies and benthic macroinvertebrates. Several different metrics from both of these methodologies were utilized. The second objective of this research was to determine what differences were found in the two types of samples. Additionally, algal sampling was utilized as a method of determining if the waters from Black Creek were having an influence on the Powell River.

3.2 METHODS

3.2.1 Station Selection

This research focused primarily on ten sampling locations within the Black Creek watershed. These sampling stations were used specifically for benthic macroinvertebrate and leaf pack studies. Some information from other sampling locations is included in this study, but the focus was on nine primary stations. These stations are described in Table 3-1.

Table 3-1 Station descriptions of sampling locations in the Black Creek Watershed.

Station Identification	Description
U1	Reference - located upstream of Black Creek Lake in forested region above picnic and recreational area.
U2	Located on large tributary of Black Creek that now flows into the man made Black Creek Lake. Station located directly upstream of road culvert. Tributary originates in large field that has been stripped and re-seeded.
U5	Located in the same tributary of Black Creek as U4. Downstream of U4 in a wetland area boarded by pine.
U6	Located in Black Creek upstream of the confluence of the U4/U5 tributary.
U7	Located in Black Creek downstream of the confluence of the U4/U5 tributary.
U9	Old “Seep 1.” Located approximately 2 km below Black Creek Gate. Seep whose source was unreclaimed highwall.
U10	Old “Seep 2.” Located along roadside below steep slope approximately 150 ft from tributary’s origin. The tributary originated in an abandoned deep mine.
L1	Located in Black Creek at the corner of White Oak Gap and Betty “B” Mine Road. A large wetland surrounded the creek at this location.
BBM	Located in Black Creek near the end of White Oak Gap Road over a hillside at the base of the wetland.
L11	Located in Black Creek at the mouth.

3.2.2 Benthic Macroinvertebrate Sampling

Benthic macroinvertebrate samples were collected at nine sampling stations in the watershed with two different sampling techniques. The first two initial samplings were completed using a modified Rapid Bioassessment Protocol (RBP) technique from the earliest versions of this sampling method (Plafkin, 1989). Samples were collected in the field using a D-framed net (580 um mesh) in a riffle/run m^2 sampling area. These samples were preserved using 70% ethyl alcohol and returned to the lab for identification (Edmunds et al. 1976; Merritt and Cummins, 1984; Stewart and Stark, 1993; Wiggins, 1977). Samples were initially only identified to the family level. Metrics were calculated when each station was scored against the reference using the RBP II metrics (family level), resulting in a determination of Moderate, Severely, or Non-Impaired.

The second method of invertebrate sampling was more quantitative but still qualitative. Three replicates per station were collected using a D-framed net. The samples were taken from a measured m^2 area, moving from left to right, bottomed to top in the grid and were preserved in 70% ethyl alcohol. After samples were returned to the lab, they were identified to the lowest practical taxonomic level (Edmunds et al. 1976; Merritt and Cummins, 1984; Stewart and Stark, 1993; Wiggins, 1977). Statistical analysis was completed on the following six metrics: Richness, % Mayfly, % Shredders, EPT Richness, % EPT, and Midge/EPT Ratio. Number Cruncher Statistical Software 97 (NCSS97) was then used to determine if the metrics were significantly different at the $p<0.05$ level as compared to the reference site. The significance was tested using a One-way ANOVA and Fishers LSD (multiple comparison test) if the data was normal or a Kruskal-Wallis Multiple Comparison Z test if the data were non-normal. Samples were collected on five occasions: three times in 1996 and twice in 1997.

3.2.3 Leaf Pack Breakdown

Sugar maple leaves, *Acer saccharum*, were placed in 3-mm mesh pecan bags attached to a brick after being dried in 5 gram pre-weighed allotments. The sugar maple leaves were used because of their moderate rate of breakdown in streams (Webster and Benfield, 1985). Each station had four bricks with four bags attached to each brick. A bag was removed from each brick after 24-hrs, 7, 21 and 35 days, and samples were brought back to the lab where they were sorted for invertebrates. The bags were then stored in a refrigerator at 4°C until analysis could be completed. Four leaves from each bag were measured four times using the penetrometer method (Feeney, 1970; Lynde, 1994). The leaves and all leaf material were returned and air-dried at room temperature. Leaves were measured for dry weight and then samples were ashed at 500C for one hour to obtain ash-free dry weight. Exponential breakdown rates (k values), dry weight, % loss, and % loss/day were calculated (Benfield and Webster, 1985). These results as well as penetrance were compared using the statistical analysis (NCSS 97 Program) at the $p<0.05$ level as compared to the reference site. The significance was tested using a One-way ANOVA and Fishers LSD (multiple comparison test) if the data were normal or a Kruskal-Wallis Multiple Comparison Z test if the data were non-normal.

3.2.4 Periphyton Analysis

Unglazed ceramic tiles were collected from six locations: five located in the Powell River and one in Black Creek for periphyton analysis. This was done in an effort to determine if the algal community at the mouth of Black Creek was different from that within the Powell River and to see if Black Creek's discharge was having an effect on the algal community in the Powell. These station locations do not correspond with the sampling stations used for benthic macroinvertebrate sampling and leaf pack studies and are listed in Table 3-2.

Table 3-2. Algal station locations.

Station Identification	Location
UP1	Located upstream of Black Creek confluence in the Powell River.
BC1	Located within Black Creek approximately 5m from the mouth.
DN1	Located 7m downstream of the confluence of Black Creek in the Powell River.
DN2	Located 17m downstream of the confluence of Black Creek in the Powell River.
DN3	Located 200m downstream of the confluence of Black Creek in the Powell River.
DN4	Located 300m downstream of the confluence of Black Creek in the Powell River.

The unglazed ceramic tiles (6.45 cm^2), which were mounted on bricks, were placed instream for three weeks at stations in lower Black Creek and in the Powell River. Each brick contained eight tiles, with four bricks at each station. These samples were removed from the creek without breaking the surface of the water, which could have caused sloughing of the algal material from the tiles, then transported back to the lab where the tiles were promptly scraped. Using methods outlined in Standard Methods (APHA, 1992), chlorophyll *a*, ash-free dry weight (AFDW), species diversity, and cell counts were determined. Methods used in Standard

Methods were similar to those used by other researches to determine this information and were referenced during the study designed (Bott, 1978; Cattanoe, 1995; Peterson et al. 1994; Steinman, 1992). Species identification was completed by mounting and identifying samples under a microscope using Prescott's freshwater algae key (1978). This information was then compared using statistical analysis (One-way ANOVA) at the $p<0.05$ level. The significance was tested using a One-way ANOVA and Fishers LSD (multiple comparison test) if the data were normal or a Kruskal-Wallis Multiple Comparison Z test if the data were non-normal. Comparisons were made between the upstream station in the Powell to the stations in Black Creek and the four downstream in the Powell River.

3.3 RESULTS

3.3.1 Benthic Macroinvertebrates

Using the RBP II method, all stations were Moderately to Severely Impaired when compared to U1 (reference)(Table 3-3). Moderately and Severely Impaired are both determined based on a percentage score calculated using RBP metrics (Plakin et al. 1989). Samples were collected on August 8, 1995 and October 12, 1995. Twelve benthic macroinvertebrate families were collected at Station U1. Stations U2 and L1 were Moderately Impaired and had eight families present. Stations U2, U7, U8, and U11 were also Moderately Impaired and had four, five, two, and six families present, respectively. Station U9, which had no organisms present, was Severely Impaired. Hydropsychidae was the dominant family at most stations, while Leuctridae was the dominant taxa at U1.

The second RBP sampling date, October 21, 1995, had similar results. All stations were Moderately to Severely Impaired when compared to Station U1 (Table 3-2). Eleven taxa were collected at U1. Stations BC-2, U5, U6, U7, L1, and BBM were all Moderately Impaired. The remaining stations (U2, U4, U9, U10, and U11) were Severely Impaired, and there were no taxa collected at U9, U10, and U11. Leuctridae again dominated the reference sample (U1), while Chironomidae and Hydropsychidae dominated most of the remaining samples.

Table 3-3. RBP scores for samples collected on 08/08/95 and 10/21/95 .

Station Sampling Event	U1	U2	BC-2	U4	U5	U6	U7	U8	U9	U10	U11	L1	BBM
August 8, 1995	100	26.3	---	---	---	15.8	26.3	26.3	0	---	42.1	26.3	---
	---	MI	---	---	---	SI	MI	MI	SI	---	MI	MI	---
October21, 1995	100	10.5	21.1	15.8	36.8	26.3	26.3	15.8	0	0	0	42.1	47.4
	---	SI	MI	SI	MI	MI	MI	SI	SI	SI	SI	MI	MI

SI – Severely Impaired
MI – Moderately Impaired

Replicate benthic macroinvertebrate samples (three) were collected on 03/22/96, 04/26/96, 10/25/96, 11/17/96 and 04/19/97. Samples collected at Stations U9 and U10 did not contain benthic organisms during any of the sampling events (Tables 3-4 and 3-5). Taxa richness ranged from 3 to 25 at the other stations. Stations U1, L1, and L11 had the highest taxa richness values (greater than 18). This result was consistent for all five sampling events, with ranges at U1 slightly lower than L1 and L11. Stations U2, U6, and U7 had taxa richness that ranged from 6.0 to 14 and was significantly less ($p<0.05$ level) than U1 during the 03/22/96, 10/25/96, and 11/17/96 sampling events. Taxa richness at U5 was significantly less ($p<0.05$ level) than U1 and had an average of 2.0 for all sampling events.

The metric percent mayflies had higher values at U1 than all other stations (Table 3-4). At Station U1, percent mayflies was highest in the March 1996 sample, with 45% of the organisms being mayflies (Table 3-4). Station U5 did not have any mayflies present in the sample during any of the sampling events. Stations U2, U6, and U7 had less than ten percent mayflies during the five sampling events (Tables 3-4 and 3-5). Stations L1 and L11 had significantly less ($p<0.05$ level) mayflies during the 11/17/96 sampling event.

The metrics EPT richness and percent EPT had similar values at Stations U1 and L11 (Tables 3-4 and 3-5). Values at U5, U6, and U7 were lower than U1 for both metrics during all five sampling efforts. These values were often significantly different ($p<0.05$ level) from U1 (Tables 3-4 and 3-5). Station L1 had values that were only slightly lower than U1. Percent EPT values at U2 were significantly lower than U1 during the 04/19/97 and 10/25/96 sampling events, and EPT richness was significantly lower during the 04/26/96, 04/19/97, and 10/25/96 sampling events. The metric midge/EPT ratio was also utilized, and this metric had the highest values at Stations U5 and U7 (Tables 3-4 and 3-5). The midge/EPT ratio was significantly higher at U5 during all five sampling events and at U7 during the 03/22/96 and 10/25/96 sampling events. These two stations also had a higher number of Chironomidae.

Percent shredders was also examined, and the predominant shredder at Station U1 was *Leuctra*. Station U2 had similar values to U1 during the 03/26/96 and 11/17/96 sampling events and values were only significantly less ($p<0.05$ level) during the 04/26/01 sampling event (Tables 3-4 and 3-5). Station U5 had no shredders. Stations U6, U7, and L1 had shredder populations, but at low numbers relative to U1. These values were significantly less than U1 at U6 and U7 during the 03/26/96 and 04/22/96 sampling events and at U7 and L1 during the 10/25/96 sampling event. Station L11 had consistently higher percentages of shredders than all other sampling stations. At L11, the shredder population was dominated by Trichoptera, while at U1, Plecoptera dominated.

Table 3-4. Mean values of benthic macroinvertebrate samples collected in the spring of 1996 and 1997.

		Stations								
Metric	**P value**	**U1**	**U2**	**U5**	**U6**	**U7**	**U9**	**U10**	**L1**	**L11**
	3/22/96									
Richness	0.000000*	20.33	**11**[A]	**3**[A]	**11**[A]	**7**[A]	**0**[A]	**0**[A]	22	21
% Mayfly	0.000000*	45.3	**4.4**[A]	**0**[A]	**5.1**[A]	**0.6**[A]	**0**[A]	**0**[A]	40.9	**18.3**[A]
% EPT	0.000000*	65.6	67.9	**5.0**[A]	**33.9**[A]	**15.0**[A]	**0**[A]	**0**[A]	65.7	80.7
EPT Richness	0.000000*	10.7	6.7	**0.7**[B]	7.7	**3.7**	**0**[B]	**0**[B]	13.0	15.7
Midge/EPT Ratio	0.0001	0.1	0.4	**16.9**[B]	1.9	**5.7**[B]	---	---	0.2	0.1
% Shredders	0.000000*	15.7	15.5	**0.0**[A]	**6.1**[A]	**0.9**[A]	**0**[A]	**0**[A]	14.4	23.8
	4/26/96									
Richness	0.000000*	18.7	10.7	**2.3**[B]	11.0	8.3	**0**[B]	**0**[B]	20.7	19.7
% Mayfly	0.000000*	40.4	**1.1**[B]	**0**[B]	4.9	1.7	**0**[B]	**0**[B]	37.3	13.9
% EPT	0.000000*	72.0	47.9	**8.8**[B]	33.1	13.0	**0**[B]	**0**[B]	70.1	80.3
EPT Richness	0.000000*	10.7	**6.0**[A]	**1.0**[A]	**7.7**[A]	**3.3**[A]	**0**[A]	**0**[A]	13.00	14.7
Midge/EPT Ratio	0.0003	0.1	0.6	**11.5**[B]	1.7	6.7	---	---	0.2	0.1
% Shredders	0.000000*	17.7	**11.4**[A]	**0**[A]	**10.2**[A]	**1.6**[A]	**0**[A]	**0**[A]	14.3	32.6
	4/19/97									
Richness	0.000000*	21.0	11.7	**2.7**[A]	11.0	8.0	**0**[A]	**0**[A]	21.0	22.3
% Mayfly	0.000000*	38.8	**1.9**[B]	**0**[B]	2.3	**0.8**[B]	**0**[B]	**0**[B]	25.5	21.8
% EPT	0.000000*	68.8	**47.9**[A]	**7.8**[A]	**35.7**[A]	**15.1**[A]	**0**[A]	**0**[A]	60.6[A]	81.9
EPT Richness	0.000000*	11.0	**6.7**[A]	**1.0**[A]	**7.0**[A]	**4.0**[A]	**0**[A]	**0**[A]	10.7	15.7
Midge/EPT Ratio	0.0001	0.2	0.9	**13.6**[B]	1.7	5.4	---	---	0.2	0.1
% Shredders	0.000000*	18.9	5.6	**0**[B]	9.1	1.3	**0**[B]	**0**[B]	13.5	32.1

[A] **Normal data with values significantly lower than U1 using Fishers LSD Test.**

[B] **Non-Normal data with values significantly lower than U1 using Kruskal-Wallis Multiple Comparison Z test**.

Alpha = 0.05

* = <0.0000001

Table 3-5. Mean values of benthic macroinvertebrate samples collected in the fall of 1996.

		Stations								
Metric	**P value**	**U1**	**U2**	**U5**	**U6**	**U7**	**U9**	**U10**	**L1**	**L11**
	10/25/96									
Richness	0.000000*	19.0	**14.0**^{A}	**2.7**^{A}	**11.0**^{A}	**7.3**^{A}	**0**^{A}	**0**^{A}	21.3	21.0
% Mayfly	0.000000*	36.6	**9.1**^{A}	**0**^{A}	**3.2**^{A}	**2.4**^{A}	**0**^{A}	**0**^{A}	**29.5**^{A}	**16.5**^{A}
% EPT	0.000000*	55.6	67.5	**7.7**^{A}	**31.9**^{A}	**13.5**^{A}	**0**^{A}	**0**^{A}	59.6	81.1
EPT Richness	0.000000*	7.3	8.0	**1.0**^{A}	**7.0**^{A}	**3.7**^{A}	**0**^{A}	**0**^{A}	10.0	15.7
Midge/EPT Ratio	0.0001	0.2	0.3	**14.7**^{B}	2.1	8.6	**---**	**---**	0.2	0.11
% Shredders	0.000000*	12.8	15.0	**0**^{B}	8.8	3.5	**0**^{B}	**0**^{B}	10.8	32.3
	11/17/96									
Richness	0.000000*	19.0	**14.0**^{A}	**2.7**^{A}	**11.0**^{A}	**7.3**^{A}	**0**^{A}	**0**^{A}	21.3	21.0
% Mayfly	0.000000*	36.6	**9.1**^{A}	**0**^{A}	**3.2**^{A}	**2.4**^{A}	**0**^{A}	**0**^{A}	**29.5**^{A}	**16.5**^{A}
% EPT	0.000000*	55.6	67.5	**7.7**^{A}	**31.9**^{A}	**13.5**^{A}	**0**^{A}	**0**^{A}	59.6	81.1
EPT Richness	0.000000*	7.3	8.0	**1.0**^{A}	**7.0**^{A}	**3.7**^{A}	**0**^{A}	**0**^{A}	10.0	15.7
Midge/EPT Ratio	0.0003	0.2	0.3	**14.7**^{B}	2.1	8.6	**---**	**---**	0.2	0.11
% Shredders	0.000000*	12.8	15.0	**0**^{B}	8.8	3.5	**0**^{B}	**0**^{B}	10.8	32.3

^{A} Normal data with values significantly lower than U1 using Fishers LSD Test.

