VIRGINIA WATER RESOURCES RESEARCH CENTER

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Research Opportunities in Interdisciplinary Watershed Sciences and Engineering

Edited

By

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Acknowledgments

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Dedicated to the memory of our friend and colleague
Dr. G. V. Loganathan who passed away on April 16, 2007

Summary of Research Program

A unique aspect of our REU site was that REU fellows were placed in different academic departments doing research on diverse aspects of water sciences and engineering. The research program was designed in such a manner that REU fellows be involved in individual research but also learn techniques of a good researcher, and how to communicate research results verbally and in writing by attending mandatory weekly forums. Weekly forums provided an opportunity for fellows to present their research to their peers and advisors. This approach served several purposes: 1) it provided an opportunity to program directors to gauge fellows' progress; 2) fellows gained self confidence by making these presentations (almost all did not have previous experience); 3) this process facilitated the opportunity to learn from each other and also broaden the participants' horizon about various research topics; and 4) gave participants practical experience in communicating scientific research to peers and outsiders.

Another unique feature of our REU site was that research advisors were not limited to principal investigators and senior personnel listed in the submitted proposal. Principal investigators made an attempt to identify other researchers and laboratories on Virginia Tech campus that were in good match with selected REU participants' research interests. These additional advisors included Dr. Jack Webster (Biological Sciences), Dr. Marc Edwards (Civil and Environmental Engineering) and Dr. Thomas Burbey (Geosciences).

About two weeks before program completion, fellows were provided with a set of guidelines for writing research papers and example journal article. Fellows submitted their first draft of paper to program directors and research advisors on July 30, 2007. Research advisors and mentors reviewed draft reports and made suggestions for improvement. This document is a compilation of research papers submitted to program directors.

Program directors provided clear guidance to REU fellows regarding preparation of their PowerPoint presentations and presentation of research results. The following strategy was adopted for making fellows comfortable in communicating their research objectives/results: 1) The first time, fellows were asked to make a 3- minute presentation and discuss objectives of their research; duration of presentations were gradually increased to 5 minutes, 10 minutes, and 15 minutes (final presentation at the culmination of program); 2) About midway in the program, fellows were provided with an evaluation sheet and asked to rate each other for clarity and content of presentation; and 3) On one occasion names were randomly drawn and each fellow was asked to make a presentation about the other person's research. This was a way to make sure they are attentive to each other's presentation and learn something about each other's research and to provide feedback to participants regarding their success in communicating their research. On August 3, NSF REU fellows made a 15-minute final presentation. Fellows presented their research objectives and results to research advisors and invited guests. About 25 persons including the fellows attended these presentations. Each presentation was followed by 4-5 minutes of question/answer and discussion. One research advisor (Dr. Kachroo) joined the forum via Web Cam from UNLV.

This document contains research reports prepared by the REU fellows under direction of their research mentors/advisors.

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Solubility as a Mechanism for CSMR Effects on Lead Leaching

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Abstract

The solution chemistry of Pb(II) at relatively low pH (≈ 3-5) was examined in the presence of chloride, sulfate, and phosphate. Lead sulfate solids are relatively insoluble even at pH 3. Pb(II) forms soluble complexes with Cl- that can significantly increase the solubility of lead. Since lead solubility can be correlated to lead contamination problems in drinking water, the data suggest that chloride will worsen lead problems at low pH, whereas sulfate will decrease lead problems. The contradictory trends for the two anions may provide a mechanistic explanation for the success of the empirical chloride:sulfate mass ratio in explaining lead contamination of potable water. Specifically, the trends explain why lead contamination sometimes worsens when changing from alum (higher sulfate) to ferric chloride (higher chloride) coagulants.

Keywords: coagulant changeover; galvanic corrosion; CSMR; lead sulfate solubility; lead chloride complexes

Introduction

The widespread occurrence of lead-copper connections in water distribution systems is a potential public health hazard. Examples of common lead bearing plumbing-copper pipe connections include 1) brass fixtures connected to copper pipe, 2) Pb:Sn solder joints in copper pipe, and 3) copper pipe-lead pipe connections at service lines. Lead contamination of water in these systems can be exacerbated by the connection to copper, which results in accelerated corrosion of the lead material due to galvanic effects (Petrucci 1997).

The copper surface acts as the cathode and is the site of numerous reduction reactions including the reduction of oxygen:

$$O_2 + 4e^- + 2H_2O \rightarrow 4OH^- \quad \text{(R1)}$$

The corresponding anodic reaction is the oxidation of lead to lead (II):

$$Pb \rightarrow Pb^{2+} + 2e^- \quad \text{(R2)}$$

The production of lead (II), a Lewis acid, causes a local pH drop at the anode (i.e., the lead bearing pipe surface). Since lower pH often increases corrosivity and lead leaching, this is a significant concern. A pH drop of 3 units was observed near the lead surface of a 50:50 lead-tin solder connection to copper pipe (Triantafillydou 2006).

It has been demonstrated in both case studies and bench scale work that a high chloride to sulfate mass ratio (CSMR) can cause increased lead contamination of potable water (Triantafillydou 2006; Dudi 2004). High CSMR in drinking water has been attributed to utilities switching from aluminum sulfate coagulants to chloride-based coagulants such as ferric chloride or poly-aluminum chloride for more effective removal of particulates. In some cases, the lead problems caused by this changeover have not been resolved by the addition of orthophosphate, which is the standard procedure for dealing with lead leaching issues.

The low pH observed at the surface of the lead anode may be the key to unraveling this mystery. Most prior research has examined lead solubility at the pH range of bulk water, which is much higher than at the lead surface during these contamination events and is therefore unrepresentative. Moreover, chloride and sulfate concentrations tend to increase markedly at the lead anode surface, and effects of these higher anion concentrations on lead solubility has not been thoroughly explored.

In this work, the solubility of Pb(II) at lower pH in the presence of sulfate, chloride and phosphate is examined to better understand lead contamination that occurs in drinking water with galvanically coupled lead-copper connections.

Research Methods

Lead speciation was quantified by a variety of techniques. Soluble lead was determined after membrane filtration. Particulate lead was determined as the difference between known total lead and measured soluble lead, or by dissolution of the solids collected on the surface of the membrane filter. At several fixed pHs lead speciation was determined in the presence and absence of chloride, sulfate, and phosphate. Observations are compared to existing models.

Existing models predicted that a lead sulfate solid can form on the surface of lead anodes even at very low pH when SO_4^{2-} levels become high enough, tending to decrease leaching at the lead surface. Higher Cl^- is predicted to increase lead solubility by formation of $PbCl_n^{(2-n)}$ complexes; $PbCl^+$ was specifically investigated in this study and an equilibrium constant for its formation at pH 3 was estimated. Phosphate corrosion inhibitors are very unlikely to be effective at this lower pH range, because lead phosphates tend to form at higher pHs.

Low pH Solubility Studies – Sulfate Addition

Chemicals were added as reagent grade salts. All solutions were prepared by first dissolving 0.48 mM $Pb(NO_3)_2$ in distilled and deionized water and adding HNO_3 to bring the solution to pH 3 or 4. The sulfate concentrations in these solutions were varied from 0 to 2.66 mM SO_4^{2-} by adding Na_2SO_4. $NaNO_3$ was added in variable amounts to maintain a constant ionic strength of 0.01 for each solution (Table 1). The pH was then finalized to pH 3.00 or 4.00 ± 0.05. After 24 hours, unfiltered and filtered (through 0.45 μm pore size nylon filters) samples were collected for each solution and preserved with 2% nitric acid. The lead and sulfate concentrations were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Low pH Solubility Studies – Chloride Addition

All chemicals used in this study were added as reagent grade salts. All solutions were prepared by first dissolving 0.48 mM $Pb(NO_3)_2$ in distilled and deionized water and adding HNO_3 to bring the solution to pH 3. The chloride concentrations in these solutions were then varied from 0 to 8 mM Cl^- by adding NaCl. $NaNO_3$ was added in variable amounts to maintain a constant ionic strength of 0.01 for each solution (Table 2). The final pH was adjusted to 3.00 ± 0.05. After 24 hours, the free Pb concentration was measured, and a sample was collected and preserved with 2% nitric acid to determine the total soluble Pb concentration. Free Pb was measured using a Pb^{2+} ion specific electrode (ISE). Total soluble Pb was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) after passing the solutions through a nylon filter with 0.45 μm pore size.

Table 1. Sulfate and Nitrate Concentrations

Na_2SO_4 added (mM)	$NaNO_3$ added (mM)
0	8
0.33	7
0.66	6
1.33	4
2	2
2.33	1
2.66	0

Table 2. Chloride and Nitrate Concentrations

NaCl added (mM)	$NaNO_3$ added (mM)
0	8
2	6
4	4
6	2
8	0

Low pH Solubility Studies – Phosphate Addition

Chemicals were added as reagent grade salts. All solutions were prepared by first dissolving 0.48 mM $Pb(NO_3)_2$ in distilled and deionized water. The Na_2HPO_4 concentration in these solutions was adjusted to 1.26 mM. The pH was varied from 3 to 5 by adding HNO_3. After 24 hours, unfiltered and filtered (through 0.45 μm pore size nylon filters) samples were collected for each solution and preserved with 2% nitric acid. The lead and phosphate concentrations were measured using Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Results and Discussion

Low pH Solubility Studies – Sulfate Addition

The addition of sulfate at pH 3 and 4 caused a large decrease in soluble lead as the amount of sulfate added increased. The results at pH 3 were not always consistent in the magnitude of the change, but the overall trend remained the same across all trials (Figure 1). The results at pH 4 were very consistent (Figure 2).

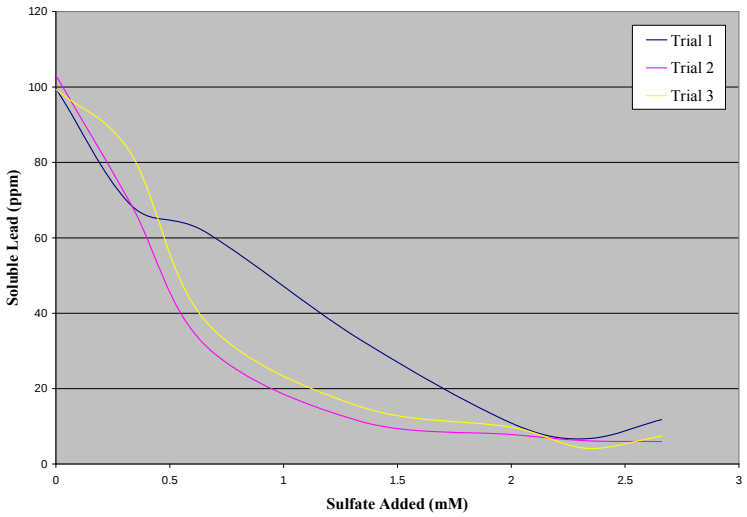

Figure 1. **Effect of Sulfate Addition on Soluble Lead at pH 3**

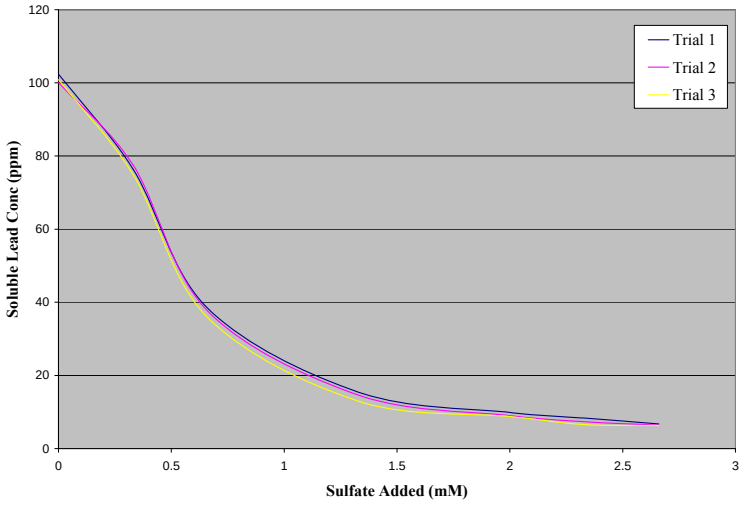

Figure 2. Effect of Sulfate Addition on Soluble Lead at pH 4

Analysis of the precipitate revealed a ratio of lead to sulfate in the precipitate of 0.930 ± .088.

When these trials were averaged and the two pH values were compared, very little difference was apparent in the solubility of lead sulfate from pH 3 to pH 4 (Figure 3). Though the formation of a hydroxide-containing precipitate is being investigated, the data in Figure 3 seems to suggest that hydroxide is not part of the solid given the similarity in solubility as pH increases.

Combining these two pieces of evidence, the precipitate is assumed to be $PbSO_4$ (s), which has a K_{sp} expression

$$K_{sp} = [Pb^{2+}][SO_4^{2-}] \quad (1)$$

Optimized constants were determined as $K_{sp} = 2.44 \text{ X } 10^{-8}$ ($R^2 = 0.67$) and $K_{sp} = 2.53 \text{ X } 10^{-8}$ ($R^2 = 0.97$) for pH 3 and 4 respectively.

Low pH Solubility Studies – Chloride Addition

When the concentration of chloride was varied at pH 3, very little decrease was seen in the soluble lead concentration, whereas the free lead (determined by ion specific electrode) decreased (Figure 4). The explanation is that chloride reacts with the Pb(II) to form soluble complexes with lead. Using the free and total lead concentrations measured, a K value can be estimated for the formation of $PbCl^+$.

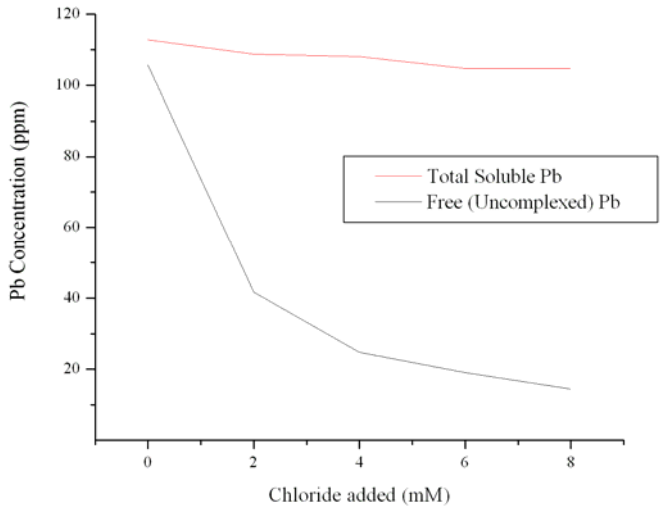

Figure 4. Effect of Chloride Addition on Soluble/Free Lead

The preliminary data collected are consistent with formation of $PbCl^+$. The molar concentration of the lead chloride complex is equal to the difference between the total soluble lead and the Pb^{2+}. The formation reaction:

$$Pb^{2+} + Cl^- \rightarrow PbCl^+ \quad (R3)$$

has an equilibrium expression:

$$K = [PbCl^+]/[Pb^{2+}][Cl^-] \quad (2)$$

An optimized constant of K = 8.6 X 10^2 was determined by the method of least squares (R^2 = 0.991), (Figure 5).

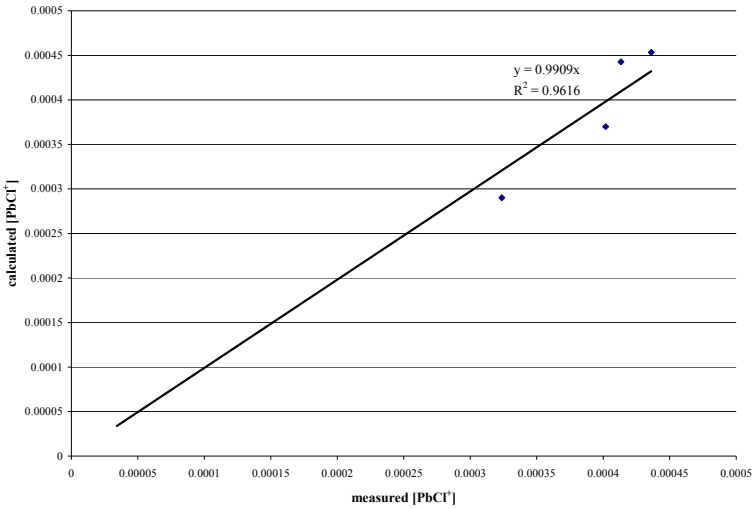

Figure 5. Evaluation of K for the Formation of [$PbCl^+$]

Low pH Solubility Studies – Phosphate Addition

As the pH was lowered from 4 to 3, there was a sudden increase in soluble lead concentration. This suggests that phosphate does not effectively passivate lead surfaces at low pH (Figure 6).

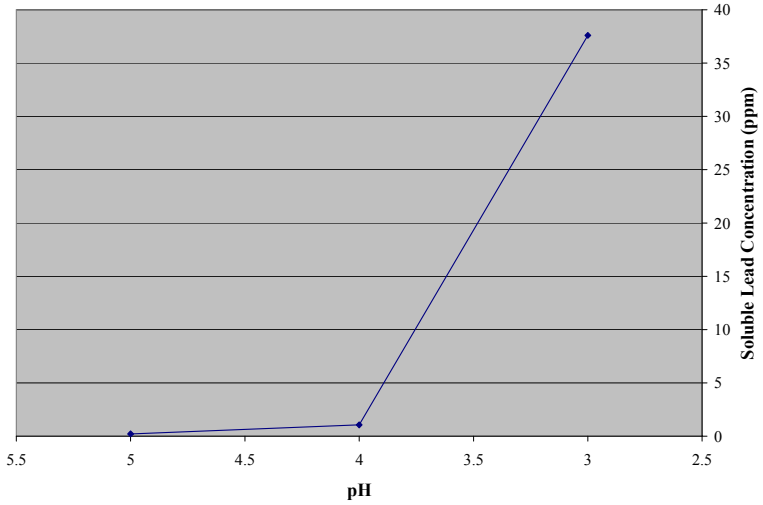

Figure 6. Effect of pH on Lead Phosphate Solubility

Conclusions

- At pH 3 and pH 4, the concentration of soluble lead decreases with the addition of sulfate.
- At low pHs of 3-4, changes in the pH have little effect on the solubility of lead sulfate.
- According to the data collected, the K_{sp} at pH 3 for $PbSO_4$ (s) is 2.44×10^{-8} and the K_{sp} at pH 4 for $PbSO_4$ (s) is 2.53×10^{-8}
- At pH 3, the uncomplexed lead concentration decreases with the addition of chloride, presumable as a lead chloride complex forms.
- According to the data obtained from a lead (II) ISE, the equilibrium constant (K) for the formation of $PbCl^+$ is 8.6×10^2.
- Decreasing the pH with a constant phosphate concentration increases the solubility of lead phosphate compounds and limits the effectiveness of phosphate as a corrosion inhibitor.

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Determination of the Taste Threshold of Iron in Water

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Abstract

The effect of iron on human health is a relevant issue in the food and beverage, and the drinking water industries. The impact of iron taste is important as many food and beverage products are being fortified with iron. Iron can also be found in drinking water as a naturally occurring mineral in groundwater or resulting from the corrosion of metal piping in the water distribution system. This study determined the taste threshold for ferrous iron at near neutral pH. This is of particular importance for the drinking water industry, which provides water at or near pH 7.0 and attempts to avoid customer complaints from aesthetic problems. A one-of-five test was used to determine a threshold with solutions containing 0.002 – 5 mg/L of Fe^{2+} in Nanopure water. Individual thresholds for iron ranged from 0.003 mg/L to 5 mg/L Fe^{2+} with population thresholds calculated by geometric mean and logistic regression being 0.052 and 0.031 mg/L respectively.

