

2008 NSF REU Proceedings of Research

**Research Opportunities in Interdisciplinary Watershed Sciences and
Engineering at Virginia Tech**



Edited by

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Disclaimer

The findings, conclusions or recommendations presented in this report are those of the authors and do not necessarily reflect the views of the National Science Foundation or Virginia Tech.

Summary

The goal of our NSF REU program is to provide a diverse group of undergraduate students a stimulating interdisciplinary environment, where critical research questions related to water are addressed. The program intent is to nourish analytical skills and creativity of future scientists/engineers and potential graduate students.

In summer of 2008, eight students with diverse academic and cultural backgrounds were accepted as NSF REU fellows to the program. Program participants, home institutions and research topics were as follows:

Corrie Rose Campbell, Rose-Hulman Institute of Technology, water chemistry of plumbing pipes

Monica Neely, Oregon State University, biogeochemical controls

MaryTheresa Pendergast, Columbia University, oxygen depletion in reservoirs

Krista Williams, Virginia Tech, microbial source tracking

Frank Gronwald, North Carolina Agricultural & Technical State University, watershed monitoring & instrumentation

John Kenny, University of Kansas, watershed instrumentation & monitoring

Brittany Bogle, University of Arkansas, evaluation of decentralized and small-scale drinking water systems in Mexico

Ini Li, Columbia University, green buildings and rainwater harvesting

Upon completion of the program, fellows prepared research reports (this document) and presented their research results to research advisors/mentors and guests.

During the 10 week program, NSF REU fellows were required to attend weekly forums/seminars that were held on Friday afternoons offered by Virginia Tech experts. These forums were designed to meet several objectives of the NSF REU program: 1) gain a broader view of water issues; 2) learn how to be a good researcher; 2) learn how to communicate research results verbally and through research reports; and 3) learn about ethics in science and research. At these forums, fellow also learned about conflict resolution, graduate student experiences, and citizen scholar program and how to engage society in scientific exploration.

This document is a compilation of the research papers of our 2008 NSF REU program in interdisciplinary watershed sciences and engineering.

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The Characteristics and Chemical Performance of Polyethylene and Poly(1-butene) Pipes Removed from a Water Distribution System

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ABSTRACT

Four polymer pipes were removed from a buried potable distribution system in Florida and were examined to determine their physical, mechanical, and surface integrity and chemical interactions with neat toluene and trichloromethane. All specimens were characterized using digital scanning calorimetry (DSC), thermogravimetric analysis, infrared spectroscopy (IR), durometer hardness, and sorption testing. Analytical testing revealed that three HDPE pipes and one PB pipe were received, and that the PB pipe properties and contaminant interactions were very different from HDPE materials as confirmed by DSC, IR, and sorption testing. IR and oxidation induction time results demonstrated that all aged pipes exhibited oxidation. Toluene and trichloromethane diffusivity through some, but not all, HDPE pipes varied based on wall thicknesses and ages and contaminants penetrated through new (control) HDPE pipe the slowest. Residue was found on the inner walls of three pipes (0.010-0.097 mg/cm²), but did not affect contaminant interactions. Results of this study enable PE water pipe manufacturers, users, and regulators to better understand the condition of buried polymeric pipe and their resistivity to toluene and trichloromethane.

Keywords: polyethylene, poly(1-butene), aging, pipe, sorption, oxidation

Introduction

Polymeric water pipes are increasingly being installed in North American potable water distribution systems primarily due to their ease of installation, low cost, corrosion immunity, and assumed lifespan of 50 to 100 years. Historically, poly(vinyl chloride) (PVC), polyethylene (PE), and to a lesser extent poly(1-butene) (PB) have played a key role in the expansion of the North American polymeric potable water pipe market. In 1996, PVC was ranked first in the world's plastic pipe demand accounting for 62% of the global market with PE ranking second accounting for 33.5% of the world's plastic pipe demand (Raynaud, 2004). In contrast, PB pipe was heavily installed in the 1980s but was discontinued due to the large number of mechanical failures caused by chlorinated water exposure (Park et al., 1989; Park et al., 1991). As of 2008, \$1B has been spent to remediate PB pipe failures clearly demonstrating that failure to understand how polymeric pipes age and interact with water can be catastrophic.

As the two potable water pipes studied in this research, PE and PB are semi-crystalline thermoplastics that contain amorphous and crystalline regions. Crystalline regions are those areas of the polymer where the polymer chains are more densely packed and lined up in parallel. Packed polymer chains consequently make chemical permeation more difficult. Amorphous regions are vulnerable to permeation due to the less structured polymer chain formations. Another major difference between PE and PB is their melting (T_m) and glass-transition (T_g) temperatures. Below a polymer's T_g , amorphous regions take on a glass-like or rigid condition. The T_m is the temperature at which crystallites disappear, the polymer begins to flow, and is dependent on the polymer's molecular weight (Fried, 2003). Finally, additives used for manufacturing these polymers are relatively similar. Carbon black acts as a shield against the degrading effects of ultraviolet light. A variety of antioxidants are added to these materials during manufacture and these additives prevent oxidation of carbon-hydrogen bonds.

Oxidation is the prime reason for cessation of PB pipe installations in the 1980s. Conditions such as increased free chlorine concentration, pH, water temperature, exposure time, and water chemistry have been reported to accelerate polymer oxidation (Karlsson et al., 1992; Karlsson et al., 1993; Gedde, 1994) and material properties such as antioxidant content have been shown to prevent oxidation (Park et al., 1989). Chemical interaction with polymer pipes has also caused many problems in the past. Environmental contamination incidents such as leaking underground storage tanks and above ground chemical spills have resulted in the migration of petroleum compounds and chlorinated solvents in the surrounding soil and groundwater. These contaminants have been found to penetrate buried polymer pipes and contaminate drinking water resulting in maximum contaminant level exceedances, human illness, and bad taste and odor (Holsen et al., 1991). Diffusion is the movement of a chemical from a higher to a lower concentration. The diffusion coefficient and the solubility of the chemicals are important they determine a measure of how fast and to what degree contaminants interact with polymers.

The purpose of this research is to better understand the integrity of PE pipes installed in buried potable water distribution systems. The objectives of this research were to (1) compare the physical, mechanical, and surface characteristics of polyethylene pipes removed from a buried water distribution systems to one new PE pipe, (2) determine diffusion coefficients for toluene and trichloromethane through each material, and (3) analyze which polymer characterization techniques are useful for identifying polymer pipe and assessing their integrity. Results of this work will determine the mechanical, physical, and surface characteristics of buried polymeric pipe and their resistivity to certain chemicals which have the potential to affect drinking water.

Materials and Methods

Three groups of polymeric materials were analyzed during this work: (1) four polymeric pipes removed from a buried potable water distribution system operated by Pasco County Utilities, Florida; (2) one new HDPE standard inside dimension ratio (SIDR)-9 potable water pipe obtained from a local plumbing

supply store, and (3) one HDPE and one LDPE prepressed resin sheet obtained from McMaster Carr, Inc. (Atlanta, GA).

A differential scanning calorimeter (DSC) (New Castle, DE) was used for calorimetric measurements including the melting temperature (T_m), oxygen induction time (OIT), and glass transition temperature (T_g) for select samples in accordance with ASTM E794 (2001). Crystalline content for polyethylene and polybutylene was calculated using the formula $w_c = (\Delta H_{m[\text{Experimental}]} / \Delta H_{m[100\% \text{ Theoretical HDPE}]})$ where $\Delta H_{m[100\% \text{ Theoretical HDPE}]}$ was assumed to be 293 J/g (Karlsson et al., 1992) and $\Delta H_{m[100\% \text{ Theoretical PB}]}$ was assumed to be 125 J/g. The $\Delta H_{m[\text{Experimental}]}$ was found through using the area under the melting trace of the first heating. (Karlsson et al., 1993). OIT was found as outlined in ASTM 3895 (2004). Polymer composition was examined using a TA Instrument Q₅₀₀ thermogravimetric analyzer (TGA) (New Castle, DE) in accordance with ASTM E1131 (1998). A Perkin Elmer Spectrum One Fourier Transform (FT-IR) spectrometer (Waltham, MA) in Attenuated Transform Reference (ATR) mode with a ZnSe crystal at 4 cm^{-1} resolution was used to characterize sample surface chemistry.

Toluene and trichloromethane sorption diffusion coefficients were determined using the immersion method similar to Chao et al. (2006). Dog-bone shaped samples were immersed in 99.0% pure toluene and trichloromethane solutions. Samples were removed from contaminant intermittently, quickly blotted with KIMWIPES™, weighed, and reimmersed in contaminant solution. This sequence continued over a 250 hour period in order to make certain equilibrium was achieved. An absorption curve was then generated using percent weight gain (%) versus immersion time (hr). The equation: $D = 0.049 * (l^2 / t_{0.5})$ was used to calculate the diffusion coefficient of each pipe sample. The variables are as follows: the time it takes for the polymer to gain half of its equilibrium weight ($t_{0.5}$, s), thickness (l , cm) is the measured polymer thickness, and the contaminant diffusion coefficient (D , cm^2/s).

