Determination of Acidity, Aluminum, Calcium, Iron, and Manganese Concentrations in Turtle Creek, Westmoreland and Allegheny Counties, PA.

Travis Bickmore

University of Pittsburgh at Greensburg

Department of Natural Science, Mathematics, and Engineering

Greensburg, PA 15601

Research Advisor: Matthew Luderer

Spring 2016

Abstract:

The concentrations of iron, manganese, aluminum, calcium, and acidity have been determined from samples taken at 1.53 miles, 2.80 miles, 3.06 miles, 4.30 miles, and 4.58 miles in Turtle Creek, Westmoreland and Allegheny Counties, PA. Samples were obtained in triplicate from each access point biweekly over 12 weeks (for a total of six sample collections) and then analyzed using flame atomic spectrophotometry (FAAS) techniques. The purpose of this experiment was to provide an analysis of common pollutants pertinent to trout health and acid mine drainage in Turtle Creek at different access points in order to assess the stream's ability to host trout populations.

Introduction:

This study focuses on sampling the Upper Turtle Creek because there is a large concentration of abandoned mines in that area which heavily pollute this section of stream. The upper section of Turtle Creek is approximately 29.0 miles, stretching from its origin in Delmont, PA and flowing into Penn Township, PA.[1] Historically, this section of the stream is in a hotbed of coal mines that were in operation, starting in the early to mid-1800s.[1] This section of the stream has depressed populations of pollution sensitive organisms, such as trout.[1] The importance of accessing the pollutant levels in this section of the stream is important not only because it will provide a more recent assessment of the pollution levels but it may also be used as a reference when determining areas producing the most pollutants.

Sample points were chosen in order to provide the best insight into the impact of AMD on the stream. Sample points were also chosen with respect to those used in the Turtle Creek Watershed TMDL report so that easy comparisons could be made between the two reports. The sample point at mile 1.53 is chosen due to it being close to the origin of the stream which also is the location of the first source of acid mine drainage.[1] The second sample point at mile 2.80, is taken after where Turtle Creek is met by two unnamed tributaries which are both affected by their own AMD sources, none of which are listed as major sources of pollutants by the Civil & Environmental Consultants 2002 report. Directly after this sample point mile 2.80 there is one major source of AMD as well as the confluence of a tributary affected by two other sources of AMD.[1] Samples will be taken at mile 3.06 to determine the change in levels of the above listed pollutants from these three sources. Finally, samples at miles 4.30 and 4.58 are to be taken to assess the levels of pollutants being deposited into the stream by another major source of AMD approximately 4.45 miles into the stream. The location of the sources of AMD are approximated using the 2002 report by the Civil & Environmental Consultants report.[1] Figure 1 provides an illustration of the location of sample points on the stream.

The Watershed:

The Turtle Creek Watershed encompasses roughly 150 square miles of forested, farmed, and urbanized land, stretching from its origin in Westmoreland County to the Monongahela River in Allegheny County in southwest Pennsylvania.[2] The stream winds through a hotbed of coal mines, which operated for roughly 100 years from the mid-1800s to the mid-1900s.[2] Since their closing, the mines have filled with ground water, becoming laden with pollutants which eventually flow into the creek. Areas in the creek that had once been rich in various species of trout have recently been uninhabitable to trout due to AMD. [1] The stream has historically been sporadic in its accommodation to freshwater wildlife as detailed by the Turtle Creek Watershed River Conservation Plan. As outlined in the report, the stream has a history of high levels of pollution, causing trout stocking in the upper section of Turtle Creek to stop after multiple attempts to remedy the issue. The mine drainage and other local sources of pollution laden the creek with iron, manganese, aluminum, and calcium in addition to depressing the pH of the stream water.[1]

Previous Studies of the Turtle Creek Watershed:

In 2002, Civil & Environmental Consultants Incorporated composed the Turtle Creek Watershed River Conservation Plan, which includes a comprehensive history, detail of all known previous assessments of the stream pollutants, and assessment of the watershed in the then current condition.[1] This study was commissioned by the Turtle Creek Watershed Association (TCWA) to provide a detail of the characteristics of the watershed, to identify sources of pollutants, and to provide a recommended plan of action to begin to remedy the pollutants. The plan discusses a major fish kill that occurred in 1989, which killed approximately 3,200 hatchery bred brown trout (*Salmo trutta*) and rainbow trout (*Oncorhynchus mykiss*), as well as 2,500 forage fish.[1] The reason for the fish kill was that there was an extreme lowering of the stream pH, approximately 4.06, as well as acutely high levels of aluminum.[1] The fish developed mucus over their gills, thus preventing the exchange of dissolved gases and ions with the surrounding water.[1] Fish were stocked again in 1991 but another fish kill experienced in 1993 ceased stocking efforts until later when reclamation efforts were implemented.[1]