Keywords: iron, taste, threshold, drinking water

Introduction

Iron is a critical nutrient for which the World Health Organization (WHO) recommends a daily intake of 10 to 50 mg depending on age, gender, and bioavailability of dietary iron (WHO 1996). Iron is critical to the performance of several internal functions including oxygen transport and DNA synthesis. A deficiency of iron can cause impaired mental and motor development, altered behavior, and most commonly, anaemia. Iron deficiency is the most common nutritional disorder worldwide and is most likely the only significant nutritional deficiency found in industrialized countries. The food and beverage industry has been attempting to aid in the solution of the problem by fortifying some foods and drinks with iron. Recently the drinking water industry has also begun to provide iron fortification in order to help prevent or eliminate Iron deficiency as a problem in industrialized countries (Allen and Casterline-Sable 2001).

Iron in drinking water generally comes from two main sources. In the case of groundwater usage as drinking water, iron may be present as a naturally occurring element at higher than normal levels. If the drinking water provided is surface water, and has been treated prior to being piped to users, iron present has most likely been removed, however, corrosion of metal pipes may introduce iron to the drinking water (Volk et al. 2000). Iron piping should not be used if the water being distributed is heavily acidic or alkaline because these waters tend to corrode iron piping.

There are only a few studies on the taste of iron in drinking water. Iron has been described as having a bitter, bloody, astringent or metallic flavor. Compounds such as $FeSO_4$ have been proposed as a prototypical metallic compound and as a reference standard in food sensory evaluation (Civille and Lyon

1996). It has been proposed that iron perception is not only gustatory, and the detection may rely heavily on olfaction (Hettinger et al. 1990; Lawless et al. 2004).

Drinking water health standards have not been established for Iron due to its low toxicity, however, it can cause severe illness at consumption levels above 1 mg/kg of body weight per day (WHO 1996). The United States Environmental Protection Agency (USEPA) has established a Secondary Maximum Contaminant Level (SMCL) for iron as 0.3 mg/L to prevent aesthetic problems (color, taste, odor) in drinking water. Above this level drinking water utilities may expect consumer complaints about a metallic taste or sensation upon drinking the contaminated water or complaints about the discoloration of water due to the presence of ferric iron.

Previous studies show a wide variation in the individual threshold values of Iron in drinking water. Determined threshold concentrations have ranged from 0.04 to above 20 ppm (Cohen et al. 1960). The goal of this research was to determine a more concise flavor threshold for iron concentration in drinking water, in order to aid the drinking water industry in providing acceptable water to the general public.

Iron Chemistry

Iron flavor thresholds determine the concentrations of ferrous iron at which 50 % of the panelists can detect the presence of iron in their water. In water, iron can exist as ferrous or ferric cations with its speciation controlled by pH and pE, the later being a measure of the redox potential of the water. In ground waters that contain low oxygen, the reduced ferrous species tends to dominate and is generally present as a colorless dissolved ion.. In oxygenated surface waters or drinking water treated with an oxidant, the ferric form is more prevalent. Ferric iron has a low solubility as its hydroxide salts and consequently forms an orange-brown precipitate that discolors water if not removed by treatment. Iron can also enter drinking water from corroding iron pipes.

Redox cycling between ferrous and ferric iron occurs naturally in water and in the human mouth/skin, depending on pH, pE, and the presence of reducing bacteria (Nealson and Saffarini 1994), and chemical agents such as ascorbic acid (Vitamin C) which is common in many foods and beverages (Glindemann et al. 2006). Thus, even though ferric iron may be more prevalent in water distributed to peoples homes, ferrous iron can still form in the actual water that is ingested. Depending on the concentration, consumers may be able to taste or smell the iron in the water and it may create customer dissatisfaction. If the consumer is unable to detect iron and the level present in the drinking water is high (> 3.0 mg/L) it may cause illness.

Previous research has demonstrated that iron in the mouth has both taste and odor components, thus people perceive a “flavor”. The perception of ferrous iron is reduced if the nose is occluded, indicating that ferrous iron produces a retronasal odor (Lawless et al. 2004). Ferrous iron reacts with skin lipids to produce the “metallic” odor and a reproducible series of n-aldehydes, including n-hexanal, and ketones, including 1-octene-3-one. The odor threshold of 1-octen-3-one in air to be 0.05 ppb, which indicates humans are extremely sensitive (Glindemann et al. 2006).

Materials and Methods

Panel Description

Thirty healthy (volunteer) panelists were tested for the study. None had participated in iron tasting studies before, although some had participated in controlled taste testing in a study with copper prior to this study. The panelists consisted of 15 male and 15 females. Their ages ranged from 18 to 60 years.

The sensory protocol used was approved by the Institutional Review Board at Virginia Tech and all panelists read and signed an informed consent form.

Panelists were asked to complete a questionnaire about their personal habits to help in the development of reasoning for their ability or inability to taste iron. As a group, the panelists drank a varying range of water per day, with some drinking very little (0 to 1 8 oz. glass) and some drinking a considerable amount (4 or more 8 oz. glasses). Their water preferences were also variable, with some preferring tap water, and others bottled or mineral water. Several panelists were occasional smokers, none were heavy smokers.

Iron Stimuli
A 100 mg/l iron stock was prepared from Iron (II) Sulfate (Sigma-Aldrich, PA, CAS # 13463-43-g). The stock solution was then diluted to obtain concentrations in the range of .002 – 10 mg/l Fe. All iron samples were prepared fresh daily in an effort to prevent the oxidation of ferrous Iron to ferric iron, which would then precipitate.

Thirteen concentrations (0.002, 0.005, 0.01, 0.02, 0.05, 0.1 0.2 0.5 1, 2, 3, 4, and 5 mg/l total Fe) were used for threshold testing. Concentration intervals were not uniform but generally followed a doubling pattern. The concentrations were verified using either a flame atomic adsorption spectrometry (Perkin Elmer 5100) or and inductively coupled plasma – mass spectroscopy (Thermo X-Series).

Test water sample preparation
A Barnstead Nanopure system was fed distilled water and subsequently deionized and carbon filtered it to produce distilled-deionized water. This system produced water with a chemical resistivity of approximately 18 MΩ/cm and a pH of approximately 6.

Nanopure water was used both as the standard water and to prepare the iron solutions. 1 L glass bottles were used to produce the solution by adding the appropriate amount of iron stock solution to obtain the desired sample water concentration. This was done by performing the required calculations based on the concentration of the stock solution and the concentration of the sample water desired. The appropriate amount of water was then removed from the distilled deionized water, to be replaced by stock solution. An eppendorf dispenser was used to measure the stock solution and add it to the Nanopure water.

Sample water concentrations were then verified using either the atomic adsorption spectrometry or the inductively coupled plasma – mass spectroscopy. If the concentration was not within a tenth of the desired concentration the sample water was adjusted and re-tested.

Threshold determination of ferrous iron in Nanopure water

The experiment determined the concentration of iron that consumers would be able to taste in water. The taste thresholds were determined for each panelist individually. The panelists tested in the study demonstrated taste thresholds ranging from 2 ppb to 5 ppm. The individual thresholds that were found were used to determine a panel wide threshold concentration, and the panel wide threshold may be projected onto the entire population.

Sensory Procedure
All test waters were presented at room temperature (22 – 24^{o} C). Panels were conducted in a closed room with minimal noise or odor influence. For each test, the control, rinse water and iron solution were presented at the same pH.

In order to decrease the likelihood of correctly guessing, a one-of-five forced choice test was used instead of a triangle test. This decreased the guessing probability from 33 to 20%. The one-of-five test consisted

of four cups filled with control water and one with sample water. A 1 of 5 test was also used by Beguin-Bruhin et al. (1983), Zacarias et al. (2001) and Cuppett et al. (2006) when testing for copper threshold concentrations.

Only one session was performed per day to avoid any effect of aftertaste. Although there is very little information on the aftertaste of iron, it has been noted that copper, another common metal produces a prevalent aftertaste. Beguin-Bruhin et al. (1983), Zacarias et al. (2001) and Cuppet et al. (2006) administered only one copper concentration per session to prevent the effect of aftertaste.

Five 3 oz. white plastic sample cups were coded with randomly chosen three digit numbers. Three digit numbers were used to reduce bias. They were then filled with 20 ml of sample and/or control water and presented to panelists in a randomized order. Panelists were also provided with two larger (9 oz.) cups, one filled with control or Nanopure water, the other empty to be used for expectoration. Also provided was a simple score card that consisted of five boxes labeled with the numbers used on the cups and with a place for name and date of the panelists.

Panelists were instructed first to rinse with the water provided. They were then told to taste the samples from left to right. Each sample was to be swished around the mouth for approximately 20 seconds and then either swallowed or expectorated. A one minute rest was required between samples in order to prevent the possible effect of an aftertaste. Panelists were asked to taste each cup only once and not to go back and re-taste.

Panelists were allowed to keep notes about their perceptions of each cup, but were not allowed to collaborate with any of the other panelists. They were asked to pick the 'odd' sample from the five and mark the appropriate box accordingly on their score-card. The odd sample was the sample containing iron.

Panelists were exposed either to increasing or decreasing concentrations depending on the necessity of the panelist in question. A positive report was defined when a panelist correctly identified three samples in a row, preceded by an incorrect identification.

Thresholds were calculated by both geometric mean and logistic regression methods. The geometric mean is based on the highest concentration that a panelist is unable to taste along with the lowest concentration the panelist is able to taste followed by two other correct choices. The geometric mean is then calculated using the last incorrect iron concentration and the first correct iron concentration. If a panelist is unable to taste iron above 5 mg/l they were given a taste threshold of 5 mg/l. The group threshold was calculated as the geometric mean of individual geometric mean values. Logistic regression was used to calculate the population threshold. The population threshold concentration for this research was calculated using the Abbot's formula [equation(1)] with 50% as the adjusted proportion correct and a probability of guessing by chance of 20% (one-of-five). This lead to the probability of defining the population threshold of 0.60.

$0.5 = x - 0.20/0.20 - 1 \rightarrow x = 0.60$ (1)

Results and Discussion

The majority of panelists described the taste of iron as metallic or bloody. Astringent was also used as a descriptor, particularly at the lower concentration levels. Approximately half of the panelists could taste iron at or below 0.05 ppm and nearly 90% of the panelists could detect iron at or below the USEPA SMCL of 0.3 ppm. Results are summarized in Figure 1 and Table 1.

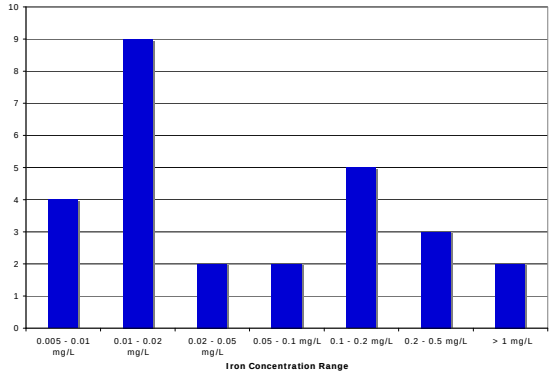

Figure 1. Number of panelist whose threshold falls between each concentration range.

Analysis of individual threshold results did not demonstrate a trend based on any external factors including age or the use of tobacco products. Panelists with the least ability to taste iron, with threshold concentrations of above 5 ppm, ranged in age from 24 – 60 yrs and consisted both of occasional tobacco users and non-users. Likewise, panelists with the strongest ability to taste iron range in age from 21 to 40 and consisted of both occasional tobacco users and non-users.

Table 1. Eight individual panelist thresholds found by the geometric mean method.

Panelist	Threshold (mg/l)
1	.014
2	.031
3	.007
4	.141
5	.003
6	.014
7	.014
8	5

The group threshold by geometric mean of the individual threshold values for each panelist (n=22) was 0.052 mg/L. The logistic regression threshold of the panel was 0.031 mg/l. The slight difference between the geometric mean thresholds and logistic regression thresholds is not significant given the differences in criteria for calculating the group thresholds. The logistic regression threshold was based on the concentration at which 50% of the panelists could taste iron, whereas the geometric mean group threshold is based on individual thresholds which required three correct panelist choices in a row.

In summary, threshold results from this research were much lower than previous studies. This research also demonstrated that a majority of the population would be able to detect iron at or below the SMCL as recommended by the USEPA. This may indicate a need for a lower level recommendation in order to

prevent aesthetic problems, and to allow drinking water utilities to be able to prevent customer complaints about the quality of the drinking water provided.

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Managing Manganese in Drinking Water: An Assessment for Microbes and Metals

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Abstract

Manganese can be released and cause aesthetic problems in the drinking water systems worldwide. The relationships between metals and microbes; at the sediment-water interface; have already been established to assess microbiological implications in natural water systems. Studies have been conducted on the oxidation and reduction of manganese by microbes, but their implications in drinking water systems have not been understood thoroughly. Five microbial strains that were recovered from the filtration and sedimentation basins of a water treatment plant in Blacksburg, Virginia were selected to conduct this research. The five selected strains were found to be capable of performing oxidation of Mn (II) to Mn (IV) when inoculated in an Mn-oxidation selective broth medium. Mn (II) present at concentrations equal or less than 0.05 mg/l in the broth medium used for this study had no effect on the growth of the five strains evaluated. Mn (II) inhibited growth of these strains when present at a concentration of 0.08mg/l. The results from this research suggest that microbial manganese oxidation takes place in drinking water systems and that it could have an effect on manganese release and deposition. This research also serves as a model for the proper investigation of other metal contaminants, which would improve the principles of the water treatment systems and ensure quality drinking water to consumers.

Keywords: Manganese, Microbes, Oxidation, Water Treatment, Drinking Water

Introduction

Manganese is one of the most abundant elements on Earth. It can be found in terrestrial; as well as; aquatic environments and naturally occurs in the form of hydroxides (OH^-). Manganese causes an aesthetic problem in drinking water when it is oxidized from Mn^{2+} (water soluble); to Mn^{4+} (water insoluble); forming black particulates that stain drinking water black, brown, or yellow. There are specific microbes that specialize in the oxidation of manganese and have been documented since the beginning of the century (Beyerinck 1913). These microbes can be found in water treatment plants because the plants pump water directly from source, whether it is a river, lake, or groundwater, where these microbes inhabit. During the seasonal months, there is a high level of soluble manganese (Mn^{2+}) released into the water column. This is due in part by algae that grow in abundance near surface water and absorb dissolved oxygen that cannot be reached at the lower levels of the water column. Microbial populations at the sediment-water interface can use alternative electron acceptors such as manganese and iron. The influent water to the treatment plant receives the same amount of soluble manganese that was present in the source water (Sly et al. 1988).

Many studies have been conducted in the past few decades to understand the role that microorganisms play on geochemical cycling of manganese in nature (Nealson and Saffarini 1994). Little is known about the effect that microorganisms have on manganese cycling in drinking water systems. Even though manganese is very difficult to remove in water treatment (Sly et al. 1994), most treatment plants use

conventional processes with an aid of an oxidant to remove it. Oxidants, such as chlorine, potassium permanganate, and ozone are used to chemically oxidize soluble manganese to its insoluble form. The precipitated particles formed are separated from the water by filtration and sedimentation basins, commonly made of coarse materials such as sand and anthracite (Knocke et al. 1988; Knocke et al. 1991). Low levels of manganese have been detected in distributing systems and have led to consumer complaints of "black water". The USEPA has set the secondary maximum contaminant level (MCL) for manganese at 0.05mg/l (USEPA 2004). Measures have been taken to manage the amount of manganese released in drinking water.

The objectives of this research are to 1) Determine if Mn(II) can be oxidized by microorganisms recovered from a drinking water treatment plant and measure the amount of Mn(II) oxidized to Mn (IV), and 2) Evaluate the effect of Mn(II) has on the growth of Mn-oxidizing microorganisms. This research will lead to a better understanding of manganese cycling and help prevent aesthetic problems associated with it from reoccurring in drinking water.

Methods

Origin of the Selected Microbial Strains: Microbial strains 1, 2, and 3 were discovered in the in the filtration basin in the waster treatment plant. The media was predominately anthracite. Microbial strain 4 was discovered in a sludge media on top of the sedimentation basin. The last strain was also discovered in the sedimentation basin, but in a sludge media at the bottom of the basin.

Detection and enumeration of Mn(II) Oxidizing microorganisms: The Mn-oxidizing medium was incubated at 30° C for 2-4 weeks. The Mn-oxidation agar was examined for colonies with dark centers or dark edges as evidence of deposition of dark (oxidized) Mn. Each putative isolate was picked, streaked for isolation and a broth culture archived by freezing (-70° C).

Measurement of Growth of Individual Isolates: Created four different concentrations of Mn for each microbe. Prepared different mediums with the four concentrations of Mn at a pH level of 7.0-7.4 to avoid turbidity. Autoclaved samples for 15 minutes. Used 0.2mL of Mn(II) free growth cultures to inoculate 18mL of Mn-oxidation broth in a sterile side arm flask and incubate at 30°C. Measured the turbidity of Mn cultures as an indicator of cell density (growth) using a spectrophotometer at 540nm, as an indicator of Mn-oxidation.

Measurement of Mn-oxidation of Individual Isolates: Picked single isolated colonies of each type and inoculate into 5mL of Mn-oxidation broth (per liter of 10mM HEPES buffer (pH 7-7.4), 0.001g $FeSO_4$ per $7H_2O$, 0.2g $MnSO_4$ per $4H_2O$, 2g Peptone, and 0.5g Yeast Extract. In a micro-centrifuge tube, mixed 0.2mL of cultures at early stationary phase with 1mL of Leucoberbelin Blue as an indicator of the presence of Mn(IV). Immediately centrifuged for 5 minutes and then transferred 1mL of the supernatant liquid into a spectrophotometer cuvette. Measured absorbance at 620nm as an indicator of the formation of oxidized Mn.

Determination of Mn release from filtration media using Chlorine: Created four treatments of 10g of sand and anthracite in a 125mL flask. Next, 100mL of tap water was added to each flask. Using the calculations, added 0, 10,100, and 200mg/L of chlorine to each of the flasks. The Chlorine HACH Kit was used to measure the amount of chlorine for the 0 and 10mg/L samples. For the 100 and 200mg/L samples, the Iodometric Method I was followed to titrate $NA_2S_2O_3$ with KI crystals and a starch indicator (Standard Methods 4-49). Lastly, the samples with chlorine were soaked for 24 hours and then streaked using R2A and Mn-Oxidation media. Titrated samples again for the chlorine lost residual.