^{B} Non-Normal data with values significantly lower than U1 using Kruskal-Wallis Multiple Comparison Z test.

Alpha = 0.05

*** = <0.0000001**

3.3.2 Leaf Penetrance

Penetrance values, over time, had a steady decline at Stations U1, L1, and L11 (Table 3-6). There was little difference in penetrance values for Station U1 and the remaining stations at 24-hrs. At seven days, Stations U2, U5, U6, U7, and U9 had values that were significantly ($p<0.05$ level) higher than the values for Station U1. This margin widened between U1, L1, and L11 and all other stations after 21 days. By 35 days, penetrance values from all stations were significantly higher than these stations. Stations U5 and U9 had little change in penetrance over the entire period, with mean values dropping approximately 50 mgs throughout the entire study. Stations with acidic waters (U5 and U9) were the first stations to be significantly different from U1 as well as L1 and L11 (21 days). These stations had penetrance values that were over 100 grams higher than U1 at 35 days. Station U10 was also significantly higher than U1 at 35 days but by a much smaller margin (~50 mgs). These three stations were significantly different consistently from U1 for all benthic macroinvertebrates metrics.

Table 3-6. Changes in leaf penetrance (mg) over 35 days at stations in Black Creek Watershed in the fall of 1996.

		Stations								
	P value	**U1**	**U2**	**U5**	**U6**	**U7**	**U9**	**U10**	**L1**	**L11**
24 HRS		379.5 (370.7-389.8)	379.5 (370.2-390.3)	380.2 (370.0-389.9)	380.6 (370.4-390.0)	379.9 (370.6-389.9)	379.7 (370.1-390.0)	380.7 (370.4-389.1)	379.4 (370.1-389.9)	380.9 (371.0-389.8)
7 DAYS	0.000000**	345.1 (330.1-359.6)	353.8 (340.5-369.7)	379.2* (370.1-389.0)	349.7 (336.4-363.3)	355.4 (341.0-369.1)	380.2* (370.2-390.0)	349.1 (335.1-364.3)	345.8 (332.1-359.4)	345.7 (331.1-360.0)
21 DAYS	0.000000**	222.8 (201.1-249.6)	270.1* (246.2-294.1)	343.4* (320.1-368.8)	263.9 (235.6-289.9)	269.2* (247.1-294.9)	344.4* (320.7-369.6)	272.5* (245.7-294.6)	223.4 (200.4-248.0)	226.8 (205.6-252.5)
35 DAYS	0.000000**	204.0 (180.5-229.8)	256.9* (235.0-274.7)	329.9* (300.5-357.3)	241.6* (225.6-259.5)	251.9* (235.4-264.9)	326.4* (300.1-349.9)	253.6* (231.5-270.4)	201.9 (190.7-238.8)	204.5 (186.9-234.2)

*** Significantly higher values than Station U1.**

**** $p<0.0000001$**

Alpha = 0.05

3.3.3 Leaf Breakdown

Initial (24-hour) breakdown at all stations was high at ~0.5 grams of weight loss (Table 3-6), presumably due to leaching of soluble components that occurs prior to colonization. Stations U1, U2, U5, U6, U7, L1, and L11 had a steady decline in % loss/day over 35 days. By Day 35, losses at U1, L1, and L11 were significantly ($p<0.05$ level) higher than all other stations. After initial breakdown, Station U5 had minimal additional loss, and Stations U9 and U10 gained mass over the 35-day period. These leaves were coated with an iron floc that deposited continually in the streambed. Stations U2, U6, and U7 had similar breakdown rates, with values for % loss after 35 days ranging from 8.14% to 9.63%. Stations U1, L1, and L11 had similar k values throughout the study. L1 and L11 had significantly ($p<0.05$ level) lower percent loss and percent loss per day on Day 21, but the values were similar on Day 35. Stations U2, U5, U6, U7, U9, and U10 had k values that were significantly ($p<0.05$ level) different from U1 after 7 days, and these stations remained significant at Day 21 and Day 35. Stations U5, U9, and U10 were the most dramatically different from Station U1, and similar trends were found in the benthic macroinvertebrate and penetrance data (Tables 3-3, 3-4, 3-5 and 3-6).

Table 3-7. Leaf breakdown rates measured as % loss at each station in the fall of 1996.

		Stations								
	P value	**U1**	**U2**	**U5**	**U6**	**U7**	**U9**	**U10**	**L1**	**L11**
Sample Weight (g)										
24 HOURS		4.49	4.55	4.46	4.46	4.51	4.5	4.57	4.59	4.48
7 DAYS		4.34	4.47	4.55	4.48	4.51	4.55	4.56	4.28	4.34
21 DAYS		3.94	4.25	4.50	4.13	4.18	4.54	4.54	4.11	4.13
35 DAYS		3.50	4.12	4.45	4.04	4.14	4.62	4.69	3.62	3.63
% loss										
24 HOURS	0.133960	10.07	8.93	10.77	10.65	9.95	10.02	8.67	8.11	11.45
7 DAYS	0.000013	3.43	1.77*[A]	-1.99*[A]	-0.37*[A]	-0.07*[A]	-1.17*[A]	0.15*[A]	6.78	1.91
21 DAYS	0.00000*	12.32	6.74*[A]	-0.18*[A]	7.49*[A]	7.25*[A]	-0.99*	0.60*[A]	10.44*[A]	6.76*[A]
35 DAYS	0.00000*	21.25	9.62*[A]	0.33*[A]	9.63*[A]	8.14*[A]	-2.64*	-2.62*[A]	21.10	17.98
%loss /day										
7 DAYS	0.000013	1.88	1.51*[A]	0.57*[A]	-0.06*[A]	-0.01*[A]	-0.19*[A]	0.02*[A]	1.13	0.32
21 DAYS	0.00000*	0.62	0.34*[A]	-0.01*[A]	0.37*[A]	0.36*[A]	-0.05*[A]	0.03*[A]	0.52*[A]	0.34*[A]
35 DAYS	0.00000*	0.62	0.28*[A]	0.01*[A]	0.28*[A]	0.24*[A]	-0.08*[A]	-0.08*[A]	0.62	0.53
k values										
24 HOURS	1.59	-0.0415	-0.0439	-0.0405	-0.0409	-0.0419	-0.0420	-0.0443	-0.0455	-0.0395
7 DAYS	0.00000*	-0.0106	-0.0127*[A]	-0.0036*[A]	-0.0101*[A]	-0.0118*[A]	-0.0038*[A]	-0.0030*[A]	-0.0081	-0.0087
21 DAYS	0.00000*	-0.0020	-0.0025*[A]	-0.0018*[A]	-0.0024	-0.0025*[A]	0*[A]	-0.0031*[A]	-0.0021	-0.0025
35 DAYS	0.00000*	-0.0008	-0.0013*[A]	-0.0011*[A]	-0.0013*[A]	-0.0014*[A]	0*	0*	-0.0008	-0.0009

[A] Normal data with values significantly lower than U1 using Fishers LSD Test.

Alpha -= 0.05

*** indicates significantly different from Station U1.**

**** = <0.0000001**

3.3.4 Periphyton Colonization

Station UP1 had significantly higher cell count and chlorophyll *a* values than BC1 and DN1 (Table 3-8). The upstream station, UP1, had a mean value of 3585 cells/mm^2. The station within Black Creek (BC1) and DN1, the first station within the Powell River, had mean cell counts of 1523.4 and 1905.6 cells/mm^2, respectively. Cell counts at the upstream reference station, UP1, were also significantly higher than DN2, the second downstream station in the Powell River. Mean values for chlorophyll were 12.2 mg/m^2 at UP1 to 1.29 mg/m^2 at BC1 and 3.2 mg/m^2 at DN2. Values for AFDW ranged from a mean value of 325.61 to 408.32 g, and there were no significant differences among stations (including the reference station, U1) for this parameter.

A total of 13 different algal taxa were collected at these six stations (Table 3-8). Four of the algal species were green algae, and the remaining nine species were diatoms. Station DN2 had the highest number (10) of different species. Stations UP1, DN3, and DN4 each had nine species, while BC1 and DN1 each had eight. The genera, *Eunotia* and *Tabellaria,* were found at BC1 and DN1 (*Eunotia* was also found at DN2). Based on the cell counts and chlorophyll *a* values, Black Creek is having an influence on the Powell River at least as far as the first few downstream sampling stations. This would extend the area of influence in the Powell River to at least 17 meters downstream of the discharge of Black Creek but no further than 100 m downstream, where the next station was located.

Table 3-8. Algal species present, cell counts, chlorophyll a, and AFDW of samples collected in Black Creek and the Powell River in the fall of 1996.

Station	UP	BC1	DN1	DN2	DN3	DN4
Species	*Chlamydomonas* sp. *Ankistrodesmus* sp *Selenastrum* sp *Spirogyra* sp *Achnanthes sp.* *Navicula* sp. *Rhopalodia* sp. *Nitzschia* sp. *Fragilaria* sp.	*Chlamydomonas* sp. *Spirogyra* sp *Eunotia* sp. *Meridion* sp. *Tabellaria* sp. *Nitzschia* sp. *Anomoeneis* sp. *Fragilaria* sp.	*Chlamydomonas* sp. *Selenastrum* sp *Spirogyra* sp *Eunotia* sp. *Meridion* sp. *Tabellaria* sp. *Nitzschia* sp. *Fragilaria* sp.	*Chlamydomonas* sp. *Selenastrum* sp *Spirogyra* sp Achnanthes sp. *Eunotia* sp. *Meridion* sp. *Navicula* sp. *Nitzschia* sp. *Anomoeneis* sp. *Fragilaria* sp.	*Chlamydomonas* sp. *Ankistrodesmus* sp *Selenastrum* sp *Spirogyra* sp Achnanthes sp *Navicula* sp. *Rhopalodia* sp. *Nitzschia* sp. *Anomoeneis* sp. *Fragilaria* sp.	*Chlamydomonas* sp. *Ankistrodesmus* sp *Selenastrum* sp *Spirogyra* sp Achnanthes sp *Navicula* sp. *Rhopalodia* sp. *Nitzschia* sp. *Anomoeneis* sp. *Fragilaria* sp.
Cell Counts (Organisms /mm2)	3585.8	1523.4*	1905.6*	2262.5*	2944.7	3030.6
Chlorophyll a (mg/M2)	12.2	1.3*	3.2*	7.4	5.8	4.6
Ash-Free Dry Wt.	367.9	383.9	408.3	361.1	325.6	397.2

*** indicates significantly different from Station U1.**

Alpha - .05

3.4 DISCUSSION

3.4.1 Benthic Macroinvertebrates

Literature regarding AMD and benthic macroinvertebrates generally report very similar trends to those found in the Black Creek watershed. In 1969, Roback and Richardson (1969) observed that under acid mine drainage conditions (pH < 3.5), Odontata, Ephemeroptera and Plecoptera were completely eliminated. Ephemeroptera and Plecoptera were also absent from samples collected at stations where the pH fell below 5.0 SU and the conductivity was greater than 500 uhmos. In Black Creek, the station that had a pH of less than 3.5 had no benthic macroinvertebrates present in the samples (U9) and the station with a pH below 5.0 SU (U5) had only Trichoptera and Chironomidae present. Station U10 had elevated conductivity and no benthic macroinvertebrates present in the samples collected.

Others (Carbone et al. 1998; Guerold et al. 2000; Nalmqvist and Hoffsten, 1999) reported similar trends under acidic conditions, as well as decreases in diversity and taxa richness (Dills and Rogers, 1974). Courtney and Clements (1998) reported similar findings using stream microcosms. However, several authors reported these findings with one dramatic difference (Kimmel et al. 1996; Ventura and Harper, 1996; Winerbourn and McDiffett, 1996). These researchers found that species of the order Plecoptera (stoneflies) were present in stream, and often dominant. In a 1993 study, Whipple and Dunson found larval stoneflies to be more tolerant of acidic waters than trout (Whipple and Dunson, 1993). This study also indicated that life expectancy (in days) of the stoneflies was lower in waters with low pH, high conductivity, and low hardness (12.5 as mg/l $CaCO_3$) than those with low pH, high conductivity, and high hardness (24.0 as mg/l $CaCO_3$) as well as low ph and low conductivity (28.8 uhoms). In a review of observation and experimental approaches of stream organism response to heavy metals Clements and others (1991) reported, that Plecoptera have moderate tolerance to heavy metals.

A 1989-90 study in a West Virginia headwater stream concluded that *Amphinemura sp., Luectra sp., Eurylophella funeralis,* and *Paracapnia angulata* were all characteristic of a stream with a pH of 4.3 (Griffith et al. 1995). None of these species were present in samples collected at U5 in the Black Creek watershed (pH range from 3.62 - 4.78). A striking difference between the two data sets are the conductivity measures. Conductivity in the West Virginia stream was less than 100 umhos/cm, while values in Black Creek were greater than 1000 uhmos/cm.

Exposure to low pH and some metals can cause ionoregulatory dysfunction in some organisms (Cole et al. 2001). Perhaps the presence of external gills makes most Ephemeroptera less tolerant than Plecoptera to this type of distress.

3.4.2 Leaf Breakdown

Due to increased acidification of rainwater, there is a large body of literature on leaf breakdown in acidified lakes. All reviewed literature had the same conclusion: low pH values resulted in slower leaf breakdown (Bermingham et al. 1996; Brock et al. 1985; Jenkins and Suberkropp, 1995; Kok and Van Der Velde, 1994). There are much fewer studies exploring the effects of AMD on leaf pack decomposition. In streams with pH 4.2, leaf pack breakdown was significantly lower than in reference streams (Meegan et al. 1996). This condition is similar to those found by Scheiring (1993) where breakdown rates were two times faster in control streams (pH was < 4.0 in acid stream). In Black Creek Station U9, which consistently had a pH below 4.0 SU, little or no breakdown after initial leaching occurred. Station U5, which had pH values ranging from 3.62 to 4.78, had an average percent loss after 35 days of 0.33%. The reference station (U1) had an average of 21.25%, an almost 10-fold difference. In the Meegan et al (1996) study, conductivity was below 100 uhmos. In the Scheiring (1995) study, hardness was 396 mg/l $CaCO_3$. In both studies, some breakdown was experienced under AMD conditions. In Black Creek streams, the pH was similar at a few locations, but these stations had no hardness and conductivity values over 800 uhmos (U5 and U9). The sampling station, U10, had high conductivity and pH within the 6.0 – 9.0 range; however, this station, like U9 had virtually no leaf breakdown after initial leaching. While it is evident that high conductivity may play some role in reduced leaf pack breakdown in this watershed, it is not evident as to which factor may be limited, microbial/fungal colonization or benthic macroinvertebrate colonization, particularly since no benthic macroinvertebrates were found in samples at their locations. Additionally, there were no sampling stations in the Black Creek watershed that exhibited low pH and low conductivity values (< 100 umhos).

Two other phenomena which are occurring in the watershed may be reducing leaf pack breakdown. During the study, two of the leaf pack stations also had weight gain. The leaves collected from these stations had a coating of iron material, iron hydroxide (yellow-boy), much like that on the stream bottom. It is possible that this material, much like other floc, may inhibit leaf colonization (Gray and Ward, 1983). Additionally, instream metal concentrations and their effects on leaf colonization were not assessed, but may have been influencing breakdown rates (Schultheis and Hendricks, 1999).

3.4.3 Periphyton

It is well known that algae accumulate heavy metals (Clements, 1991; Vymazal, 1984)). Natural attenuation of some algal species may reduce elemental concentrations in the water column 5 to 10 fold (Lawrence et al. 1998). Due to this characteristic, there is ongoing research to utilize algae as treatment for environmental purification of wastewater (Matheickal and Yu, 1999).