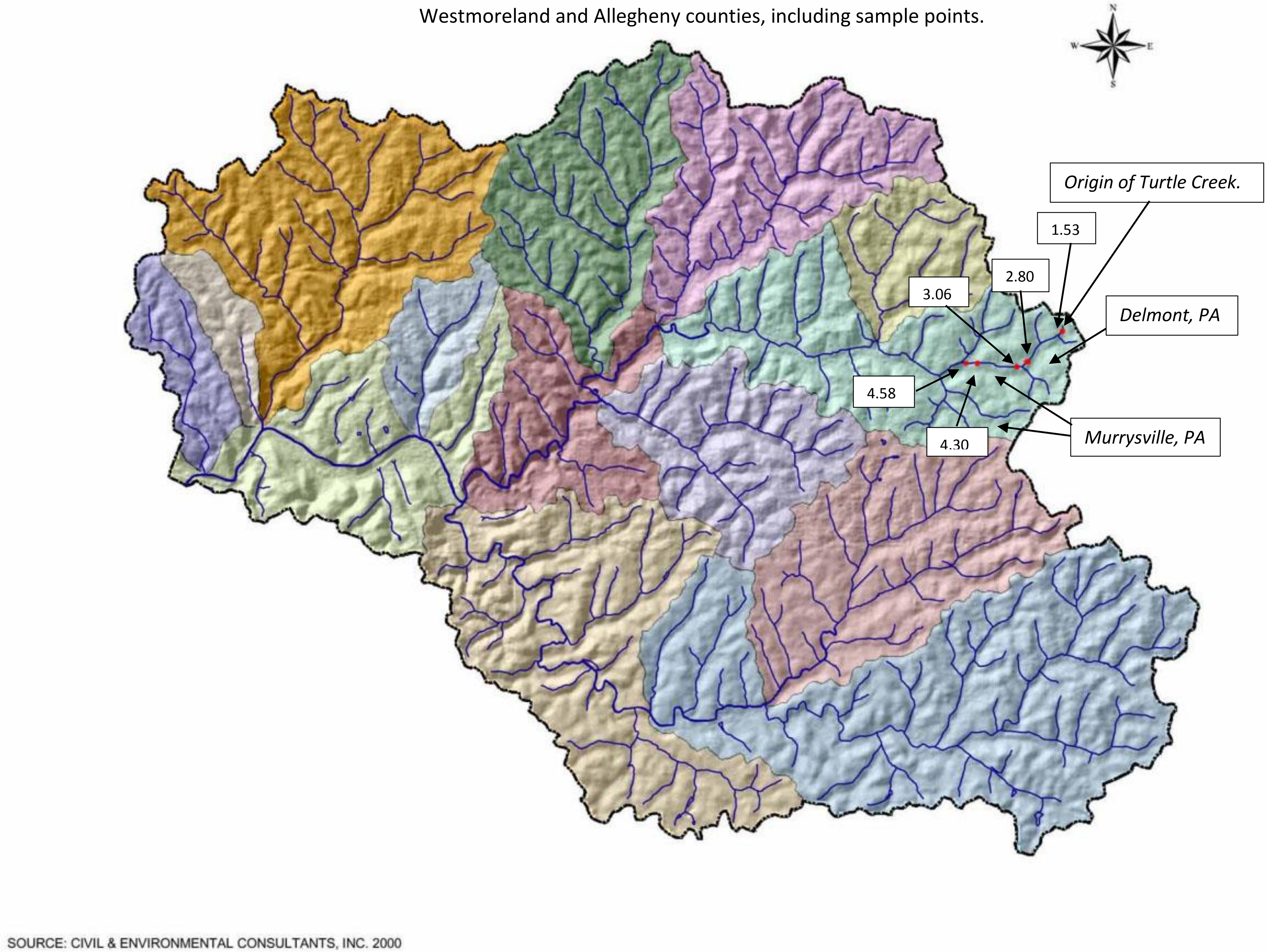
The most recent survey of stream metals was prepared by the Pennsylvania Department of Environmental Protection in 2009.[2] This report is especially beneficial for this study's purposes because it considers the long term exposure the creek has experienced to the AMD sources occurring in the upper section of the creek by stating its recorded values as long term total maximum daily loads. For the Delmont discharges (sample points 1.53 and 2.80) the report focuses on the elevated levels of aluminum and iron that are in the stream, reporting the need for a 59% decrease in aluminum and a 98% decrease in iron (Table 1).[2] Concentrations reported were

0.67 mg/L of aluminum and 27.08 mg/L of iron. The report also states that pH levels ranging from 3.4 to 5.3 were found in this section.[2] The Export discharge between sample points 4.30 and 4.58 was found to have a pH ranging from 2.8 to 3.2.[2] Also in this section aluminum concentrations of 10.32 mg/L were found along with a concentration of 2.06 mg/L of iron.[2] The levels of manganese were not observed in the upper section of the stream. The levels of calcium was not reported in this study.

Table 1: Percent reduction of Aluminum, Iron, and Acidity necessary to meet water quality standards in the Delmont mine drainage section of Turtle Creek.[2]

Parameter	Existing Load (lbs/day)	TMDL Allowable Load (lbs/day)	NPS % Reduction required
Delmont- Delmont Deep Mine Discharge			
Aluminum (lbs/day)	6.44	2.64	59%
Iron (lbs/day)	280.81	5.22	98%
Acidity (lbs/day)	1733.71	121.36	93%

Figure 1: Map of the Turtle Creek Watershed[1], encompassing portions of Westmoreland and Allegheny counties, including sample points.



Detail of Pollutants:

Calcium, a bleached grey, soft metal is of extreme importance in the health of vertebrates.[3] The metal is essential to vertebrate bone health, muscle contraction, nerve function, osmotic pressure maintenance, flow of ions and electrolytes, intracellular regulatory functions, and plays roles in reproduction.[4] Calcium is naturally found in freshwater streams because when the water flows over rocks, like limestone, it dissolves calcium where it may be taken in by trout and/or other organisms[5]. Normal freshwater streams and watersheds contain approximately 4-100 mg/L of calcium; for trout at least 25 mg/L are needed.[5] When trout inhabit streams with low calcium, their reproduction slows, muscle contractions may be interfered with, electrolyte flow is disrupted, their bones may become weak and brittle, and their nerve function is impaired.[4] Waters with less than 10 mg/L of calcium usually can only support sparse plant and algae life.[5] Two common pollutants, calcium sulfite and calcium sulfate, are byproducts of coal production.[6] These pollutants will cause a stream to become more acidic and harmful to the stream's wildlife, which will be discussed in greater detail further in this introduction.[6] It is also notable that calcium plays a role in the lethality of aluminum pollution, as discussed in the previous paragraph. Because of its role in the regulation of electrolytes in fish it can hinder the effects of aluminum toxicity.[4] In soft acids calcium will negate the electrolyte draining effects of aluminum.[4] Be that as it may, the aluminum will still be toxic in this case because it will cause the fish to be anemic.[4]

The pH, or the logarithmic concentration of hydrogen ion concentration, of a stream not only directly influences the growth and overall health of stream wildlife but it also is a great factor in the availability of nutrients as well as lessening or heightening the toxicity of dissolved metals.[7,8] In 2011 Raymond Menendez conducted an 11 month analysis of hatchery brook trout (*Salvelinus fontinalis*), utilizing all levels of trout development, in varying levels of pH depression. The pH levels studied were 4.5, 5.0, 5.5, 6.0, 6.5, and a control of 7.1.[9] The study found that embryos' survival rates were reduced significantly as the pH was depressed beyond 6.5.[9] The trout in the depressed pH environments also did not grow as large as the control fish as well as producing less viable eggs, most severely at 5.0 and lower.[9] The results of this study demonstrated that at pH levels below 6.5 trout development is stunted as well as reduced viability of their embryos.[9]