Results and Discussion

Determination and Measurement of Mn-Oxidation performed by Microorganisms

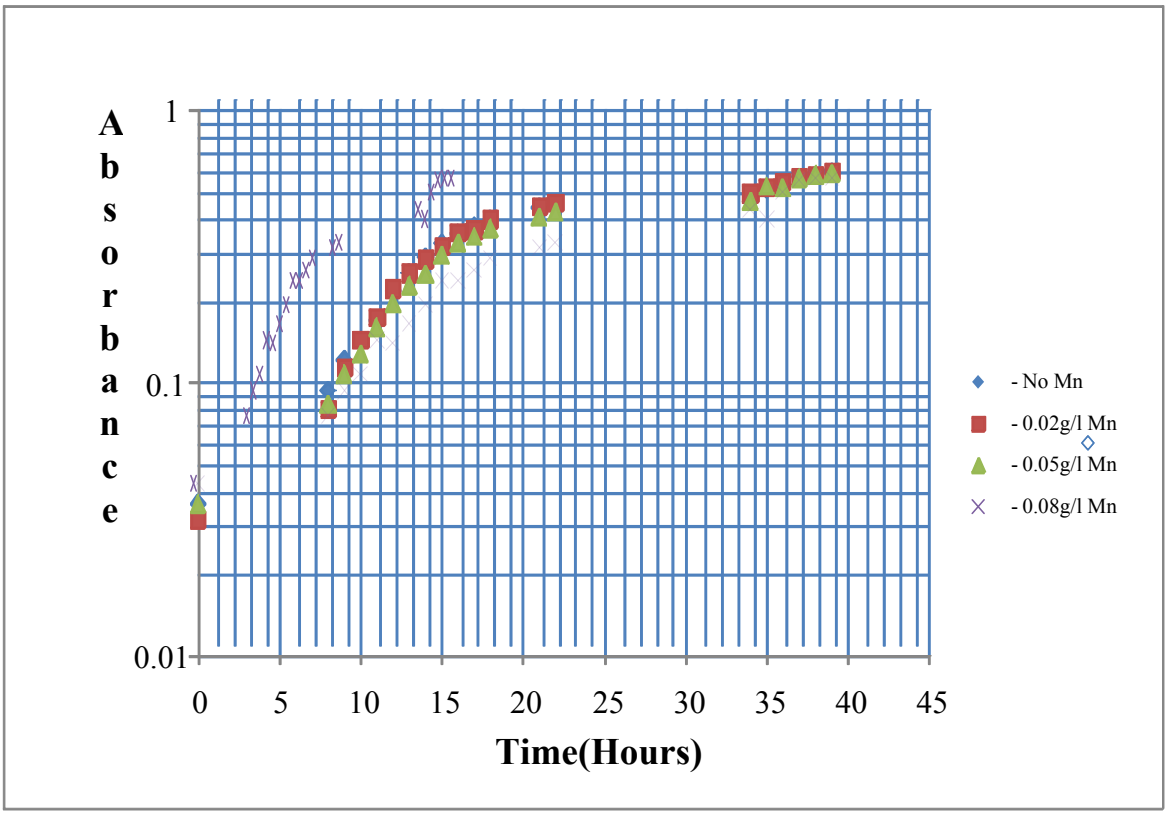
It is very well understood and documented that these microorganisms can oxidize and reduce manganese. Even though their role in the water treatment process has yet to be determined, the understanding of the biogeochemical role in metals could shed light on this aesthetic problem in drinking water. This experiment could help in manganese management in water treatment plants. Determining which basin, filtration or sedimentation, harbors the most microbes that oxidize manganese faster and quantitatively could be beneficial since most water treatment plants use conventional methods to eliminate microorganisms such as chlorine to apply for disinfection. Table 1 shows each of the five selected strains that were obtained for this research experiment, along with two samples of tap water that were controls for this experiment. Mn-oxidation was measured at the end of every week to see which strain oxidizes the most. After the first two weeks, Mn-oxidation was observed in bacteria strains 3 and 4, meaning that these strains could oxidize manganese is the shortest amount of time. At the end of the experimental duration, it was observed that strain 2 had the highest capacity for Mn-oxidation at an absorbance level of 1.760.

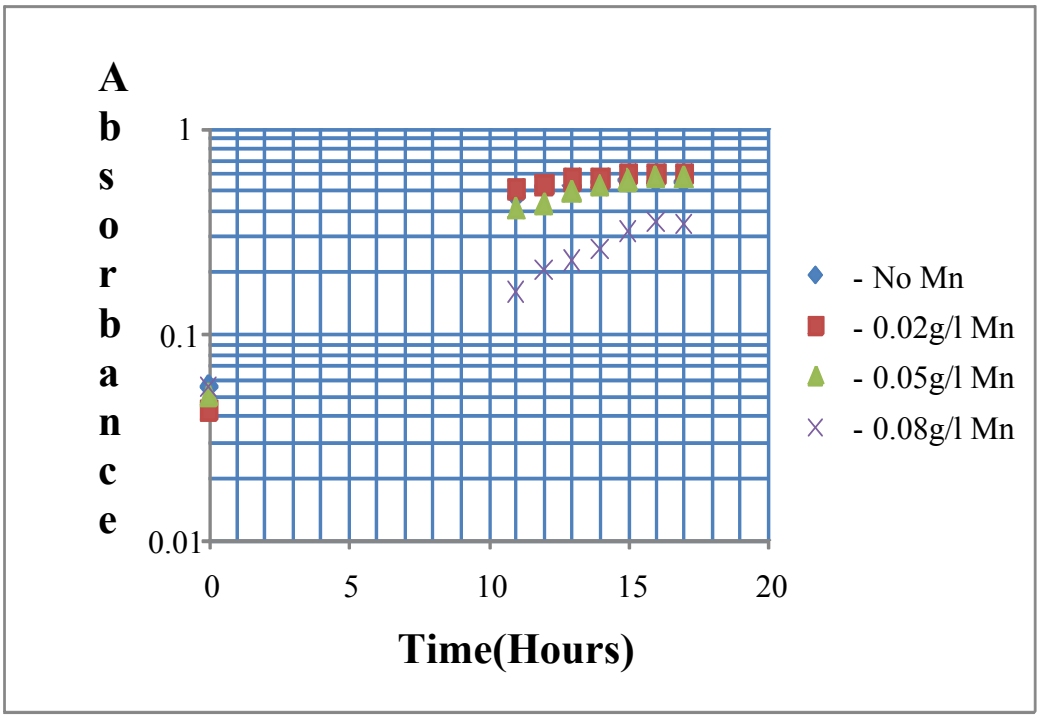
Absorbance at 620nm							
Isolate	**Day 2**	**Day 8**	**Day 11**	**Day 15**	**Day 22**	**Day 25**	**Day 30**
Microbe Strain 1	0.21	0.375	0.246	0.48	1.42	0.886	1.05
Microbe Strain 2	0.207	0.438	0.212	0.217	0.346	0.293	0.383
Microbe Strain 3	0.207	0.225	0.225	0.284	0.525	0.655	1.001
Microbe Strain 4	0.2	0.235	0.206	0.216	0.319	0.353	0.373
Microbe Strain 5	0.223	0.256	0.303	0.589	1.175	1.457	1.76
Control 1	0.219	0.216	0.219	0.227	0.225	0.223	0.224
Control 2	0.215	0.228	0.221	0.211	0.216	0.225	0.213

Table 1. Mn oxidation measurements for the selected strains over a 30-day duration

Effect of Mn (II) on microbial growth

It has been documented that some microorganisms experience vast amounts of heterotrophic growth in the presence of metals such as manganese, lead, iron, and copper (Nealson and Saffarini 1994). It was desired to evaluate if Mn-oxidizing microorganisms benefit from Mn (II) in a way that affects their growth. Figures 1a-c illustrates the growth curves obtained for a microorganism isolated from the anthracite material in the filtration basin of a water treatment plant at different manganese concentrations. The experimental treatment of "no manganese" serves as a control for this experiment. The results shown in Figures 1a-1c suggest that manganese present at concentrations equal or less than 0.05 g/l has no effect on the growth of these microorganisms. The trend shows that these microbes can grow well with and without manganese. This could be attested to the fact that there are other compounds, such as inorganic and organic, could be responsible for heterotrophic growth. Interestingly, growth of these microbes was inhibited by the presence of manganese at 0.08 g/l Mn. Such result suggests that microbial growth is inhibited at high concentrations of manganese for which metabolic functions could be altered.


Absorbance
1
0.1
0.01
0
5
10
15
20
25
30
35
40
45
Time(Hours)
- No Mn
- 0.02g/l Mn
- 0.05g/l Mn
- 0.08g/l Mn


Absorbance
1
0.1
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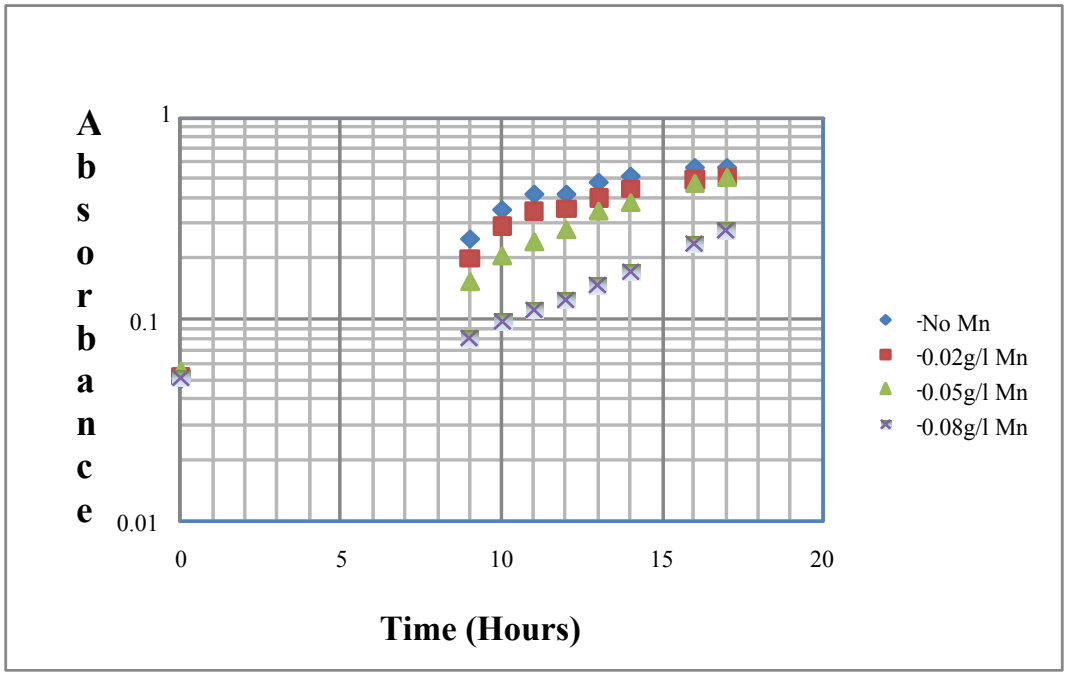
Time(Hours)
- No Mn
- 0.02g/l Mn
- 0.05g/l Mn
- 0.08g/l Mn



Figures 1a-c. Replicate growth curves at different concentrations of Mn for microbe strain 3 isolated from the anthracite material at the water treatment plant.

Conclusions

The presence of Mn-Oxidizing microorganisms in the filtration and sedimentation basins of water treatment plants gives an edge into understanding the role that microbes play in the Mn cycling through the treatment process. Although the focus of this project was solely based on the microbes that oxidize Mn(II) to Mn(IV), this research could also serve as a template to understanding the microbes that are responsible for the reduction of Mn(IV) to Mn(II), which would give a comprehensive outlook on the particulate (oxidized) and soluble (reduced) states of manganese. The results from this research coincide with earlier predictions that microbes play a role in the release of manganese into drinking water (Beyerinck 1913).

The biogeochemical cycling of manganese has only been documented and observed in natural systems such as lakes, rivers, and streams. The fact that microorganisms isolated from the water treatment plant were able to perform Mn –oxidation suggests that biogeochemical cycling of manganese could be a key factor affecting manganese release and deposition in drinking water systems. This research would have a greater impact on the management of metals in water treatment plants and perpetuate the importance of microorganisms in the deposition and reduction of metals. This research could also aid the USEPA in making new guidelines for manganese and introducing new conventional methods to reduce the number of microbes that specialize in the biogeochemical cycling of metals and ensure the quality of drinking water from the tap.

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Antibiotic Resistance Patterns in *Escherichia coli* to Differentiate Between Human and Non-human Sources

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Abstract

In this study, seven antibiotics: ampicillin, cefalexin, doxycycline, gentamicin, lincomycin, penicillin, and trimethoprim were used in Antibiotic Resistance Analysis (ARA) to help create a clearer distinction between isolates of *Escherichia coli* (*E. coli*) from humans and non-human sources. Through statistical analysis, three antibiotics (doxycycline, gentamicin, and penicillin) were identified that allowed nearly an 80% rate of correct classification (RCC) between human and non-human sources, while the other four antibiotics did not contribute to the human and non-human RCC.

Keywords: antibiotic resistance analysis (ARA), *Escherichia coli*, microbial source tracking (MST)

Introduction

Antibiotic Resistance Analysis (ARA) is one of the many 30+ methods of Microbial Source Tracking (MST). MST is a general term for the process of using bacteria or other microorganisms found in feces to find the sources of contamination within a watershed. This process becomes extremely important when fecal contamination occurs at beaches or other public swimming areas. The first questions that are always asked when a site is contaminated is "is the source human?" and "if so, where is it coming from?" If the source is human, then human enteric viruses (there are over 120 human enteric viruses, all pathogenic in small doses) might also be present in the water. These viruses are known to cause a variety of unpleasant symptoms such as ear infections, skin rashes, swimmer's diarrhea, and more serious illnesses such as polio and hepatitis. The ultimate goal of using MST within a contaminated area is to determine the sources of the pollution whether it is human or non-human fecal contamination and then use appropriate corrective measures. There are many methods for tracking fecal bacteria in water, but there is no "best method." Each of the types of MST approaches has their own pros and cons and the most convincing approach at present is one that provides "multiple lines of evidence," which means using at least 2 or more MST methods to determine the source of the contamination. The more proof that a certain source is contributing to the contamination, the less problem authorities have with spending money to investigate and fix the problem. However, as there are many types of MST it can take time and money to produce these multiple lines of evidence. The cheaper and easier it is to perform MST the more contaminated sites can be identified from the perspective of identifying the sources, and then cleaned up.

In order to perform ARA on a contaminated waterbody, several steps must be taken. First, samples of fecal matter from sources surrounding the contaminated area must be collected. Samples from dog, cow, horse, human, etc., and other organisms that might contribute to the contamination must be gathered and analyzed before water samples are taken. After collection of samples, the fecal matter is suspended in sterile buffer and filtered onto synthetic membranes which are then placed on growth agar and incubated. After incubation, individual colonies of bacteria are harvested and grown. These bacteria are then plated

onto agar, spiked with different concentrations of antibiotics, and incubated. The growth of the bacteria is recorded and placed into what is known as a "source library." After the collection and analyzing of the individual strains of *E. coli* for different sources of fecal matter, water samples can then be taken.

After collecting water samples from the contaminated areas, they are put through the same process of filtering, harvesting, and incubation. The growth of the sample bacteria is compared to the source library and run through a statistical program which analyzes the probability of a sample being from a particular source. However, in order for this method to work, a large source library must be created and maintained throughout the sampling and analyzing period. This can be a difficult and expensive process. Because domestic animals, like humans, are often given antibiotics, most fecal bacteria within the animals will show some level of resistance to them. However, some antibiotics are for human use only. It stands to reason that bacteria from a human source might be able to grow when exposed to these antibiotics, but animal bacteria will not. This study focused on finding antibiotics which give a large distinction between human and non-human sources, with the hopes of discovering a set of antibiotics and growth patterns that can be used for any contaminated area without the creation of a source library.

Materials and Methods

Isolation of *E. coli*

All isolates used in this study were acquired from freeze storage where isolates from previous studies were kept after completion. The isolates chosen for the study were refrigerated until needed. The frozen isolates were grown on Eosin Methylene Blue Agar (EMB) and Trypticase Soy Agar BBL (TSA). The isolates that showed green-metallic growth on EMB were considered positive for *E. coli*. The isolates positive for *E. coli* were then picked from the TSA using sterile toothpicks and placed into 96-microwell plates containing Colilert (a commercial product that measures the presence-absence of *E. coli*). The microwell plates were incubated for 24h at 37°C, and after incubation were checked for fluorescence under UV light (positive Colilert test for *E. coli*). Any wells that did not exhibit fluorescence were not considered in the study.

Antibiotics

Seven antibiotics were evaluated: ampicillin, cefalexin, doxycycline, gentamicin, lincomycin, penicillin, and trimethoprim. All antibiotics and reagents were acquired through Fischer Scientific and used without further purification. These seven antibiotics were chosen due to their use in the human and animal health industries. Several sources were consulted to determine the effectiveness and usage of the antibiotics and Table 1 shows which reference materials were consulted to determine their suitability for this study:

Table 1. Sources Consulted for Determination of Antibiotics

Antiobiotic	Sources Consulted
ampicillin	Raymond *et al.* 2006
cefalexin	Cox 2001
doxycycline	Anderson *et al.* 2006 Cox 2001
gentamicin	Garofalo *et al.* 2006 Raymond *et al.* 2006
lincomycin	Gilchrist *et al.* 2007
penicillin	Garofalo *et al.* 2006 Raymond *et al.* 2006 Sayah *et al.* 2005
trimethoprim	Anderson *et al.* 2006

Stock solutions of antibiotics were created and stored in the refrigerator or freezer until needed.

Antibiotic Resistance Analysis (ARA)
This procedure was adapted from Hagedorn et al. (1999) and Wiggins (1996). TSA was mixed and autoclaved at 115°C for fifteen minutes. Known amounts of stock solutions of antibiotics were added to the TSA and mixed thoroughly. The TSA was then poured into 100x15mm Petri dishes and allowed to solidify. *E. coli* grown in 96-microwell plates were transferred to the TSA using a 48 prong replica-plater. The Petri dishes were then incubated for 24h at 37°C and stored in a refrigerator until analyzed. A binary system was used to record the growth of each individual colony. A "1" indicated growth while a "0" indicated no growth.

Statistical Analysis
After recording the growth of the bacteria the data was placed into a statistical program (JMP v. 6.0.2, SAS Inc., Cary, NC) and the Multiple Analysis of Variance (MANOVA) test was performed on the data. After running the MANOVA test, the results were compared to the actual source of the bacteria (human or non-human) and a comparison of those data was performed. This gives an accurate depiction of how well the antibiotics would actually perform had the source of the bacteria been unknown. This is also helpful in determining which antibiotics and concentrations are significant in determining the source. By excluding a certain antibiotic or concentration and comparing the Rate of Correct Classification (RCC) with the original RCC, the JMP program will show whether or not the difference is significant.

Results and Discussion

The attempts at growing *E. coli* and finding antibiotics that show distinction between the isolates were split up into several trials over the summer of 2007.

Trial 1
The first attempt produced poor results. Forty-eight isolates were chosen from the freezer and were defrosted and placed directly into 96-microwell plates using sterile toothpicks containing Colilert. Twelve of the 48 isolates did not show fluorescence under UV light after incubation. This could possibly be due to the fact that the isolates were not first allowed to propagate on a growth media and then transferred to the Colilert. Because 25% of the wells did not show presence of *E. coli*, all non-fluorescent isolates were discarded and a new set of isolates were obtained.

Trial 2
In the second attempt, the isolates from the freezer were plated onto EMB for growth and allowed to incubate at 27°C for 24h. Any isolates that showed green growth on the EMB were transferred to the microwell plate using an inoculating loop. Only one plating onto antibiotics was performed using these isolates. Between the first and second plating, the wells were contaminated while refilling the wells with more Colilert, so they had to be discarded.

Trial 3
Isolates were again plated onto EMB, incubated, and transferred to the microwell plate. Several platings were performed onto antibiotics, but the main goal of this trial was to determine the concentrations of antibiotics that would give the most distinction between the isolates. After the ranges of antibiotic concentrations were determined, the isolates were then plated onto all antibiotics using the newly determined ranges. However, when analyzing the growth responses, some of the isolates began to show a growth pattern that did not look like *E. coli*. After performing the same trial again, it was determined that the isolates had likely been contaminated with another type of bacterium. These too were discarded.