Chlorophyll *a* and cell counts were the only two measures that had a significant difference between the algal community upstream in the Powell River (UP1) and at the mouth of Black Creek. These two measures were also significantly different at the downstream Powell River station, which is 7 m below the mouth of Black Creek (DN1) and 17 m below the mouth of Black Creek (DN2). While these data infer that Black Creek is having an influence on the instream biota of the Powell River, what specific factor may be influencing the biota may be difficult to pinpoint (AMD or other non-point source inputs). For example, the decrease in chlorophyll *a* and cell counts could easily be related to sedimentation (characteristic of AML), not AMD pollution (Vasquez et al. 1999). However, this parameter was not measured and, therefore, cannot be ruled out or credited as a possible source of impairment. Metal hydroxides also may be limiting production (Niyogi et al. 1999). Other research indicates that total algal abundance and the number of species are likely to be depressed at high metal sites (Foster, 1982).

The segments of Black Creek and the Powell River utilized during the periphyton study did not have any significant sources of AMD, and while *Ulothrix* (a known acid tolerant taxa*)* was not present in any of the algal samples, several acid tolerant species were present at all sampling stations (Warner, 1970). Samples contained the genus *Achnanthes* (UP1 and DN2)*, Navicula (*UP1, DN2, DN3, and DN4) and *Nitzschia* (UP1, BC1, DN1, DN2, DN3, and DN4), which have acid tolerant species within the genus (Rushforth et al. 1981). Other pollution tolerant organisms present include *Chlamydomonas* and *Eunotia* species (Havas et al. 1982; Lampkin and Sommerfeld, 1982). Unlike the cell counts and chlorophyll a results, the algal taxa do clearly indicate Black Creek as a source of impairment in the Powell River. Some research indicates that the degree of the pollution rather than the type of pollutant present helps determine what algal species are present (Foster, 1982). It should be noted that the periphyton study, while yielding useful information, is not comparable to the benthic macroinvertebrate studies.

Similar trends were found in the benthic macroinvertebrate sampling and the leaf pack study. As expected, areas with the least amount of AMD had higher numbers of shredders and faster rates of decomposition. Stations U5, U9, and U10, where no shredders were collected, had very little associated weight loss (% loss). Stations U2, U6, and U7 had some shredders present, but often not as high of a percentage of the community compared to that found in the reference or furthest downstream station. The leaf packs at these stations did have weight loss, but it was not as dramatic as that associated with the reference location (U1). While not proven empirically, it appears that AMD has similar influences on benthic communities and leaf pack breakdown. In both cases, metal toxicity as well as water chemistry (pH and conductivity) may be influencing factors.

It is evident from this study that several areas of the watershed are being influenced by AMD. Both the benthic macroinvertebrate sampling and the leaf pack studies identified areas with varying degrees of impairment. Periphyton sampling was also successful in identifying some degree of impairment. Each of the methods that were used to identify impairment, had several associated metrics (i.e., taxa richness, % loss, etc). Further, statistical analysis as well as a weight of evidence approach were used to determine if the stream segment was being influenced by AMD. Hence, AMD impairment may be detected by carefully tested multi-metric indices. It is likely that, of the three methods, benthic macroinvertebrates would be more useful for monitoring impacts and then recovery in an AMD system that is undergoing substantial restoration. A large pool of information already exists on stream recovery monitoring using this method. Additionally, over the two past decades, multiple biotic indices have been developed with varying degrees of success (Barbour et al, 1996; Karr et al, 1986; Ohio EPA, 1987; Plafkin et al, 1989). Each index's goal is to measure change as a result of some human influence. While fish are often highlighted, benthic macroinvertebrates, and, to a lesser degree, periphyton, have been utilized. A classic example of this approach is the stream condition index developed for the state of West Virginia (Gerritsen et al, 2000). Biological data was collected from streams throughout the state using standardized techniques (RBP, 1999) and entered into a database from which a stream condition index was produced. The state is using this index in the watershed assessment program to help identify streams that need to be listed on the state's 303(d) list (WVDEP, 2001). It should be noted that a number of streams on this state's 303(d) list are indeed impaired as a result of AMD.

3.5 LITERATURE CITED

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4.0 CHAPTER FOUR

Analysis and Discussion of Methods Utilized for Identifying Acid Mine Drainage in The Black Creek Watershed

(ABSTRACT)

Black Creek, a small watershed located in Wise County, Virginia, is an area that has been mined historically using both surface and deep mining techniques. The watershed was identified in previous studies as an area impacted by acid mine drainage or AMD (Cherry et al. 1995). In the present study, the watershed was evaluated using several assessment techniques to determine which areas were the most severely impacted by AMD. The assessment methods were evaluated to determine which method was most practical. Sampling stations along the creek were classified according to the degree of impairment (Non-Impaired, Slightly, Moderately, Severely, and Severely pH Impaired). The Non-Impaired station served as the reference and was located in the upper reaches of the watershed. The Slightly Impaired locations occurred at various points in the watershed and typically had normal pH and elevated (greater than 500 uhoms) conductivity. The Moderately Impaired locations had pH values below 5.0 SU and conductivity greater than 1000 uhoms. Severely and Severely pH Impaired locations had conductivity greater than 1500 uhoms and pH values that were less than 3.0 SU. Once the locations were categorized, the level of impact was evaluated using basic water chemistry; metals analysis of sediments and water column; acute toxicity testing using both *Daphnia magna* and *Pimephales promelas*; short-term elutriant and sediment tests; chronic sediment test using *Chironomus tentans* and *D. magna*; a purge study; benthic macroinvertebrate sampling; leaf pack and algal tile studies. After evaluating these methods, it was determined that basic water chemistry and benthic macroinvertebrate sampling were the best procedures for characterizing the impacts of acid mine drainage in this watershed. Based on this information as well as the other test methods used in this evaluation, the reference station was identified as not impaired. Two stations located in the lower portions of Black Creek (L11 and L1) were also not impaired or only Slightly Impaired, with the benthic macroinvertebrate results indicating little impairment. Stations U2, U6, U7, and BBM were also found to be Slightly Impaired. The station on the margin of the wetland, U5, was Moderately Impaired. Two previously identified areas of impairment, U9 and U10, (Cherry et al. 1995) were identified as Severely pH Impaired and Severely Impaired.

4.1 INTRODUCTION

Black Creek, a watershed located in Wise County, Virginia, is a third-order stream with multiple tributaries. Many of the tributaries, as well as portions of the main stem of Black Creek, have been affected by either historical or post-Surface Mining Control and Reclamation Act (SMCRA) mining practices. The stream, which flows for 6.7 kilometers from headwaters to mouth, has many areas that are impacted by acid mine drainage (AMD) (Cherry et al. 1995). The watershed's historic mining practices include the old "shoot and shove" method of contour mining and some deep mining. In the upper watershed, two primary sources of acid mine drainage are present. The first is a seep from an old contour, while the second is from an abandoned deep mine. This source is not acidic but does have high metal content and very high conductivity. This study was completed in an effort to identify impairment resulting from AMD using several different assessment methods. Major sampling stations along the creek are identified in Figure 2-1.

The stream segments surveyed were broken into five broad categories based on water chemistry. This was done in an effort to better classify sampling stations so that water chemistry and biotic sampling could be examined concurrently. All sampling locations were placed into one of these five categories, which are similar to those utilized in Soucek (2000) but specific for this watershed.

1. Non-Impaired
2. Slightly Impaired (slightly elevated conductivity and metals)
3. Moderately Impaired (depressed pH, elevated conductivity and metals)
4. Severely Impaired (extremely high conductivity, elevated metals)
5. Severely pH Impaired (extremely acidic pH, high conductivity, elevated metals)

Streams that are Non-Impaired are those that have similar characteristics to the reference station in this study. The reference station had normal pH (6-9 SU): had conductivity below 500 umhos; and exhibited alkalinity, not acidity. The metals present in the water column and sediment were occasionally elevated, but there was no evidence that aquatic life was impaired.

Streams or stream segments that were Slightly Impaired were those that had elevated conductivity (greater than 500 umhos but usually less than 1000 umhos) and elevated metals in the water column and sediments. Some of these sampling locations had iron floc and other types of precipitate deposited on the streambed.

One area that was sampled in the Black Creek watershed was classified as Moderately Impaired. The Moderately Impaired condition included consistently depressed pH (< 4.0 SU) and elevated conductivity (> 1000 umhos). Additionally, the metals in the water column and sediments at this location were elevated.

There were two types of Severely Impaired sampling locations. The first type was Severely Impaired due to elevated conductivity and metal content of both the water column and stream sediments. This sampling location had pH values that consistently fell within the normal range (6.0-9.0 SU). The second type of impairment was Severely pH Impaired. This classification included stations that had pH values that were below 3.0 SU. These stations also had elevated conductivity and high metal content in the water column and sediments.

Chapter One utilized several short-term toxicity tests, chronic sediment tests, a purge study, field reconnaissance, and chemical analyses to identify AMD. Chapter Two used methods that were not solely laboratory based to identify impairment (benthic macroinvertebrate studies, leaf packs and algae tiles). In this chapter, all methods utilized in this study were compared to one another in an effort to determine if they produced similar results when identifying impairment resulting from acid mine drainage.

4.2 METHODS

Field water chemistry was measured using standard meters. Temperature and pH were measured using a Hanna Model 9024 pH meter. Dissolved oxygen was measured using a YSI Model 57 meter, and conductivity was measured using an Orion Model 122 meter. Samples for alkalinity, acidity, and hardness were collected in the field and returned to the laboratory for analysis using Standard Methods (APHA, 1992) and laboratory SOPs on record at 2006 Derring Hall, Virginia Tech. Metal analyses were completed on water column and sediment samples. This material was then sent to an accredited laboratory for analysis where Standard Methods or appropriate EPA methods were utilized.

Acute toxicity tests were completed on water samples collected in the field using the fathead minnow, *Pimephales promelas,* and the daphnid, *Daphnia magna*. The acute toxicity tests were 48 hrs in duration and completed according to the USEPA Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Webber, 1993). The elutriant tests (Nebeker et al. 1984) and short-term and chronic sediment tests were completed with *D. magna* (Nebeker, 1984) and *Chironomus tentans* (EPA, 1994). *P. promelas* were 13 days old at time of testing and *D. magna* were less than 24 hours old, except for those used in the chronic sediment tests which were 5 days old.

Benthic macroinvertebrate samples were collected using qualitative (Plafkin et al. 1989) and semi-quantitative techniques. The samples were analyzed using a series of metrics that are commonly used in Appalachian streams (Smith and Voshell, 1997). Leaf pack samples were removed from the stream at 24 hour, 7, 21 and 35-day intervals and analyzed for breakdown (using methods described by Benfield and Webster (1985)) and penetrance (Feeny, 1970). Periphyton tiles were also collected at six locations. Using methods outlined in Standard Methods (APHA, 1992), chlorophyll a, AFDW, species diversity, and cell counts were determined.

Significance testing was completed using a One-way ANOVA at the $p<0.05$ level comparing impacted sites to the reference. If the data were found to be normal, Fishers LSD test was utilized. If the data were non-normal, then a Kruskal-Wallis Multiple Comparison Z test was used to determine significance.

4.3 RESULTS

Water chemistry and sediment samples were collected at various locations in the Black Creek watershed (Tables 4-1, 4-2, and 4-3). These locations were selected based on field reconnaissance and water chemistry. As a result of these efforts, it is possible to differentiate each of the sampling locations using the impairment based categories: Non-Impaired, Slightly Impaired, Moderately Impaired, Severely Impaired, and Severely pH Impaired.

Table 4-1. Range of pH, conductivity, alkalinity, and acidity values collected in 1995-1997 at select sampling stations in the Black Creek watershed.

Station	pH (SU)	Conductivity(umhos/cm)	Alkalinity *	Acidity *
U1 (Non-Impaired)	6.69 - 7.84	196 – 497	27 - 150	
U2 (Slightly)	6.15 - 7.58	747 – 923	56 - 100	
U4 (Severely pH)	3.40 - 4.40	1395 - 1864		85 - 118
U5 (Moderately)	3.62 - 4.78	1087 - 1795		64 - 157
U6 (Slightly)	6.43 - 6.98	542 – 1107		15 – 62
U7 (Slightly)	4.70 - 6.89	650 – 1435		40 – 62
U8 (Severely pH)	3.10 - 5.72	929 – 1938		213 - 336
U9 (Severely pH)	2.75 - 3.60	1510 - 2040		270 - 1785
U10 (Severely)	6.21 - 7.67	800 – 1722		149 - 412
U11 (Moderately)	3.10 - 6.46	745 – 1980		42 - 200
L1 (Slightly)	6.10 - 7.81	668 – 1376	50 - 198	
BBM (Slightly)	6.54 - 7.03	802 – 1222	56	
L11 (Slightly)	7.12 - 7.87	750 – 997	43 - 72	

* as mg $CaCO_3$/L

Table 4-2. Water column metals (mg/L) in October and November 1995 at select sampling stations in the Black Creek watershed.

Station	Al		Cu		Fe		Mg		Mn		Zn	
	Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov	Oct	Nov
U1 (Non-Impaired)	0.486	0.250	0.012	BDL	0.237	0.390	23.9	16.5	0.064	BDL	0.017	BDL
U2 (Slightly)	0.521	0.730	0.002	BDL	2.76	20.2	51.2	28.5	2.36	2.37	BDL	BDL
U4 (Severely pH)	12.7	10.1	0.023	BDL	0.204	0.17	117.0	74.0	2.38	1.87	0.285	0.23
U5 (Moderately)	11.2	2.31	0.019	---	2.49	0.47	116.0	49.0	2.64	1.86	0.257	0.08
U6 (Slightly)	2.12	---	BDL	---	1.31	---	58.9	---	1.27	---	0.053	---
U7 (Slightly)	6.54	---	0.004	---	1.87	---	85.9	---	2.01	---	0.175	---
U8 (Severely pH)	31.9	28.1	0.456	BDL	22.9	11.3	107	81.0	5.71	5.31	0.459	0.34
U9 (Severely pH)	31.7	22.3	0.435	BDL	6.75	13.6	104	55.0	4.26	2.01	0.435	0.30
U10 (Severely)	0.556	0.94	BDL	BDL	14.3	8.98	35.9	23.5	2.99	2.82	BDL	0.02
U11 (Moderately)	1.08	14.7	0.018	BDL	11.0	3.25	48.1	130	3.49	2.22	0.018	0.27
U11* (Severely)	---	0.55	---	BDL		3.59		33.0		6.75		0.05

*** sampled at Origin of U11**

Table 4.3. Concentrations of metals from sediments (mg/kg) removed from the stream at select sampling stations in the Black Creek watershed in November 1995.

Station	Al	Cu	Fe	Mg	Mn	Zn
U1 (Non-Impaired)	2800	2.7	9500	300	450	18.5
U2 (Slightly)	1980	12.5	39000	1700	4500	53.1
U8 (Severely pH)	1000	2.5	176000	150	10.9	20.2
U9 (Severely pH)	950	5.1	19600	170	7.2	17.6
U10 (Severely)	2600	19.3	77600	360	1520	22.0
U11 (Moderately)	2700	7.6	15300	760	110	26.8

Station U1 was the reference station and was therefore identified as Non-Impaired, although some concentrations of metals in both the sediments and water column were at times elevated (Tables 4-2 and 4-3). Stations U2, U6, U7, L1, and L11 were grouped together as Slightly Impaired. During most sampling events, these stations had pH values that ranged from 6.0 to 9.0 SU (Table 4-1). The exception to this was U7, which occasionally had pH values below 5.0 SU. Conductivity for this group was predominately below 1000 but above 500 umhos. Again, there were a few exceptions to this statement. For example, U6, U7, and L1 had at least one measurement above 1000 uhoms. Station U5 was listed as Moderately Impaired. This sampling location had extremely high conductivity with values consistently above 1000 umhos and often over 1500 umhos; the highest value measured in the field was1795 umhos. Stations U8 and possibly U4 may have been only Moderately Impaired (as opposed to Severely pH Impaired); however, not enough data were collected at these two sites to effectively discern between the two categories. Additionally, these sampling locations were not included in the biological studies. Station U10 was the only station that could be described as Severely Impaired. While pH always fell within the normal range, conductivity, acidity, and metal content in both the water column and sediments were extremely high. Copper, on one occasion, was 19.3 mg/Kg in the sediments. Station U9 was classified as Severely pH Impaired. The field measurement for pH often fell below 3.0 SU, indicating extremely acidic conditions.