Another study published in the Canadian Journal of Zoology aimed at determining a lethal pH for brook trout (*Salvelinus fontinalis*).[10] Fingerling trout were subjected to acute and chronic exposure to dilutions of sulfuric acid and sodium hydroxide to determine lethality in temperatures of 10^{o}C and 20^{o}C.[10] The study found that a pH of 3.5 and below as well as 9.8 and above proved lethal to trout with no difference between temperature groups as well as without regard to trout length or weight.[10] Heath describes in his book *Water Pollution and Fish Physiology* that the lethality is due to the acidity inhibiting the intake of electrolytes through the gills while promoting the loss of electrolytes. He states when the water is at a pH of 4 the intake of electrolytes stops almost completely and the loss of electrolytes is greater than fish in an environment with a neutral pH.[4]

Iron is a silver grey metal that is most abundant element in the earth's crust.[11] Iron has an atomic mass of 55.84 g/mol and when non-oxidized has a solubility of 60 mg/L.[12] However, when streams are heavily laden with the metal, the water and surrounding oxygen will oxidize the iron producing ferric hydroxide, $Fe(OH)_3$.[13] The heavily concentrated ferric hydroxide forms an orange-red precipitate, as seen in Figure 2, which is commonly seen in streams heavily polluted with AMD.[13] The precipitate forms a film over everything it comes in contact with, including fish eggs and gills.[13] The precipitate also creates a film over the entire stream bed thus eliminating habitat for spawning fish and insects as well as eliminating invertebrates that are vital to trout feeding.[13] For these reasons, in extreme excess, iron is hazardous to fish populations.

Figure 2: A stream in Pennsylvania with obvious discoloration caused by iron pollution.[14]

Manganese, atomic number 25, is a silver metal and is brittle in its metallic form.[15] While it has little industrial use in its metallic form it is, however, very useful as a component in alloys.[15] The metal is the 5th most abundant element in the earth's crust.[15] Because of its high abundance, it is also commonly found in AMD and, consequently, is a common pollutant in streams. Many streams with manganese as the most abundant pollutant are visually black because of the manganese precipitate covering the stream bed.[4] The high concentrations are toxic to trout life, causing their blood sodium levels to drastically diminish and eventually causing death.[4] The manganese pollution in trout is also hazardous to any life form higher in the food chain. Continued consumption of trout with high manganese levels or consumption of water with extremely high levels of manganese can cause Chronic Manganese Poisoning (CMP).[16] CMP results in severe neurological symptoms and disorders, degrading the basal ganglia and possibly resulting in death.[17]

Aluminum, the 3rd most abundant element in the earth's crust is also a common pollutant in streams affected by mine drainage, acid rain, and land that has been strip mined.[4] The lethality of high aluminum concentrations for trout populations is a complex issue. High concentrations of aluminum are lethal to fish, but the methodology behind their death relies on the concentration of calcium and pH of the water.[4] Water with a low pH and high concentrations of aluminum cause death in fish by making them rapidly lose electrolytes while also severely reducing the amount the fish can take in through their gills, eventually causing death.[4] As the pH of the water rises to approximately 4.8 the loss of electrolytes by aluminum inhibition decreases although the aluminum now causes hypoxia, also resulting in death.[4] High levels of calcium appear to decrease the effects of the excess aluminum except in water with hard acids, where the aluminum causes hypoxia.[4] One adaption trout possess, however, is that in chronic exposure to high aluminum concentrations, the gills appear to decrease the binding of aluminum, thus combating their high blood volumes of the metal.[4]

<u>Aims of Proposed Study:</u>

The purpose of this study is to determine if the upper Turtle Creek is safe for fish stocking. The study will determine the concentrations of Aluminum (Al), Calcium (Ca), Iron (Fe), acidity, and manganese (Mn) concentrations in water at five access point in the upper Turtle Creek over a period of twelve weeks. I hypothesize that the concentrations of the metallic pollutants will be abnormally high and unsafe. I believe that the acidity of the stream will be too depressed for aquatic wildlife to thrive.

Materials and Methods:

Determination of Aluminum, Calcium, Iron, and Manganese Concentrations:

Stock nitric acid was diluted to 5% nitric acid as needed by using the dilution formula ($M_1V_1=M_2V_2$). A magnetic stir bar was placed carefully placed in a 1000 mL graduated cylinder and placed on a magnetic stir plate. First, approximately 800 mL of distilled water is added to the graduated cylinder. Next, the calculated amount of stock nitric acid (71.5 mL) was carefully and slowly added to the graduated cylinder while stirring. Then, the solution is diluted to the 1000 mL mark with distilled water while still on the magnetic stir plate.

The solution was then transferred to a 1000 mL Nalgene storage bottle.

The metals being analyzed in this experiment are Aluminum, Calcium, Iron, and Manganese. Standard solutions will be prepared for each metal. Linear limits and standard concentrations of each metal are shown in Table 2. The metal stock solutions used to prepare these standard solutions have a concentration of 1000 parts per million (ppm). The dilution equation, $M_1V_1=M_2V_2$, will then be used in the preparation of these standard solutions for each metal. First, approximately 50 mL of 5% nitric acid will be added to a 100 mL volumetric flask using a funnel. The appropriate amount of stock metal solution will be transferred to the volumetric flask using a micropipette. The solution will then be diluted to the 100mL mark using the 5% nitric acid. Then the standard solutions will be transferred to a 125 mL Nalgene storage bottle.

Table 2: Linear Limits and Standard Concentrations of Metals

Metal	Linear Limit (ppm)	Standard Concentrations (ppm)
Aluminum (Al)	100	0.00, 1.00, 2.00, 3.00, 4.00, 5.00

Calcium (Ca)	5.00	0.00, 1.00, 2.00, 3.00, 4.00, 5.00
Iron (Fe)	10.0	0.00, 1.00, 2.00, 3.00, 5.00, 10.0
Manganese (Mn)	2.00	0.00, 0.25, 0.50, 1.00, 1.50, 2.00

The Perkin Elmer Atomic Absorption Spectrometer AAnalyst 200 will be prepared by setting the correct position for the flame and lamp. After the instrument has been prepared for analysis, a reagent blank will be aspirated and the absorbance will be triplicated, averaged, and recorded. The AMD samples will then be aspirated and the absorbance will be triplicated, averaged, and recorded. After each AMD sample has been analyzed, the instrument will be reset for the next metal and the samples will be reanalyzed for the new selected metal. The concentration of each metal will be determined by the absorbance and related to the calibration curves for each metal and presented in parts per million (ppm).

Determination of Acidity:

Before the samples were acidified the pH was to be determined. An electronic pH meter was calibrated and kept moist throughout the experimentation. The pH meter's probe was rinsed thoroughly with distilled water before testing each sample to prevent contamination falsifying the results. The pH meter was dipped in the sample until a stable reading was reached. The pH meter would then be rinsed with distilled water again to prevent future contamination.