Results

The collaborating water utility removed four polymeric water pipes from the ground, and then mailed them overnight to the authors for analysis. With the exception of one pipe removed from the utility distribution system, manufacturers of the three other pipes could not be identified because no visual labeling markings were observed. The age and disinfectant exposure history of all pipes analyzed are shown in **Table 1**.

Pipe outer diameter, wall thickness, inner wall residue quantification, and visual attributes were recorded (**Table 2**). Residue was visually detected on the inner wall of the PE 3, PB 1, and PE 4 pipes and not detected on either PE 2 or PE 1 pipes. To determine if inner wall residue impacted pipe contaminant interaction characteristics, both PE 3 and PB 1 pipes were cut in half, where one half was scraped and cleaned of all residue and the other half was left in its natural state and both halves underwent sorption testing. The PB 1 pipe contained the greatest amount of residue (0.097 mg/cm^2).

Mechanical and thermal characteristic results of the polymer samples are shown in **Table 3** and compositional analysis results are shown in **Table 4**. A Turkey-Kramer multiple comparison test with a q value of 0.05 was used. Several differences within T_m and ΔH_m results were detected. The PE 3 Pipe had the greatest T_m while the LDPE Resin sample had the least T_m : $\text{PE 3} > \text{PE 2} = \text{PE 1} = \text{PE 4} > \text{PB 1} > \text{LDPE Resin}$. A comparison of OIT results revealed that little antioxidant remained in all field pipe samples, while the new PE 1 pipe had the greatest level by a factor of ten. Interestingly, the PB 1 pipe had a very different T_m and ΔH_m compared to all other pipes and PE standards analyzed. Due to this great difference, the glass-transition temperature for the PB 1 sample was determined and the result (-24°C) more closely resembles that of PB than PE. Based on the stark thermal differences between PB 1 pipe and all others analyzed, the PB 1 pipe was thought to be PB. All pipes analyzed contained significantly less than 2% carbon black except for the PB 1 pipe. Two percent carbon black is required according to ASTM D3350 (2004). The carbon black content of 0.63% was the lowest in the PE 2 pipe.

Infrared spectroscopy was useful in identifying chemical bond types on the polymer surface. Specifically, IR quantifies the frequency of absorption which is dependent on the atomic bonds, as well as the mass and geometry of the atoms (Silverstein, 1998). For instance the difference between PE ($[-\text{CH}_2-\text{CH}_2-]_n$) and PB ($[-\text{CH}_2-\text{C}(\text{CH}_3)_2-]_n$) can be easily seen due to the presence of methylene groups in a PB IR spectrum and lack of these groups in PE. **Figure 1** shows infrared spectroscopy results for PE 3, Timber Oak, PB 1, PE 1, and PE 4 water pipe inner wall surfaces. Notably, surface oxidation was detected on all field pipe inner wall surfaces to varying degrees and not detected on the new (control) pipe. Hydroxyl bonds (occurring between 3384 cm^{-1} to 3359 cm^{-1}), vinyl bonds (1632 cm^{-1} to 1595 cm^{-1}), and carbonyl bonds (1124 cm^{-1} to 1099 cm^{-1}) were most common. The PB 1 pipe spectrum significantly differs from all other pipe and controls tested due to the presence of both C-H and C-H₂ bonds. These bonds are present from 2958 cm^{-1} to 2848 cm^{-1} and more closely resemble a PB spectrum.

To determine if residue coating on the inner pipe wall played any role in the absorption of organic chemicals for PE 3 and PB 1 pipes, contaminant interaction results for the pipe halves that were scraped of residue and contained residue were compared using a 2-way ANOVA (α of 0.05). Results shown in **Tables 5** and **6** indicate that the diffusion coefficient and change in mass when immersed in toluene and trichloromethane were similar.

Chemical interaction results for toluene are provided in **Table 7**. A Turkey-Kramer multiple comparison test was used to compare diffusion coefficients and total weight absorbed for all pipes ($q=0.05$). Statistical testing determined that PE 1 and PE 3 pipe wall thicknesses were similar. In addition, the amount of toluene sorbed in each sample was only different for PE 2 pipe which had the thickest pipe wall. Interestingly, the PB 1 pipe absorbed a much greater percent of its mass (12%) compared to all other pipes tested (7-8%) (**Figure 2**). Further, the PB 1 pipe attained equilibrium after only 2.7 hours compared to all other pipes which achieved equilibrium after 9 and 18 hrs. Toluene diffusion through PE 3 and PE 2 pipe were similar, while diffusion through PE 1 was the slowest. Similar to the toluene exposed pipe samples, the pipe wall thickness and amount of trichloromethane sorbed of the PE 3, PE 1, and PE 4 pipes were similar (**Table 8**). The PB 1 sample had the thinnest wall thickness, though it had the largest diffusion coefficient by a factor of ten. It achieved a much greater percent of weight (23%) in a shorter amount of time (1.85 hours) compared to the other pipes (12-13% weight in 12-19 hours) (**Figure 3**).

Discussion

The residue coating on the PB 1 and PE 3 pipes did not affect organic chemical diffusivity or the amount of contaminant sorbed (**Table 5** and **6**). However, specimens were fully immersed in contaminant and all sides were exposed (coated and uncoated). Approximately 40% of each specimen's surface area was coated. If coating truly affects PE and PB contaminant interactions, it was undetectable in the current study due to method limitations.

Toluene and trichloromethane diffusivity through PE pipes of different ages varied. The diffusion coefficient for both toluene and trichloromethane of the control pipe was less than that of all aged PE pipes tested except for the PE 4 pipe when absorbing toluene. This finding contradicts the study by Park et al. (1989) in which no difference was found between the diffusion coefficient of a 12-year old PB water pipe (Wesflex, $\frac{3}{4}$ in) and new PB water pipe. In this present study, the PB pipe specimen exhibited a much greater diffusion coefficient than all PE materials examined. Park et al. (1989) also found the PB toluene diffusion coefficients were approximately twice as large as the LDPE diffusion coefficients. For the current study, PB diffusion coefficients were between two to four times greater than the HDPE diffusion coefficients.

As shown in **Table 3**, varying degrees of oxidation were detected in all of the water utility pipes. The OIT of the new PE 1 PE (97.2 min.) was drastically different from the OIT of all aged pipes (0 to 8.27 min.). Results of this work revealed that the age of pipe does not relate well to antioxidant level. The variation in oxidative resistance could be due to the difference in the amount and type of antioxidants initially manufactured into the pipe and exposure conditions. The differing OIT results of different aged pipes clearly demonstrate the complexity of understanding how polymer pipes age in a real water distribution system. Results from this work contradict a previous study by Gedde et al. (1994) in terms of long term behavior of polyolefin pipes. Gedde et al. (1994) state that rapid polymer degradation begins as soon as the antioxidants are depleted and after that “Regime C” begins which constitutes 5-10% of the pipes’ total lifetime. This logic does not compare well to results of water pipes removed from the water utility distribution system. The PE 2 pipe, the youngest of the pipes studied, had no oxidative resistance (OIT=0) though no evidence of extreme thermal oxidation was found in this pipe. If Gedde et al. (1994) reasoning is accurate, then the PE 2 pipe will experience failure within the next 2 years (total 9 year service life). Results from measuring oxidative resistance of existing installed polymer pipes puts into question the application of models created through the experimentation of artificially aged PE and their difficulty and unrealistic nature when applied to naturally aged polyolefin piping.

IR spectroscopy was very helpful in analyzing the condition of utility water pipes. IR results also support the conclusion that the water utility polymer pipe surfaces were oxidized and that surface oxidation can occur on polymer pipes even though OIT is not zero. The presence of carbonyl ($>C=O$), vinyl ($C=C$), and hydroxyl ($-OH$) bonds on the surface agreed with the results of the aged pipes in this study agreed with a study by Karlsson et al. (1992, 1993). Infrared spectroscopy was also helpful in differentiating between PE and PB pipe. Specifically, the presence of methylene and methyl bonds on PB 1 pipe signified a PB spectrum. DSC thermal transition and contaminant interaction testing were valuable in determining polymer type. The great difference between the PB 1 pipe’s T_m compared to all other PE pipes implied that a difference existed. A follow-up DSC test revealed that the PB 1 pipe had a T_g similar to that of PB ($-24^\circ C$). Further, the ten fold difference in diffusion coefficients, as well as the time required to reach half-equilibrium (**Table 7** and **8**) were also helpful in determining that the PB 1 pipe was not PE. A literature review revealed that Park et al. (1989) found that the toluene diffusion coefficients for PB pipe were greater than those for LDPE pipes.