Bioassays with *D. magna* and *P. promelas* indicated that water or sediments from only one location were acutely toxic: the Moderately Impaired Station U5 (Table 4-4). The conductivity of the sample tested was 1100 uhoms/cm while the pH was 4.16 SU. None of the Severely Impaired sampling locations were tested in this study, although they were tested previously and were found to be acutely toxic (Cherry et al, 1995).

Table 4-4. Acute, elutriant and short-term sediment toxicity test results.

Station (Level of Impairment)	Acute Water Column Toxicity Test		Elutriant Toxicity Test	Short-term Sediment Toxicity Test
	48-hr Survival		96-hr Survival	96-hr Survival
	D. magna	*P. promelas*		
U1 (Non-Impaired)	100	100	95	100
U5 (Moderately)	0	0	85	85
U6 (Slightly)	100	100	60	90
U7 (Slightly)	100	100	55	90
U9 (Severely pH)	---	---	0	0
U10 (Severely)	---	---	90	85
L1 (Slightly)	100	100	15	100
BBM (Slightly)	100	100	15	90
L11 (Slightly)	100	100	80	100

In the elutriant tests, the Non-Impaired station had very little toxicity, with 95 percent survival of *D. magna*. Severely pH Impaired station, U9, had high toxicity associated with the elutriant sample (Table 4-4). Survival of daphnids in the 96-hour test was zero, with a pH below 3.0 SU throughout the test. Slightly Impaired stations, L1 and BBM, also had high toxicity, with 15 percent survival in this test. Moderately Impaired station, U5, had 85 percent survival, and Slightly Impaired stations, U6, U7, and L11, had 60, 55, and 80 percent survival, respectively. Severely Impaired station, U10, had minimal elutriant toxicity, with 90 percent survival in this test.

All sampling locations had 85 percent survival or greater in the short-term sediment test (Table 4-4). The only sampling location with any significant mortality (100%) was Station U9, which was categorized as Severely pH Impaired. The pH of the water column in this sample at the end of 24 hours was 2.97 SU.

In the initial test using the midge, *C. tentans*, sediments from all sampling locations were significantly more toxic than the Non-Impaired location (Table 4-5). Survival was impaired for Slightly Impaired station, U6, at eight months and for Severely Impaired (including Severely pH Impaired) stations at eight weeks and seven months. Growth was significantly different from the Non-Impaired station for all locations at eight weeks. By seven months, only Slightly Impaired station, L1, Moderately Impaired station, U5 and Severely Impaired stations were significantly different from the Non-Impaired station. At 15 months, there was no significant difference for growth (or survival) at any of the sampling locations.

Table 4-5. Survival and growth of C. tentans in four sediment tests during a sediment purge study.

Station	Test 1 **(0 Week)**	Test 2 **(8 Weeks)**	Test 3 **(7 Months)**	Test 4 **(15 Months)**	Test 2 **(8 Weeks)**	Test 3 **(7 Months)**	Test 4 **(15 Months)**
	Survival				Growth		
U1 (Non-Impaired)	90.0	97.5	85.0	75.0	5.20	4.80	3.65
U5 (Moderately)	**50.0***	77.5	77.5	52.5	**2.30***	**3.41***	3.66
U6 (Slightly)	**55.0***	**72.5***	65.0	47.5	**---**	4.26	3.84
U7 (Slightly)	**45.0***	82.5	80.0	65.0	**3.42***	3.83	3.24
U9 (Severely pH)	**0***	**70.0***	**55.0***	55.0	**---**	---	2.93
U10 (Severely)	**20.0***	**72.5***	**57.5***	55.0	**---**	---	2.97
L1 (Slightly)	**50.0***	95.0	82.5	50.0	**3.66***	**3.06***	3.67
BBM (Slightly)	**37.5***	77.5	70.0	72.5	**3.56***	3.88	2.93
L11 (Slightly)	**50.0***	85.5	85.0	60.0	**2.45***	3.96	3.34

*Values significantly different from U1

In the initial *D. magna* test, survival was significantly different between the Non-Impaired station and Severely pH Impaired station, U9 (Table 4-6). Reproduction was significantly different between the Non-Impaired location and all other stations for both the initial test and after eight weeks. The third test at seven months failed (survival in the controls was below the 80% minimum). The fourth test at week 15 did not have any significant differences between the Non-Impaired station and all other locations for both survival and reproduction. Initial metal concentrations for water column and sediments in the first series of sediment tests are listed in Tables 4-7 and 4-8. Tap water that had been run through a carbon filter system was used in these studies.

Table 4-6. Survival and growth of D. magna in three sediment toxicity tests during a sediment purge study.

Station	Test 1	Test 2	Test 4	Test 1	Test 2	Test 4
	Survival			Reproduction		
U1 (Non-Impaired)	80.0	85.0	90.0	33.1	46.0	22.5
U5 (Moderately)	80.0	85.0	82.5	**13.3***	**23.3***	20.8
U6 (Slightly)	97.5	80.0	82.5	**13.8***	**28.5***	22.9
U7 (Slightly)	92.5	100	87.5	**15.7***	**31.1***	24.0
U9 (Severely pH)	**0.0***	60.0	80.0	---	**13.9***	27.2
U10 (Severely)	75.0	90.0	77.5	**8.6***	**31.0***	22.9
L1 (Slightly)	77.5	85.0	77.5	**15.1***	**31.1***	25.5
BBM (Slightly)	70.0	82.5	75.0	**16.2***	**23.9***	27.6
L11 (Slightly)	85.0	90.0	80.0	**15.2***	**19.3***	24.8

*Values significantly different from U1

Table 4-7. Metals in the water column at the end of the first series of sediment toxicity tests.

Metal	**Al**	**Cu**	**Fe**	**Mg**	**Mn**	**Zn**
U1 (Non-Impaired)	.0020	.0020	3.600	6.300	.8700	.1000
U5 (Moderately)	1.440	.0020	7.900	23.00	1.280	.0200
U6 (Slightly)	.6900	.0020	2.520	20.00	.6000	.2100
U7 (Slightly)	1.2700	0.4650	3.6900	35.3000	1.8100	0.1003
U9 (Severely pH)	1.9900	0.0347	23.0000	42.0100	5.9000	0.4500
U10 (Severely)	2.9770	0.1561	2.0800	29.3100	0.1966	0.2708
L1 (Slightly)	1.1300	2.0000e-3	2.4400	25.0000	3.3000	0.0500
BBM (Slightly)	0.6000	2.0000e-3	1.6600	23.0000	5.3000	0.0200
L11 (Slightly)	0.4600	0.002	10.5000	20.5000	3.1800	0.003

Table 4-8. Metals in the sediments of the first series of sediment toxicity tests.

Metal	**Al**	**Cu**	**Fe**	**Mg**	**Mn**	**Zn**
U1 (Non-Impaired)	3041	18.2	12500	677	995	40.8
U5 (Moderately)	16520	76.5	20064	3451	417	308
U6 (Slightly)	8613	280	22832	2416	981	224
U7 (Slightly)	9510	46.0	12700	2203	782	351
U9 (Severely pH)	5219	51.2	89800	1167	168	308
U10 (Severely)	4580	37.7	52500	1977	1264	62
L1 (Slightly)	4190	28.1	14000	2400	954	79
BBM (Slightly)	7860	64.6	69500	1374	3250	142
L11 (Slightly)	3680	9.6	21400	1100	3800	90

Benthic macroinvertebrates were collected at nine sampling stations ranging from Non-Impaired to Severely pH Impaired (Table 4-9). During the spring sampling events, samples collected at the Severely pH Impaired (U9) location did not have any benthic macroinvertebrates present. The samples collected at Severely Impaired station, U10, were also void of benthic macroinvertebrate life. Samples collected at Moderately Impaired station, U5, were consistently significantly different from the Non-Impaired station for all metrics that were analyzed. Slightly Impaired stations, U2 and U6, were significantly different for either two or three of the six metrics for all spring sampling events. Slightly Impaired station, U7, was significant for all metrics during one spring sampling event and significant for two and three of the six metrics for the other two spring events, respectively. Both L1 and L11, which were both Slightly Impaired stations, had only one significantly different metric each during all three spring sampling events.

Table 4-9. Mean values of benthic macroinvertebrate samples collected in the spring of 1996 and 1997.

Metric	U1 Non-Impaired	U2 Slightly	U5 Moderate	U6 Slightly	U7 Slightly	U9 SpH	U10 S	L1 Slightly	L11 Slightly
3/22/96									
Richness	20.33	**11**[A]	**3**[A]	**11**[A]	**7**[A]	**0**[A]	**0**[A]	22	21
% Mayfly	45.3	**4.4**[A]	**0**[A]	**5.1**[A]	**0.6**[A]	**0**[A]	**0**[A]	40.9	**18.3**[A]
% EPT	65.6	67.9	**5.0**[A]	**33.9**[A]	**15.0**[A]	**0**[A]	**0**[A]	65.7	80.7
EPT Richness	10.7	6.7	**0.7**[B]	7.7	**3.7**	**0**[B]	**0**[B]	13.0	15.7
Midge/EPT Ratio	0.1	0.4	**16.9**[B]	1.9	**5.7**[B]	---	---	0.2	0.1
% Shredders	15.7	15.5	**0.0**[A]	**6.1**[A]	**0.9**[A]	**0**[A]	**0**[A]	14.4	23.8
4/26/96									
Richness	18.7	10.7	**2.3**[B]	11.0	8.3	**0**[B]	**0**[B]	20.7	19.7
% Mayfly	40.4	**1.1**[B]	**0**[B]	4.9	1.7	**0**[B]	**0**[B]	37.3	13.9
% EPT	72.0	47.9	**8.8**[B]	33.1	13.0	**0**[B]	**0**[B]	70.1	80.3
EPT Richness	10.7	**6.0**[A]	**1.0**[A]	**7.7**[A]	**3.3**[A]	**0**[A]	**0**[A]	13.00	14.7
Midge/EPT Ratio	0.1	0.6	**11.5**[B]	1.7	6.7	---	---	0.2	0.1
% Shredders	17.7	**11.4**[A]	**0**[A]	**10.2**[A]	**1.6**[A]	**0**[A]	**0**[A]	14.3	32.6
4/19/97									
Richness	21.0	11.7	**2.7**[A]	11.0	8.0	**0**[A]	**0**[A]	21.0	22.3
% Mayfly	38.8	**1.9**[B]	**0**[B]	2.3	**0.8**[B]	**0**[B]	**0**[B]	25.5	21.8
% EPT	68.8	**47.9**[A]	**7.8**[A]	**35.7**[A]	**15.1**[A]	**0**[A]	**0**[A]	60.6[A]	81.9
EPT Richness	11.0	**6.7**[A]	**1.0**[A]	**7.0**[A]	**4.0**[A]	**0**[A]	**0**[A]	10.7	15.7
Midge/EPT Ratio	0.2	0.9	**13.6**[B]	1.7	5.4	---	---	0.2	0.1
% Shredders	18.9	5.6	**0**[B]	9.1	1.3	**0**[B]	**0**[B]	13.5	32.1

[A] **Normal data with values significantly lower than U1 using Fishers LSD Test.**

[B] **Non-Normal data with values significantly lower than U1 using Kruskal-Wallis Multiple Comparison Z test.**

SpH – Severely pH Impaired
S – Severely Impaired

Benthic macroinvertebrate samples were also collected during two fall events. The Severely Impaired and the Severely pH Impaired sampling locations did not have any benthic macroinvertebrates present in the samples (Table 10). Samples collected at Moderately

Impaired station, U5, were significant for all metrics when compared to the Non-Impaired reference station (U1). Slightly Impaired station, U7, was significant for all metrics during the first fall sampling and for four of six during the second. Slightly Impaired stations, U2 and U6, were significant for two, three, or four of six metrics during the two fall sampling events. L1 had two significantly different metrics during the first fall sampling event, and, like L11, one significant difference during the later fall event. Both of these stations were listed as Slightly Impaired.

Table 4-10. Mean values of benthic macroinvertebrate samples collected in the fall of 1996.

Metric	**U1** Non-Impaired	**U2 Slightly**	**U5** Moderately	**U6 Slightly**	**U7 Slightly**	**U9 SpH**	**U10** S	**L1 Slightly**	**L11 Slightly**
	10/25/96								
Richness	21.7	**10.7**[A]	**2.3**[A]	**10.0**[A]	**5.7**[A]	**0**[A]	**0**[A]	18.7	21.7
% Mayfly	32.0	1.4	**0**[B]	3.3	**0**[B]	**0**[B]	**0**[B]	23.0	19.6
% EPT	67.2	**55.9**[A]	**3.1**[A]	**28.3**[A]	**5.9**[A]	**0**[A]	**0**[A]	**57.9**[A]	85.7
EPT Richness	12.3	**5.7**[A]	**0.7**[A]	**6.3**[A]	**2.0**[A]	**0**[A]	**0**[A]	9.0	16.0
Midge/EPT Ratio	0.1	0.6	**22.7**[B]	2.4	**18.2**[B]	**---**	**---**	0.3	0.1
% Shredders	21.3	15.3	**0**[A]	12.9	**4.7**[A]	**0**[A]	**0**[A]	**10.8**[A]	20.4
	11/17/96								
Richness	19.0	**14.0**[A]	**2.7**[A]	**11.0**[A]	**7.3**[A]	**0**[A]	**0**[A]	21.3	21.0
% Mayfly	36.6	**9.1**[A]	**0**[A]	**3.2**[A]	**2.4**[A]	**0**[A]	**0**[A]	**29.5**[A]	**16.5**[A]
% EPT	55.6	67.5	**7.7**[A]	**31.9**[A]	**13.5**[A]	**0**[A]	**0**[A]	59.6	81.1
EPT Richness	7.3	8.0	**1.0**[A]	**7.0**[A]	**3.7**[A]	**0**[A]	**0**[A]	10.0	15.7
Midge/EPT Ratio	0.2	0.3	**14.7**[B]	2.1	8.6	**---**	**---**	0.2	0.11
% Shredders	12.8	15.0	**0**[B]	8.8	3.5	**0**[B]	**0**[B]	10.8	32.3

[A] **Normal data with values significantly lower than U1 using Fishers LSD Test.**

[B] **Non-Normal data with values significantly lower than U1 using Kruskal-Wallis Multiple Comparison Z test.**
SpH – Severely pH Impaired
S – Severely Impaired

4.3.1 Results Summary

Table 4-11 is a summary of the successfulness of each method that was used in this study in detecting the multiple levels of impairment that resulted from AMD. As indicated, the presence of iron floc proved to be a good indicator of acid mine drainage. Iron flocs presence even had a noted effect in Slightly Impaired streams where the crayfish, Camberidae, could be caught by hand with ease. It is uncertain if the slowed response of the organisms was a result of the floc material that coated the organisms, as well as the stream bed, or overall impact of high iron loads and elevated conductivity.

Table 4-11. Results of multiple methods of measuring impacts of acid mine drainage in the Black Creek watershed.

	Non-Impaired	**Slightly Impaired**	**Moderately Impaired**	**Severely Impaired**	**Severely pH Impaired**
Iron Floc Present	---	+	+	+	+
Acute Toxicity Test					
D. magna	---	-	+	---	---
P. promulas	---	-	+	---	---
Elutriant Test	---	**+**	**+**	**+**	**+**
Short-term (96-hr) Sediment Test	---	-	-	-	+
Ten-day Sediment Test					
D. magna (survival/reproduction)	---	(-/+)	(-/+)	(-/+)	(+/+)
C. tentans *(survival)*	---	**+**	**+**	**+**	**+**
Benthic Macroinvertebrate Sampling					
Richness	---	**+**	**+**	**+**	**+**
% Mayfly	---	**+**	**+**	**+**	**+**
% EPT	---	**+**	**+**	**+**	**+**
EPT Richness	---	**+**	**+**	**+**	**+**
Midge/EPT Ratio	---	**+**	**+**	**+**	**+**
% Shredders	---	**+**	**+**	**+**	**+**
Penetrance		**+**	**+**	**+**	**+**
Leaf Breakdown Rates					
% loss	---	**+**	**+**	**+**	**+**
% loss/day	---	**+**	**+**	**+**	**+**
k value	---	**+**	**+**	**+**	**+**
Algal Counts					
Cell Counts (Organisms/mm^2)	---	**+**	---	---	---
Chlorophyll a (mg/mm^2)	---	**+**	---	---	---
Ash Free Dry Wt.	---	**+**	---	---	---

Acute water column toxicity testing was successful in identifying impairment at Moderately Impaired stations, but not those that had only slight impairment. Short-term sediment tests only identified the most impaired stations while elutriant tests were more successful, indicating all stations tested had some level of impairment. Long-term sediment tests had a similar level of success, with *C. tentans* being slightly more sensitive than *D. magna*.