List of Equipment:

Equipment	Quantity
Magnetic Stir Plate	1
Magnetic Stir Bar	1
Micropipette (20-200 µL)	1
Micropipette (100-1000 µL)	1
Perkin Elmer Atomic Absorption Spectrometer AAnalyst 200	1
pH meter	1

List of Glassware:

Glassware	Quantity
Nalgene Storage Bottles (300 mL)	90
Nalgene Storage Bottles (1000 mL)	4
Volumetric Flask (100 mL)	6
Graduated Cylinder (1000 mL)	1
Graduated Cylinder (10 mL)	1
Stirring Rods	3
Short Stem Glass Funnel	3
Beaker (250 mL)	3

List of Chemicals:

Glassware	Quantity
Calcium AA Standard (1,000 ppm Ca) in 3% HNO_3 100 mL	1
Manganese AA Standard (1,000 ppm Mn) in 3% HNO_3 100 mL	1
Iron AA Standard (1,000 ppm Fe) in 3% HNO_3 100 mL	1
Aluminum AA Standard (1,000 ppm Al) in 3% HNO_3 100 mL	1
HCl 6M (0.1 L)	1
Deionized water (3.0 L)	1

Budget:

Item	Quantity	Price
Magnetic Stir Plate	1	UPG
Magnetic Stir Bar	1	UPG
Micropipette (20-200 µL)	1	UPG
Micropipette (100-1000 µL)	1	UPG
Perkin Elmer Atomic Absorption Spectrometer AAnalyst 200	1	UPG
pH meter	1	UPG
Nalgene Storage Bottles (300 mL)	90	UPG
Nalgene Storage Bottles (1000 mL)	4	UPG
Volumetric Flask (100 mL)	6	UPG
Graduated Cylinder (1000 mL)	1	UPG
Graduated Cylinder (10 mL)	1	UPG
Stirring Rods	3	UPG
Short Stem Glass Funnel	3	UPG
Beaker (250 mL)	3	UPG
Calcium AA Standard (1,000 ppm Ca) in 3% HNO_3 100 mL	1	$14.02
Manganese AA Standard (1,000 ppm Mn) in 3% HNO_3 100 mL	1	$14.02
Iron AA Standard (1,000 ppm Fe) in 3% HNO_3 100 mL	1	$14.51
Aluminum AA Standard (1,000 ppm Al) in 3% HNO_3 100 mL	1	$14.86
HCl 6M (0.1 L)	1	UPG
Deionized water (3.0 L)	1	UPG

Results:

It was hypothesized that the pH levels of all samples would be depressed beyond acceptable limits and the levels of metal contaminants would be elevated beyond safe levels. The pH levels were found to be as expected, far too depressed for any chance at supporting trout or any other freshwater wildlife. The metal concentrations were found to be partly as expected. Aluminum was found to be almost entirely within normal limits, mostly ranging within the middle of acceptable freshwater levels. Only at sample points 2 and 3 was Aluminum found to be above acceptable limits, reaching at its peak 11.1ppm. Calcium was found to be within acceptable limits, even being acceptable for normal drinking water. Iron was found to be in extreme excess in all points of the creek. These levels however were found to be acceptable during the colder months when the mines from which the AMD is sourced from may have frozen over. Manganese levels were found to be elevated beyond acceptable limits for chronic exposure in all areas sampled in every week sampled. The results for all metals and pH are listed in the below tables 3-7. Each table, with the exception of table 3, includes the absorbance, concentration ppm, standard deviation (SD), and relative standard deviation (RSD) along with the calibration curve equation. There were 92 water samples collected over 12 weeks, all of which were tested. In total there were 18 samples collected at each sample point with the exception of the Borland Farm Road Tributary which only had 2 samples collected on 03/28/2016. The Borland Farm Road Tributary was tested with excess materials to obtain additional data about its contents it brought into Turtle Creek. Each sample was approximately 125mL in volume.

Table 3: pH Results

Sample Date: 01/14/2016	Sample Point		pH
	1	a	4.3
		b	4.3
		c	4.3
	2	a	3.5
		b	3.5
		c	3.4
	3	a	3.5
		b	3.5
		c	3.5
	4	a	4.3
		b	4.4
		c	4.4
	5	a	4.3

		b	4.3
		c	4.3
Sample Date: 01/28/2016	Sample Point		pH
	1	a	4.3
		b	4.3
		c	4.3
	2	a	3.5
		b	3.5
		c	3.5
	3	a	3.5
		b	3.5
		c	3.5
	4	a	4.4
		b	4.4
		c	4.4
	5	a	4.4
		b	4.4
		c	4.4
Sample Date: 02/11/2016	Sample Point		pH
	1	a	4.1
		b	4.2
		c	4.1
	2	a	3.2
		b	3.2
		c	3.2
	3	a	3.2
		b	3.2
		c	3.2
	4	a	4.2
		b	4.2
		c	4.2
	5	a	4.1
		b	4.1
		c	4.1
Sample Date: 02/28/2016	Sample Point		pH
	1	a	4.2
		b	4.2
		c	4.1
	2	a	3.4
		b	3.3
		c	3.2
	3	a	3.1
		b	3.2

		c	3.2
	4	a	4.0
		b	4.0
		c	4.0
	5	a	4.3
		b	4.3
		c	4.2
Sample Date: 03/10/2016	Sample Point		pH
	1	a	4.1
		b	4.1
		c	4.1
	2	a	3.3
		b	3.3
		c	3.3
	3	a	3.3
		b	3.3
		c	3.3
	4	a	4.3
		b	4.3
		c	4.4
	5	a	4.6
		b	4.6
		c	4.6
Sample Date: 03/28/2016	Sample Point		pH
	1	a	4.2
		b	4.2
		c	4.2
	2	a	3.3
		b	3.3
		c	3.3
	3	a	3.3
		b	3.3
		c	3.3
	4	a	4.2
		b	4.3
		c	4.4
	5	a	4.6
		b	4.6
		c	4.6
	Borland Farm Rd Tributary	a	5.0
		b	5.1