Conclusions

Residue coating on the water utility pipe inner walls did not affect the toluene or trichloromethane diffusivity or mass of contaminant sorbed. Approximately 40% of the tested pipe samples were covered with residue during immersion testing. Toluene and trichloromethane diffusivity through the collaborating water utility HDPE water pipes differed for some, but not all pipes. Even when pipe thicknesses were equal, contaminant diffusivity was different indicating that HDPE pipes of the same thickness interact with contaminants differently. Similar to literature findings, the utility PB pipe absorbed a much greater percent of its weight in a shorter period of time in contrast to all PE pipe samples. The toluene and trichloromethane and diffusion coefficients for the PB pipe were much greater than those of all PE pipes. All water utility pipes exhibited signs of surface and bulk polymer oxidation, but the degree of oxidation varied between pipes. The new control PE pipe did not exhibit any signs of oxidation. The prediction by Gedde et al. (1994) that the final 5-10% of PE pipe service life occurs after antioxidants are depleted may not be realistic. Methods that were most useful in pipe identification included IR and DSC analysis as well as sorption testing.

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Figures and Tables:

Table 1. Attributes of Potable Water Pipes Examined

Name of Pipe	Removal Date	Utility Reported Age, Water Disinfectant Type, and Exposure Period
PE 1	--	New
PE 2	June 4, 2008	7 years in service (combined chlorine only)
PE 3	February 14, 2008	20 years in service (18 years free available chlorinated water and 2 years exposure to combined chlorine)
PE 4	August 15, 2008	25 years in service (25 years free available chlorinated water only)
PB 1	June 4, 2008	30 years in service (28 years free available chlorinated and 2 years combined chlorine)

Table 2. Experimentally Measured Pipe Characteristics¹

Name of Pipe	Outer Diameter (mm)	Thickness (mm)	Inner Wall Residue (mg/cm ²)	Visual Description
PE 1	25.8 ± 0.4	2.57 ± 0.06	--	Pipe wall black in color; thin silver line on outer pipe wall in axial direction.
PE 2	28.7 ± 0.1	3.37 ± 0.06	--	Outer 0.70 mm of pipe wall blue in color; remaining 2.66 mm of pipe wall black in color.
PE 3	24.5 ± 0.5	2.52 ± 0.03	0.010	Pipe wall entirely black in color; small brown dots and equally spaced indentations found on inner pipe wall.
PE 4	22.2 ± 2.6	2.65 ± 0.03	0.041	Pipe wall entirely black in color; both ends of pipe section slightly deformed and ovular.
PB 1	23.0 ± 1.0	1.74 ± 0.07	0.097	Pipe wall entirely black in color; pipe walls were very thin and brittle; heavy orange-reddish coating on inner wall.

Table 3. Mechanical and Thermal Properties of Polymeric Materials Analyzed¹

Name of Material	Durometer Hardness	T_m (°C)	ΔH_m (J/g)	OIT (min.)	Crystallinity (%)
PE 1	65.2 ± 2.15 ^a	128.7 ± 0.5 ^{ab}	149.1 ± 5.5	97.2 ± 1.8 ^a	50.9 ± 1.9 ^a
PE 2	65.4 ± 2.39 ^a	130.4 ± 1.1 ^a	156.9 ± 5.6	0.0 ± 0.0 ^d	53.6 ± 1.9 ^a
PE 3	65.2 ± 1.54 ^a	132.5 ± 0.4 ^c	148.8 ± 5.1	4.8 ± 4.2 ^c	50.8 ± 1.8 ^{ab}
PE 4	66.2 ± 1.14 ^a	128.5 ± 0.3 ^b	143.7 ± 6.3	8.27 ± 4.06 ^b	49.1 ± 2.1 ^b
PB 1 ²	64.1 ± 2.66 ^a	123.7 ± 0.8 ^d	56.6 ± 0.3	0.0 ± 0.0 ^d	44.2 ± 0.2 ^c
LDPE Resin	56.7 ± 1.47 ^b	110.9 ± 0.2 ^e	64.2 ± 1.6	--	21.9 ± 0.5 ^d

1. Durometer hardness results represent the mean and standard deviation of five replicates. All other results represent the mean and standard deviation of three replicates. Letters in each column refer to similar materials based on a Turkey-Kramer multiple comparison test using q=0.05.
2. The T_g was experimentally determined to be -24.08 °C

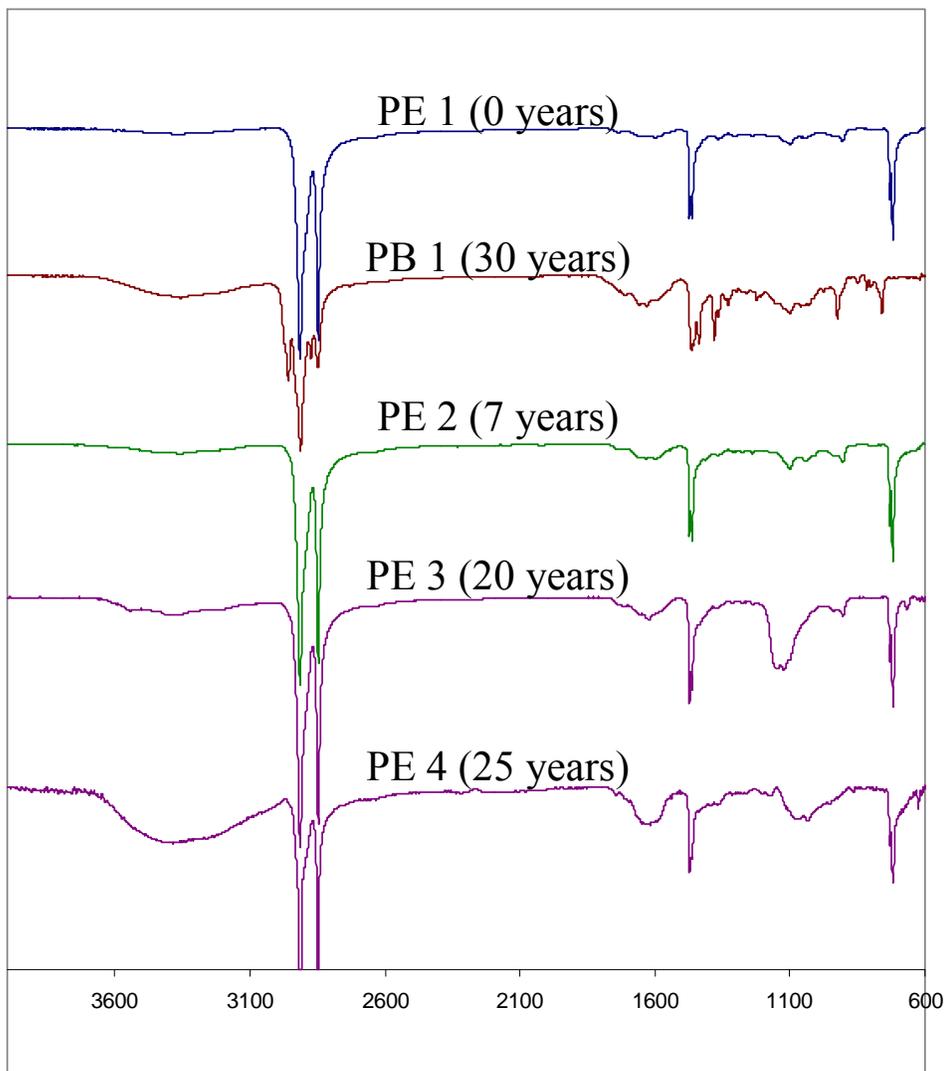


Figure 1. Infrared Spectrum for Four Polymeric Water Utility Pipe Samples and One New Pipe Sample. Numbers next to pipe represent years in service.

Table 4. Results from Pipe Compositional Analysis

Name of Pipe	Percentage of Polymer				Onset of Degradation Temperature (°C)
	Volatile Loss (%)	Polymer (%)	Carbon Black and Char (%)	Ash Content (%)	
PE 1 (new control)	0.52	98.52	1.86	0.01	450.7
PE 2 (7 yrs. old)	0.41	98.96	0.63	0.00	460.4
PE 3 (20 yrs. old)	0.79	97.69	1.52	0.00	457.6
PE 4 (25 yrs. old)	0.80	98.23	2.11	0.02	452.5
PB 1 (30 yrs. old)	0.78	97.16	2.06	0.00	430.4

Table 5. Toluene Interaction Results for Coated and Uncoated Pipes¹

Pipe Description	Thickness (mm)	Amount of Contaminant Sorbed at Equilibrium (mg)	Time to Half Equilibrium (hr)	Diffusion Coefficient (cm ² /sec)
PE 3 Uncoated	2.55 ± 0.03 ^a	2.38 ± 0.04	9.95 ± 0.35	8.9 ± 0.3 x 10 ^{-8a}
PE 3 Coated	2.48 ± 0.02 ^a	2.54 ± 0.04	9.63 ± 0.13	8.8 ± 0.8 x 10 ^{-8 a}
PB 1 Uncoated	1.75 ± 0.10 ^b	2.65 ± 0.12	3.08 ± 0.57	1.4 ± 0.1 x 10 ^{-7b}
PB 1 Coated	1.74 ± 0.09 ^b	2.64 ± 0.15	2.72 ± 0.32	1.5 ± 0.1 x 10 ^{-7b}

1. Letters in upper right correspond to values in same column which are not different according to a two-factor ANOVA (q=0.05).