Trial 4

Forty-eight new isolates from 12 sources were obtained from the freezer. These isolates were harvested using the procedure described in the Methods section. However, in this trial 4 replicates (clones) of each *E. coli* strain were placed into the microwell plates instead of one per *E. coli* strain to ensure precision. No matter what antibiotic concentration was used, ampicillin, cefalexin and lincomycin did not provide any distinction. Either all the bacteria grew on the antibiotic, or none of the bacteria grew. Therefore, when performing a statistical analysis, these data were not included. Figure 1 below shows a statistical analysis on the remaining four antibiotics:

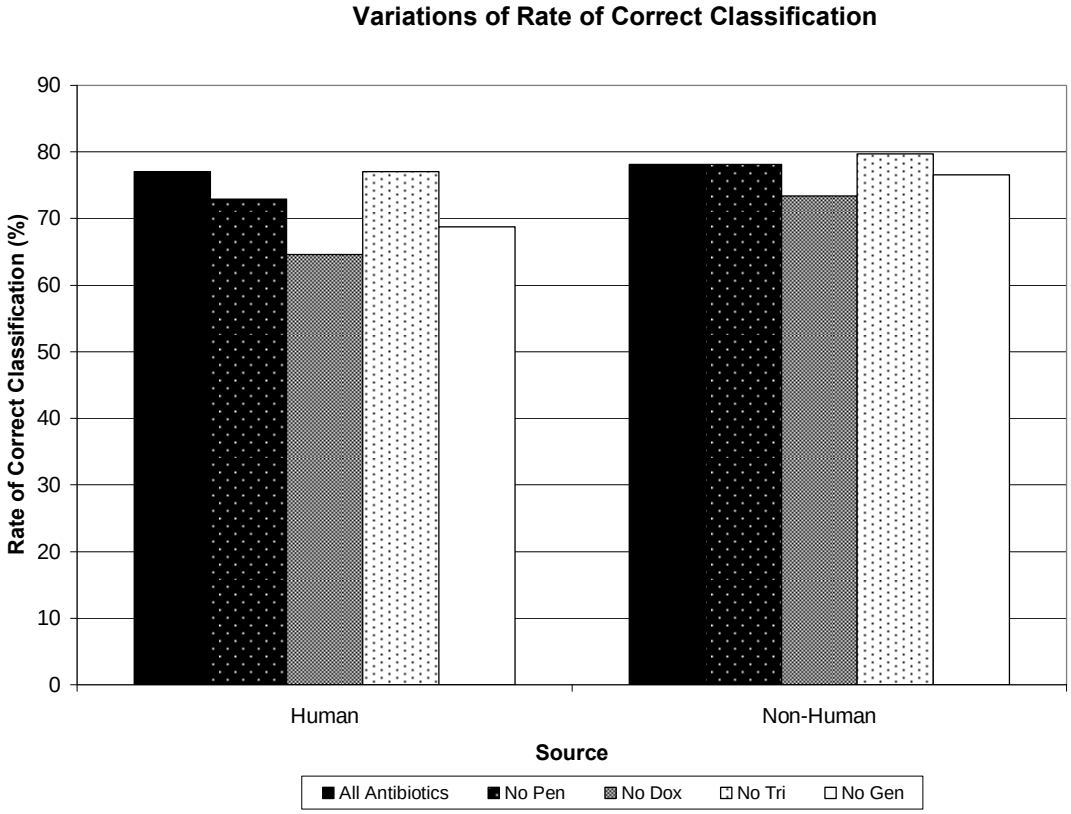

Figure 1. RCC for Four Antibiotics

Figure 1 shows that almost 80% of the time the antibiotics were capable of classifying a bacterium as either human or non-human. Also, when three out of the four antibiotics were removed the RCC decreased, indicating that those antibiotics were necessary for creating a human/non-human differentiation. Trimethoprim was the only antibiotic that did not decrease the RCC when removed. In fact, it increased the RCC for non-human when not included. Therefore, it is clear that the three antibiotics: doxycycline, gentamicin, and penicillin were all capable of showing a human/non-human distinction with the isolates and concentrations tested in this study.

However, when a statistical analysis of the individual concentrations was performed, interesting results appear. These are shown below in Figure 2:

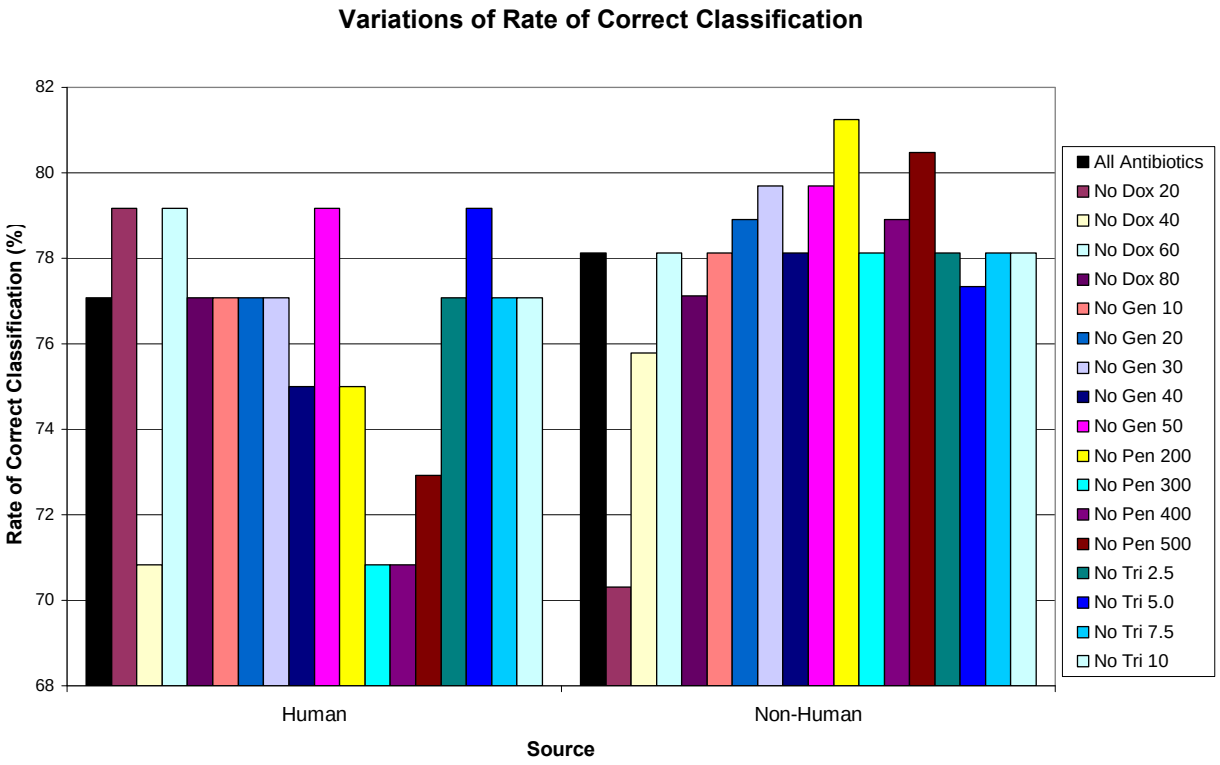

Figure 2. RCC for Individual Concentrations of Four Antibiotics

In Figure 2 it is clear that there is a large amount of variation occurring between concentrations, but there are several constant reactions that can be observed. First, penicillin is clearly an important antibiotic to use. In all concentrations the RCC for humans dropped when it was not included. Second, trimethoprim again does not appear to contribute to the RCC when removed. In fact, in the case of 5.0 µg/ml, the RCC for humans increased significantly. Finally, the results for both doxycycline and gentamicin were extremely variable. For example, doxycycline, at a concentration of 20 µg/ml, increased the RCC for human when removed, decreased the RCC at 40 µg/ml, and increased the RCC at 60 µg/ml. Either the data was incorrectly recorded or 40 µg/ml is an extremely good predictor of human/non-human sources. This last test should be repeated. The same type of trend occurred with gentamicin. For concentrations of 10, 20, 30, and 50 µg/ml, the RCC was either unchanged or increased, but for 40 µg/ml there was a large decline in the RCC. This again could be contributed to the fact that at times discerning between growth and no growth was difficult and the final decision can be affected by human error.

The same results appeared: doxycycline, gentamicin, and penicillin were all able to create a distinction between human and non-human sources of *E. coli*. In the future, the results from gentamicin should be carefully analyzed and recorded in order to ensure correct classification.

Conclusion

The three antibiotics doxycycline, gentamicin, and penicillin were able to show a human/non-human RCC approaching 40%. Even though there was considerable variability within the different concentrations of the antibiotics (Fig. 2), in total there was a clear difference (Fig. 1). However, there was the problem of the sample size. Only 72 isolates were used throughout the entire study, but the results did indicate that the three aforementioned antibiotics showed a good potential for distinguishing between *E. coli* form different sources. While the results are promising, more research should be performed before a complete

answer can be given. Such research should focus on testing more isolates from a broader geographical collection area, testing additional concentrations of the antibiotics, and trying the procedure on *Enterococcus,* the indicator of marine water quality.

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The Hydrology of the Timber Ridge Quarry Site, Botetourt County, Virginia

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Abstract

Rockydale Quarries intend to mine a limestone adjacent to Catawba Creek to a depth of 38m on Timber Ridge Properties, near Fincastle in Botetourt County, Virginia. The goals include determining areas of significant groundwater flow and evaluating the optimum placement of pumps in the event of quarry operations. The methods used include measuring the streamflow of the creek to determine areas of losing or gaining streamflow, performing resistivity surveys to locate karst or other structural features that may enhance fluid flow, and well logging to discover the possible locations of hydraulically connected fractures. The results of this study show that flow occurs in narrow channels that can be easily contained and maintained. The quarry operation should have negligible effect on Catawba Creek.

Keywords: groundwater, karst, resistivity surveys, mining

Introduction

Rockydale Quarries owns the land occupied by high-grade limestone on Timber Ridge Properties nine miles north of Fincastle in Botetourt County, Virginia, which they intend to mine. The proposed quarry lies adjacent to Catawba Creek. One objective of this investigation is to find the nature and quantity of groundwater flow that could potentially interfere with the quarry operations and to determine whether the flow system is connected to Catawba Creek. Another objective is to determine the optimum placement for pumps for dewatering the proposed quarry. Limestone is a soluble rock. In most cases the limestone at the study site is highly competent and does not contain water or permit water to flow through it. However, this is a mature karst area with sinkholes, sinking streams, and solution enhanced fractures and bedding planes (Fitts 2002). Locating karst features is important because they can potentially carry large quantities of groundwater. Some of the more obvious karst features were identified and are shown in Figure 1.

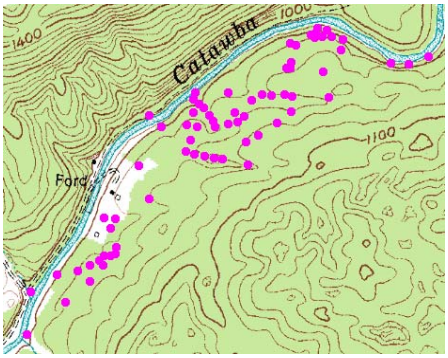

Figure 1. Locations of known karst features.

Methods

The methods used during this investigation include: 1) estimating the changes in stream flow along Catawba Creek by measuring the depths and average velocities across the creek at four separate monitoring locations 2) performing resistivity surveys using an AGI Supersting resistivity system. The system measures the difference in voltage at potential electrodes after current has been injected into current electrodes, and 3) installing and logging wells to obtain a better understanding of the possible locations of hydraulically connected fractures.

Study Site (from ATS International, Inc. 2004).
"The quarry site is located approximately 9 miles north of the Town of Fincastle in central Boutetourt County, Virginia. The site and surrounding areas are underlain by sedimentary bedrock units of the Valley and Ridge Province. The Valley and Ridge Province consists of elongate parallel mountain ridges and valleys that are underlain by folded and faulted Paleozoic sedimentary bedrock. These parallel ridges and valleys are the result compressional tectonics and subsequent differential weathering of layered clastic and carbonate rocks.

The study site and immediate surrounding areas are underlain by Ordovician and Cambrian carbonate rocks of varying lithologies. Along its frontage with U.S. 220 the site is underlain by the Edinburg Formation (Oe, limestone and shale) which is covered with a veneer of quaternary alluvium (Qal). Down-section from the Edinburg Formation is the Lincolnshire and New Market Limestone unit (Oln, limestone with chert) which comprises a narrow band on the western edge of the site and which represents the primary units that will be quarried. The bulk of the property is underlain by the Beekmantown Formation (Ob, dolomite, chert, and limestone). The easternmost portion of the site is underlain by the Chepultepec and Conococheague Formations (OCr, limestone, conglomerate, dolomite, and sandstone).

Thrust faults are common to the west of the site. One of these faults trends northeast along Catawba creek and passes through the northern portion of the property. The site lies on the downthrown side of this fault. Another thrust fault occurs west of that fault and the two join approximately 3000 feet north of U. S. 220.

Beneath the proposed quarry the rocks dip gently to the northwest and strike toward the northeast. However northwest of the faults the strike and dips are very different. In this area the dip angles range from 302 degrees and 320 degrees in a northwesterly direction. A map of the quarry site is shown in Figure 2 with the outline of the quarry shown in red.

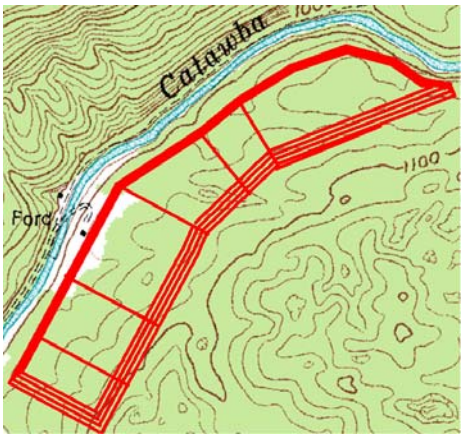

Figure 2. The quarry site which is located 6 miles north of Fincastle in central Boutetourt County, Virginia.

Within the fault complex the units are more complex. Some are overturned as indicated by the overturned syncline between the two faults. Other map notations indicate vertical to steeply dipping beds. Thus, the geologic structure is significantly different across the fault. The structural relationships on the site and across the faults are depicted in Figure 2 constructed from the geologic map."

Streamflow

Streamflow can be defined as the discharge (Q) of a river at a specific point. The discharge equals the cross-sectional area multiplied by the average velocity (Q = VA). Streamflow is significant in this investigation because a considerable change in streamflow could possibly indicate that the creek is losing or gaining water due to hydraulic connection with fractures or karst features in the adjacent aquifer. If this were the case, the creek could be connected to the proposed quarry site and could make it more difficult to pump the ground-water out of that area, without adversely affecting the creek.

Measurements were taken at four locations along Catawba Creek shown in Figure 3. At each location, average velocity and depth were measured at a distance of every meter across the creek. In this case, the depth of the river for each one meter section is equivalent to the cross-sectional area of that section. The sum of all the sections is equal to the cross-sectional area of the stream. The streamflow was computed for each of the four sites using the equation Q = VA.

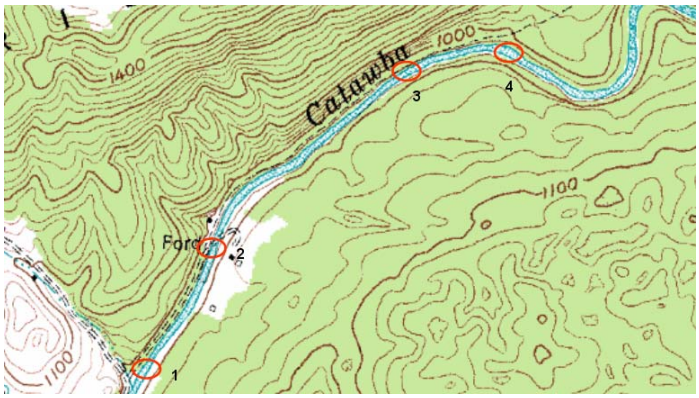

Figure 3. Average velocity and depth measurements were taken at these four locations.

Resistivity Surveys

A resistivity survey involves extending 640 meters of cable that has electrodes attached to the cable at every 10 m interval. The electrodes are pounded into the ground to make electrical contact with the underlying geologic units. A control box sends current into two of the electrodes and measures the potential at two other electrodes at a time until all electrodes have both been used as current and potential electrodes. The potential electrodes measure the difference in the resulting voltage (Loke 2001). The dipole-dipole array configuration is used in these tests because it has been shown to best identify vertical and horizontal fractures.

Using the resistivity information collected, a program is used to invert the resistance data to obtain an apparent resistivity map and ultimately a color-coded resistivity map of the resistance of the units beneath the survey. The red side of the spectrum represents rock that is most resistive (no water or fractures), and the blue end of the spectrum represents rock that is least resistive (most conductive). When a rock is shown as least resistive on the visual, it means that it is a more conductive area that has higher moisture content and may indicate the presence of karst features.

Borehole Testing

Air rotary drilling was used to install 10 wells 130 ft deep on Timber Ridge Properties, 8 of which are shown in Figure 4. This is done by attaching a drill bit to a number of hollow drill stems. As the drill is spun, air and sometimes water is circulated down the drill stem to the bit in order to blow out the cuttings as drilling progresses. The cuttings are carried inside the drill stem, and back up to the surface where the materials collect and can be identified. All the wells were drilled to a 6-inch diameter. A surface conductor casing was used for the top 37 feet of each well to prevent the hole from caving in (Fitts 2002).

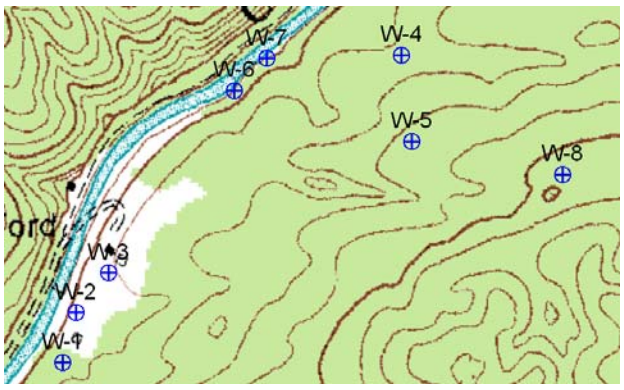

Figure 4. Well locations

Only two of the ten wells constructed produced a significant quantity of water, wells W-2 and W-7. The following equipment was used to log W-7: 3-arm caliper, optical televiewer, heat-pulse flow meter, and probes that record natural gamma radiation, spontaneous potential, resistivity, temperature, and drawdown. Well W-2 experienced significant "silting" due to mud seams encountered during drilling. Therefore, only caliper, gamma, and single-point resistivity data were collected at this site.

The 3-arm caliper is used to measure the diameter along the length of the borehole. Fractures can be mapped using the caliper by taking note of the depths at which the diameter of the borehole gets significantly larger.

The optical televiewer takes a 360˚ picture of the inside of the borehole. Using these pictures, fractures and large openings in the rock can be found, lithology changes can be identified, and fracture directions and orientations mapped.

The velocity of flow up or down the borehole is recorded with a heat-pulse flow meter. The probe consists of a heating grid that is located between two heat sensors. After a pulse of heat is emitted from the heating grid, the time elapsed for temperature change is used to calculate the velocity of flow and in what direction that flow is moving (Hearst et al. 2000). Tests were run every meter along the borehole, once without a pump running, and once with a pump running at approximately 2.1 gallons per minute.