Table 4: Aluminum Results

Sample Date 01/14/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.015	0.0001	0.5	5.54
	b	0.015	0.0002	1.6	5.54
	c	0.015	0.0002	1.5	5.54
2	a	0.025	0.0002	0.6	9.23
	b	0.028	0.0001	0.5	10.3
	c	0.028	0.0002	0.6	10.3
3	a	0.028	0.0002	0.9	10.3
	b	0.028	0.0002	0.6	10.3
	c	0.028	0.0003	1.2	10.3
4	a	0.013	0.0002	1.7	4.80
	b	0.014	0.0001	0.9	5.17
	c	0.014	0.0003	2.4	5.17
5	a	0.011	0.0001	1.1	4.06
	b	0.011	0.0002	1.4	4.06
	c	0.011	0.0002	1.4	4.06
Sample Date 01/28/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.016	0.0002	1.0	5.90
	b	0.016	0.0001	0.3	5.90
	c	0.016	0.0002	1.4	5.90
2	a	0.029	0.0004	1.5	10.7
	b	0.028	0.0001	0.2	10.3
	c	0.029	0.0002	0.8	10.7
3	a	0.029	0.0002	0.6	10.7
	b	0.029	0.0002	0.8	10.7
	c	0.029	0.0006	2.0	10.7
4	a	0.015	0.0001	0.9	5.54
	b	0.015	0.0003	2.0	5.54
	c	0.016	0.0003	2.2	5.90
5	a	0.011	0.0001	1.2	4.06
	b	0.011	0.0001	1.2	4.06
	c	0.011	0.0002	1.9	4.06
Sample Date 02/11/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.024	0.0002	0.6	8.86
	b	0.024	0.0001	2.1	8.86
	c	0.024	0.0002	1.3	8.86
2	a	0.029	0.0004	0.4	10.7

	b	0.029	0.0001	0.1	10.7
	c	0.029	0.0002	0.4	10.7
3	a	0.029	0.0002	0.7	10.7
	b	0.029	0.0002	1.0	10.7
	c	0.030	0.0006	1.6	11.1
4	a	0.017	0.0001	0.6	6.27
	b	0.018	0.0003	0.2	6.64
	c	0.018	0.0003	0.1	6.64
5	a	0.009	0.0001	1.0	3.32
	b	0.008	0.0001	2.1	2.95
	c	0.008	0.0002	1.5	2.95
Sample Date 02/25/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.025	0.0003	1.1	9.23
	b	0.025	0.0004	1.6	9.23
	c	0.026	0.0002	0.8	9.59
2	a	0.028	0.0002	0.7	10.3
	b	0.027	0.0003	0.9	9.96
	c	0.027	0.0002	0.7	9.96
3	a	0.028	0.0001	0.5	10.3
	b	0.028	0.0006	2.2	10.3
	c	0.028	0.0001	0.5	10.3
4	a	0.016	0.0003	1.7	5.90
	b	0.016	0.0002	0.9	5.90
	c	0.016	0.0001	0.6	5.90
5	a	0.011	0.0002	2.2	4.06
	b	0.011	0.0002	2.2	4.06
	c	0.011	0.0001	0.5	4.06
Sample Date 03/10/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.025	0.0003	1.2	9.23
	b	0.023	0.0002	0.9	8.49
	c	0.023	0.0002	1.0	8.49
2	a	0.028	0.0003	1.1	10.3
	b	0.028	0.0002	0.7	10.3
	c	0.027	0.0002	0.7	9.96
3	a	0.028	0.0004	1.3	10.3
	b	0.028	0.0001	0.4	10.3
	c	0.027	0.0002	0.7	9.96
4	a	0.016	0.0002	1.6	5.90
	b	0.016	0.0003	1.9	5.90
	c	0.016	0.0002	1.4	5.90
5	a	0.012	0.0000	0.2	4.43

	b	0.012	0.0001	1.1	4.43
	c	0.013	0.0001	1.1	4.80
Sample Date 03/24/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.021	0.0001	0.7	7.75
	b	0.022	0.0003	1.2	8.12
	c	0.022	0.0003	1.4	8.12
2	a	0.027	0.0002	0.7	9.96
	b	0.027	0.0002	0.7	9.96
	c	0.028	0.0002	0.8	10.3
3	a	0.028	0.0006	2.2	10.3
	b	0.028	0.0005	1.9	10.3
	c	0.029	0.0001	0.5	10.7
4	a	0.015	0.0005	3.9	5.54
	b	0.016	0.0003	2.0	5.90
	c	0.015	0.0000	0.3	5.54
5	a	0.012	0.0002	1.5	4.43
	b	0.012	0.0001	1.2	4.43
	c	0.012	0.0000	0.2	4.43
Borland Farm Rd Tributary	a	0.006	0.0001	2.3	2.21
	b	0.006	0.0000	0.8	2.21
Slope: .00271		Y intercept: 0.0000	Equation: y=.00271x	R2: .985967	

Table 5: Calcium Results

Sample Date: 01/14/2016					
Sample ID		Mean	SD	RSD%	PPM
1	A	1.635	0.0096	0.6	61.98
	B	1.637	0.0079	0.5	62.05
	C	1.623	0.0050	0.3	61.52
2	A	1.380	0.0056	0.4	52.31
	B	1.393	0.0008	0.1	52.81
	C	1.392	0.0008	0.1	52.77
3	A	1.400	0.0051	0.4	53.07
	B	1.394	0.0048	0.3	52.84
	C	1.399	0.0032	0.2	53.03
4	A	1.545	0.0034	0.2	58.57
	B	1.548	0.0067	0.4	58.68
	C	1.561	0.0052	0.3	59.17

5	A	1.511	0.0049	0.3	57.28
	B	1.517	0.0021	0.1	57.51
	C	1.501	0.0049	0.3	56.90
Sample Date – 01/28/2016					
Sample ID		Mean	SD	RSD%	PPM
1	A	1.646	0.0085	0.5	62.40
	B	1.642	0.0034	0.2	62.24
	C	1.646	0.0102	0.6	62.40
2	A	1.396	0.0071	0.5	52.92
	B	1.399	0.0131	0.9	53.03
	C	1.393	0.0052	0.4	52.81
3	A	1.399	0.0037	0.3	53.03
	B	1.392	0.0025	0.2	52.77
	C	1.398	0.0030	0.2	52.99
4	A	1.545	0.0012	0.1	58.57
	B	1.542	0.0030	0.2	58.45
	C	1.551	0.0070	0.5	58.79
5	A	1.521	0.0015	0.1	57.66
	B	1.521	0.0035	0.2	57.66
	C	1.518	0.0101	0.7	57.54
Sample Date – 02/11/2016					
Sample ID		Mean	SD	RSD%	PPM
1	A	1.487	0.0019	0.1	56.37
	B	1.492	0.0007	0.0	56.56
	C	1.493	0.0013	0.1	56.60
2	A	1.320	0.0042	0.3	50.04
	B	1.326	0.0061	0.5	50.27
	C	1.303	0.0073	0.6	49.39
3	A	1.323	0.0064	0.5	50.15
	B	1.325	0.0035	0.3	50.23
	C	1.323	0.0016	0.1	50.15
4	A	1.433	0.0060	0.4	54.32
	B	1.441	0.0028	0.2	54.62
	C	1.446	0.0024	0.2	54.81
5	A	1.471	0.0062	0.4	55.76
	B	1.467	0.0063	0.4	55.61
	C	1.477	0.0061	0.4	55.99
Sample Date – 02/25/2016					
Sample ID		Mean	SD	RSD%	PPM
1	A	1.346	0.0020	0.2	51.02
	B	1.343	0.0047	0.4	50.91