Table 6. Trichloromethane Interaction Results for Coated and Uncoated Pipes¹

Pipe Description	Thickness (mm)	Amount of Contaminant Sorbed at Equilibrium (mg)	Time to Half Equilibrium (hr)	Diffusion Coefficient (cm ² /sec)
PE 3 Uncoated	2.55 ± 0.02 ^a	4.17 ± 0.02	11.60 ± 0.13	7.6 ± 0.3 x 10 ^{-8a}
PE 3 Coated	2.45 ± 0.02 ^a	3.96 ± 0.04	11.88 ± 0.45	7.2 ± 0.1 x 10 ^{-8a}
PB 1 Uncoated	1.77 ± 0.05 ^b	6.07 ± 0.19	1.96 ± 0.04	2.2 ± 0.1 x 10 ^{-7b}
PB 1 Coated	1.71 ± 0.08 ^b	5.92 ± 0.01	1.85 ± 0.05	2.1 ± 0.1 x 10 ^{-7b}

1. Letters in upper right correspond to values in same column which are not different according to a two-factor ANOVA (q=0.05).

Table 7. Toluene Interaction Results for Aged Pipes¹

Pipe Name (Age, year)	Thickness (mm)	Amount of Contaminant Sorbed at Equilibrium (mg)	Time to Half Equilibrium (hr)	Diffusion Coefficient (cm ² /sec)
PE 1 (0)	2.59 ± 0.00 ^a	2.58 ± 0.04 ^a	17.22 ± 1.22	5.3 ± 0.3 x 10 ^{-8a}
PE 2 (7)	3.39 ± 0.01 ^b	3.17 ± 0.02 ^b	18.67 ± 0.35	8.4 ± 0.3 x 10 ^{-8b}
PE 3 (20)	2.48 ± 0.02 ^a	2.54 ± 0.04 ^a	9.63 ± 0.13	8.8 ± 0.8 x 10 ^{-8b}
PE 4 (25)	2.63 ± 0.02 ^a	2.57 ± 0.02 ^a	14.08 ± 0.19	6.7 ± 0.0 x 10 ^{-8a}
PB 1 (30)	1.74 ± 0.09 ^c	2.64 ± 0.15 ^a	2.72 ± 0.32	1.5 ± 0.1 x 10 ^{-7c}

1. Letters in upper right correspond to values in same column which are not different according to a Turkey Kramer Test (q=0.05).

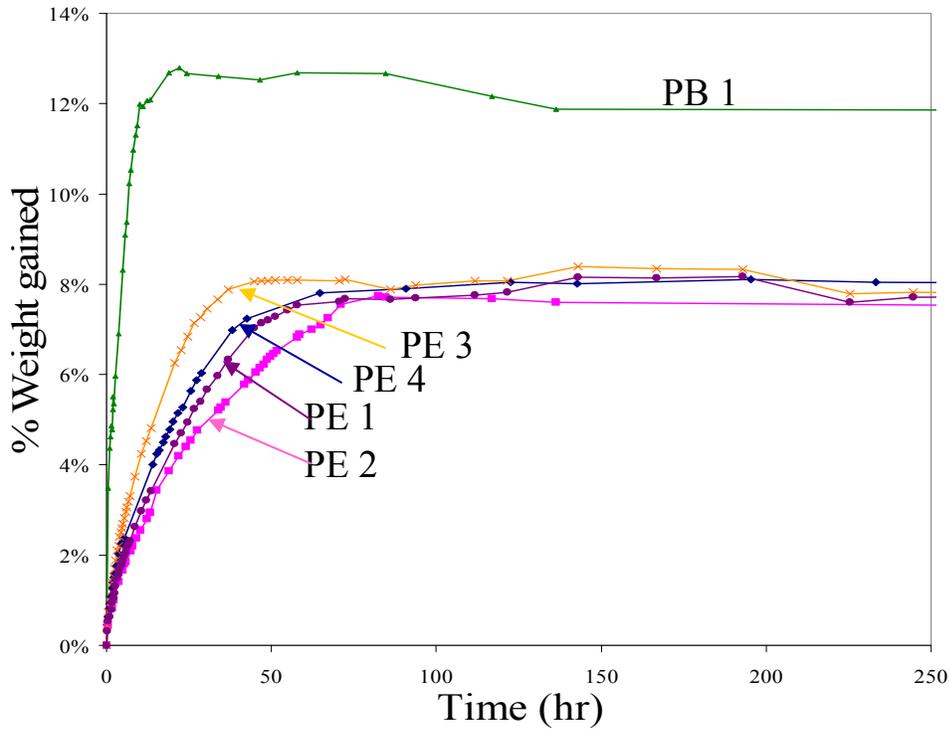


Figure 2. Toluene Sorption Curve for PE 1, PE 3 , PE 2, PB 1 , and PE 4 Pipes. Lines connect data points representing the mean of three replicate measurements at each time interval.

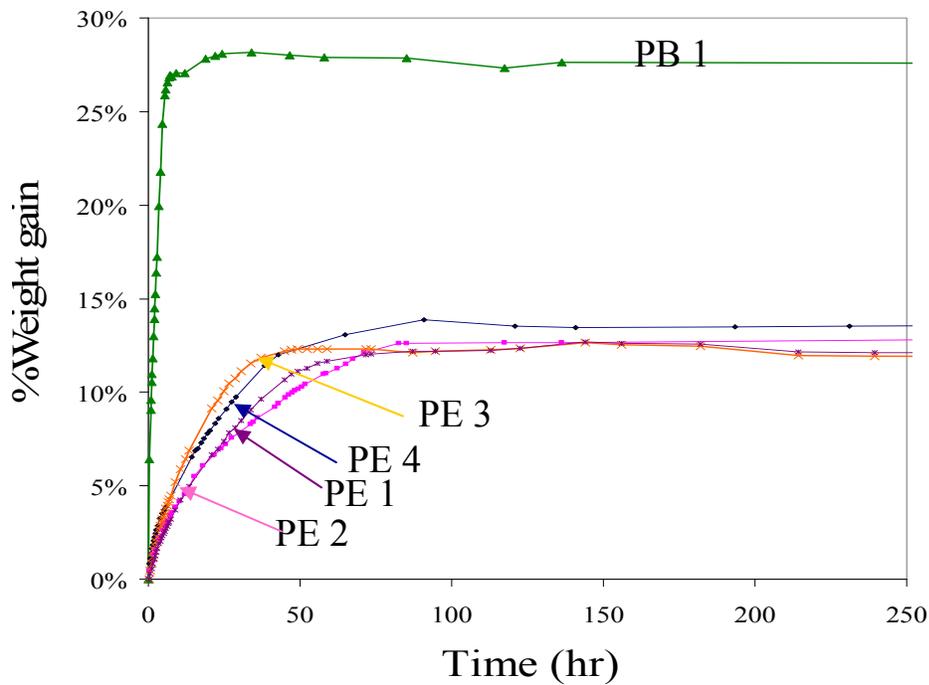


Figure 3. Trichloromethane Sorption Curve for PE 1, PE 3 , PE 2, PB 1 and PE 4 Pipes Lines connect data points representing the mean of three replicate measurements at each time interval.

Table 8. Trichloromethane Interaction Results for Aged Pipes¹

Pipe Name (Age, year)	Thickness (mm)	Amount of Contaminant Sorbed at Equilibrium (mg)	Time (hr)	Diffusion Coefficient (cm ² /sec)
PE 1 (0)	2.59 ± 0.00 ^{ab}	4.25 ± 0.02 ^a	18.04 ± 0.42	4.8 ± 0.3 x 10 ^{-8a}
PE 2 (7)	3.35 ± 0.03 ^b	5.03 ± 0.01 ^b	19.17 ± 0.17	8.5 ± 0.2 x 10 ^{-8b}
PE 3 (20)	2.45 ± 0.02 ^a	3.96 ± 0.04 ^a	11.88 ± 0.45	7.2 ± 0.1 x 10 ^{-8b}
PE 4 (25)	2.66 ± 0.04 ^b	4.34 ± 0.04 ^a	15.05 ± 0.50	6.4 ± 0.1 x 10 ^{-8c}
PB 1 (30)	1.71 ± 0.08 ^c	5.92 ± 0.01 ^c	1.85 ± 0.05	2.1 ± 0.1 x 10 ^{-7c}

1. Letters in upper right correspond to values in same column which are not different according to a Turkey Kramer Test (q=0.05).