Benthic macroinvertebrate studies, leaf pack studies, and algal studies were all successfully used to identify AMD impaired stations. As expected, trends found in the benthic studies were duplicated in the leaf pack studies. Overall, algal studies may have been the most sensitive indicator amongst these three study methods.

Table 4-12 summarizes the stations that were successfully identified as AMD sites with the methods used in this study. Station U2, which was identified as Slightly Impaired, yielded positive results for AMD impacts in 13 of the 18 metrics (methods) used. Stations U6 and U7 were also identified as Slightly Impaired. These two stations had results that indicated impairment in 14 of the 18 metrics used. The two stations, L1 and L11, which were identified as Slightly Impaired, were positive for AMD impacts (as compared to the reference) in six and four of the metrics, respectively. The Moderately Impaired station, U5, was identified as impaired in 16 of the 18 methods used. The Severely Impaired and Severely pH Impaired stations, U10 and U9, were identified as impaired in 14 and 16 of the 16 metrics utilized for these two stations, respectively. Algal tiles were not used at the primary Black Creek sampling stations and therefore was not included in Table 4-12. However, this method was successful in identifying slight levels of impairment that were not detected using benthic macroinvertebrate sampling.

Table 4-12. Stations were multiple methods of measuring impacts of acid mine drainage in the Black Creek watershed yielded successful identification of impacts.

	Non-Impaired	**Slightly Impaired**	**Moderately Impaired**	**Severely Impaired**	**Severely pH Impaired**
Iron Floc Present	---	U2, U6, U7	U5	U10	U9
Acute Toxicity Test					
D. magna	---	-	U5	---	---
P. promulas	---	-	U5	---	---
Elutriant Test	---	**U6, U7, L1**	**U5**	**U10**	**U9**
Short-term (96-hr) Sediment Test	---	-	-	-	U9
Ten-day Sediment Test					
D. magna (survival/reproduction)	---	(-/U2, U6, U7, L1, BBM, L11)	(-/U5)	(-/U10)	(U9/U9)
C. tentans *(survival)*	---	**U2, U6, U7, L1, BBM, L11**	**U5**	**U10**	**U9**
Benthic Macroinvertebrate Sampling					
Richness	---	**U2, U6, U7**	**U5**	**U10**	**U9**
% Mayfly	---	**U2, U6, U7, L1**	**U5**	**U10**	**U9**
% EPT	---	**U2, U6, U7, L1, L11**	**U5**	**U10**	U9
EPT Richness	---	**U2, U6, U7**	**U5**	**U10**	U9
Midge/EPT Ratio	---	**U2, U6, U7**	**U5**	**U10**	**U9**
% Shredders	---	**U2, U6, U7**	**U5**	**U10**	U9
Penetrance		**U2, U6, U7**	**U5**	**U10**	**U9**
Leaf Breakdown Rates					
% loss	---	**U2, U6, U7**	**U5**	**U10**	**U9**
% loss/day	---	**U2, U6, U7**	**U5**	**U10**	**U9**
k value	---	**U2, U6, U7, L1, L11**	**U5**	**U10**	**U9**

4.4 DISCUSSION

4.4.1 General Discussion

Water and sediment sampling, toxicity testing, benthic macroinvertebrate sampling, leaf pack, and algal studies were all completed as part of this study in an attempt to better identify impairment as a result of AMD in the Black Creek watershed. Basic water chemistry is included in most biotic sampling protocols (US EPA, 1999). These parameters (conductivity, pH) were used to group sampling stations. Metals analysis of both surface water and sediments was also completed during this study.

Surface water analysis was used to indicate the extent to which AMD may alter surface waters. High metals were expected to be found throughout the watershed as well as low pH and high conductivity. The acidic nature of AMD as well as its prevailing high conductivity is well documented (Skousen and Ziemkiewicz 1995; USGS 1996,1997). Gray (1996a;b), who has written extensively in the AMD literature, has also used conductivity (as well as sulfates) and visual assessments to indicate AMD.

Metals in the water column and sediments were also measured. Aluminum, copper, iron, manganese are often evaluated in AMD-impacted watersheds in addition to other heavy metals (Brake et al. 2001; Tiwary, 2001). Values for sediment and water column contamination by these metals were within the range of those found in the literature (Banks et al. 1997; Brake et al. 2001; Grout and Levings 2001; Mascaro et al. 2001; Soucek et al 2000a,b). Toxicity of these metals, individually or together, were not evaluated as part of this study. Total metals were used in this study, which indicates presence/absence of metals, but will yield only limited information about potential toxicity associated with these sediments. Therefore, while it is not necessary to discuss, it should be iterated that any sediment data should be viewed with some caution; metal contamination of sediments is very spatial and may not represent true metal content of surrounding particles (Herr and Gray, 1997). Additionally, recent studies in AMD watersheds suggest it is difficult to use metals as a interpreter of associated AMD impacts (Cherry et al. 2001). It should be stressed that sediment characterization is an important component of what may be bioavailable to the surrounding benthic community, but results should be interpreted with great care. Therefore, these parameters (metal content) were not considered when devising the initial Non-Impaired through Severely Impaired characterizations.

Additionally, only total metals were evaluated which for most metal species indicates little about bioavailability

Acute toxicity tests are often used by industry and state agencies to monitor effluents for NPDES reporting requirements. In theory, if the test does not exhibit toxicity, the effluent is not acutely toxic to the most sensitive receptor in the receiving system (Webber, 1993). Recently, it has been argued that whole effluent toxicity tests, or WET tests, similar to the ones used in this study, are often not protective of most species in the system being monitored (La Point and Waller, 2000). In this study, samples collected at stations that were slightly impaired were not acutely toxic. The sample collected at the station that was Moderately Impaired was acutely toxic. The range from Slightly toxic to Moderately toxic was 100 percent to zero survival with none of the stations exhibiting mild impairment. This dramatic range indicates that this type of test might not be the best method for screening AMD stations (Lopes et al.1999; Pereira et al.1999). However, it is difficult to argue the utility of this very simple test. In acid mine drainage environments, physiochemical parameters, such as pH and metal content, may be acting in combination where measured pH and metal content as individual components of the environment are not acutely toxic, but, in combination, may cause impairment. Simply stated, metal speciation, which is influenced by water chemistry, controls what is bioavailable and therefore toxic in the system (Brezonick et al. 1991). This in turn makes it very difficult to identify the most toxic components (McIntosh, 1991). So while the moderately toxic sample had a pH, which was low enough to cause some impairment (5.0-6.0 SU), some other constituent of the sample or a combination of constituents may have caused the acute toxicity of this sample. If water quality analysis had been the only method used to identify acute toxicity, the areas of Moderate Impairment would likely have been excluded from a list of locations that may exhibit acute toxicity. Conversely, some researchers have used acute toxicity testing to successfully identify intermittent and neutral AMD (Soucek et al. 2000a,b). The variability of these results may be associated with collection methods, organisms' health, or the stochastic nature of the AMD environment. Acute toxicity tests are snapshots of what is occurring at an instant in time in the system and may not be representative of instream conditions.

Elutriant tests and short-term sediment tests had a wide range of results, with only the Severely pH Impaired Station U9 having 100 percent mortality. Slightly Impaired stations, like L1 and BBM, had only 15 percent survival in the elutriant tests, while the short-term sediment tests had only slight impairment for both of these sampling stations. Due to the dramatic differences in results and a lack of replicate testing, it is difficult to determine what these results are indicating. Sediments collected at both L1 and BBM were high in metal contaminants. The availability of these metals in the elutriant is the likely source of impairment (Nebeker et al.1984), but which metal and to what degree it was available is unknown. The most obvious differences in these two test types is the test media. In the elutriant test, porewater removed from the interstitial spaces of the sediments was used as the test media. In the sediment test, sediments were overlaid by a laboratory-created water. It could be inferred at Stations L1 and BBM that the sediments and associated porewater exhibit toxicity, but the laboratory water used in the test sufficiently diluted the toxic materials, alleviating any short-term toxicity. How this relates to instream conditions at these two locations is difficult to discern. It could be supposed that the constant flushing of the sediments with an upstream water source would reduce instream toxicity. However, the elutriant samples indicate that porewater from sediments is acutely toxic and the instream community would be continually exposed to similar constituents.

Initial sediment toxicity tests indicated that all sampling stations had sediments that were, to some degree, toxic to the test species utilized. In the initial test, *C.tentans* had 50 percent survival at both the Moderately Impaired and some of the Slightly Impaired stations (L1 and L11). This test indicated that the most impaired sampling locations were highly toxic, with no survival of organisms. While the test successfully identified impairment, there are some additional drawbacks to this type of testing. *Daphnia magna, Hylella azteca, Chironomus tentans, Gammarus lacustis* and *Hexaginia limbata* are species that are recommended for sediment toxicity testing (Nebecker et al.1984; USEPA, 1994; ASTM, 1995). *Chironomus*, which was utilized in this study, was selected by testing agencies due to the simplicity in which it can be reared in a laboratory environment and not due to its sensitivity. Many chironomid genera are given tolerance values of ten on a scale of one to ten (USEPA, 1999). *Daphnia magna*, which were used in this study, are very sensitive to metals but are not sediment-dwelling organisms. Other issues are the dilution of toxic materials by the addition of laboratory water at a 1:4 ratio (Cherry et al. 2001) and the physical handling of the sediment material that may reduce toxicity. Many researchers continue to use sediment testing and have found sediment tests to be useful in a series of applications, which includes sediment testing as one phase of a larger study (Leppanen et al.1998; Soucek et al.2000a;b; Cherry et al.2001). Other studies in the same area did not yield similar results (Soucek et al. 2000a). In these tests, results were more variable, which suggested trends but no clear significant differences among treatment groups. Cherry et al (2001) found *D. magna* to be more sensitive than chironomids, which is opposite of what was observed in this study. It should be noted that there are several possible sources for the differences in these studies. Metals are spatial and unpredictable, with differing levels of contamination from samples collected only inches apart. These may or may not be influencing results. For example, Leppanen et al (1998) found metal concentrations in sediments highest in the spring but toxicity higher in the fall. As with acute toxicity testing, results can be influenced by collection techniques and organism health. Another source of variability may be the presence of acid volatile sulfides (AVS). While not sampled for in this study, the presence of AVS can both reduce toxicity by controlling availability through binding toxic metals or may be toxic itself (Leonard et al. 1999; Wang and Chatman, 1999). This further confounds the issue of AMD sediment toxicity.

The second, third, and final sediment toxicity tests showed a continual decline in impairment at each of the sampling locations, with the final test having no associated toxicity at any sampling location. It should be noted that this segment of the study indicated that clean flowing water through contaminated sediments may indeed reduce the amount of toxicity associated with the sediments. The sediment purge study completed in this research has not been duplicated in the literature; however, similar studies can be found in acid mine drainage treatment literature. Passive treatment of AMD removes metals from water by physical and/or chemical processes. This improves water quality (Skousen et al. 1995), which is expected to create a more hospitable environment for benthic macroinvertebrates. Additionally, substrates that are coated with AMD flocculates and precipitations are suspected to be a source of habitat destruction for benthic macroinvertebrates (Robbins et al. 1997). Substrates coated with AMD precipitants and placed in experimental streams was swept clean in as little as four weeks in experiments that used laboratory water (DeNicola and Stapleton 2002). This may be important for researchers who wish to complete or suggest restoration alternatives. Once restoration activities have been completed, this type of testing may indicate how long the sediments at the location remain toxic to the surrounding fauna.

Benthic macroinvertebrates have long been a useful tool in biomonitoring (Cairns et al.1971; Merritt and Cummins, 1988; Karr and Chu, 1999). The use of benthic macroinvertebrates for the evaluation of acid streams is certainly not new, with Dills and Rogers (1974) finding an association with species diversity and hydrogen ion concentration in the early 70's. In this study, benthic macroinvertebrates collected instream were good indicators of stream condition. Based on the metrics selected in this study, U9 and U10 were the most severely impaired sampling locations. This information mirrored what was suggested by chemical analysis at these sampling locations. Station U5 was labeled as Moderately Impaired. This was also reflected in the benthic macroinvertebrate data when every metric was significantly different from the reference. What distinguished the Moderately Imparied stations from the Severely Impaired is that no benthic macroinvertebrates were ever collected at U9 and U10, while a community did exist at U5. Stations U2, U6, U7, L1, and L11 were all classified as Slightly Impaired sampling locations. What made the analysis of benthic macroinvertebrates different from the other types of analysis is that the degrees of slight impairment were evident when using benthic macroinvertebrates as an indicator. Benthic macroinvertebrates at Station U7 indicated that this station may be slightly more impaired than U6. This scenario is likely because Station U7 lies below the discharge of U5 and is likely receiving some of its toxic inputs. Benthic macroinvertebrates also indicated that L11 is the least impaired of all the macroinvertebrate sampling locations when compared to the control. This is the furthest downstream sampling station and is located some distance (approximately 3.0 kilometers) from the most toxic AMD inputs.

Various levels of impairment were clearly discernable using the metrics selected in this study. Other researchers have found benthic macroinvertebrates to be a very useful tool in similar field applications with both heavy metals (Clements, 1991; Clements et al.1992; Poulton et al.1995; Clements, 1999; Carlisle and Clements, 1999; Beltman et al.1999) and acid mine drainage (Kimmel et al. 1996; Ventura and Harper, 1996; Winterbourn and McDiffett, 1996; Carbone et al.1998). Benthic macroinvertebrate biomonitoring is well established in other fields of investigations (Reynoldson et al.1997; Charvet et al.1998; O'Connor and O'Connor, 1999; Charvet et al. 2000). An important factor that sets field studies, like benthic macroinvertebrate sampling or fish sampling, apart from laboratory testing is that the organisms collected in the field are exposed to a variety of conditions in their environments. For example, because of the nature of AML landscapes, stream organisms influenced by AMD may be exposed to extremes in both pH and metals (Curtis, 1973). These conditions would be difficult to detect without continuous instream monitoring and would be very difficult to reproduce in the laboratory. Flow-through and mesocosm testing methods have been developed by various researchers to mimic some instream conditions. Standard laboratory testing still lacks environmental realism where organisms are all tested at a set temperature and given a set amount of nutrients. This is not to fault the testing methods which were in fact designed to reduce variability, but to note that the benthic or instream community is a more likely indicator of actual instream conditions.

Additionally, where they have been used to identify sources and/or locations of biological impairment, they also may be used to monitor activities during restoration and recovery.

Warner (1971), who studied benthic macroinvertebrates from Roaring Creek in West Virginia, found an abrupt drop in total taxa present in acid mine drainage streams once pH fell below 4.5 SU. The researcher determined that because of the complex environment, it was not possible to precisely assess the modes of toxicity, but found that pH was a reliable index to gage the effects of acid mine drainage on aquatic life. While a dramatic drop in taxa richness was not evident in this research, the correlation between taxa richness and pH was found to be significant. Dills and Rogers (1974) also found this strong relationship between pH and taxa richness in studies related to AMD treatment, where stonefly nymphs were found to have elevated drift rates and depressed feeding in AMD treatments (Cole et al. 2001). Other researchers found stoneflies to be more tolerant of acid mine effluent than trout and were useful indicators of toxicity of highly acidic or metals mixtures (Whipple and Dunson, 1993). Hickey and Clements (1998) studied the relationship between metals and benthic macroinvertebrates and found agreement between results from AMD streams in this study and those found in New Zealand metal-impacted streams, particularly with reference to mayflies. Kiffney and Clements (1994) found that metal mixtures were also toxic to benthic macroinvertebrates in Rocky Mountain streams.

Two other analyses were utilized in this study: algal tiles and leaf packs (Table 4-11). Leaf pack investigations were not presented because results mirrored those found in the benthic community. This is likely not a coincidence due to the link between leaf pack breakdown and benthic macroinvertebrate communities. The algal tiles were not included because the sampling locations were not similar to those used in other aspects of this study. Both methods, particularly the algal studies, will yield useful, site specific results (Bott et al, 1978; Benfield, 1981; Benfield and Webster, 1985; Jenkins and Suberkropp, 1995). In AMD studies, noted differences have been found in detrital processing rates. Meegan et al. (1996) found reduced rates, as well as a shift in the type of shredders present instream. In circumneutral streams, large-particle detritivores dominated, while in acidic streams, smaller shredders were present. Another study found increased concentrations of zinc and increased deposition rates of metal oxides were most closely related to decreased rates of litter breakdown (Niyogi et al. 1999). This decreased breakdown rate and increased deposition rate occurred in the Black Creek watershed at locations that had the most severe AMD. Other authors have found reduced rates associated with decreased pH (Kok and Van Der Velde, 1994; Carpenter et al.1987). In this research, sites that had low values for pH also exhibited the coating phenomenon, therefore, making it difficult to discern between the two effects, but overall AMD impacts were apparent.