Potassium 40 is common in clay minerals and tends to be the most dominant source of gamma radiation in sedimentary formations (Fitts 2002). Because of this, natural gamma radiation is found in areas at which there is more sediment than competent rock and thus indicates the location of fractures.
When measuring spontaneous potential, the natural voltage difference between two electrodes is measured. Spontaneous potential is used to find where permeable formations are and the boundaries of these formations (Fitts 2002).

Each resistivity test measures the resistivity of the surrounding area, but each uses a different spacing and therefore measures different volumes of rock. The tests help to locate fractures surrounding the borehole. The resistivity tests used include single-point resistance, short normal resistivity, and long normal resistivity. Single point resistance and short normal resistivity measure the resistance between two electrodes in the well. For long normal resistivy, the resistivity measurements are taken between an electrode at the land surface and an electrode in the well (Burbey 2007).

The temperature of river water is always a warmer temperature than groundwater. At this site the creek temperature was 22^{o} C. and the groundwater temperature was 16^{o} C. Temperature was measured in the borehole continuously while the pump was discharging water at a rate of 30 gal/min. If, while the pump is running, an increase in ambient groundwater temperature is observed, it can be concluded that water from the creek is being pumped from the well and the fracture system is in hydraulic connection with the creek.

When the water in a well is being pumped, the drawdown is the change in the depth of the water. This is measured using a Solinst level logger. The level logger is lowered into the water of the well, and left in the same location throughout pumping. Every minute it takes measurements of pressure and temperature. Using the pressure measurements, the drawdown can be readily calculated.

Results and Discussion

Streamflow

The average stream flow for each location is shown in Table 1. According to these data, there is a significant increase in stream flow between locations 1 and 2. This implies the river is gaining water somewhere between those two points and is likely the result of tributary flow from the northwest side of the creek and not from groundwater.

Table 1. Streamflow measurements taken at each location.
(Notice the stream flow of location one is nearly 30,000 ft^3/day less than all locations following)

Location	Streamflow (m^3/day)
1	79,824.96
2	109,576.80
3	103,602.24
4	101,234.88

Resistivity Surveys

Resistivity surveys were obtained at two locations, R1 and R2, shown in Figure 5. The results of R1shown in Figure 6, reveal three areas of low resistivity near R1-1, R1-6, and R1-8. These areas coincide with karst lineaments and associated fractures already known to exist and shown in Figure 7 (Burbey, 2007). The results of R2, shown in Figure 8, display only one main area of concern between 300 and 400 m, or between R2-2 and R2-4 on the map. Upon drilling a well at this location, it was concluded to be dry. The area of low resistivity is deep enough that it will not be a concern during quarry operations.

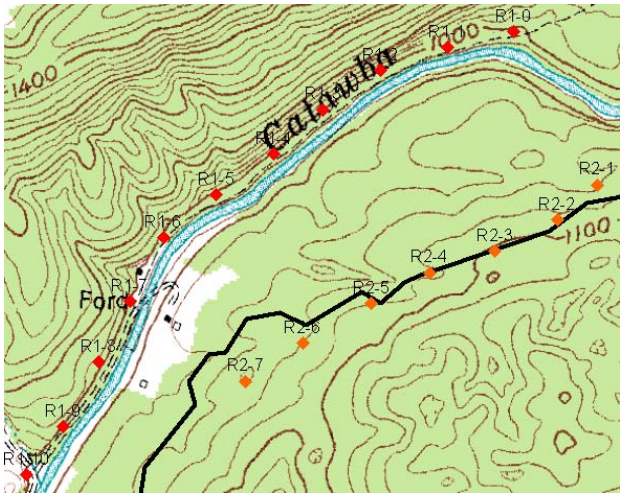

Figure 5. The two locations at which resistivity tests were done.

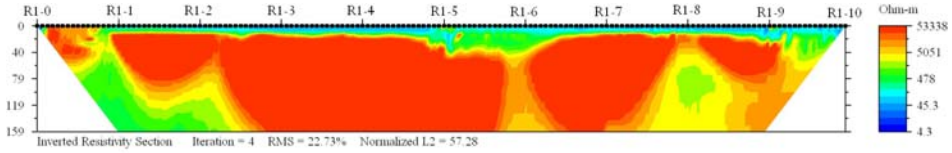

Figure 6. Resistivity line R1 results

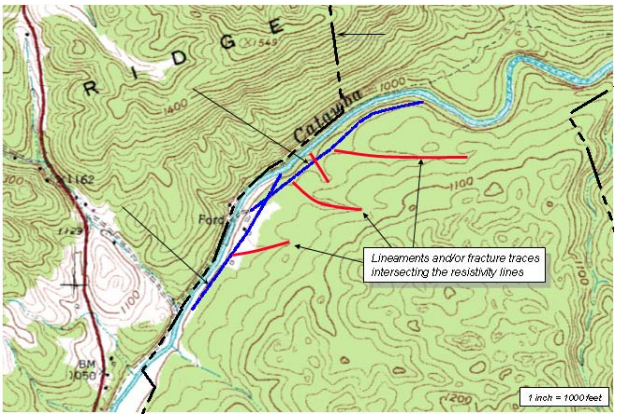

Figure 7. Lineaments intersecting the resistivity line

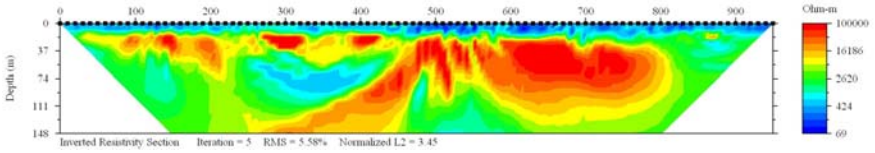

Figure 8. Resistivity Line R2 results

Borehole Testing

Well W-7

The horizontal fractures identified by wellbore logging seem to be following the bedding planes of the formations of the area, the Lincolnshire Formation and the Beekmantown Formation. By studying the data produced from the optical televiewer, one can see that the boundary between Lincolnshire Formation ends and Beekmantown Formation occurs at a shallow depth of 15 meters. (The Lincolnshire Formation has a darker color than the Beekmantown Formation.) Because it is known the Beekmantown Formation dips 15˚-30˚ to a depth much below the river, it is believed the fractures do not connect with the river (Burbey 2007). The fractures found on the image produced by the optical televiewer correspond to the increases in diameter found by the 3-arm caliper. This is shown in Figure 9.

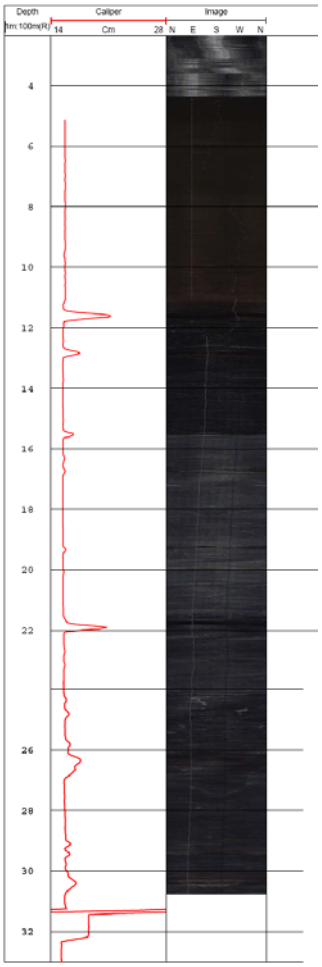

Figure 9. The 3-arm caliper data and the optical televiewer image correspond about the locations of fractures

Very little change in flow was found with the heat-pulse flowmeter when run in ambient conditions. While running the pump at 2.1 gal/min, however, there was significant flow found at 11 meters depth and still little change anywhere else as shown in Figure 10. This large change coincides with a 7 cm wide fracture and is the main fracture producing water at this site.

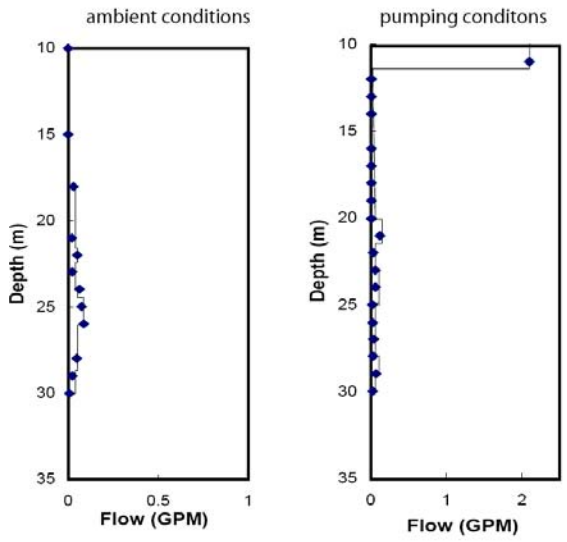

Figure 10. The ambient and pumping conditions of well W-7.

Gamma radiation, spontaneous potential, and all of the resistivity measurements correspond to each other,shown in Figure 11. Where there are dips of low resistivity, there is more conductivity, and there are fractures. The smallest resistivity measurement is located at a depth of 11.5 meters, which confirms the fractures found in the heat-pulse flowmeter test. The gamma radiation also coincides with the resistivity measurements, peaking when the resistivity is at a low.

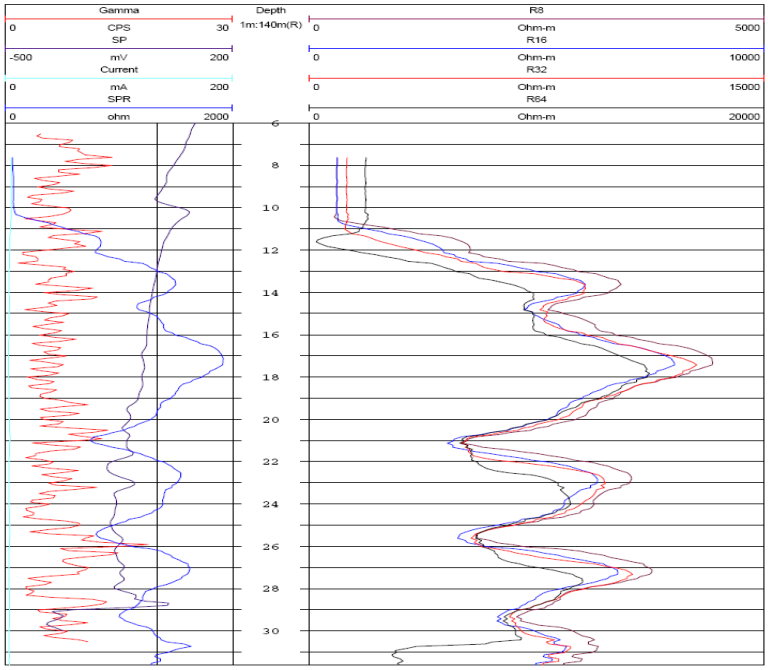

Figure 11. Gamma, spontaneous potential, and resistivity measurements

The temperature of the water in the well was about 16˚ C while the temperature in the river was approximately 22˚ C. Figure 12 shows that throughout 4 hours of pumping 30 gal/min of water from the well, the temperature varied by less than half a degree, never reaching above 16.1˚ C. From this, one can only postulate that the fractures in the well do not draw water from Catawba Creek.

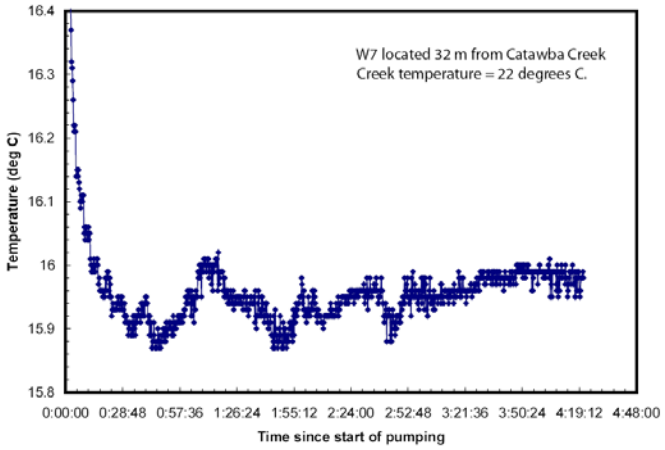

Figure 12. Temperature measured over a length of 4 hours

While the pump was running, drawdown was also being measured with a level logger as shown in Figure 13. The fact that the time-drawdown plot is linear is indicative of a fracture system (Burbey 2007) and not porous media flow. One can see three peaks on the graph that do not match the linear system which can be explained by three incidents, each at which the pump needed to be restarted and the well started to recover. These incidents are as follows: 1) a voltage overload caused the pump to shut down, 2) the system was trying to pull more power than was available and shut down, and 3) the generator ran out of gas.

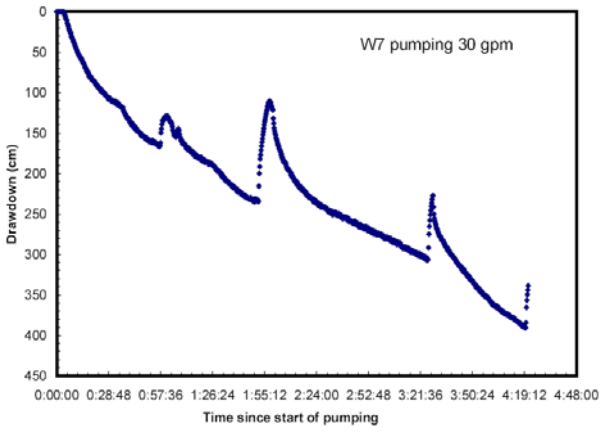

Figure 13. Drawdown of W-7 over a 4 hour period of pumping.

Well W-6

During the four hours of pumping, W-6, located approximately 88 meters from W-7, showed a similar downward trend. This demonstrates the connection between W-6 and W-7 through a fracture system. The drawdown, shown in Figure 14, also displays a linear trend, which likely indicates that W6 is connected to W7 by a single fracture.

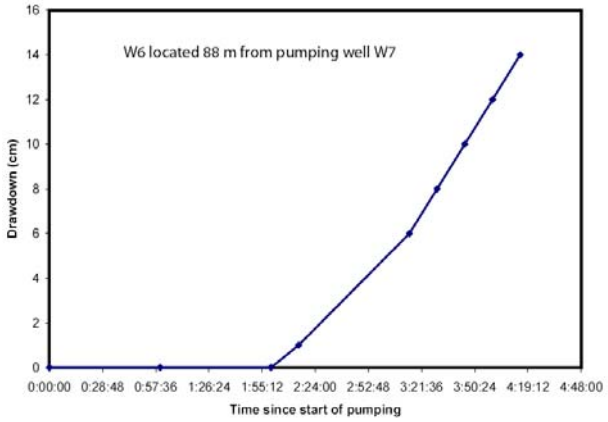

Figure 14. Drawdown of W-6, located 88 meters from W-7

Conclusions

This study shows the complexity of groundwater movement through fractures in a karst dominated system. There are areas of weathered karst zones in the proposed quarry area, shown in Figure 8. The zones are approximately 1 to 2 meters wide. The fractures have a dual system of shallow and deep water production. The deep system is locally connected to the shallow system but appears to have no connection to Catawba Creek as a result of dipping geology while the shallow system, only present at times of intense precipitation, does flow into the river.

Because the deep system appears to have no connection to the creek, quarry operations will likely have a minimal impact on the creek and will primarily draw groundwater from recharge areas to the east. Pumps should be placed along the four fracture system shown in Figure 8 in order to minimize the interference of the groundwater.

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The Ecological Stoichiometry of *Pycnopsyche gentilis* and Its Resources

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Abstract

Ecological stoichiometry is a branch of ecological study which traces elements along the various components of an ecosystem. If applying this concept on an organismal level, a flow of nutrients can be traced between an organism and its resource. Oftentimes, an organism eats matter that is not an exact fit to the chemical composition of its body (i.e. the nutritional requirements of that organism). This study was formed to explore how that consumer-resource imbalance affects the physiological processes of that organism such as excretion and assimilation. While data indicated distinct relationships between an organism and nutrient content of its food, sample sizes were simply not adequate for providing statistically reliable answers. However, this study provokes many new questions and provides a foundation for further studies of this fairly unexplored area.

Keywords: *stoichiometry, resource-consumer imbalance, excretion rates*

Introduction

Ecological stoichiometry is a relatively new area in the pursuits of ecological study. Most studies use a broad definition which involves study of energy and flow of elements and the proportions of that energy and matter relative to the organisms and resources involved in the flow (Vanni et al 2002; Cross 2003; Frost 2002). Sterner and Elser defined this area of study as "the balance of multiple chemical substances in ecological interactions and processes." In synopsis, ecologists study the stoichiometry of ecosystems or organisms as a chemist studies the stoichiometry of a chemical reaction: the balance of elements within an ecosystem, community, population, or individual is investigated while questioning factors which may disrupt these imbalances.

The interaction between an organism and its environment, one of the central tenets of ecology, can be thought of as a chemical reaction in which each component (i.e. the organism, its food, its excretion and egestion) can be thought of as a molecule of the reaction. To illustrate this idea, Sterner and Elser (2002) gave an example of the "human molecule": $H_{375,000,000}$ $O_{132,000,000}$ $C_{85,700,000}$ $N_{6,430,000}$ $Ca_{1,500,000}$ $P_{1,020,000}$ $S_{206,000}$ $Na_{183,000}$ $K_{177,000}$ $Cl_{127,000}$ $Mg_{40,000}$ $Si_{38,600}$ $Fe_{2,680}$ $Zn_{2,110}$ Cu_{76} I_{14} Mn_{13} F_{13} Cr_{7} Mo_{3} Co_{1}. Because the human has a specific body chemistry with certain elemental needs certain elements will be selected and used by the body when ingested, or eaten. Carbon, for instance could be traced through the monitoring of a human's food cycle; by measuring the C content of food, body, excretion, and egestion then a flow of matter will be observed, just as the flow of energy observed in thermodynamic chemistry.

As one might imagine, the reaction between an organism and its environment is not always a straight-forward, simple calculation. By using the feeding habits of an organism as a model of flow of matter, one can think of consumers in terms of the balance, or imbalance of matter shared in each component of the reaction. For instance, a cannibalistic organism by definition consumes matter which meets exactly the organism's biological needs (*i.e.* it is exactly the same as the organism itself), and therefore, a perfect balance between the organism and its food source is achieved. Most consumers, however, are not cannibalistic, which presents issue of *elemental imbalance*.

Elemental imbalance was defined by Sterner and Elser as "a measure of the dissimilarity in relative supply of an element between an organism and its resources" (2002). When an organism is not eating exactly what its body requires, the organism must "select" which elements are retained relative to which are simply egested or excreted. *Egestion* involves the food passing straight through the digestive system in organic form while *excretion* occurs after the matter has been assimilated through the gut wall and incorporated into an inorganic form. If either of these two processes occurs, the matter was unneeded. For instance, if an organism with a relatively low C:N ratio in its body is exposed to a food source with also a high C:N ratio, then the organism will require a larger quantity of food to meet the N requirements of its body, as N would be a very scarce source of food.

The objective of this study was to investigate the degree of consumer-resource imbalance between caddisflies *Pycnopsyche gentilis* and their food. Stoichiometric theory was applied to the food cycle of these organisms in order to trace an elemental flow and to investigate how the quality (N content) of food affects physiological processes such as excretion and assimilation of nutrients while using consumer-resource stoichiometry as a measure of nutrient flow.