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Investigation of Roxarsone Biotransformation under Aerobic Conditions

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ABSTRACT

This investigation sought to determine the capacity of a historically unamended (no litter application) surface soil to biotransform roxarsone. Thirteen day experiments contained soil, media, and roxarsone. Samples were analyzed for As species (roxarsone, As(V), As(III)) with high performance liquid chromatography in series with inductively coupled plasma mass spectrometry and total arsenic with graphite furnace absorption spectroscopy. Results showed no aerobic roxarsone biotransformation in the experiments. These results suggest that native microorganisms within unamended soils are not capable of roxarsone biotransformation, and that biotransformation capability is only gained through the introduction of poultry litter, containing different microorganisms, to soil systems.

Keywords: roxarsone, arsenic, biotransformation, aerobic

Introduction

The organoarsenical roxarsone (3-nitro-4-hydroxybenzene arsonic acid) is employed by the poultry industry as a feed additive. Approximately 70% of broiler chickens in the United States are fed roxarsone (Chapman and Johnson 2002). Its use controls the intestinal parasitic infection, coccidiosis, while promoting growth, feed conversion, feathering, egg production, and pigmentation of broiler chickens (Anderson 1983). Roxarsone is mixed at 25-50 mg per kg feed (Anderson 1983) and passes through poultry mostly untransformed (Morrison 1969; Abdo, Elwell et al. 1989; Moore, Daniel et al. 1998), resulting in total As concentrations in poultry litter (PL) ranging from <1 to 40 mg kg⁻¹ (Anderson and Chamblee 2001; Jackson and Bertsch 2001; Garbarino, Bednar et al. 2003; Rutherford, Bednar et al. 2003). In addition to elevated As concentrations, PL is high in organic material, nitrogen, and phosphorus, making it an ideal source of fertilizer for agriculture. An estimated 250,000 to 350,000 kg of As is applied annually in the United States (Rutherford, Bednar et al. 2003). PL application usually occurs no more than a few miles from where it is produced (Daniel, Moore et al. 1995), which has led to intensive PL application within regions containing concentrated poultry farming operations. The Delmarva Peninsula, encompassing portions of Delaware, Maryland, and Virginia, is one of such regions, as it produces 7% of U.S. broiler chickens (Nachman 2005). Agricultural land on the Delmarva has the potential to receive application of 1.5 billion kg of poultry litter annually, which would result in the addition of 50,000 kg of As from roxarsone (Sims and Wolf 1994). One of the concerns about PL application is the potential for introduction of As into water supplies, as As is easily leached from PL. (Rutherford, Bednar et al. 2003) found that 70-75% of As in PL is water soluble. In the Pocomoke River

watershed, which is on the Delmarva, the USGS found As concentrations up to 27 µg/L in groundwater near fields that have received PL application (Hancock, Denver et al. 2002).

Due to the recent determination that arsenic is a carcinogen (NRC 2001), the EPA has lowered the maximum contaminant level (MCL) for As in drinking water to 10 µg/L from 50 µg/L (EPA 2007). Many studies have been conducted on arsenic toxicity showing links to cancer of the bladder, lungs, skin, kidney, nasal passages, liver, and prostate. The toxicity of As varies with species, where inorganic forms such as As(III) and As(V) are classified as human carcinogens and are more toxic than organoarsenicals (Lasky, Sun et al. 2004).

Microorganisms have the capability to biotransform roxarsone, releasing the more toxic inorganic forms As(V) and As(III) (Garbarino, Bednar et al. 2003; Brown, Slaughter et al. 2005; Cortinas, Field et al. 2006; Stolz, Perera et al. 2007). Other transformation products include 3-amino-4-hydroxybenzene arsonic acid (3A4HBAA), monomethylarsenate (MMA), and dimethylarsinate (DMA), among others (Cortinas, Field et al. 2006; Stolz, Perera et al. 2007). Studies have shown that roxarsone biotransformation can occur under both aerobic and anaerobic conditions. Biotic investigations have shown aerobic roxarsone biotransformation in topsoils (Ap) to which poultry litter had been historically applied, whereas transformation was not observed in Ap soils of the same series that had not been previously exposed to PL (Brown, Slaughter et al. 2005). Anaerobic studies under methanogenic and sulfate-reducing conditions using sewage sludge as the inoculum showed rapid decreases in roxarsone concentrations, achieving 96% roxarsone removal in 16 days (Cortinas, Field et al. 2006). Transformation of roxarsone to predominantly 3-amino-4-hydroxybenzene arsonic and inorganic As in chicken litter enrichments has also been observed and *Clostridium* species have been identified as predominantly responsible for such anaerobic transformations (Stolz, Perera et al. 2007)

Most of the previous research has considered soil and PL that have been exposed to roxarsone. Limited investigation has been done on the ability of a historically unamended soil to biotransform roxarsone. Unamended soils may have different microbial populations than amended soils. This is of importance because any resulting biotransformation of roxarsone in the two may be dissimilar. Such differences could result in higher concentrations of an As species in either the amended or unamended soil relative to the other. There are unique water quality and health implications associated with each As species.

The goal of this investigation was to determine the capacity of microbes native to surface soils of the unamended Beaverdam Sand formation of the Delmarva Peninsula to biotransform roxarsone. As surface soils are exposed to the atmosphere, my experiments were conducted under aerobic conditions.

Methods

Field Sampling

Soil samples were taken from a 2-acre plot in Sussex Co., DE, on the Delmarva Peninsula. Poultry litter has not been applied to the site for more than five years which has resulted in As concentrations of less than 0.3 µg/L from shallow, on-site wells. The site lies within an agricultural watershed and is underlain by weathered sands and gravels of the Beaverdam Sand (Shedlock, Denver et al. 1999; Ator, Denver et al. 2005). Surface soil samples were collected at four locations within the plot using a methanol-sterilized 2” auger to a depth of two feet. The soils were stored in sterile glass jars, transported to the laboratory on ice, and were refrigerated until experimentation. The four soil samples were homogenized before use in experiments.

Experimental Design

Six experimental Erlenmeyer flasks (500 mL) were assembled along with five control flasks (Table 1). To each of the six experimental flasks was added 25.0 g of homogenized soil, 250 mL media, and 625 µL roxarsone (80 mg/L) to achieve 200 µg/L Rox-As. The media contained 2.5 g/L NaHCO₃ buffer, 0.25 g/L NH₄Cl as a nitrogen source, 0.6 g/L NaH₂PO₄·H₂O as a phosphorous source, 0.1 g/L KCl to provide

Table 1. Experimental design showing additions to experimental and control trials.

	Flask	ADDITION				
		Media	Live Soil	Killed Soil	ROX	LAC
EXPERIMENTAL	A	X	X		X	X
	B	X	X		X	X
	C	X	X		X	X
	X	X	X		X	
	Y	X	X		X	
	Z	X	X		X	
CONTROL	KL	X		X	X	X
	K	X		X	X	
	R	X			X	
	S	X	X			
	DO	X	X			

for ionoregulation, and was adjusted to pH 6 after autoclaving. The roxarsone standard was preserved with EDTA (0.01 M). To three of these six experimental flasks, which will be referred to as the lactate series (Flasks A, B, C), 250 mL of sodium lactate was added after autoclaving (to prevent volatilization/polymerization) to achieve 10 mg/L lactate and served as a possible electron donor. The remaining three of the experimental flasks will be referred to as the media series (X, Y, Z). Both roxarsone and lactate were filter-sterilized through a 0.2 μm membrane before addition.

The first two controls consisted of a lactate series (KL) and a media series (K) control, and were established by adding autoclaved (killed) soil while keeping all other respective additions unchanged. The third control monitored dissolved oxygen (DO), and was assumed to be representative of the DO in all flasks. A DO probe (Orion Sensorlink Dissolved Oxygen Probe, Model PCM8000) was affixed in the neck of the flask with a perforated rubber stopper to allow sufficient airflow. The fourth control (R) monitored possible glass sorption of As and contained only media and roxarsone additions identical to that of the experimental flasks. The fifth (S) monitored for possible release of As from the soil and contained only soil and media additions identical to that of the experimental flasks. All were kept at 25-30°C, covered with a 0.2 μm BugStopper filter that allowed for air flow, positioned on orbital shaker tables to provide aerobic conditions, and kept covered to prevent photodegradation.