Tease and Coler (1984) found that pH and aluminum reduce net productivity and movement of diatoms and cyanobacteria. The researchers also determined that pH alone does not restrict these movements. Diatoms have also been used to identify AMD (Verb and Vis, 2000). Like this research, the authors used a shift in species to identify recovery in AMD areas, but total number of species in this study was higher than those found in Black Creek or the Powell River. The same authors found that macroalgal communities were not a good indicator of recovery, as they identified only the most severely impacted stations (Verb and Vis, 2001). Niyogi et al. (1999) found that deposition rates and types of metal hydroxide influenced periphyton communities present in AMD streams.

A review of the literature produced study results that varied both dramatically and slightly from those in Black Creek. Overall, the differences found in the literature and this study are easily explained by variability in the nature of the method or in the parameter (such as sediments) being used. Researchers found few relationships between metal concentrations in the sediments and the overall impacts of AMD. Strong similarities also existed in AMD streams benthic macroinvertebrate assemblages. Mayflies (Ephemeroptera) were consistently the most sensitive taxa, and the metric associated with mayflies were the best at discerning impacts. Relationships between pH and deposition were also evident in other leaf pack studies in AMD streams. While few AMD periphyton studies exist in the literature, those that do indicate that AMD can influence periphyton in a variety of ways with pH, metal concentration, and deposition rates all playing important roles.

4.4.2 Other Studies in the Powell River Watershed

Black Creek flows into the Powell River downstream of the town of Norton and above the community of Appalachia. This portion of the Powell River drains a relatively small portion of the total watershed. In Kent Junction, Virginia, the Roaring Fork joins with the Powell River. Approximately 8 miles downstream, at Three Forks in Big Stone Gap, the Powell River mainstem merges with the South Fork of the Powell River and becomes a substantially large river referred to as the mainstem of the Powell River. The river then flows through the remainder of Wise County, then Lee County south-westerly towards the Virginia-Tennessee state line. Various other studies have been completed in the Powell River basin (Soucek et al. 2000a,b; Cherry et al. 2001; Soucek et al. 2001; Schmidt et al. 2002a,b). These studies were completed in the North Fork of the Powell River sub-watersheds specifically several small sub-watersheds in the Stone Creek/Straight Creek drainage and in Reed's Creek in Summers Fork of the North Fork of the Powell River. The North Fork of the Powell watershed joins the mainstem of the Powell downstream of Pennington Gap, Virginia and upstream of Jonesville, Virginia between river mile 156 and 157. Studies completed in these watersheds used similar methods so that some of the results were comparable.

Water quality was recorded in the Ely Creek watershed by the aforementioned researchers in 1997. The pH measurements in the watersheds had ranges similar to those found in Black Creek. The pH range of 2.9 to 7.7 mirrored those found throughout Black Creek (Schmidt et al. 2002b). A notable difference between these two watersheds is the conductivity values. In the four months that AMD was evaluated in Ely Creek, extremely high conductivity values, in excess of 3,000 uhmos, were detected in Ely Creek at locations that had severely depressed pH (Cherry et al, 2001). This is much greater than the highest value recorded in Black Creek, which was 2040 uhmos at U9, a Severely pH Impaired station. There was also a lack of sites with near-neutral pH and high conductivity in Ely Creek watershed. In Black Creek, there were stations that had neutral pH and conductivity of greater than 1,000 umhos.

In Puckett's Creek, the acidic AMD sites had ranges for conductivity and pH that were more similar to those found in the Black Creek watershed (Soucek et al. 2000a,b). The highest conductivity values recorded in these studies was between 1,581 and 1,720 uhmos, respectively. There were no neutral pH sites with conductivities as high as those recorded in Black Creek in this watershed. Data ranges recorded for Reed's Creek (Schmidt et al. 2002b) appears too also lack data with a high conductivity (greater than 1,000 uhmos) and neutral pH.

Water quality samples were collected in Ely Creek, Puckett's Creek, and Reed's Creek and analyzed for aluminum, iron, and manganese (Schmidt et al. 2002a,b). Mean values found in the water column for aluminum at upstream stations were higher in Black Creek than all three North Fork watersheds. The mean aluminum values found at acidic AMD stations in Puckett's Creek were similar to Black Creek's Severely pH Impaired stations, while Ely Creek values were at least 10 mg/l lower than Black Creek's. Iron and manganese values in the water column were not notably different in these four watersheds.

Sediment samples were collected in Ely Creek, Puckett's Creek, and Reed's Creek and analyzed for the five metals (aluminum, copper, iron, manganese, and zinc) (Schmidt et al. 2002). Mean aluminum values at all three station categories reported by Schmidt and others had values similar to their associated reference. The same was true of the Black Creek watershed. The mean values reported for Black Creek did not indicate any noticeable trend upstream to downstream or amongst the different categories of sampling stations. Mean values for aluminum in Puckett's Creek and Reed's Creek were higher than those found in the Black Creek watershed in November of 1995 but similar to those found in the sediments used for toxicity testing. Mean copper values in Black Creek in November, 1995, were similar to those in Ely Creek and Puckett's Creek with one exception of a high reading of 19.5 mg/kg in Black Creek. The values recorded for sediments used in toxicity tests were much higher in Black Creek than any reported in the three North Fork watersheds. For example, a value of 260 mg/kg was reported in a sample collected at the Moderately Impaired station, U5. Some higher values were also found in Reed's Creek. However, these were recorded in upstream stations.

Iron values in the Black Creek watershed ranged from 9,500 to 176,000 mg/kg in both the November 1995 and sediment test samples (collected in December 1995). These values were dramatically higher than those reported in Ely Creek, but likely similar to the ranges found throughout the Puckett's Creek and Reed's Creek watersheds (Schmidt et al. 2002a,b). The same trend was observed for manganese and zinc when comparing Black Creek results to Ely Creek, Puckett's Creek, and Reed's Creek, with the exceptions of manganese values in AMD-influenced stations in Puckett's Creek. These values were considerably lower than other sampling stations in all three of the other watersheds.

Survival of *C. dubia* in toxicity tests from samples collected in upstream (reference) and acidic AMD stations were similar in the Black Creek and North Fork sub-watersheds. Upstream samples had acceptable survival, while low pH stations had zero survival. The neutral AMD stations in Ely Creek, Puckett's Creek, and Reed's Creek had more variability than those found in the Moderately or Severely Impaired stations in Black Creek. These stations had no acute toxicity in short-term tests, while the North Fork sub-watershed reported some level of impairment. Sediment toxicity test results reported for Ely Creek (Cherry et al. 2001) differed from Black Creek in that all AMD influenced stations were impaired as compared to the reference station. Another notable difference in these two studies were the impacts to *C. tentans* in the Black Creek test. This test organism, which resides in the sediments, was more sensitive than *D. magna*. These results were opposite those found in Ely Creek. In Puckett's Creek, acidic samples had some percentage of survival for both test organisms, while those found in Black Creek did not.

Benthic macroinvertebrate samples were collected in the North Fork sub-watersheds and were collected in a slightly different manner than those in Black Creek (Schmidt et al. 2002b). However, some of the information gathered from these studies can be compared to those found in Black Creek. In Black Creek, taxa richness was zero at the Severely pH Impaired station, which was similar in water quality to Ely Creek's and Puckett's Creek's acidic AMD stations. In Ely Creek and in Puckett's Creek, these locations did support some aquatic benthic macroinvertebrate organisms (Schmidt et al. 2002b). Neutral AMD type stations in Black Creek had benthic taxa richness values more similar to those found in Puckett's Creek and Reed's Creek than those in Ely Creek. EPT taxa richness values appear higher in Black Creek at AMD neutral stations than those found in the three North Fork sub-watersheds while percent mayfly values appear similar to slightly lower than those found in stations with similar water quality in Puckett's Creek (Soucek et al. 2000).

Both the studies completed in Puckett's Creek and Ely Creek (Soucek et al. 2000a; Cherry et al. 2001) had similar results with regard to identifying impairment associated with AMD. The Puckett's Creek study concluded that stations receiving continuous acidic or neutral AMD had significantly fewer EPT organisms and percent Ephemeroptera abundances than unimpacted or intermittent AMD stations. This same trend is noted in the Black Creek data. A study completed in the Ely Creek watershed also had some of the same conclusions. Specifically, benthic macroinvertebrates appear to be a sensitive sampling approach when investigating AMD.

Black Creek had two locations with severe impairment. One station had low pH and high conductivity (U9), and one had high conductivity (U10). While the impacts at these stations were dramatic, they were not as extensive as those found in the Ely Creek watershed. The impacts in Ely Creek extended further downstream and covered a larger area than those found in the Black Creek watershed. Results from studies conducted in Puckett's Creek were more similar to Black Creek in their levels of impairment, but, of the two, Black Creek is likely the watershed with more impacts from AMD. This statement is based upon organism survival in toxicity tests and benthic communities in areas that did not support biological communities in Black Creek. Reed's Creek did not have the acidic nature that Black Creek did and was likely the least impaired of the four watersheds discussed above.

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Appendix A - Site Classification

Appendix A - Site Classification

Upper Stations

Initial Site Designation	Final Site Designation	Description
U1	UBC -1	Reference Site - Located above Black Creek Lake and the recreational facilities.
U2	UD-1	First major tributary entering Black Creek Lack approximately 0.6 km from UBC-1.
BC-2	UBC-2	Just below Black Creek Lake dam.
U3		Road side ditch south of Black Creek Lake caretaker's cottage.
U4	UD-2	Originates on eastern side of road to Black Creek Lake just at entrance to Black Creek Lake.
U4b	UD-3	Roadside seep along road to Black Creek Lake, 1.3 km from UBC-1.
U5	UD-2a	Tributary formed by discharge from UD-2.
U6	UBC-3	In Black Creek before tributary from UD-2 enters.
U7	UBC-4	In Black Creek after UD-2 tributary enters stream.
U8	UD-4	Roadside seep which runs along road to Black Creek Lake, approximately 1.8 km south from UBC-1.
U9 'Old Seep 1'	UD-5	Seep originating from bench east of Black Creek Lake Road, approximately 50 m south of UD-4.
U10 'Old Seep 2'	UD-6	Drainage from deep mine located on the east side of road (20 m to origin), approximately 1.9 km south of UBC-1.
U11	UD-7	Stream originating approximately 0.8 km from road, located approximately 2.0 km south of UBC-1
BC-3	UBC-5	At road to Paramount Coal holdings.
U12		Drainage ditch under road to Black Creek Lake.

Specifications

U - Upper. Refers to sites located above the intersection of White Oak Gap Road and Black Creek Lake Road.

UBC - Refers to site located directly in Black Creek in the upper region of the watershed.

UD - Refers to site located in a tributary or is the tributary in the upper region of the watershed.

Lowercase letters - Site is located in reference to an other site. Example: UD-2a is located downstream on the same tributary as UD-2.

Lower Stations

Initial Designation	Final Designation	Description
L1	LBC-1	Just after Black Creek passes under White Oak Gap Road.
L2	LD-1	Stream running under VA 618 just before junction with road to Black Creek Lake.
White Oak Gap Seep	LD-2	Approximately .25 km from intersection of Black Creek Lake Road and White Oak Gap Road on west side.
	LBC-2	Black Creek at White Oak Gap Seep, below input from LD-2 on western side of creek.
L3		Second tributary , after LD-1, entering Black Creek on the east side.
L4		Third tributary , entering Black Creek on the east side.
L5		Fourth tributary, runs through large wetland before entering Black Creek on the east side.
L6		Fifth tributary, breaks into three streamlets which drain into wetland and then seep into Black Creek.
BC-4 (BBM)	LBC-3	Just above holding pond near Betty B Mine.
L7		Sixth tributary, entering Black Creek on the east side.
L8		Seventh tributary, entering Black Creek on the east side.
L9		Eighth tributary, entering Black Creek on the east side.
BC-5	LBC-4	Just above input from LD-3 and LD-4.
L10 (L10a)	LD-3	Runoff from wetland area east of Black Creek and just south of the Betty B Mine shop. Formed by LD-4 and 5.
L10b	LD-4	Drainage from deep mine approximately 50 m south of LD-3.
L10c	LD-5	Drainage from deep mine approximately 70 m south of LD-3.
L11	LBC-5	Just above US 58.

Specifications

L - Lower. Refers to sites located below the intersection of White Oak Gap Road and Black Creek Lake Road.

LBC - Refers to site located directly in Black Creek in the lower region of the watershed.

LD - Refers to site located in a tributary or is the tributary in the lower region of the watershed.

Lowercase letters - Site is located in reference to an other site. Example: UD-2a is located downstream on the same tributary as UD-2.

Appendix B – Water Quality Data

Appendix B – Water Quality Data

Conductivity

Site	6/28/95	8/8/95	8/31/95	10/4/95	11/15/95	12/5/95	12/14/95	2/23/96	3/7/96	4/2/96
U1	497	370	474	518	480			196	246	220
U2	812	843	889	848				816	809	747
UBC-2								392	369	592
U3	781	845	868	888				1395		
U4	1560	1716	1802	1864	1530			1395	1347	1410
U4-b								1088		1016
U5	1483	1624	1755	1795				1244	1087	1180
U6	664	699	1107	931	590			542	561	
U7	876	945	1435	1341	740			680	650	
U8	1867	1048	Dry	1938				1071	929	1493
U9	1835	2010	2040	2040				1706	1510	1622
U10	865	848	848	854				827	1722	800
U11	1867	1980	Dry	974				1680	1583	745
UBC-5									647	
U12	1396									
L1	1293	1269	1376	1328	829			738	755	
L2	868							1203		
LD-2								881	930	1067
LBC-2								820		809
LBC-3									802	1222
L3	580									
L4	993									
L5	1315									
L6	801									
L7	1170									
L8	979									
L9	964									
LBC-4								794		780
L10	395				610					1050
LD-4					862			912		712
LD-5										1057
L11	957				868			780	770	750

Measurements made instream using a Orion Model 122 meter.

pH

Site	6/28/95	8/8/95	8/31/95	10/4/95	11/15/95	12/5/95	12/14/95	2/23/96	3/7/96	4/2/96
U1	6.69	7.31	7.36	7.43	6.83	6.80		6.80	7.84	6.98
U2	7.21	7.37	7.47	7.53		6.15		7.41	7.58	6.93
UBC-2								7.14	7.28	7.24
U3	8.05	8.55	8.58	7.83						
U4	3.40	3.78	3.57	3.62	4.40	3.66		3.73	3.72	3.37
U4-b							3.2	3.91	4.56	4.11
U5	3.62	4.12	3.66	3.64		4.00		4.78	4.78	4.61
U6	6.43	6.77	6.70	6.82	7.03			6.83	6.52	
U7	5.88	6.00	4.70	5.22	5.23			6.30	6.03	
U8	5.84	5.72	Dry	3.10		3.30		3.39	3.48	3.22
U9	2.75	3.20	3.09	2.97		3.31		3.60	3.18	2.88
U10	6.21	7.17	7.10	7.67		6.80		7.43	7.19	6.29
U11	3.84	3.80	Dry	6.46		3.10		3.92	4.37	6.20
UBC-5									6.13	
U12	7.33									
L1	7.81	7.37	7.31	7.27	7.11			6.94	6.10	
L2	7.04							7.48	6.05	
LD-2								7.10		5.86
LBC-2								7.33		6.63
LBC-3									6.54	7.03
L3	6.86									
L4	6.84									
L5	7.06									
L6	7.01									
L7	7.44									
L8	3.41									
L9	3.60									
LBC-4										7.15
L10	5.72				7.30			6.69		6.92
LD-4										6.60
LD-5										6.93
L11	7.37				7.87			7.83	7.75	7.30

Measurements were made using a Hanna Model 9024 pH meter with calibrations made according to manufactures suggestions.