	C	1.351	0.0016	0.1	51.21
2	A	1.255	0.0016	0.1	47.57
	B	1.244	0.0038	0.3	47.16
	C	1.249	0.0073	0.6	47.35
3	A	1.246	0.0008	0.1	47.23
	B	1.252	0.0018	0.1	47.46
	C	1.258	0.0076	0.6	47.69
4	A	1.322	0.0092	0.7	50.11
	B	1.329	0.0027	0.2	50.38
	C	1.328	0.0118	0.9	50.34
5	A	1.352	0.0028	0.2	51.25
	B	1.364	0.0032	0.2	51.71
	C	1.357	0.0022	0.2	51.44
Sample Date – 03/10/2016					
Sample ID		Mean	SD	RSD%	PPM
1	A	1.448	0.0034	0.2	54.89
	B	1.436	0.0044	0.3	54.44
	C	1.447	0.0019	0.1	54.85
2	A	1.348	0.0064	0.5	51.10
	B	1.340	0.0072	0.5	50.80
	C	1.315	0.0241	1.8	49.85
3	A	1.342	0.0020	0.1	50.87
	B	1.346	0.0020	0.2	51.02
	C	1.344	0.0063	0.5	50.95
4	A	1.388	0.0065	0.5	52.62
	B	1.388	0.0029	0.2	52.62
	C	1.385	0.0050	0.4	52.50
5	A	1.421	0.0053	0.4	53.87
	B	1.423	0.0014	0.1	53.94
	C	1.439	0.0089	0.6	54.55
Sample Date – 03/24/2016					
Sample ID		Mean	SD	RSD%	PPM
1	A	1.456	0.0133	0.9	55.19
	B	1.456	0.0017	0.1	55.19
	C	1.454	0.0079	0.5	55.12
2	A	1.347	0.0012	0.1	51.06
	B	1.337	0.0175	1.3	50.68
	C	1.329	0.0079	0.6	50.38
3	A	1.342	0.0044	0.3	50.87
	B	1.345	0.0048	0.4	50.99
	C	1.345	0.0060	0.4	50.99

4	A	1.399	0.0025	0.2	53.03
	B	1.399	0.0072	0.5	53.03
	C	1.386	0.0060	0.4	52.54
5	A	1.421	0.0023	0.2	53.87
	B	1.424	0.0030	0.2	53.98
	C	1.425	0.0040	0.3	54.02
Borland Farm Rd Tributary	A	1.480	0.0005	0.0	56.10
	B	1.486	0.0005	0.0	56.33
Slope: .02638		Y intercept: 0.0000	Equation: Y=.02638x	R^2=.994008	

Table 6: Iron Results

Sample Date 01/14/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.000	0.0001	100.2	0.000
	b	0.000	0.0001	24.5	0.000
	c	0.002	0.0001	8.1	0.145
2	a	0.000	0.0001	17.4	0.000
	b	0.000	0.0002	76.9	0.000
	c	0.000	0.0002	474.3	0.000
3	a	0.000	0.0002	51.2	0.000
	b	0.000	0.0001	662.1	0.000
	c	0.000	0.0002	234.7	0.000
4	a	0.001	0.0003	22.2	0.073
	b	0.003	0.0004	16.5	0.218
	c	0.001	0.0005	36.8	0.073
5	a	0.039	0.0001	0.3	2.83
	b	0.013	0.0003	2.5	0.945
	c	0.039	0.0006	1.6	2.83
Sample Date 01/28/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.002	0.00	4.30	0.145
	b	0.001	0.00	24.40	0.073
	c	0.002	0.00	16.80	0.145
2	a	0.000	0.00	120.10	0.000
	b	0.000	0.00	133.20	0.000
	c	0.000	0.00	163.70	0.000
3	a	0.000	0.00	104.10	0.000
	b	0.000	0.00	238.90	0.000

	c	0.000	0.00	124.40	0.000
4	a	0.004	0.00	3.10	0.291
	b	0.005	0.00	4.70	0.363
	c	0.002	0.00	14.40	0.145
5	a	0.000	0.00	20.00	0.000
	b	0.000	0.00	233.00	0.000
	c	0.000	0.00	87.00	0.000
Sample Date 02/11/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.047	0.00	0.60	3.42
	b	0.046	0.00	0.30	3.34
	c	0.031	0.00	0.70	2.25
2	a	0.006	0.00	2.80	0.436
	b	0.005	0.00	4.90	0.363
	c	0.010	0.00	4.80	0.727
3	a	0.004	0.00	1.40	0.291
	b	0.003	0.00	8.00	0.218
	c	0.004	0.00	7.40	0.291
4	a	0.061	0.00	1.20	4.43
	b	0.051	0.00	0.40	3.71
	c	0.054	0.00	0.50	3.92
5	a	0.063	0.00	0.40	4.58
	b	0.070	0.00	0.30	5.09
	c	0.071	0.00	0.90	5.16
Sample Date 02/25/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.047	0.0004	0.9	3.42
	b	0.045	0.0006	1.3	3.27
	c	0.045	0.0001	0.3	3.27
2	a	0.030	0.0004	1.4	2.18
	b	0.029	0.0002	0.5	2.11
	c	0.030	0.0002	0.5	2.18
3	a	0.030	0.0002	0.7	2.18
	b	0.030	0.0003	0.9	2.18
	c	0.030	0.0002	0.2	2.18
4	a	0.116	0.0004	0.3	8.43
	b	0.116	0.0002	0.2	8.43
	c	0.118	0.0007	0.6	8.58
5	a	0.172	0.0009	0.5	12.5
	b	0.170	0.001	0.6	12.4
	c	0.171	0.0011	0.7	12.4
Sample Date 03/10/2016					
Sample ID		Mean	SD	RSD%	PPM