Laboratory Sampling

All flasks were shaken for 13 days and 4 mL were aseptically pipetted from all flasks (excluding the DO control) every other day. This lent seven sampling periods. Each sampled volume was centrifuged for 10-20 min to remove suspended solids and the supernatant was filtered through a 0.2 μm membrane. Approximately 1 mL of each was preserved with 25 μL HNO₃ (16 M) and was centrifuged an additional 5-10 min for total As concentration. The remaining 2 mL was preserved with 20 μL EDTA (0.25 M) in opaque bottles for speciation.

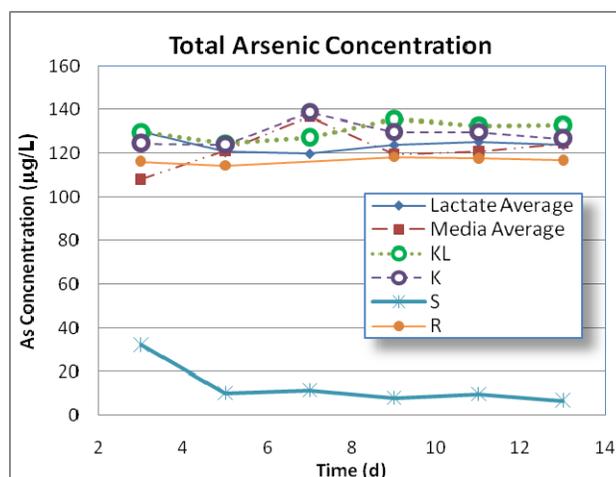
Chemical Analysis

Graphite Furnace Atomic Absorption Spectroscopy (GFAAS, Varian SpectrAA 220Z) with Zeeman background correction was used to analyze for total As concentrations. The matrix modifier used was a mixture of Pd and Mg(NO₃)₂. Concentrations were validated using a check standard. The detection limit for this method is approximately 3 $\mu\text{g/L}$.

To analyze for As species (roxarsone, As(V), and As(III)), high performance liquid chromatography using an AS7 column (Dionex IonPac 4x250 mm) coupled with inductively coupled plasma mass spectrometry (ICPMS, X-series, Serial #X-0458) was used. The HPLC was utilized to separate the species, and the ICPMS was used to determine the speciated As concentrations. To separate the species, the HPLC eluent program was as follows. From 0 to 1 min, the eluent (Eluent 1) consisted of 2.5 mM HNO₃ in 0.5% methanol. From 1 to 6 min, the eluent (Eluent 2) consisted of 50 mM HNO₃ in 0.5% methanol. From 6 to 9 min, the program switched back to Eluent 1. Due to high cost of using the ICPMS, not all samples were analyzed for As species.

Microbial Analysis

Upon completion of the 13 day experiment, the remaining slurry from each flask, containing soil and solution, were frozen. These samples were shipped to microbial ecologist Dr. John F. Stolz at Duquesne University, who will use molecular analysis techniques to determine the presence of genes that allow for the use of arsenic species in microbial respiration.



Results

Total Arsenic

Total As concentrations, as measured by GFAA, remained constant in all flasks throughout the experiment (Figure 1). The lactate series and killed (KL) control averaged 124 $\mu\text{g/L}$ As and 130 $\mu\text{g/L}$ As, respectively. The media series and killed (K) control averaged 122 $\mu\text{g/L}$ As and 129 $\mu\text{g/L}$ As, respectively. Control R averaged 117 $\mu\text{g/L}$ As and S, representative of background As concentrations, averaged 16 $\mu\text{g/L}$ As.

Speciated Arsenic

The HPLC-ICPMS method was used to separate and analyzed arsenic species, including As(III), As(V), and Roxarsone. After HPLC separation, the individual arsenic species were analyzed by ICPMS. Analysis of mixed standards containing all three species gave the following retention times: 1.5 min for As(III), 5.3 min for As(V), and 7.7 min for Rox-As (Figure 2 top panel)

Figure 1. Total As concentrations determined by GFAA showing no As loss over time.

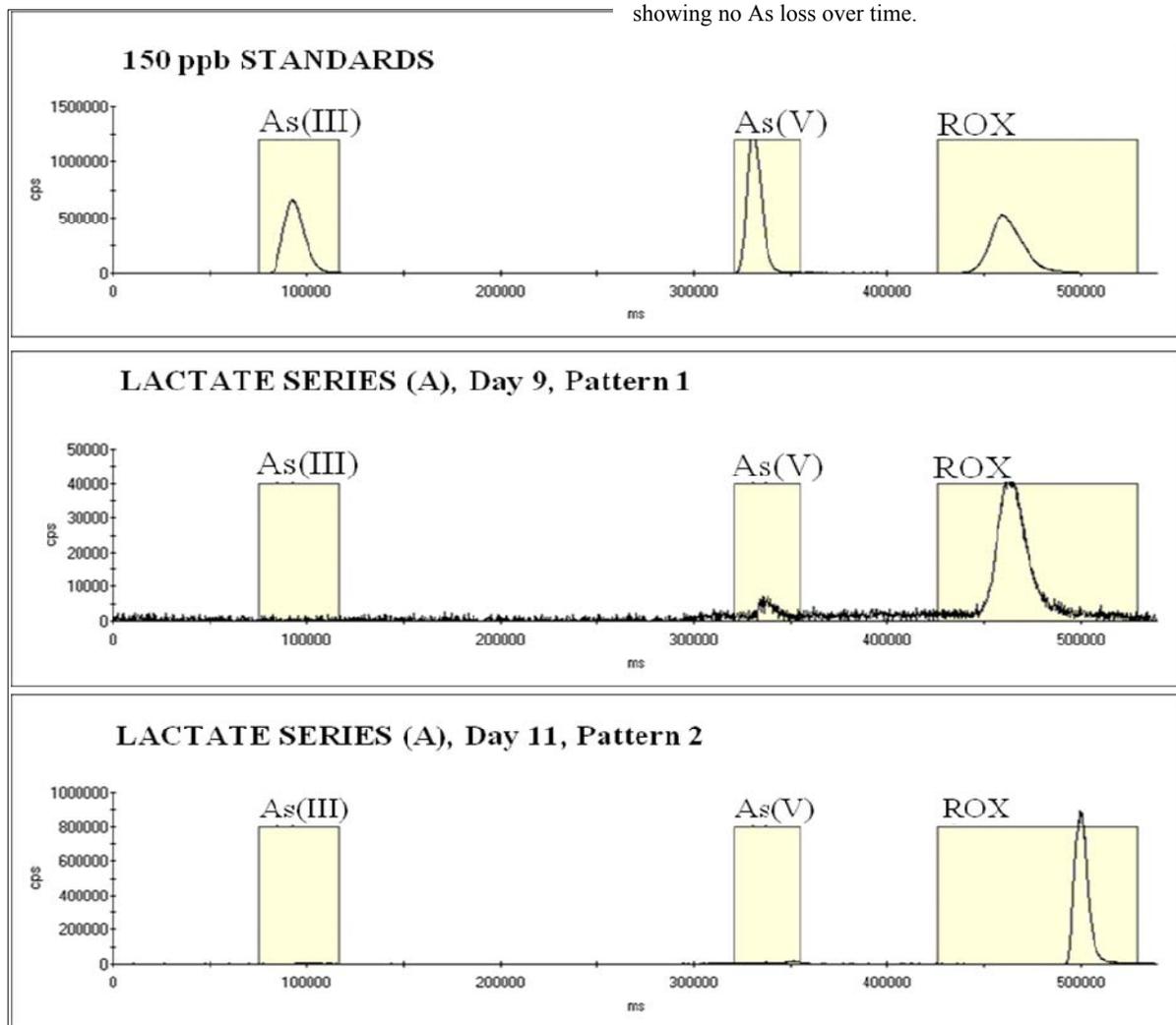


Figure 2. HPLC-ICPMS speciation chromatographs analyzed on July 2, 2008 showing both elution pattern 1 and elution pattern 2.

Arsenic speciation was conducted on two separate days. On the first day (July 2), samples from series A, X, K, and R were analyzed. It was noted that the Ar pressure was low on this day, running close to 60 psi, which is lower than what is recommended. Because Ar forms the plasma for the ICPMS, the low pressure may have caused problems with atomization of the samples. Two different elution patterns

were seen on July 2; occurrences of the two were random. Both patterns showed two peaks eluting 130 s apart, with the first eluting considerably smaller than the second eluting. In the first pattern (Figure 2 middle panel), the peaks eluted at the same time as As(V) and roxarsone standards, and the concentrations from these peak areas were calculated as such. However, the peaks were much smaller than expected, yielding 15-31 $\mu\text{g/L}$ Rox-As, and did not agree with the total As data.

The second pattern (Figure 2 bottom panel) showed As(V) and Rox-As peaks with the same 130 s separation in retention time; however, these peaks were eluting consistently 40 s later than the standards. Concentrations as determined by identifying these peaks as As(V) and roxarsone yielded values that ranged from 67-213 $\mu\text{g/L}$ Rox-As, which were more consistent with the total As data. No time dependence was seen in the variation of concentrations within this range. Days 3 and 13 of control R followed elution pattern 2.