Methods

Macroinvertebrates comprise a wide variety of consumers in lotic systems and may be classified in according to the manner in which they obtain food (Wallace and Webster 1996). These functional feeding groups are termed shredders, collectors, scrapers, and predators (Cummins 1973). Often, a large consumer-resource imbalance exists among shredders and their food supplies, therefore requiring the organisms to process relatively large amounts of detrital matter in order to obtain adequate nutrition (Balseiro and Albariño 2006). Because this study sought to characterize the C:N:P in all components of food flow, shredders were optimal because were expected to produce a larger amount of FPOM.

Larvae Collection

Caddisfly larvae *P. gentilis* were chosen as the model organism of this experiment for several reasons: a relatively large size compared to other macroinvertebrates facilitates handling of the organism, the larvae seemed to fairly abundant in most streams surveyed as potential collection sites, research had already been conducted regarding lifecycles and maintenance of *P. gentilis* larvae. The larvae have also been classified as "shredders", meaning they gather food by shredding detritus and eating microorganisms; shredders usually exhibit the greatest consumer-resource imbalance. *P. gentilis* larvae Stonecrop Creek, a hardwater stream with a fair amount of riparian coverage and shady regions (Earl et al. 2006). Larvae were collected streams with conditioned leaf packs in late June; one study, however, shows that larvae at this late stage may be experiencing aestivation, which slows metabolic processes(Mackay 1976). In order to work with active larvae and avoid this period, studies were conducted shortly (the following day) after collection.

Experimental Procedure

The first of two trials began on 12 June 2007. Conditioned leaves of unknown species were retrieved from Stone Crop Creek and used as the food source in the first trial. The tank in which the larvae were

kept was filled with aerated well water, contained gravel for substrate, and was supplied with leaves conditioned in Stone Crop Creek. Thirteen recirculation chambers (80 cm^2 by 5 cm) were then filled with 300 mL of well water and fitted with netting along the bottom to provide a substrate for the larvae to crawl. Mackay (1975) indicated that *P. gentilis* has no preference for substrata size, though organic substrata may be preferred. The temperature of water for both the tank and recirculation chambers was approximately 14.5 °C after consistent measurements; studies from Gallepp (1977) confirmed this as an optimal temperature for metabolic processes. Approximately 500 mg of wet leaves were placed in each of the chambers. One larva was placed in 10 of the chambers, with the remaining three chambers as randomly chosen controls. In order to generate the amount of FPOM needed for analysis, two additional chambers were set up with approximately 15g of leaves and five larvae each.

Chambers were run from 1400 hr,12 June to 1400 hr, 15 June totaling 36 hours. Afterward, caddisfly larvae were transferred from their recirculation chambers to a corresponding Falcon tube equipped with approximately 45 mL of well water, a small piece screen for substrate, and a tube running through a hole in the lid to aerate the water. Each larva was then left in the Falcon tube overnight to allow it to clear its gut. The following day, larvae were removed from the aerated Falcon tubes and frozen for further analysis.

After the 36 hour run, leaves were removed from each chamber and placed in a drying oven. All leaves and particles approximately greater than 1 mm were removed from the thirteen recirculation chambers. The remaining contents, or FPOM, were separated from the water via suction filtration on pre-weighed 25 mm FFG filters and placed in a drying oven. Each component of the filtering apparatus was rinsed with 10% HCL solution and DI water before the next sample was processed. After filtration, the filtrate was separated into three 50 mL Falcon tubes marked with its corresponding recirculation chamber number. Each tube was then frozen for further analysis.

The same procedures were followed during the second run of the experiment, with the exception of using Box Elder, *Acer Negundo*, leaves conditioned in Stone Crop Creek as the food source.

Water Analysis

Seventeen days later, filtrate samples from both experimental runs were thawed and analyzed with a LaChat to determine concentrations of NH_4^+, NO_3^-, and PO_4^{3-}. Carbon concentration was determined with a O.I. Analytical 1010 total organic carbon analyzer.

Tissue Analysis

Each tissue sample from larvae, leaves, and FPOM was be analyzed for C, N, and P content. First, dried tissue from larvae, leaves, and FPOM were ground into a fine powder. Five composites of each tissue type were made; for example, tissues from recirculation chambers 1 and 3 were combined into Composite 1. Thirty composites, which had then been separated by the experimental run, tissue type, and chamber number, were placed in Kartell snap containers with appropriate labels. An amount from each snap container was then analyzed in a C:N Analyzer; approximately 10 mg of ground larva, 15 – 40 mg leaf, and 15 – 40 mg FPOM were used. All instruments and surfaces were washed with methanol between use to avoid contamination.

To determine P levels, a technique employed by Rosemond (1993) was used. Sample sizes similar those of the C:N analyses were placed in acid-washed, ashed, and uniquely labeled beakers. These were then ashed in a muffle furnace at 550 °C for one hour. After the beakers had cooled, 20 mL of 1 N hydrochloric acid was added to digest contents of ash. P content was then analyzed with the absorbic acid technique.

Results and Discussion

Two experimental runs were used in this study. During Experiment I, we used an unknown quantity of conditioned leaves as the food source for *P. gentilis* larvae, while throughout Experiment II larvae were provided with conditioned Box Elder leaves. The larvae exhibited a strong affinity for Box Elder leaves; NH_4^+ excretion rate (U), PO_4^{3-} excretion rate, and assimilation rate (A) were higher in Experiment II (Table 1). This could be due to the higher nutrient content of Experiment II leaves, which are nearly half the C:N ratio of Experiment I leaves. Though a substantial difference in the leaf nutrient content exists between the experimental runs, these two will be both be used as a model of study.

Table 1. Differences of Rates among Experiment I and Experiment II

Value	Experiment I	Experiment II
U NH_4^+ - N (µmol N · g dry mass^{-1} · h^{-1})	1.7730	4.7884
U PO_4^{3-} (µmol P · g dry mass^{-1} · h^{-1})	0.0247	0.0855
U NO_3^- - N (µmol N · g dry mass^{-1} · h^{-1})	2.8311	2.8347
I (g leaf/g larva/hr)	0.0928	0.8250
E (g FPOM/g larva/hr)	0.0038	0.0220
A (g matter/g larva/hr)	0.0892	0.8030
Molar C:N of leaves	**63**	**37**

Water and tissue from larvae, leaves, and FPOM were analyzed for C:N values (Figure 1). Leaves from Experiment II were better quality in terms of N content. However, the organism and excretion C:N ratio is much lower. Note that while there is a large discrepancy of food C:N between Experiments I and II, the C:N values of the organisms from both experiments are very similar. This indicates that despite varying food sources, these organisms do indeed maintain a uniform body chemistry. The larvae from Experiment I would have had to process more low quality food to meet their bodily requirements, which demonstrates the direct relationship between the quality of a food source and an organism's physiological processes.

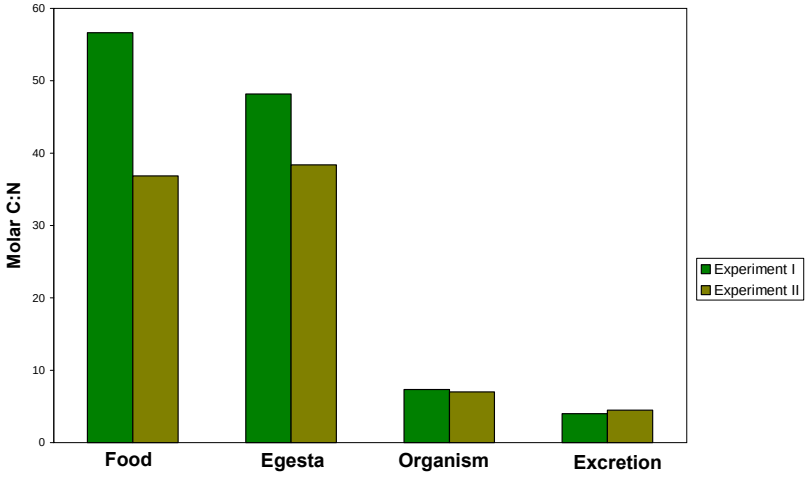

Figure 1. C:N Values for Experiments I and II

Figures 2 and Figure 3 further illustrate that the elemental requirements of an organism directly affect its biological processes—excretion in this instant. Excretion rates of an element exhibit a negative relationship with the amount of that element required by the organism for survival (Vanni et al. 2002; Cross et al. 2003). In other words, if an organism is maintaining homeostasis at a higher level of N% in its body, then one could expect that organism to exhibit higher N retention, thus forming a negative relationship with its excretion rate of N. These figures demonstrate that as the amount of element required by an organism increases, excretion of that element decreases. This relationship is rather abstract in Experiment I. Experiment II, however, shows a more distinct relationship between excretion and percent body mass of an element.

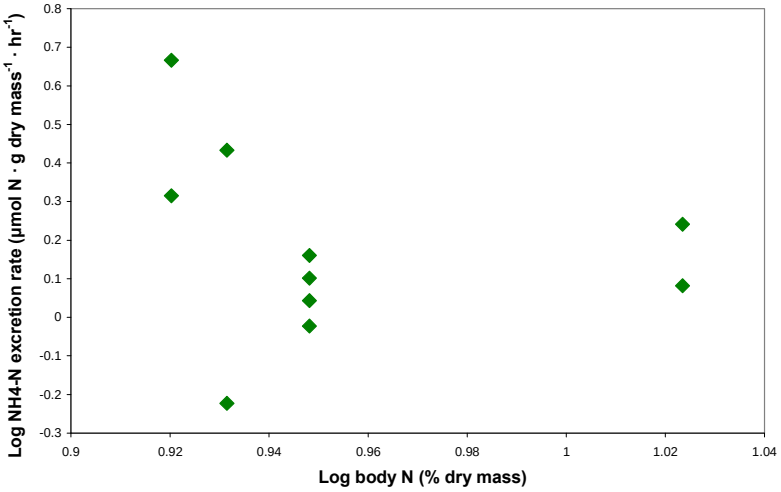

Figure 2. Experiment I: N-NH4 Excretion rate vs. Percent body N

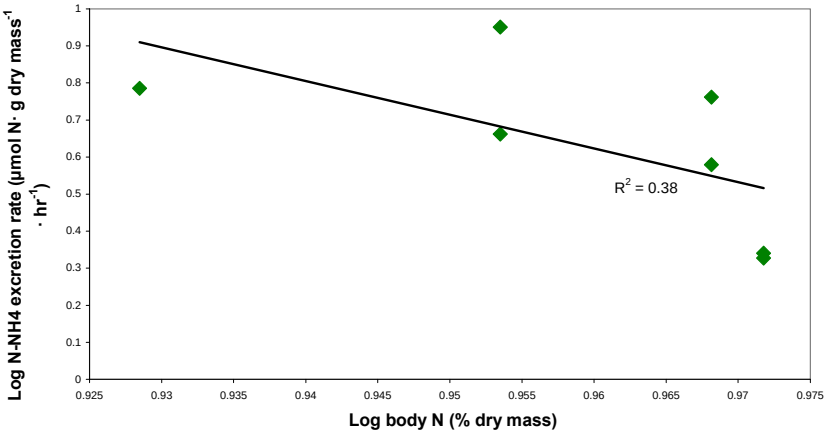

Figure 3. Experiment II: N-NH4 Excretion rate vs. Percent body N

Conclusion

This study tracks only C:N values in organisms, while many other ecological investigations have traced C:N:P as part of the stoichiometric equation. While the initial objectives of this study were to trace P values as well, time simply did not allow for this investigation. However, by tracing C:N variances in a consumer, its resource, and its environment, the data shows a distinct relationship between all pairs, or an "equation" in terms of the stoichiometric ecologist. Though this vague equation is had has been established, many components of that equation are still missing, providing an outlet for further research in this area.

In this study, for the sake of simplicity, the nutrient flow of an organism was classified into four primary components: input (leaf), body (larva), and output in the forms of excretion and egestion. This simplified version, however, does not take into account other factors such as nutrient leaching and respiration. I assumed, for instance, that all disappearance of mass of the food source is due to ingestion by larvae, while in fact some of this may be lost as dissolved organic carbon. Also, when nutrients are consumed by an organism, they can either be used for growth or respiration. At what level is the selection of nutrients occurring?

Rather than answer questions, this study has been a source for many new inquiries into consumer-resource imbalance investigations, and the concept of ecological stoichiometry in general. How does the C:N:P ratio between input, body, and output vary among different functional feeding groups? How does the addition or loss of one of these groups affect the C:N:P balance within a particular ecosystem? How is anthropic input affecting the balance of these stoichiometric equations? More research in this area is required would not only allow us to answer these important questions, but also to promote further research and restoration of our streams and watersheds.

Acknowledgements
I express my appreciation of all the hours and helped offered by Beth Cheever, who helped direct this study and Dr. Jackson Webster, my research advisor and other members of the Virginia Tech Sream Team: Bobbie Niederlehner, Dr. Fred Benfield, and Ryan McManamay. Last, but perhaps most important, I want to thank Dr. Tamim Younos and Dr. Vinod Lohani for establishing this REU fellowship here at Virginia Tech.

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A Study of Energy Consumption by Water Supplies and Wastewater Infrastructure in Blacksburg, Virginia

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Abstract

Understanding the energy and water relationship is a fundamental step toward developing technology and strategy that will help sustain the supply of both resources. Rising energy costs and increased water demand due to population growth will affect availability of safe and adequate water to meet societal needs. Information on energy consumption through water infrastructure is scarce. The objective of this study was to estimate energy consumption associated with treating drinking water, wastewater treatment, and water distribution using Blacksburg, Virginia as a case study site. Data was collected from water and wastewater treatment plants, Town of Blacksburg, and Virginia Tech. Energy consumption (kWh) for various systems components were estimated for the years 2000, 2003, and 2006. Results show fairly consistent seasonal and annual energy consumption for water and wastewater treatment plants with some noticeable peaks and valleys. Per volume basis, slightly more energy is needed for wastewater treatment compared to water treatment for drinking water supplies. For drinking water supplies, more than 60% of energy use is attributed to pumping and distribution. Paper is concluded with future research needs.

Keywords: utilities, water and wastewater treatment, water distribution, energy consumption, Virginia

Introduction

Energy and water are two indispensable and interrelated commodities that are necessary for everyday life. The intimate relationship between energy and water is two-fold: (1) Water is used for energy production, and (2) energy is used to treat and deliver water. The production of energy involves large volumes of water, consuming over 1/3 or our fresh water supplies (Hutson et al. 2005). The flipside reveals that the production of clean water and distribution consumes nearly 2-10% of the country's total energy use (Pelli and Hitz 2000). Our heavy reliance on these resources in various aspects of our lives (food, water, lighting, human health, manufacturing, agriculture, and recreation) shows the importance of better understanding the relationship between energy and water. This research focuses on the energy use for water supplies (treatment, storage and pumping related to drinking water) and wastewater infrastructure (treatment and pumping related to wastewater.

Review of Literature

There are more than 60,000 water supply plants and 15,000 wastewater treatment plants in operation in the United States (Oliver and Putnam 1997). The demand for electricity at these facilities is 75 billion kWh per year. Of that, up to 80% of these electrical energy costs are for moving water by pumping system (Oliver and Putnam 1997). Several "energy audits" and "Energy Conservation Opportunities" studies have been conducted around the United States. Lystra et al. (1981) investigated four drinking water and wastewater treatment plants in Michigan. Their studies located areas in the system that had the greatest potential for saving energy, which usually corresponds to the area which demands the most energy. According to the article, pumping was the area in which the most energy was consumed in the drinking water system, whereas the sludge handling and aeration consumes the largest amount of energy in wastewater treatment.

Pumping contributes to more than half the energy use in water systems for both drinking water and wastewater. Thus, many articles point out that the most effective way to reduce energy consumption in water systems is to improve the efficiencies of pumps. Lowell Water-Works of Lowell, Massachusetts observed an average energy savings of 40% because of improved efficiencies of raw water pumps. Likewise, the Highlands Pump Station in Denver, Colorado saved $3,000 per year after installing high efficiency motors (Oliver and Putnam 1997). In general, "potential energy savings by optimizing the performance of pumping systems range from 20-50%." (Oliver and Putnam 1997). A better understanding of the energy and water nexus would further improve efficiency.

Study Site

The site of our study is Blacksburg, a town within Montgomery County, Virginia. Blacksburg is home for Virginia Tech, state's largest university. The Blacksburg population in 2000 was 39,573 excluding on-campus Virginia Tech students. The town is dominated demographically by Virginia Tech, which enrolls more than 25,000 students. Consequently, Blacksburg is a unique university town owing to the changing seasonal population based upon the university. Lower population happens in the summer months (June – August) when students are on summer break, and higher population is observed during football season (September – November) when as many as 20,000 alumni and fans gather in Blacksburg.

Blacksburg is located in the mountainous areas of Southwest Virginia (elevation is about 2000 ft above sea level). The mountainous terrain is a notable factor in this study, as energy use for water distribution and pumping is highly dependent on the topography of the area. The source of water supplies for Blacksburg is the New River. The New River flows upstream from North Carolina, 155 miles through Virginia and into West Virginia and is considered one of the cleaner rivers in Virginia. Some segments of the New River are designated as impaired (does not meet water quality standards) (VA-DEQ 2007). However, water quality in the intake for Blacksburg water supplies which is located right below the bridge on Route 114 is considered good quality water. The wastewater from Blacksburg after treatment is returned to the New River several miles downstream from the water intake. Figure 1 shows the path for water supplies from the New River to Blacksburg and the wastewater path from Blacksburg to the New River.