1	a	0.051	0.0005	1	3.71
	b	0.034	0.0004	1.2	2.47
	c	0.034	0.0003	1.0	2.47
2	a	0.02	0.0001	0.8	1.45
	b	0.019	0.0003	1.6	1.38
	c	0.02	0.0003	1.5	1.45
3	a	0.019	0.0007	3.7	1.38
	b	0.019	0.0005	2.5	1.38
	c	0.019	0.0001	0.7	1.38
4	a	0.147	0.0006	0.4	10.7
	b	0.147	0.0001	0.1	10.7
	c	0.145	0.0007	0.5	10.5
5	a	0.235	0.0014	0.6	17.1
	b	0.235	0.0011	0.5	17.1
	c	0.237	0.0008	0.3	17.2
Sample Date 03/24/2016					
Sample ID		Mean	SD	RSD%	PPM
1	a	0.035	0.0005	1.5	2.54
	b	0.037	0.0001	0.4	2.69
	c	0.033	0.0002	0.7	2.40
2	a	0.018	0.0003	1.5	1.31
	b	0.019	0.0003	1.7	1.38
	c	0.019	0.0002	1.2	1.38
3	a	0.019	0.0001	0.7	1.38
	b	0.018	0.0002	1.1	1.31
	c	0.018	0.0001	0.7	1.31
4	a	0.146	0.0004	0.3	10.6
	b	0.146	0.0007	0.5	10.6
	c	0.147	0.0005	0.3	10.7
5	a	0.235	0.0007	0.3	17.1
	b	0.234	0.0009	0.4	17.0
	c	0.236	0.0008	0.3	17.2
Borland Farm Rd Tributary	a	0.395	0.0016	0.4	28.7
	b	0.380	0.0006	0.2	27.6
Slope: .01376		Y intercept: 0.0000	Equation: y=.01376x	R2: .985967	

Table 7: Manganese Results

Sample Date 01/14/2016					
Sample Point		Mean	SD	RSD%	PPM
1	A	0.072	0.0005	0.8	2.28
	B	0.073	0.0003	0.4	2.31
	C	0.073	0.0008	1.1	2.31
2	A	0.064	0.0007	1.1	2.03
	B	0.064	0.0003	0.4	2.03
	C	0.064	0.0002	0.3	2.03
3	A	0.064	0.0004	0.7	2.03
	B	0.064	0.0003	0.5	2.03
	C	0.064	0.0001	0.1	2.03
4	A	0.055	0.0002	0.4	1.74
	B	0.056	0.0004	0.7	1.77
	C	0.056	0.0003	0.5	1.77
5	A	0.059	0.0002	0.4	1.87
	B	0.059	0.0001	0.2	1.87
	C	0.058	0.0005	0.9	1.84
Sample Date – 01/28/2016					
Sample Point		Mean	SD	RSD%	PPM
1	A	0.075	0.0003	0.5	2.38
	B	0.075	0.0002	0.3	2.38
	C	0.075	0.0006	0.7	2.38
2	A	0.065	0.0001	0.1	2.06
	B	0.066	0.0002	0.2	2.09
	C	0.066	0.0004	0.7	2.09
3	A	0.066	0.0003	0.5	2.09
	B	0.066	0.0002	0.5	2.09
	C	0.066	0.0003	0.4	2.09
4	A	0.057	0.0005	0.9	1.81
	B	0.058	0.0001	0.2	1.84
	C	0.057	0.0002	0.3	1.81
5	A	0.059	0.0003	0.5	1.87
	B	0.059	0.0001	0.2	1.87
	C	0.059	0.0003	0.4	1.87
Sample Date – 02/11/2016					
Sample Point		Mean	SD	RSD%	PPM
1	A	0.079	0.0003	0.3	2.50
	B	0.079	0.0002	0.3	2.50
	C	0.080	0.0004	0.4	2.53
2	A	0.063	0.0004	0.6	2.00

	B	0.063	0.0003	0.4	1.99
	C	0.064	0.0004	0.6	2.03
3	A	0.063	0.0003	0.5	2.00
	B	0.063	0.0002	0.3	2.00
	C	0.063	0.0004	0.6	2.00
4	A	0.060	0.0006	1.0	1.90
	B	0.061	0.0003	0.6	1.93
	C	0.060	0.0004	0.6	1.90
5	A	0.060	0.0003	0.5	1.90
	B	0.061	0.0001	0.1	1.93
	C	0.061	0.0002	0.3	1.93
Sample Date – 02/25/2016					
Sample Point		Mean	SD	RSD%	
1	A	0.071	0.0005	0.8	2.25
	B	0.072	0.0004	0.5	2.28
	C	0.072	0.0005	0.7	2.28
2	A	0.059	0.0004	0.6	1.87
	B	0.059	0.0002	0.3	1.87
	C	0.060	0.0004	0.6	1.90
3	A	0.060	0.0003	0.5	1.90
	B	0.060	0.0003	0.5	1.90
	C	0.060	0.0003	0.5	1.90
4	A	0.057	0.0002	0.4	1.81
	B	0.057	0.0002	0.4	1.81
	C	0.058	0.0002	0.4	1.84
5	A	0.053	0.0001	0.2	1.68
	B	0.053	0.0001	0.2	1.68
	C	0.053	0.0002	0.3	1.68
Sample Date – 03/10/2016					
Sample Point		Mean	SD	RSD%	PPM
1	A	0.083	0.0003	0.3	2.63
	B	0.082	0.0004	0.5	2.60
	C	0.082	0.0004	0.5	2.60
2	A	0.062	0.0002	0.4	1.96
	B	0.063	0.0004	0.6	2.00
	C	0.063	0.0005	0.9	2.00
3	A	0.062	0.0003	0.4	1.96
	B	0.063	0.0005	0.8	2.00
	C	0.063	0.0003	0.4	2.00
4	A	0.059	0.0003	0.5	1.87
	B	0.059	0.0003	0.5	1.87
	C	0.059	0.0005	0.9	1.87
5	A	0.061	0.0003	0.5	1.93