On the second day of analysis (July 11), the Ar tank had been refilled, and the pressure was 100 psi. During these runs, pattern 2 (see Figure 2, bottom panel) was observed for all samples and concentrations did not fall below 197 $\mu\text{g/L}$ Rox-As. Because the analyses conducted on July 11 were much more consistent than those on July 2, only the July 11 data (from B, Y, and KL) are used in the final analysis. Speciation of B, Y, and KL revealed no degradation of roxarsone (Figure 2, bottom panel), as the chromatographs show only a roxarsone peak and no significant peaks of As(V) nor As(III) were observed. Peaks in chromatographs from day 3 and 13 speciation (Figure 2) are representative of results seen in sampling days 3-13 of all B, Y and KL chromatograms (Appendix). Beginning (day 3), ending (day 13), and average concentrations of As as roxarsone in B were 206, 207, and 207 $\mu\text{g/L}$. Corresponding values from Y were 214, 217, and 215 $\mu\text{g/L}$ As as roxarsone. The controls KL also revealed no roxarsone degradation nor As(V) or As(III) formation, with beginning, ending, and average As as roxarsone concentrations of 208, 216, and 214 $\mu\text{g/L}$, respectively. Data not reported here may be found in the Appendix.

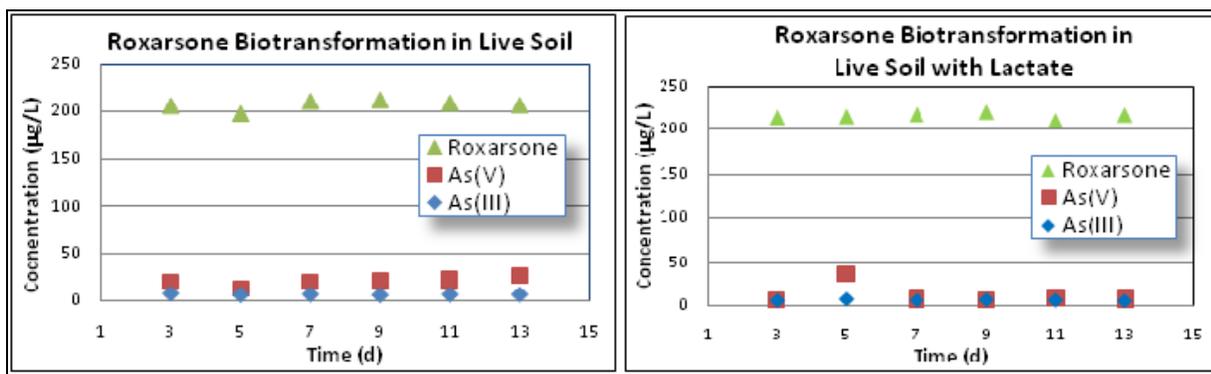


Figure 3. Left panel: Changes in roxarsone, As(V), and As(III) concentrations in live soil spiked with roxarsone (Flask Y). Right Panel: Changes in roxarsone, As(V), and As(III) concentrations in killed spiked with roxarsone and lactate (Control Flask KL). The use of killed soils excludes the possibility of microbially mediated transformation of roxarsone.

Discussion

This research required application of complex analytical techniques, and with all analytical techniques, there are challenges. For this study, there were several analytical complications. First, the HPLC-ICPMS results showed inconsistent retention times and low peaks for several of the experimental samples analyzed on July 2. Although the cause for this is unknown, we believe that the low argon pressure may have contributed to the problem. An additional complication was the 40 s delay in retention times in experimental samples as compared to standards. This could have been caused by a difference in the ionic strength between the media and the standards, but more data would need to be collected to confirm this.

For this study, I used two methods to analyze for arsenic. GFAA was used to measure total As, and the HPLC-ICPMS was used to analyze As species. To achieve mass balance, the sum of the concentrations of the individual As species should equal the total. Mass balance was not achieved for this study, as the GFAA (total) concentrations were actually lower than the sum of the species concentrations.

A possible reason for the discrepancy is using the GFAA above the 100 µg/L upper detection limit. Concentrations of 200 µg/L Rox-As were added to the trials, and since no degradation was seen in HPLC-ICPMS results, 200 µg/L total As should have been detected by GFAA. Previously, the GFAA has been able to accurately resolve concentrations up to 400 µg/L. However, standards containing what should have been 30, 75, 225, and 450 µg/L As were detected as 96%, 90%, 78%, and 57% less than expected, respectively. The standards were made by serial dilution, and if an error was made in the stock, each consecutive dilution would have the same proportional error, resulting in a linear deviation from expected values. The observed trend showed a nonlinear relationship between expected and actual concentrations, with greatest deviations above 100 µg/L. To determine if the nonlinearity was consistent above 100 µg/L, a second GFAA run was done with 0, 20, 50, 100, 250, and 500 µg/L standards. A linear dynamic range (LDR) from 0-130 µg/L was seen with nonlinearity observed above 130 µg/L. (Figure 4) with a linear dynamic range from 0-130 µg/L. This suggests the reported average total As concentrations of 120 µg/L are an underestimate due to the non-linear response of the GFAA to concentrations above 100 µg/L.

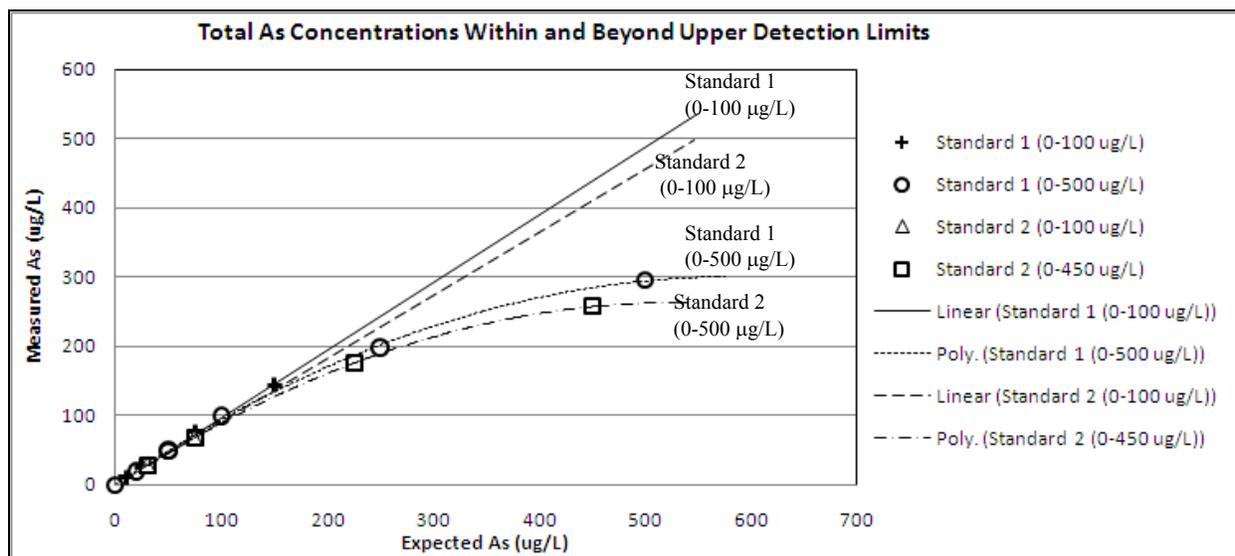


Figure 4. Expected and actual total arsenic concentrations of standards made for the HPLC-ICPMS analysis (Standard 2) and a standard made to investigate nonlinearity (Standard 1) separated into within detection limit ranges 100 µg/L and beyond detection limit ranges.

The most accurate total As concentrations in the trials can be estimated by assuming the polynomial trendline fitted to Standard 1 (0-500 µg/L) can lend a predictable relationship between known concentrations and unknown concentrations beyond the LDR. The function serving this purpose is $measured = -0.0009(expected)^2 + 1.0311(expected)$ with $R^2 = 0.9993$. Based on this relationship, the 200 µg/L Rox-As addition (an expected value) would have been detected as 170 µg/L As (a measured value). Although this corrected value is still 40 µg/L less than concentrations detected by the HPLC-ICPMS, it has explained 63% of the error between the amount of As added and the amount accounted for. The signal may also have been reduced due to high dissolved solids (EPA Method 200.9). Even though acid was added to the samples post-centrifugation and filtration, some samples turned brown-amber colored. This suggests that the pH of the samples may not have been low enough to keep iron from precipitating. The presence of particles in the analyzed samples may have been the cause of the high background interference noted (see Appendix for GFAA data and notes) and may have been another cause of decreased sensitivity of the GFAA to arsenic.