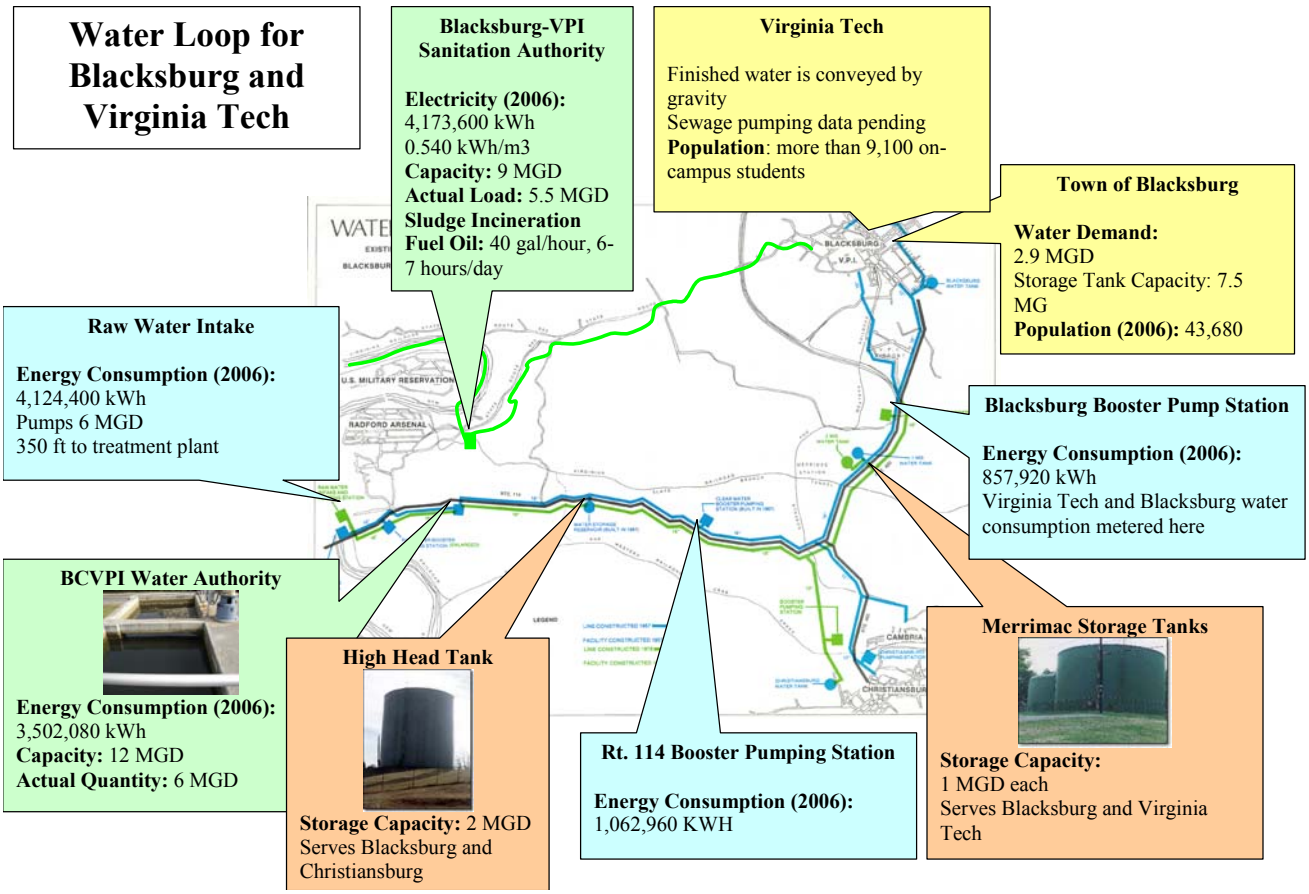

Figure 1. Location of intake, treatment, distribution and discharge for water provided to Blacksburg and Virginia Tech

The New River water intake pump station is located below bridge on Route 114. From the intake, raw water is lifted 350 ft in elevation toward the Blacksburg, Christiansburg, VPI Water Authority, which is located approximately two miles east from the raw water pump station. The Water Authority provides clean water to Blacksburg, Christiansburg, and Virginia Tech.
Current rate of raw water inflow to the water treatment plant is 6.5 MGD. The full water treatment capacity of the plant is 12.4 MGD (Higgins 2007). At the water treatment plant, the raw water goes through standard treatment processes that include flocculation, settling, filtration and disinfection. Sludge is scraped and sent to the sludge handling facilities.

The finished water from the treatment facility is pumped to the High Head tank off of Route 114 before it travels to Blacksburg and Christiansburg by the Route 114 booster pump station. The path toward Blacksburg follows Route 460 where it is stored in two 1 MG capacity tanks (Merrimac Tanks). Clean water is delivered to Blacksburg and Virginia Tech by the Blacksburg booster pump station at the rate of approximately 2,500 gallons per minute (GPM) (Schirmer and Formica 2003). There are several pumps and storage tanks in Blacksburg to deliver water to the town. According to the Annual Water Quality Report, Blacksburg has a total water storage tank capacity of 7.5 million gallons of drinking water after

two new 1 MG tanks were built in Highland Park. There are no pumping stations within the Virginia Tech campus. Pressurized water is conveyed by gravity to all Virginia Tech buildings and other uses.

Wastewater leaves Blacksburg and Virginia Tech, moving down Prices Fork Road by gravity and arrives at the Blacksburg-VPI Sanitation Authority (wastewater treatment facility). The Sanitation Authority serves Blacksburg, Virginia Tech and Montgomery County. Sixteen or seventeen pumping stations are located within Blacksburg service area to boost wastewater delivery prior to gravity flow toward the wastewater treatment facility. The Sanitation Authority typically treats loads of 6-7 million gallons per day (MGD). The Authority receives storm water runoff in addition to wastewater from Blacksburg area. During snowy weather, the load increases significantly to 20-30 MGD. In the wastewater treatment plant, influent passes through a grit chamber, sent up by artesian screw pumps, and conveyed through the rest of the plant by gravity. The majority of pumping and treating at the wastewater treatment plant is powered by electricity, except for sludge handling which uses diesel fuel (Vaught and Epperly 2007).

Research Goal

Overall Research Goal

The overall research goal is to estimate energy use by water supply and wastewater infrastructure and identify potential ways for energy efficiency and use of alternative and renewable energy sources for water treatment and delivery. This information may be used for additional research for the following: (1) integrating renewable energy sources into the water system; (2) studying the effects of energy use of water systems on the atmosphere; and (3) as a guideline for other towns and institutions to conduct their own energy audit.

The short-term research objective was to determine the amount of energy consumed for water intake, treatment, and distribution using Blacksburg, Virginia as a case study site. Specific objectives of this research were as follows: (1) estimate monthly (for seasonal variations) and annual energy usage; and (2) estimate per capita energy use for Town of Blacksburg and Virginia Tech populations.

Research Methods

Figure 2 shows the flowchart for estimating water infrastructure energy consumption for the Town of Blacksburg and Virginia Tech. Research methods included field visits and interviews, data collection from available reports, and data analysis.

Field visits and interviews

Field visits and personal interviews at the Water Authority, Sanitation Authority, Town of Blacksburg Engineering and Planning Department, and Virginia Tech Mechanical Utilities were conducted. Also, contacts were made with Appalachian Power which is the major power provider for water supply and water treatment facilities in the area of study.

Data Collection

Water use and energy data were collected for years 2000, 2003, and 2006 in order to detect possible trends. Data sets collected include: (1) monthly energy bills from the Water Authority; (2) monthly energy bills from the Sanitation Authority; (3) gallons metered at Blacksburg pump station; (4) gallons of wastewater treated by the Sanitation authority; and (5) fuel oil consumption for sludge incineration at the Sanitation Authority.

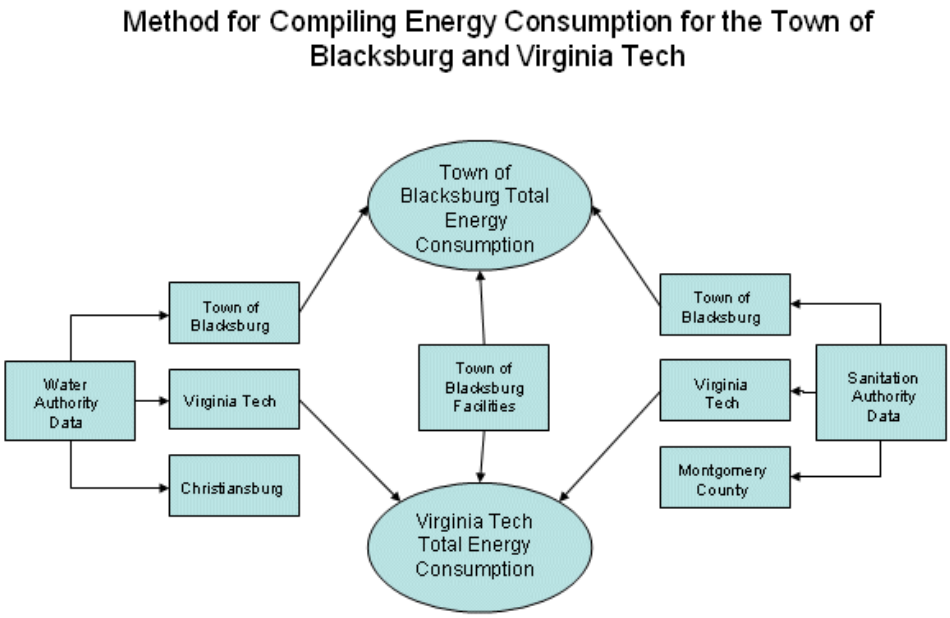

Figure 2. Flowchart for estimating energy consumption

As mentioned, the Water Authority serves Blacksburg, Christiansburg, and Virginia Tech. The Sanitation Authority serves Blacksburg, Virginia Tech, and Montgomery County. Thus, it is necessary to proportion the amount of energy used by the Town of Blacksburg and Virginia Tech separate from Christiansburg (for Water Authority energy consumption) and the County (for Sanitation Authority energy consumption) when calculating the energy consumption for water in Blacksburg. The amount of kWh the Water Authority had used for the intake, treatment, and movement of the water was broken down among Blacksburg, Christiansburg, and Virginia Tech proportional to the amount of gallons consumed by each. These calculations were made based on the assumption that the kWh used to treat and distribute the water is directly proportional to the gallons consumed. The breakdown does not consider the varying distances the water must travel, and the energy associated with head loss.

The Sanitation Authority does not have any means to measure the amount of wastewater inflow from each locality they serve. As a result, the gallons of wastewater treated from Virginia Tech, Blacksburg, and Montgomery County are based on the gallons of water metered by the Water Authority, minus estimated line loss and non-contributory sewage submitted by each locality. This breakdown was used to proportion the amount of energy consumed by the Sanitation Authority and Virginia Tech. Furthermore, the Sanitation Authority owns a single meter for all processes in the plant, making it virtually impossible to estimate the breakdown of energy for pumps and wastewater treatment. The Sanitation Authority presumably uses more energy for treatment compared to the Water Authority.

Data Analysis

After extracting metered energy consumption in kWh from Water Authority and Sanitation Authority electricity bills, graphs for monthly energy consumption were developed in order to determine seasonal variations in energy use. Comparisons for Water Authority and Sanitation Authority for both seasonal variations and kWh/volume were completed. Energy use by water and wastewater infrastructure within the Town of Blacksburg and Virginia Tech were calculated. A pie chart was developed to determine the ratio of energy consumption between pumping and treating and a map of the water path was drawn (Figure 3).

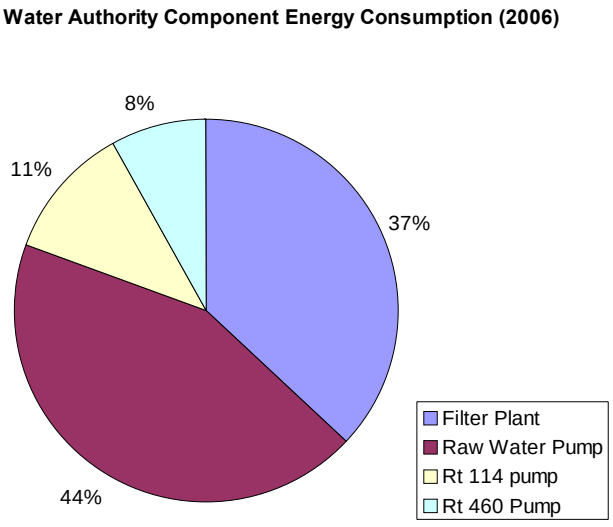

Figure 3. Breakdown of energy use for water treatment and pumping

Results and Discussion

Water Supplies

Energy usage pertaining to water supplies includes pumping and treatment by the Water Authority. Water authority energy usage fell within the range of 700,000 and 950,000 kWh in the years 2000, 2003, and 2006. Figure 3 shows the breakdown for energy use for different system components. Pumping consumed 63% of the energy in the Water Authority system in 2006, and is by far the most energy intensive component. Raw water pumping in itself consumes 44% of the energy in order to convey water 350 ft up in elevation. Treatment consumed the remaining 37% of energy.

Energy consumption at the Water Authority was relatively consistent seasonally (Figure 4). There was a visible peak in September for unknown reasons. Possible explanations could include an increase in water use due to football season and air conditioning in the dormitories. It was expected that the absence of thousands of college students in the summer months would attribute to a significant decrease in energy use, but an increase in water use for irrigation and cooling towers leveled the energy consumption.

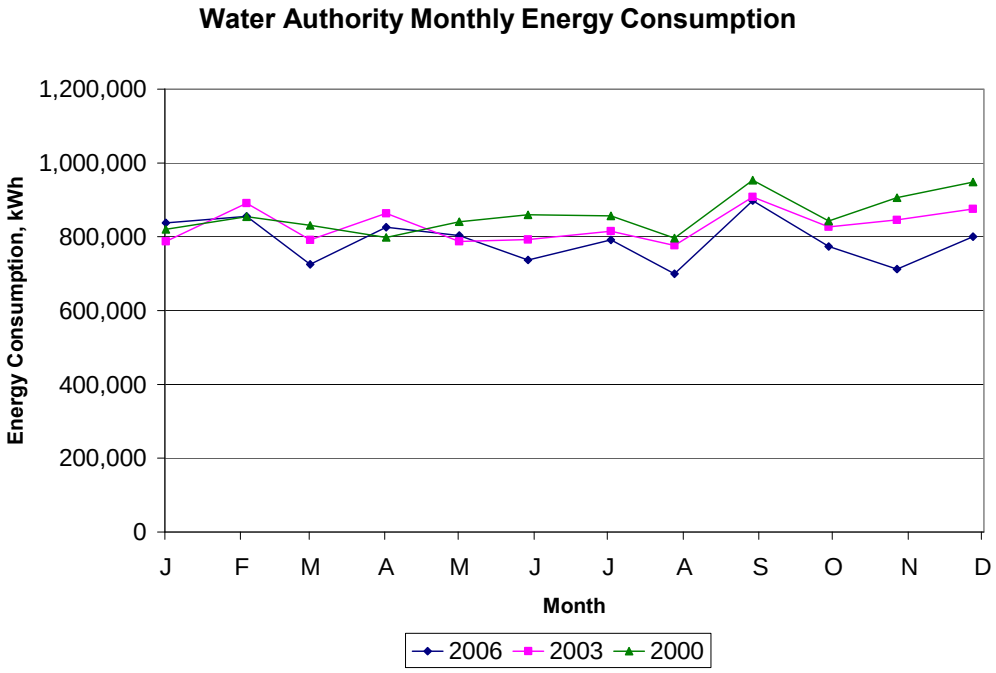

Figure 4. Seasonal variation of energy consumption reported by the Water Authority

Wastewater Treatment Facility

Figure 5 shows energy consumption at wastewater treatment facility. The Sanitation Authority operated within a range of approximately 250,000 to 425,000 kWh in the years 2000, 2003, and 2006. The wastewater treatment facility also consumes fuel oil for incineration purposes.

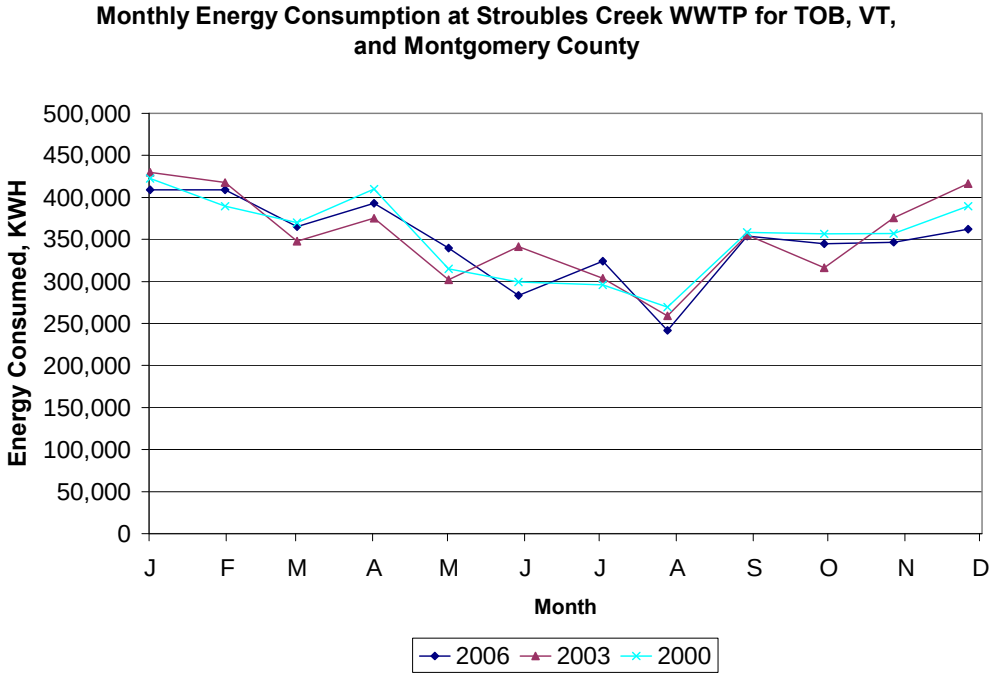

Figure 5. Seasonal variation of energy consumption by the wastewater treatment plant (Sanitation Authority)

The monthly energy consumption for years 2000, 2003, and 2006 at the Sanitation Authority was similar to water treatment plant. Less energy was consumed in the summer months. In the year 2006, there was as much as a 69% increase from August to January. Because the Sanitation Authority receives its wastewater from combined sewers, seasonal variations were possibly due to snow fall during the winter months. The decrease in wastewater could also be attributed to students leaving for summer break, resulting in a significant decrease in the Blacksburg population. The effects of summer camps and orientations that took place at Virginia Tech during the summer months were not investigated.

Figure 6 shows a comparison of energy consumption at water treatment plant (Water Authority) and wastewater treatment plant (Sanitation Authority). The wastewater treatment plant consumed as much as 140,000 kWh more than the water treatment plant in 2006. On a specific volume basis, the water treatment plant and the wastewater treatment plant consumed 0.44 kWh/m^3 and 0.54 kWh/m^3, respectively. These numbers were surprisingly close, considering the high energy intensity of wastewater treatment. The numbers for wastewater treatment include the energy consumption for the entire plant (HVAC, pumping, etc.), whereas the energy consumption for drinking water treatment is based on the filter plant's energy consumption. Thus, the specific energy used to treat wastewater may be far less than energy used to treat drinking water.

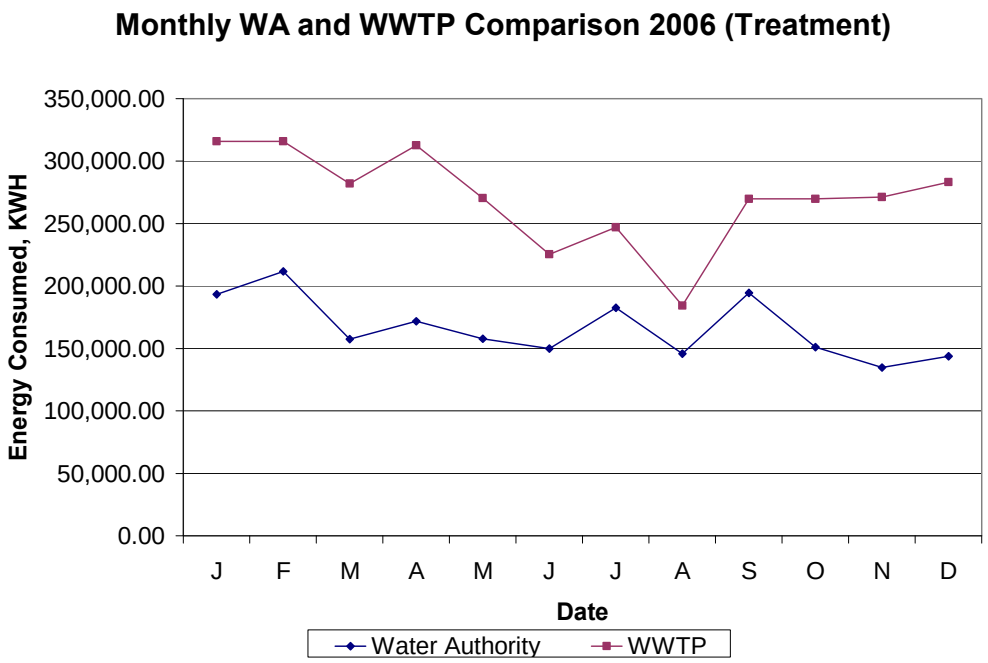

Figure 6. Comparison of monthly energy consumption by water treatment plant and wastewater treatment facility

The combined amount of electricity the Water Authority and Sanitation Authority consumed was 13,635,200 kWh in 2006 (Figure 7). The Sanitation Authority used 96,573 gallons of fuel oil to incinerate sludge. It should be noted that energy use for wastewater discharge pumps are not included in total cost as data were not available.