	B	0.061	0.0002	0.4	1.93
	C	0.061	0.0005	0.8	1.93
Sample Date – 03/24/2016					
Sample Point		Mean	SD	RSD%	PPM
1	A	0.082	0.0006	0.8	2.60
	B	0.081	0.0001	0.2	2.57
	C	0.082	0.0002	0.3	2.60
2	A	0.062	0.0002	0.3	1.96
	B	0.062	0.0002	0.4	1.96
	C	0.062	0.0002	0.3	1.96
3	A	0.062	0.0001	0.2	1.96
	B	0.061	0.0004	0.6	1.93
	C	0.064	0.0004	0.6	2.03
4	A	0.059	0.0003	0.5	1.87
	B	0.059	0.0000	0.1	1.87
	C	0.059	0.0004	0.8	1.87
5	A	0.061	0.0002	0.2	1.93
	B	0.061	0.0003	0.5	1.93
	C	0.062	0.0001	0.1	1.96
Borland Farm Rd Tributary	A	0.063	0.0003	0.6	2.00
	B	0.064	0.0004	0.6	2.02
Y intercept: 0.0000		R2= .997494	Equation: y=.03158x	Slope: 0.03158	

Discussion:

As discussed in the results the Upper Turtle Creek cannot support any freshwater wildlife with its current conditions. Excessively low pH as well as elevated metal contaminants, with the exception of calcium, make this stream unsuitable for trout stocking. This is also a concern because the contaminants found in this study flow downstream to the Lower Turtle Creek, a popular recreational fishing stream. In addition, the Upper Turtle Creek would obviously also be hazardous if consumed by humans.

Table three shows the measured pH of all 92 water samples taken over the 12 weeks of study. The pH of samples ran from 3.1 to 4.6 (5.1 if including Borland Farm Tributary). Only the

Borland Farm Road Tributary would have any chance at supporting trout life with regard to only pH. However the trout embryo viability would be severely reduced and any trout growing in the stream would be of much reduced size. The Turtle Creek would not support any trout life due to the pH even if the metals were within normal limits.

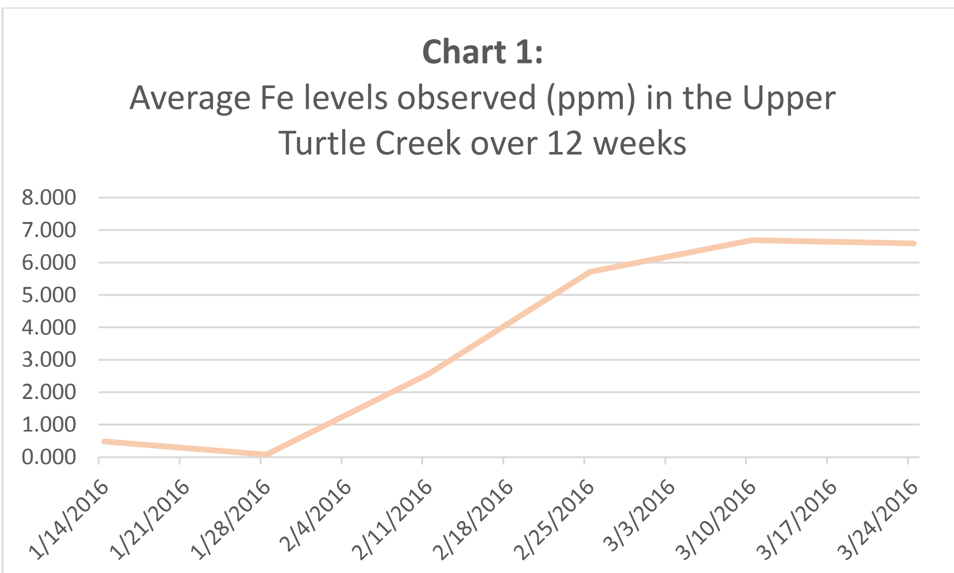
As seen in table four it can be observed that the levels of aluminum observed in the Upper Turtle Creek mostly range within normal limits except in sample points 2 and 3. The levels ran from 2.95 ppm to 11.1 ppm. Aluminum levels will increase significantly in streams with decreased pH levels like those seen in the Upper Turtle Creek.[18] If the pH of the stream was remedied then the aluminum levels may potentially fall with the rising pH due to decreased solubility of aluminum salts and aluminum oxide.[18]

Table five contains the calcium levels recorded throughout the course of the study. In all sample sites and dates the calcium levels were found to be within normal limits (4ppm to 100ppm for freshwater).[19] The calcium however would be sufficient to interact with the elevated aluminum to be toxic to trout.

Table six shows the data for iron. The iron ranged from 0.000ppm to 17.2 ppm (28.7ppm in Borland Farm Road Tributary). The acceptable range of iron in stream water is between 0.5ppm and 1.0ppm.[20] While it may seem from just this range that the stream may, in some parts, be within acceptable ranges it is not so. The same sample points contained much higher levels of iron when the weather warmed above freezing. The iron present in this section of Turtle Creek alone would make the stream unsuitable for freshwater life.

Table seven shows the data for manganese. The levels found ranged from 1.68ppm to 2.60ppm and were fairly consistent throughout all sample dates. Sample points 1 through 3 had consistently higher values than those found in sample points 4 and 5. The normal levels of manganese for freshwater though are 0.1ppm to 1.0ppm for freshwater aquatic life.[21] As can be seen by this the levels observed in the Upper Turtle Creek are much higher than what should be present in the stream.

One possible source of error for the experiment would be that the first three sample dates were in months where AMD laden tributaries may have frozen and artificially lowered the levels of contaminants in Turtle Creek. There is evidence of this when observing the collected values of iron as seen below in chart 1. As can be seen in the below graph the iron levels increase as the temperature increases, supporting that the ice stemming flow from AMD laden tributaries melted, releasing the AMD contaminants back into Turtle Creek.



Conclusion:

In conclusion, the determination of pH, aluminum, calcium, iron, and manganese in the Upper Turtle Creek was a successful experiment. The pH, aluminum, iron, and manganese all are in too much of extremes to currently support any freshwater aquatic life. Potentially substantial interventions would be necessary to remedy the stream.

Acknowledgements:

I would like to thank Dr. Luderer for being my research advisor as well as for his patience and time he took out of his personal life to help me throughout the research. Delanie Wilson also deserves special thanks for her assistance in sample collection as well as her help throughout testing. I would also like to thank Kevin Kaplan & the Turtle Creek Watershed Association for their cooperation and assistance with obtaining vital information pertinent to this project. Finally, I would like to thank Jeff Bickmore and Shannon Bickmore for their assistance in sample collection.

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