Acidity or Alkalinity

	8/31/95		10/4/95		2/31/96		3/7/96	
	Acid	Alkal	Acid	Alkal	Acid	Alkal	Acid	Alkal
UBC-1		60		57		150		27
UD-1		62		61		100		56
UBC-2								30
U3		128		122				
UD-2	104		118		92		85	
UD-3					1800		83	
UD-2a	93		113				67	
UBC-3	15		35				15	
UBC-4			40				10	
UD-4			336				213	
UD-5	334		328		1785		270	
UD-6	155		154		235		149	
UD-7			131		200		42	
UBC-5								24
LBC-1						50		86
LD-2								117
LBC-3								56
LBC-5						50		43

Acid = Acidity as mg/L $CaCO_3$

Alkal = Alkalinity as mg/L $CaCO_3$

Appendix C - Sediment Tests Data

Appendix C - Sediment Tests Data

Sediment Purge Study Metals Analysis

U1 – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	.0020	.0020	3.600	6.300	.8700	.1000
Test 2	.4600	.0020	3.080	13.20	3.480	.0040
Test 3	.7081	.0020	.0331	9.109	.0092	.0040
Test 4	7.137	.2082	11.40	9.918	1.364	.3410

U1 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	3041	18.2	12500	677	995	40.8
Test 2	2420	.003	9834	334	3558	22.6
Test 3	1738	11.4	6205	398	396	22.1
Test 4	1704	8.4	11181	415	379	85.6

U5 – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	1.440	.0020	7.900	23.00	1.280	.0200
Test 2	2.980	.0020	.4600	11.00	.1600	1.080
Test 3	.5793	.0020	.0587	10.81	.5888	.0077
Test 4	1.180	.0798	.6504	13.89	.0737	.0602

U5 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	16520	76.5	20064	3451	417	308
	11846	40.3	33408	1485	216	131
Test 2						
Test 3	9638	30.5	20230	2195	184	95
Test 4	7957	43.3	35181	1995	178	247

U6 – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	.6900	.0020	2.520	20.00	.6000	.2100
Test 2	1.570	.0020	3.050	12.00	.9400	.0700
Test 3	.5793	.0020	.0978	9.923	1.720	.0040
Test 4	.9924	.6430	.6430	11.65	.3808	.9328

U6 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	8613	280	22832	2416	981	224
Test 2	11531	15	31540	1445	1494	156
Test 3	9638	28.4	21800	2190	315	79
Test 4	7957	28.9	25880	1848	644	378

U7– Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	1.2700	0.4650	3.6900	35.3000	1.8100	0.1003
Test 2	1.5700	2.0000e-3	3.0500	12.0000	0.9400	0.0700
Test 3	0.7724	3.0000e-3	0.1146	9.9160	0.1380	4.0000e-3
Test 4	1.0310	0.4580	0.4051	13.4700	0.1843	0.0903

U7 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	9510	46.0	12700	2203	782	351
Test 2	4160	12.0	16500	555	678	70
Test 3	3247	45.6	n/a	574	107	77
Test 4	3946	21.0	n/a	936	600	416

U9 – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	1.9900	0.0347	23.0000	42.0100	5.9000	0.4500
Test 2	1.7800	2.0000e-3	3.0500	12.0000	0.9400	0.0700
Test 3	1.7060	3.0000e-3	0.0978	3.3750	0.0184	11.2900
Test 4	1.0690	0.2706	0.2676	10.1000	1.0000e-3	0.0310

U9 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	5219	51.2	89800	1167	168	308
Test 2	3930	40.0	96400	720	118	82
Test 3	5065	54.2	33770	1741	1576	191
Test 4	3889	44.0	10683	1069	283	337

U10 – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	2.9770	0.1561	2.0800	29.3100	0.1966	0.2708
Test 2	0.9600	2.0000e-3	7.0500	10.5000	3.4800	0.0600
Test 3	0.5793	2.0000e-3	0.1285	8.7530	0.0784	4.0000e-3
Test 4	1.0310	0.2012	0.4386	13.3700	0.1843	0.0802

U10 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	4580	37.7	52500	1977	1264	62
Test 2	4330	18.0	16500	900	780	112
Test 3	5192	38.7	47886	1394	1709	77
Test 4	4594	29.2	56208	1107	1003	372

L1 – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	2.9770	0.1561	2.0800	29.3100	0.1966	0.2708
Test 2	0.9600	2.0000e-3	7.0500	10.5000	3.4800	0.0600
Test 3	0.5793	2.0000e-3	0.1285	8.7530	0.0784	4.0000e-3
Test 4	1.0310	0.2012	0.4386	13.3700	0.1843	0.0802

L1 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	4190	28.1	14000	2400	954	79
Test 2	5940	12.0	25700	4720	905	99
Test 3	5064	18.8	33700	1742	582	25
Test 4	5903	17.2	29670	1691	657	270

BBM – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	0.6000	2.0000e-3	1.6600	23.0000	5.3000	0.0200
Test 2	0.9900	2.0000e-3	2.9900	13.0000	2.7300	0.0500
Test 3	0.5793	2.0000e-3	0.0503	9.5500	1.0000e-3	4.0000e-3
Test 4	1.2210	0.2741	0.3531	10.5200	1.1550	0.0602

BBM - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	7860	64.6	69500	1374	3250	142
Test 2	4430	11.0	22900	1100	4350	95
Test 3	2361	9.2	15643	320	1467	47
Test 4	n/a	54.9	58379	1443	2745	1001

L11 – Water Column

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	0.4600	0.002	10.5000	20.5000	3.1800	0.003
Test 2	1.2300	0.002	2.7200	12.0000	0.8600	0.0500
Test 3	0.0322	0.002	0.0084	5.0160	0.001	0.004
Test 4	1.2210	0.0461	0.4051	15.7100	0.5404	0.1705

L11 - Sediment

Metal	Al	Cu	Fe	Mg	Mn	Zn
Test 1	3680	9.6	21400	1100	3800	90
Test 2	13900	20.0	30400	710	1650	194
Test 3	5744	19.7	28047	1533	5855	152
Test 4	4645	18.4	27682	1310	4488	355

Appendix D – Curriculum Vita

CURRICULUM VITA

Jessica Lynn Yeager~Seagle

Potesta & Associates, Inc	Home Address
2300 MacCorkle Avenue SE	217 Rosemont Avenue
Charleston, West Virginia 25304	South Charleston, WV 25303
(304) 342-1400	(304) 744-0205
(304) 414-4755	(304) 421-1876
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EDUCATION

M.S. in Environmental Science, Marshall University, South Charleston Campus.
Completion date – Fall 2005

M.S. in Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.
Defense December 2003
Thesis: The Impacts Of Acid Mine Drainage On The Black Creek Watershed, Wise County, Virginia.

B.S. in Biology (Chemistry Minor), 1993, Fairmont State College, Fairmont, West Virginia.

EMPLOYMENT

2000 - Present

Senior Scientist. Potesta and Associates, Inc., Charleston, West Virginia

Aquatic Ecologist, Ecotoxicologist. Design and supervision of field studies, data analysis and interpretation and report writing. Stream channel restoration and creation. Environmental impact assessments. Duties: to manage and supervise a multitude of project relation to stream restoration; stream mitigation; endangered species management; ecological risk assessment; benthic macroinvertebrates; fish studies; and other areas which require input ecological assessment. Expert witness testimony, special assistant to council regarding water quality issues and other services for litigation purposes. Provide agency liaisons for clients. Mentor for junior staff.

1998 -2000

Project Manager/Scientist. Biological Monitoring, Inc., Blacksburg, Virginia

Aquatic Ecologist, Ecotoxicologist. Design and supervision of laboratory and field studies, data analysis and interpretation, and report writing. Duties include: supervision of acute and chronic toxicity test; supervision of sediment toxicity test; supervision of field studies and assessment of macroinvertebrate and fish samplings; mussel surveys; glochidia and juvenile mussel studies; report writing and cost projections; date analysis and data assessment; development of client database and client management; attend scientific meetings and present recent research material; and publication. Quality Control Officer determine if all materials entering and leaving the building meet the standards put forth in our guidance manuals. Duties include: auditing; periodic inspection of files and work place; and management of safety officer and head laboratory personnel.

1996 – 1998

Graduate Teaching Assistant. Department of Biology, Virginia Tech, Blacksburg, Virginia.

Instructional teaching laboratory sections in General and Principles Biology. Duties included: preparing, administering and grading written class material; conducting laboratory activities; and administering examinations.

1995 – 1996

Graduate Research Assistant. Department of Biology, Virginia Tech, Blacksburg, Virginia.

Conducted field and laboratory study for the Department of Mines. Duties included: conducting extensive field reconnaissance; sediment purge studies; toxicity testing; benthic macroinvertebrate studies; developing GIS database; and culturing of test organisms.

1994 – 1995

Laboratory Technician Senior, Department of Biology, Virginia Tech, Blacksburg, Virginia.

Laboratory technician for aquatic toxicology laboratory. Laboratory duties which included maintenance of algae and daphnid cultures, acute and chronic bioassays, water quality analysis, coordination of ordering supplies and equipment for laboratory processing data and QA/QC duties.

1992 – 1993

Head Resident Assistant, Fairmont State College, Fairmont, West Virginia.

Head resident in co-ed resident hall. Duties included: supervised 146 resident and seven resident assistants; ran weekly meetings which included scheduling and RA education; teaching two credit class for future resident assistants; provided educational programming for residents; counseled/advised and facilitated the development of residents.

1993

Laboratory Technician Senior, West Virginia Department of Agriculture, Plant Industries

Division, Guthrie Center, Charleston, West Virginia. 1993.
Laboratory technician in the pest identification laboratory. Duties included: sorting and identification of Central Appalachian Pest studies samples; mounting, labeling, and sorting of insects for preservation; invertebrate identification; data entry, data processing; student workshops; and field surveying of pest species (flora and fauna).

1991 – 1992

Resident Assistant, Fairmont State College, Fairmont, West Virginia.

Resident assistant in co-ed dorm during regular academic year (head resident during summer terms). Duties included: supervising of 22 women; providing educational programming; and enforcement of resident hall policies.

1990 – 1991

Laboratory Assistant/Tutor, Department of Chemistry, Fairmont State College, Fairmont, West Virginia.

Supervised 101 and 105 chemistry laboratories which included: conducting laboratory; providing assistance to students; and instruction of lab procedures. Provided assistance in chemical storeroom and data base development for Material Safety Data Sheets.

PROFESSIONAL ORGANIZATIONS

Society of Environmental Toxicology and Chemistry
North American Benthological Society
ASSMR

CONTINUING EDUCATION

Natural Stream Channel Design
Rosgen – Class 1 and 2
West Virginia University Natural Stream Channel Design Class 1, 2, 3, and 4

SPECIAL RECOGNITION

Alumni Scholarship
Honor Roll
Kappa Delta Pi Honorary
1996 SETAC Student Travel Award ($250)
“Excellent” Review – Fall 1995, Spring 1996, and Fall 1996

PUBLISHED ABSTRACTS AND PRESENTATIONS

Yeager~Seagle, J., M. Yeager Armstead and L. Emerson. 2004 Benthic Macroinvertebrate Studies Conducted in Mountaintop Mining/Valley Fill Influenced Streams in Conjunction with the USEPA Environmental Impact Study. West Virginia Academy of Science. Concorde, West Virginia (Paper Presented)

Armstead, M. Yeager, J. Yeager~Armstead and L. Emerson. 2004

Yeager, J.L., W.J. Rasnake, C.A. Smith and D. Gruber. 1999. The Effect of Fine Coal Refuse on Glochidia and Juvenile Unionid Mussels. Twentieth Annual Meeting, Society of Environmental Toxicology and Chemistry, Philadelphia, PA (Poster Presented)

Yeager, J.L., W.J. Rasnake, C.A.Smith and D. Gruber. 1999. Characterization of Exposure and Effects from a Mine Tallings Release. Twentieth Annual Meeting, Society of Environmental Toxicology and Chemistry, Philadelphia, PA (Poster Presented)

Yeager, J.L., W.J. Rasnake, M.B. Wagner, A.D. Kinney, B.D. Lorch, C.A. Smith and D. Gruber. 1999. Assessing the influence of Logging on Macroinvertebrates and Particulate Organic Matter, 25 Years Later. Twentieth Annual Meeting, Society of Environmental Toxicology and Chemistry, Philadelphia, PA. (Poster Presented)

Rasnake, W.J., J.L. Yeager and D. Gruber. 1999. The Effect of Hardness, pH, and TOC on Copper Toxicity. Twentieth Annual Meeting, Society of Environmental Toxicology and Chemistry, Philadelphia, PA (Poster Presented)

Yeager, J.L., D. Gruer, W.J. Rasnake and C.A. Smith. 1999. The Use of Glochidia and Juvenile Unionid Mussels to Determine the Potential Effect of Coal Fine Refuse on T/E Mussel Species of the Powell. 47th Annual Meeting, North American Benthological Society. Duluth, Minnesota. (Poster Presented)

Gruber, D., W.J. Rasnake, J.L. Yeager and C.A. Smith. 1999. The Effect of Fine Coal Refuse on Glochidia and Juvenile Unionid Mussels. Proceeds from the Freshwater Mollusk Conservation Society First Annual Symposium. Chattanooga, TN (In press)

Gruber, D., W.J. Rasnake and J.L. Yeager. 1998. Estimation of Exposure and Effects from a Mine Tailings Release Upstream of Endangered Species Critical Habitat. Nineteenth Annual Meeting, Society of Environmental Toxicology and Chemistry, Charlotte, NC. (Presented Poster)

Yeager, J.L., A.J. Campbell, D.S. Cherry, and J.R. Bidwell. 1998. The Use of Periphyton to Determine the Influence of Heavy Metal Laden Waters in the Powell River. Nineteenth

Annual Meeting, Society of Environmental Toxicology and Chemistry, Charlotte, NC. (Presented Poster)

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Potesta and Associates, Inc. Report on the Status of Threatened and Endangered Species in the Massey Gap Quarry Permit Area. J.F. Allen Company, June 2001.

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Potesta and Associates, Inc. Sludge Field Soils Testing Report. Hester Industries, Inc. August 2001.

Potesta and Associates, Inc. Results of the Water Chemistry Sampling Conducted in the Kanawha River, South Charleston, West Virginia in August 2001. Clearon Corporation. September 2001

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Potesta and Associates, Inc. Endangered Species Status Report for a Proposed Wind Farm in Grant County, West Virginia. NedPower Mount Storm, LLC. September 2002.

Potesta and Associates, Inc. Endangered Species Status and Phase I Risk Assessment for a Proposed Wind Farm in Grant County, West Virginia. NedPower Mount Storm, LLC. November 2002.

Potesta and Associates, Inc. Results of a Qualitative Benthic Survey in the Mainstem of Rum Creek of the Guyandotte River, Logan County, West Virginia. Logan County Mine Services, Inc. Bandmill Coal Corporation. November 2002.

Potesta and Associates, Inc. Virginia Energy Surface Mine Operation Valley Fill No. 2 Minimal Impact Determination, Hurley, Virginia. Virginia Energy Company. February 2003.

Potesta and Associates, Inc. Feasibility Study of Mitigation/Enhancement Options for the Stony River Basin. Dominium Resource Services, Inc. March 2003.

Potesta and Associates, Inc. Summary of Sediment Sampling and Particle Size Distribution Comparisons Conducted at Sites on the Ohio River for Martin County Coal Corporation February 2001 through November 2002. Martin County Coal Corporation. March 2003

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Potesta and Associates, Inc. Conceptual Mitigation Plan for Stream Restoration and Mitigation White Flame No. 10. White Flame Energy, Inc. June 2003.

Potesta and Associates, Inc. Surface Mine No. 10. Minimal Impact Determination. White Flame Energy, Inc. June 2003.

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Potesta and Associates, Inc. Draft Ephemeral Stream Channel Design Plans, a Supplement to the White Flame No. 10 Compensatory Mitigation Plan. White Flame Energy, Inc. August 2003

Potesta and Associates, Inc. Compensatory Mitigation Plan for Premium Energy Surface Mine No. 3, Amendment 1. Premium Energy, Inc. August 2003.

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Potesta and Associates, Inc. Virginia Energy Lower, Middle and Upper Elk Creek Strip Durable Rock Fills C, D, E and F, Minimal Impact Determination Study, Hurley, Virginia. Virginia Energy Company. August 2003.

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Potesta and Associates, Inc. Draft Biological Assessment for the Potential Adverse Effects of Mining on the Endangered Indiana Bat (*Myotis sodalis*) for White Flame Energy Inc. Proposed Surface Mine No. 10. White Flame Energy, Inc. November 2003.

Potesta and Associates, Inc. Draft Biological Assessment for the Potential Adverse Effects of Mining on Endangered Mussels in the Ohio River. White Flame Energy, Inc. November 2003.

Potesta and Associates, Inc. Summary of the Water Chemistry and Benthic Macroinvertebrate Studies in the Tug Fork 2001 and 2002. Martin County Coal Corporation. November 2003.