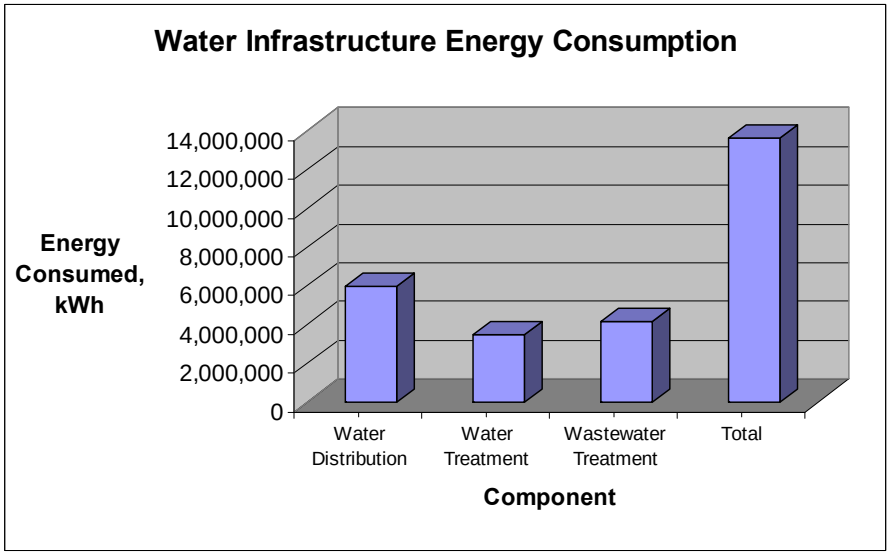

Figure 7. Components of energy consumption for water infrastructure in Blacksburg, Virginia

Figure 8 shows results for Water Authority and Sanitation Authority treatment and pumping energy consumption for Blacksburg and Virginia Tech. Water and Sanitation Authority treatment and pumping for the Town of Blacksburg was 8,594,909.85 kWh in 2006. In the same year, the proportion for Virginia Tech was 3,621,889.52 kWh. These numbers are lower than previous years. The Town experienced more than 500,000 kWh drop in energy since 2003. The ratio between the Town and Virginia Tech energy consumption remained consistent in the years 2000, 2003, and 2006.

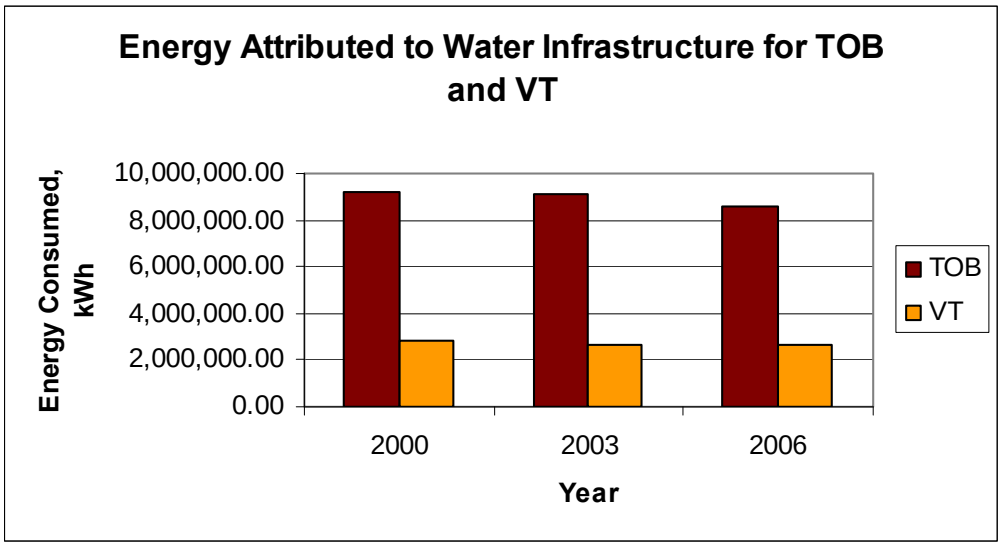

Figure 8. Energy use for treatment and pumping reported by the Water Authority and Sanitation Authority

The following does not directly relate to the results, but is important to note because it involves energy costs. The company supplying the Water Authority's electricity (American Electric Power) uses a smaller billing factor for larger and more energy demanding facilities that falls within an energy bracket at any time during the year. The Authority met that bracket by turning on all raw water pumps for a few hours, saving approximately $48,000 last year.

Total energy calculations do not include "end use"– energy used to heat water for showers or circulation inside a building, nor does it include data consumption for the production of treatment chemicals or construction (i.e. laying in pipeline, building facilities, etc). As mentioned previously, the Sanitation Authority uses only one meter for their entire operation, making it virtually impossible to break down the amount of energy for treatment only, thereby affecting results for comparisons between the Water Authority and Sanitation Authority. The energy data for the wastewater pumping within Town of Blacksburg and Virginia Tech are not included. Thus the compilation shown in Figure 2 has not yet been computed and per capita energy uses were unable to be calculated.

Conclusion

After extensive data collection from both drinking and wastewater facilities, it can be attested that more than 60% of the energy consumption is attributed to pumping alone. Energy consumption for drinking water treatment remains fairly steady throughout the year, while the wastewater treatment plant shows noticeable peaks during the winter and dips during the summer. There has also been a downward trend in total energy use by the Town and Virginia Tech for unknown reasons. Excluding the energy used for pumping within Blacksburg, it takes more than 8.5 million kWh to supply clean water and treat wastewater for the Town. It is important to develop technology and implement strategies for balancing both sides (water for energy and energy for water) to maintain sustainable sources of both energy and water.

Recommendations for Future Research

As water quality standards become more stringent, research for improving drinking water and wastewater treatment technologies without increasing energy consumption should be explored. For example, wind energy was being considered as an alternative energy source for a wastewater treatment plant in the Aegean Islands in order to meet the load of summer tourism (Haralambopoulos et al. 1997). Using solar energy for water and wastewater treatment is another attractive option. Other possibilities include using waste as a source of energy, which has been put into practice in Sweden. A wastewater treatment plant sells heat from sewage which supplies enough energy to heat 80,000 apartments. There is much research need about the reciprocal and inexplicable relationship between energy and water, and as our understanding deepens, sustainability of both resources will improve.

After interviewing several plant managers, there is research need about examining the 21% water loss, focusing on causes and preventative measures.

Acknowledgments

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Developing a Small Scale Wireless Data Collection System for use in Watershed-Based Research

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Abstract

The main focus of this research was to develop a prototype for the wireless data collection system. This involves researching numerous hydrologic and water quality sensors and modems for wireless communication and developing an understanding of the workings of microcontrollers and of the user interface software LabVIEW. During the ten week period, instruments and software were identified and assembled. Some preliminary tests were conducted to evaluate the functionality of the designed system.

Keywords: data acquisition, remote sensing, real-time monitoring, instrumentation, watershed

Introduction

Remote sensing is the gathering of data or information of properties on a given object or area by use of devices that are not in physical contact with the object or area. Techniques of remote sensing involve, but are not limited to, electromagnetic radiation, radiometers, scanners, lasers, radio frequency receivers, radar systems, sonar, thermal devices, sound detectors, seismographs, magnetometers, and other instruments. Data acquisition is the sampling of real physical properties to generate data that can be analyzed by a computer. This usually involves the use of sensors that convert a real parameter (such as humidity) to an electrical signal. The data acquisition hardware (or DAQ) receives the electrical signals and processes the signals in order to make sense of the data. Instrumentation relates to the measurement and control of attributes of physical systems. It usually involves the sort of input/output devices which can be given a command and they will give back a desired response based on what they are programmed to do.

Watershed-based research will benefit highly from the before-mentioned technologies. An ultimate goal of this study is to design an on-campus watershed sciences and engineering laboratory at Virginia Tech. This entails having instrumentation for real-time monitoring of hydrologic and water quality parameters. A wireless data receiving/transmission system is vital in order to convey the real-time measurements to a web-based user interface. The proposed location of this design will be Stroubles Creek nearing the Duck Pond at Virginia Tech.

The main focus of this study was to develop a prototype for the wireless data collection system. This involves researching numerous hydrologic and water quality sensors and modems for wireless

communication and developing an understanding of the workings of microcontrollers and of the user interface software LabVIEW.

Methods

The research study began by assessing current knowledge of electronics and computer programming skills. Being a junior level undergraduate student, one's limitations have to be made clear. Programming knowledge came from a course in Fortran 90 and an introductory course of assembly language and CPU architecture. Practical electronics were still basic, as the only hands-on electronics experience came from a laboratory section that was part of an introductory circuits course and some time spent dealing with hobby robotics (Iovine 2004). More experience was necessary. Therefore, some time was spent building a mobile robot. The motor driver IC was introduced and, more importantly, the implementation of a PIC16F84A microcontroller and its capability in several applications were explored.

Results and Discussion

The PIC16F84A microcontroller will serve to be the brains of the project as it can run programs that are used to control motor drive systems, read sensors, and communicate. A microcontroller is basically an inexpensive single-chip computer which one can program to make decisions and perform functions based on situations and events (Iovine 2004). The PIC16F84A is optimal for controlling remote sensors for its low cost and low power consumption. It is an 8-bit microcontroller which uses Reduced Instruction Set Computer (RISC) language. There are only a total of 35 different instructions with which one can program onto the PIC's flash memory. The PIC16F84A has 18 physical pins from which 13 are bi-directional input/output (I/O) pins. Figure 1 shows a detailed schematic of the PIC16F84A and its pins. The MCLR pin (4) is the Master Clear (Reset) input, and it is an active low reset to the device. This means that if the incoming voltage to that pin is a low signal (0 V in this case), then the PIC will automatically reset itself. We do not want this to occur for our purpose; therefore we will feed it with a high voltage (+5 V). The OSC1/CLKIN and OSC2/CLKOUT pins (15 and 16) are the oscillator crystal input and output pins, respectively. These are connected to an oscillatory crystal in order to keep track of time and the instruction cycle rate for the microcontroller. The VSS pin (5) and VDD pin (14) are the ground reference and positive supply, respectively, for the logic and I/O pins.

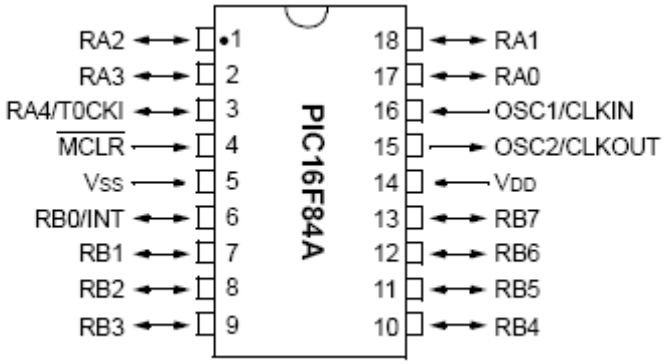

Figure 1. PIC16F84A microcontroller pin designations (e.g. see Matic 2000).

After having understood the principles of the PIC16F84A microcontroller, a research in water quality and hydrologic sensors was needed. There are several different types of sensors that will be needed to have a fully capable watershed laboratory on Stroubles Creek and the Duck Pond. There are water quality sensors that measure chemical properties of water, and some examples of these are pH, conductivity,

dissolved oxygen, turbidity, salinity probes, as well as the ion selective electrode analyzer, which can monitor nitrate, chloride, calcium, and ammonium, among others. There are also sensors that measure the physical attributes of a stream of water and the ambient area, such as temperature, flow rate, barometers, and humidity sensors. After an assessment of the different types of sensors was made, it was noted that air temperature and humidity sensors would be the most advantageous sensors for the small scale prototype of the wireless data collection system.

Research was conducted in order to acquire the most useful sensors for the project. After looking through numerous websites and online vendors, a LM34CZ temperature sensor was selected (for example see: http://www.hobbyengineering.com/H1752.html). It is a temperature sensor that turns out an output voltage which is proportional to the measured temperature. It has a temperature range of -40 to +230 degrees Fahrenheit with an error range of +/- 2 degrees. A SHT11 sensor which measures temperature and relative humidity from a company called Sensirion was also selected (for example see: http://www.parallax.com/detail.asp?product_id=28018). The latter was a bit more expensive, yet still relatively cheap. The added parameter enhances its benefit. This sensor returns either a 14-bit or 12-bit number when prompted that must be converted into the actual value for temperature or humidity by using simple formulas as shown in the SHT11 datasheet (Figures 2 and 3).

$$\text{Temperature} = d_1 + d_2 \bullet SO_T$$

VDD	d_1 [°C]	d_1 [°f]
5V	-40.00	-40.00
4V	-39.75	-39.50
3.5V	-39.66	-39.35
3V	-39.60	-39.28
2.5V	-39.55	-39.23

SO_T	d_2 [°C]	d_2 [°f]
14bit	0.01	0.018
12bit	0.04	0.072

Table 8 Temperature conversion coefficients

Figure 2. Calculation for temperature.
(http://www.parallax.com/dl/docs/prod/datast/shtx.pdf)

For improved accuracy with minimal calculation complexity the following calculation is recommended:
$RH_{real} = (a*SO + b) / 256$
Where SO denotes the 8 bit humidity sensor output signal.

Validity	a	b
$0 \leq SO \leq 107$	143	-512
$108 \leq SO \leq 255$	111	2893

With the above values the calculation can be done with a single 8 bit multiplication followed by a 16bit addition / subtraction.

For a 16 bit SO, $RH_{real} = (a'*SO' + b') / 4096$, where SO' denotes the 12bit sensor output signal and a' = a, b'= b*16. The validity limits also have to be multiplied by 16.

Figure 3. Calculations for Relative Humidity.
(http://www.parallax.com/dl/docs/prod/datast/shtx.pdf)

The hardest part of this study was how wirelessly transmits data from the PIC microcontroller to a laptop. There are several ways of doing this such as using Bluetooth technology and radio frequency modems, among others. Professor Tom Martin and two of his graduate students in the Electrical and Computer Engineering department at Virginia Tech recommended the XBee ZigBee RF modules by MaxStream.

The XBee ZigBee RF modules are great for this project because they offer a larger range of transmission than most ZigBee modules (for example see: http://www.adhocelectronics.com/Products/ZigBee). They are also not very expensive and are consume low power. The XBee Pro RF modules that are used in the prototype run off a 215 mA current and use a voltage supply of anywhere between 2.8 and 3.4 V. These radio frequency modules operate at a frequency of 2.4 GHz. Now, there was the issue of figuring out how to connect these modules to communicate with the PIC microcontroller and, the other, to connect to a remote laptop. Fortunately, there are development boards that the RF modules can easily be latched onto in order to facilitate accessibility to the pins (Figure 4). The development boards that will be used have RS-232 serial ports. In order to fully understand how the information was being communicated between components, an understanding of serial communication is required.

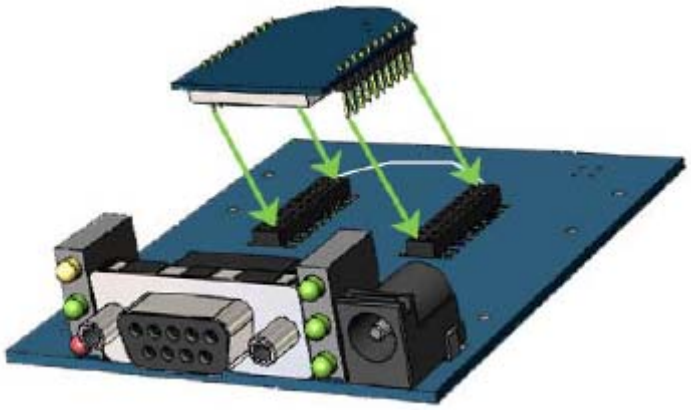

Figure 4. XBee RF module mounting to an RS-232 interface board.
(http://www.maxstream.net/products/xbee/manual_xb_oem-rf-modules_zigbee.pdf)

Serial communication is undeniably one of the simplest ways of sending data in electronic communication. Serial is the method of sending information one bit at a time. This is done by sending and receiving high and low voltage signals. The high signal is a “1” and the low signal is a “0”. There are four crucial settings that must be the same on both the transmitter and receiver: baud rate, data bits, stop bits, and parity. Baud rate is the speed at which the bits are being transferred. These must match both ends of the communication so that both know how long a signal will last for one bit. The XBee modules can run up to 250 Kbps. Data bits are the number of bits that are being delivered in a packet. Packets begins with a start bit, next are the data bits, followed by a parity bit (possibly), and ends with a stop bit (Figure 5). The number of data bits depends on what data is being sent. If the decimal number 250 is being sent, then 8 bits need to be sent because the binary representation of 250 is “11111010”. Parity is optional and is used to check whether the right number of bits has been received. The stop bit is used to identify the end of a packet and to allow for correction if the devices have become out of sync.

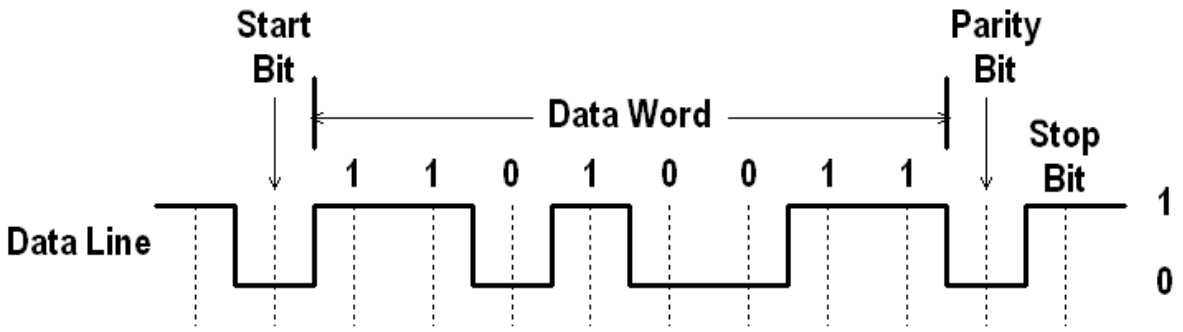

Figure 5. Example of a Packet in Serial Communication.

Depending on the logic of the serial communication, the specific voltage levels vary. According to the PIC16F84A datasheet, its I/O pins use TTL logic which recognizes 0 V as low and +5 V as high. This is a problem, because the modules will be connected via the RS-232 serial ports which recognize -12 V as low and +12 V as high signals. Therefore, we can not directly connect the microcontroller to the RF module. In order to communicate between the two devices, we need a level shifter. A MAX232CPE chip was obtained that is perfect for the job. It only requires a +5 V supply, and four 1 uF electrolytic capacitors. It has two channels for TTL to RS-232 logic conversion and two channels for RS-232 to TTL logic. In this project, only one channel will be needed of each. The TTL to RS-232 channel will be used for data sent from sensor to microcontroller to RF module, and the RS-232 to TTL channel for communicating back to the microcontroller from laptop.

Conclusions

During the ten week period, instruments and software were identified and assembled. Some preliminary tests were conducted to evaluate the functionality of the designed system. The follow up work includes interfacing and displaying the laptop information with National Instrument's programmable software, LabVIEW. Using NI VISA, it will be possible to communicate directly to the RF module that has wirelessly received data from the sensor-PIC-level shifter-RF module setup.

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