Potesta and Associates, Inc. Ephemeral Stream Channel Design Plans, a Supplement to the Premium Energy's Surface Mine No. 2 Compensatory Mitigation Plan. Premium Energy, Inc. December 2003.

Potesta and Associates, Inc. Conceptual Mitigation Plan for the King Coal Highway. Nicewonder Contracting, Inc. December 2003.

Potesta and Associates, Inc. Compensatory Mitigation Plan for Stream Restoration and Mitigation for the Red Jacket Section of the King Coal Highway. Nicewonder Contracting, Inc. January, 2004.

Potesta and Associates, Inc. Mussel Survey Plan. Fola Coal Company. January, 2004.

Potesta and Associates, Inc. Threatened and Endangered Species Study Plan. Nicewonder Contracting, Inc. February, 2004.

Potesta and Associates, Inc. Habitat Assessment for the Indiana Bat and Virginia Big-eared Bat Nicewonder Contracting, Inc. February, 2004.

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Potesta and Associates, Inc. Environmental Information Document for the Red Jacket Section Of the King Coal Highway. Nicewonder Contracting, Inc. April, 2004.

Potesta and Associates, Inc. Stream and Wetland Delineation Report for Bee Run. West ‘ Virginia Division of Natural Resources. May, 2004.

Potesta and Associates, Inc. Compensatory Mitigation Plan for Guyan Surface Mine S-5007-01 Arch Coal, Inc. Apogee Coal Company dba Arch of West Virginia. May 2004

Potesta and Associates, Inc. A Biological Review of Four U.S. Army Corps of Engineers Mitigation Protocols. National Mining Association. May 2004.

PROFESSIONAL ACTIVITY

1994

Hoechst Celanese Corporation

- Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.
- Hoechst Celanese Corporation. Fish sampling, juvenile fry sampling, thermal effluent monitoring to determine the impact of heated effluent on fish effluent on fish reproductive success in macrophyte habitat.

IBM Corporation

- Acute toxicity testing of effluent using *Daphnia pulex* and *Pimephales promelas*.

International Paper Company

- Chronic toxicity testing of effluent using *Ceriodaphnia dubia* and *Pimephales promelas*.

Sonoco Products Company

- Determination of the source of acute toxicity in settling pond effluent. Study included determination of retention time of settling ponds, acute toxicity testing and chemical analysis on influent and final effluent samples.
- Field and laboratory study including benthic macroinvertebrate surveys, water chemistry and effluent toxicity testing to determine the impact of chlorine dioxide treated effluent of the receiving system.

1995

Hoechst Celanese Corporation.

- Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.
- Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.
- Acute toxicity testing using *Ceriodaphnia dubia* and *Pimephales promeles* in treated and untreated filter tip effluent.

IBM Corporation.

- Acute toxicity testing of affluent using *Daphnia pulex* and *Pimephales promelas*.

International Paper Company

- Chronic toxicity testing of effluent using *Ceriodaphnia dubia* and *Pimephales promelas*.

Sonoco Products Company.

- Determination of the source of acute toxicity in settling pond effluent using *Ceriodaphnia dubia*.
- Field and laboratory study including benthic macroinvertebrate surveys, water chemistry and effluent toxicity testing to determine the impact of chlorine dioxide treated effluent of the receiving system.

Virginia Environmental Endowment

- Rapid bioassessment of benthic macroinvertebrate community structure in the Little River watershed to assess habitat degradation and instream community impairment due to agriculture.

1996

Eastman-Kodak Corporation, Tennessee Plant

- Acute toxicity testing of effluent using *Ceriodaphnia dubia.*

Hoechst Celanese Corporation, Celco Plant.

- Evaluation of Chronic Toxicity of 001 Effluent using *Ceriodaphnia dubia* and *Pimephales promelas*.
- Monitoring of chlorination for the control of *Corbicula fluminea* biofouling.

International Paper Company

- Chronic toxicity testing of effluent using *Ceriodaphnia dubia* and *Pimephales promelas*.

Virginia Environmental Endowment

- Rapid bioassessment of benthic macroinvertebrate community structure in the Little River watershed to assess habitat degradation and instream community impairment due to agriculture.

1997 – 1999

Lone Mountain Processing, Inc.

- Responsible for study design, data interpretation report generation and endangered species issues for a coal company on issues relating mine discharge. Specific issues include aquatic toxicity and deposition of precipitates in a receiving stream.

Jackson and Kelly, PLLC

- Development of benthic macroinvertebrate studies on Spruce Mine #1 permit area
- Provided expert testimony in federal court.

Wyatt, Terrance and Combs, PLLC

- Provided technical assistance for lawsuit, including brief and summaries regarding suit brought by the Kentuckians for the Commonwealth in an area the litigants identified as ecologically significant and therefore could not be disturbed.

2000

Anker Energy

- Vindex Facility. Evaluation of permit wasteload allocations from proposed activities located upstream of a designated trout stream.
- Provided stream delineation determination using State and Federal guidelines.

Arch Coal Corporation.

- Assistance in preparation of a report detailing benthic macroinvertebrate surveys conducted supplemental to the US EPA's Environmental Impact Statement on Mountaintop/Valley Fill Coal Mining. Project has included presentation of study findings, review of the US EPA findings, discussions with state and federal regulatory agencies, and revisions in response to comments.

Decota Consulting Company

- Provide assistance with mussel issues and Pritchard Mining Company, Inc. at the Dry Branch Surface Mine (Cabin Creek District, Kanawha County, West Virginia) permit.

Jackson and Kelly, PLLC

- Provided technical assistance regarding Phoenix II litigation.
- Provided technical assistance for Green Valley litigation.

Koppers Industries

- Provided technical assistance for emergency water withdraw.

Massey Coal Corporation.

Martin County Coal

- Spill response involving a 250 million gallon coal mine slurry release including physical, chemical, and biological monitoring, consulting relating to remediation and restoration, liaison with regulatory and emergency response agencies, assessment of damages and negotiations of fines.

Alex Energy

- Evaluate FPOM and CPOM sampling efforts and report finding for inclusion in EIS.
- Prepare biological assessment for threatened and endangered species.

Mettiki Coal

- Evaluation of ephemeral points and surveys for the presence of trout.

2001

Anker Energy

- Provided stream delineation determination using State and Federal guidelines as well as benthic community structure when necessary.
- Provided an evaluation of dredge materials and made recommendations as to whether Anker should allow disposal of these materials at their facility.

Arch Coal Corporation.

- Assistance in preparation of a report detailing benthic macroinvertebrate surveys conducted supplemental to the US EPA's Environmental Impact Statement on Mountaintop/Valley Fill Coal Mining. Project has included presentation of study findings, review of the US EPA findings, discussions with state and federal regulatory agencies, and revisions in response to comments.

Cogentrix

- Evaluation of cooling tower discharge for permitting purposes.

Columbia Gas

- Evaluation of air permit information for compressor stations in Ohio.

Eastern Associated Coal

- Provide assistance with new West Virginia anti-degradation policy and Tier 2.5 listings.

Greer Lime Company

- Provided stream delineation determination using State and Federal guidelines in streams with karst topography.

Hester Industries, Inc

- Evaluation of nitrogen-enriched waste for land application

J. F. Allen Company

- Evaluation of the status of the endangered Virginia northern flying squirrel, *Glaucomys sabrinus fuscus*; the endangered running buffalo clover, *Trifolium stoloniferum*; the threatened Cheat Mountain salamander, *Plethodon nettingi*; and the endangered Indiana bat, *Myotis sodalist* on J.F. Allen property. Provided coordination with USFWS.

Kanawha Eagle, LLC.

- Provided stream delineation determination using State and Federal guidelines.
- Provided recommendations with regard to evaluating impoundment failure.

Massey Coal Corporation

Martin County Coal

- Spill response involving a 250 million gallon coal mine slurry release including physical, chemical, and biological monitoring, consulting relating to remediation and restoration, liaison with regulatory and emergency response agencies, assessment of damages and negotiations of fines.
- Evaluation and review of Environmental Protection Agency field visit, sampling and subsequent report.

Elk Run

- Technical assistance with permit language relating to benthic macroinvertebrate communities structure.

Independence Coal Company

- Evaluation and technical support regarding containment and cleanup of a coal fine material spill.
- Evaluation of benthic macroinvertebrate community following coal fine material spill as per NOV.

Omar Mining

- Evaluation and assessment of fish kill.

Mettiki Coal

- Evaluation of ephemeral points and surveys for the presence of trout.
- Provided surveys to supplement information collected by Potesta in 2000 associated with D-mine.
- Evaluation of wetland communities as they exist in areas with and without deep mining using both plant and benthic communities.

South Putnam Solid Waste Authority

- Provided technical assistance for flow augmentation study.

Summit Engineering

- Provided technical assistance and co-authorship of an environmental impact statement for Alex Energy's Robinson North surface mine permit.

2002

Arch Coal

- Review of Spruce Mine No 1 EIS written by Michael Baker and Associates for biological validity.
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Bright Enterprises

- Technical support regarding threatened and endangered species issues associated with Mount Port Crayon properties.

Eastern Associated Coal

- Provide assistance with West Virginia anti-degradation policy and Tier 2.5 listings.

Greer Lime Company

- Provided stream delineation determination using State and Federal guidelines in streams with steep valleys and historical disturbance.

Jackson and Kelly, PLLC

- Evaluation of CHIA for Mettiki Coal permit appeal.
- Development of study to evaluate effects of flow and deep mining for Mettiki SMB appeal.
- Assistance with litigation associated with State of West Virginia versus Martin County Coal Corporation. This included review of expert reports, pre-deposition meetings, evaluation of State's charges and evaluation of States' evidence. Negotiation meetings with the State.

Massey Energy Company

Martin County Coal

- Spill response involving a 250 million gallon coal mine slurry release including physical, chemical, and biological monitoring, consulting relating to remediation and restoration, liaison with regulatory and emergency response agencies, assessment of damages and negotiations of fines.
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Bandmill Coal Corporation

- Benthic macroinvertebrate survey preformed in accordance with NOV and evaluation of WVDEP recommendations regarding stream restoration as remediation measures for coal fines release from injection pipes.

Elk Run

- Provided additional assistance for permitting of Laxare and West of Stollings.

Raul Sales

- Evaluation of coal fines release upstream of the facility near Gary, West Virginia on the Tug Fork of the Big Sandy River.

NedPower Mount Storm, LLC

- Provided technical assistance and permitting for FAA compliance at a windmill facility.
- Provided technical assistance and produced reports for expert testimony regarding federally listed threatened and endangered species.
- Provided mapping using ArcView.
- Provided 404 permit assistance.
- Provided coordination with USFWS.
- Provided review for counsel (legal services) for PSC hearings.
- Provided technical support for migratory bird issues.

Sammons Law, PLLC

- Technical support for permit reviews.
- Evaluation of Compensatory Mitigation Plans for Virginia Energy.
- Minimal Impact Statements.

2003

Eastern Associated Coal
- Provide assistance with new West Virginia anti-degradation policy and permitting (wasteload allocations) at several Eastern Facilities.
- Socio-economic justification studies.

FOLA Coal Company
- Expert testimony for Surface Mine Board.
- Stream channel design specs for ephemeral, intermittent and perennial stream channels for the compensatory mitigation plan associated with Fola 4a.
- Study plan for multi-year mussel survey.

Jackson and Kelly, PLLC
- Assistance with litigation associated with State of West Virginia versus Martin County Coal Corporation. This included review of expert reports, pre-deposition meetings, evaluation of State's charges and evaluation of States' evidence. Preparation of expert report were also provided to J&K. Extensive work done to determine states methods of determining fines.
- Expert witness testimony for trial associated with fine coal refuse release and downstream business which went bankrupt.
- Technical assistance for same case.
- Technical assistance associated with NWP 21 litigation.

Massey Energy Company

Martin County Coal
- Spill response involving a 250 million gallon coal mine slurry release including physical, chemical, and biological monitoring, consulting relating to remediation and restoration, liaison with regulatory and emergency response agencies, assessment of damages and negotiations of fines.
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Bandmill Coal Corporation
- Benthic macroinvertebrate survey preformed in accordance with NOV and evaluation of WVDEP recommendations regarding stream restoration as remediation measures for coal fines release from injection pipes.

Sammons Law, PLLC
- Technical support for permit reviews.
- Evaluation of compensatory mitigation plans for Virginia Energy.
- Minimal impact statements for White Flame projects.
- Compensatory mitigation plans for Premium Energy projects.
- Translators for multiple facilities.
- Environmental permitting and associated with highway.
- Threatened and endangered species issues.

2004

FOLA Coal Company

- Expert testimony for Surface Mine Board.
- Benthic macroinvertebrate survey reporting for Fola Coal Company
- Benthic macroinvertebrate survey reporting for Powellton's Bridge Fork West Surface Mine Permit
- Individual Permit for USACE preparation and associated components (Compensatory Mitigation Plan and Environmental Information Documents for four pending permits.

Jackson and Kelly, PLLC

- Expert witness testimony for trial associated with fine coal refuse release and downstream business which went bankrupt.
- Technical assistance for same case.
- Technical assistance associated with NWP 21 litigation and Green Valley Coal.
- Preparation of CMP for Green Valley Coal.

Massey Energy Company

Martin County Coal

- Spill response involving a 250 million gallon coal mine slurry release including physical, chemical, and biological monitoring, consulting relating to remediation and restoration, liaison with regulatory and emergency response agencies, assessment of damages and negotiations of fines.

Green Valley Coal

- Benthic macroinvertebrate survey reporting.
- Compensatory mitigation plan for Revision # 5.

Alex Energy

- Benthic macroinvertebrate survey reporting.

Sammons Law, PLLC

- Technical support for permit reviews.
- All permitting and associated support documents (Alternatives Analysis, CMP, EID, etc) for the Red Jacket Section of the King Coal Highway.
- Translators for multiple facilities.
- Threatened and endangered species issues.

RESEARCH EXPERIENCE

Laboratory Skills

Invertebrate Identification
General Water Chemistry, Routine Laboratory Analysis (Chlorophyll a, etc.)
Culturing Cladocerans, Algae, Fish, Unionids
Analytical Chemistry using Atomic Absorption, Gas Chromatography, HPLC

Salt acute and chronic toxicity testing
- *Mysid bahia*
- *Menidia beryline*

Pore water testing
- *Villosa iris*
- *Lampsllis fasciola*

Freshwater acute and chronic toxicity testing
- *Ceriodaphnia dubia*
- *Corbicula fluminea*
- *Daphnia manga*
- *Pimephales promelas*
- *Villosa Iris*
- *Lampsllis fasciola*

Sediment toxicity testing
- *Chironomus riparius*
- *Chironomus tentans*
- *Corbicula fluminea*
- *Daphnia magna*
- *Villose Iris*
- *Lampsllis fasciola*
- *Hexaginia*
- *Hyalella azteca*

Field Monitoring
- Benthic invertebrates sampling and identification
- Fish sampling
- Sediment sampling
- Ichthyoplankton studies
- Periphyton studies
- Mussel surveys
- Global Positioning

Artificial Stream Systems
- Sediment Studies
- Leaf Degradation Studies
- Mussel Continuous-flow Studies

Other Skills
- ArcView
- RiverMorph

RELEVANT COURSEWORK

Undergraduate

General Biology	Environmental Biology	Botany
Zoology	Invertebrate Zoology	Genetics
Molecular Biology	Cell Biology	Microbiology
Environmental Microbiology	Plant Physicology	Biophotography
Physics	General Chemistry	Geology
Instrumental Chemistry	Analytical Chemistry	Organic Chemistry
Physical Chemistry	Field and Laboratory Ecology	Ecology

Graduate

Biometry	Environmental Chemistry	Aquatic Ecotoxicology
Stream Habitat Management	Acid Mine Drainage	Aquatic Entomology
Environmental Fish Physiology	Topics in Freshwater Ecology	Hazard Evaluation
Endangered Species Management	Survey of Acid Mine Drainage Literature	
Environmental Law I	Environmental Law II	

ACTIVITIES AND ORGANIZATION IN COLLEGE AND GRADUATE SCHOOL

American Chemical Society
Kappa Delta Pi
Zeta Tau Alpha – Officer
Freshman Counselor – Co-director
Student Awareness Programming
Student Alumni Council
Student Ambassador
Alcohol Awareness
F.S.C. Foundation Volunteer
Homecoming Court
Judicial Board – Chairperson
United Nations Organization – Officer
Cross Country
Track (Letterman)

References Available on Request