Despite the analytical challenges, results of this research demonstrated that aerobic, microbially mediated transformation did not occur in this experiment. This conclusion has been drawn from the speciation analysis showing no roxarsone transformation in live or killed soils. The absence of biotransformation in the experiments implies the current inability of soil microbes from the surface soils at the field site to transform As from roxarsone into As(V) or As(III) through aerobic respiration. Importantly, the soil from the sample site is a sandy soil that has received no application of PL containing

As within the past 5 years. Similar results were observed by Brown et al. (2005), who measured aerobic roxarsone biotransformation in topsoils (Ap) to which poultry litter had been historically applied, whereas transformation was not observed in Ap soils of the same series that had not been previously exposed to PL. Lack of roxarsone transformation means the more toxic As species As(V) and As(III) will not be released into soils if only roxarsone is introduced. These inorganic forms are more soluble, thus more mobile in water, than roxarsone. Increased mobility may lead to leaching of inorganic As in both surface waters and unconfined groundwater. Sources such as these may be used for recreation or public drinking water and may also provide habitat to economically and ecologically valuable fish and wildlife.

Future application of PL to the site, which will occur in Spring 2009, would introduce microbes, organic matter and nutrients not present in the unamended soil. Microbes in PL that have been consistently exposed to roxarsone will likely play a role in transformation, as seen in PL water extractions showing roxarsone degradation to a variety of As species over time (Garbarino, Bednar et al. 2003; Cortinas, Field et al. 2006). Therefore, future PL application to the sample site may result in inorganic As release from roxarsone due to PL microbial respiration.

Suggestions for Future Work

Suggestions on how to more accurately and confidently assess roxarsone transformation products if this experiment were repeated include modifications to HPLC-ICPMS and GFAA methods as well as addition of electrospray ionization coupled with mass spectrometry to analyze species. In order to verify As standard concentrations used in speciation analysis, standards for all species involved should be prepared individually. This would enable each to be separately analyzed for total As, thereby determining actual concentrations of standards for use in HPLC-ICPMS calibration.

Furthermore, all observed retention times in pattern 2 were 40 s greater than standards. Since pattern 2 was used to express final concentrations, it is appropriate that one should feel confident the late eluting species indeed corresponds to a chosen standard. To confirm suspected species, use of electrospray ionization coupled with mass spectrometry would allow for mass to charge analysis, thereby supporting or disproving a hypothesized species identity.

When determining total As by GFAA, samples should first be diluted to concentrations that fall within the LDR. With dilution comes increased error, although likely less error than would be incurred by operating outside the LDR while using the LDR calibration curve. As shown, error arising from such operation can lead to concentration underestimates of almost half of actual values. The presence of particles in the samples even after centrifugation, filtration, and acid preservation could be prevented in the future by ensuring that the appropriate volume of acid is added to samples to bring the pH <2.

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Appendix

Table 2. GFAA total As concentrations in µg/L and experiment averages.

Time (d)	As Concentration (µg/L)											
	Lactate					No Lactate						
	Flask A	Flask B	Flask C	Average	Flask KL	Flask X	Flask Y	Flask Z	Average	Flask K	Flask R	Flask S
3	133	137	121	130	130	118	128	78	108	125	116	32
5	125	119	117	121	125	123	113	127	121	124	114	10
7	118	120	121	120	127	---	137	---	137	139	---	11
9	127	125	119	124	136	122	120	117	120	130	118	8
11	125	127	124	125	133	122	123	119	121	130	118	10
13	125	124	123	124	133	126	125	123	125	127	117	7
Average				124	130				122	129	117	16

Table 3. GFAA total As concentration detail including absorbance, background absorbance, and calibration (MES cal).

Analyst		NOTES
Date	7/1/2008	
Started	14:11	
Worksheet	Mn 7-1-08	Yellow highlight denotes samples where background absorbance is greater than or equal to the As absorbance -- these concentrations are suspect. The cause of the high background absorbance may be due to the presence of particles in the samples.
Comment		MES calibration conducted by hand by assuming linear relationship between absorbance and concentration.
Methods	As	

Method: As (Zeeman)	Run	Software cal ug/L	Abs	Bkg abs	MES cal ug/L	Comments
As	CAL ZERO	0	0.01	0.0028		
As	STANDARD 1	20	0.0644	0.0077		
As	STANDARD 2	50	0.1297	0.0064		
As	STANDARD 3	100	0.2387	0.0053		
As	Check 50 ppb	42.3833	0.1104	0.0025	42	
As	T1 A	134.864	0.3195	0.0311	133	
As	T1 B	139.859	0.3307	0.0037	137	
As	T1 Y	130.541	0.3097	0.0669	128	
As	T1 MS	32.944	0.0891	0.0802	32	concentration suspect
As	T2 Z	129.139	0.3065	0.0234	127	
As	T2 MS	10.3418	0.038	0.1888	10	concentration suspect
As	T3 MS	11.7122	0.0411	0.0463	11	concentration suspect
As	T3 K	141.159	0.3337	-0.0071	139	
As	T3 Y	139.529	0.33	-0.0192	137	
As	NO SAMPLE	-1.2785	0.0118	0.0011	-1	
As	T4 KL	138.008	0.3266	0.003	136	
As	T4 A	129.266	0.3068	0.0383	127	
As	T4 B	127.529	0.3029	0.0781	125	
As	T4 C	121.297	0.2888	0.0835	119	
As	T4 K	132.166	0.3134	0.0319	130	
As	T4 X	123.783	0.2944	0.058	122	
As	CAL ZERO	0	0.0138	-0.0002	0	
As	Reslope	50	0.1345	0.0187	52	

As	T4 Y	117.957	0.2914	0.0587	120	
As	T4 Z	114.418	0.2832	0.0503	117	
As	T4 RM	115.896	0.2866	-0.0416	118	
As	T4 MS	6.9537	0.033	0.0902	8	concentration suspect
As	T5 KL	130.065	0.3196	0.0075	133	
As	T5 A	122.329	0.3016	0.052	125	
As	T5 B	124.531	0.3067	0.0185	127	
As	T5 C	121.927	0.3007	0.0479	124	
As	T5 K	127.249	0.3131	0.0086	130	
As	T5 X	119.385	0.2947	0.0246	122	
As	T5 Y	120.331	0.2969	0.0478	123	
As	T5 Z	116.519	0.2881	0.0379	119	
As	T5 RM	115.342	0.2853	-0.0243	118	
As	T5 MS	8.7606	0.0372	0.0571	10	concentration suspect
As	CAL ZERO	0	0.0166	-0.0147	1	
As	Reslope	50	0.1377	0.0057	53	
As	T6 B	118.78	0.3	-0.0119	124	
As	T6 KL	127.442	0.3205	-0.0104	133	
As	T6 A	119.522	0.3018	-0.006	125	
As	T6 C	117.656	0.2973	-0.0003	123	
As	T6 K	121.508	0.3065	0.0215	127	
As	T6 X	120.594	0.3043	0.0142	126	
As	T6 Z	117.475	0.2969	0.0113	123	
As	T6 Y	119.695	0.3022	-0.0041	125	
As	T6 RM	111.754	0.2833	-0.0467	117	
As	T6 MS	4.9522	0.0302	0.038	7	concentration suspect
As	T1 KL	124.212	0.3129	-0.0358	130	
As	T1 C	115.861	0.2931	-0.0152	121	
As	T1 K	119.486	0.3017	0.0095	125	
As	T1 X	112.69	0.2856	0.0288	118	
As	T1 Z	74.3321	0.1946	0.1057	78	concentration suspect
As	T1 RM	111.191	0.282	-0.0414	116	
As	T2 KL	-2.028	0.0136	-0.0331	0	see replicate at end
As	T2 A	120.052	0.303	0.0277	125	
As	T2 B	114.069	0.2888	0.0321	119	
As	T2 C	112.081	0.2841	0.0207	117	
As	T2 RM	109.358	0.2777	-0.0565	114	
As	T2 K	118.875	0.3002	0.0059	124	
As	T2 X	118.233	0.2987	0.024	123	
As	CAL ZERO	0	0.0131	0.0069	-1	
As	Reslope	50	0.0758	-0.0059	27	
As	T2 Y	UNCAL	0.2755	0.0329	113	
As	T3 KL	UNCAL	0.3075	-0.0305	127	
As	T3 A	UNCAL	0.2856	-0.0212	118	
As	T3 B	UNCAL	0.2915	-0.0196	120	
As	T3 C	UNCAL	0.2934	-0.0198	121	
As	T2 KL REP	UNCAL	0.3012	-0.0062	125	

Table 4. HPLC-ICP-MS speciation concentrations in mg/L. ROX: roxarsone.

	Run	Day	75As	75As	75As
			As(III)	As(V)	ROX
			ppb	ppb	ppb
Standards	10 ppb		13.0	17.6	14.8
	25 ppb		23.9	22.5	23.5
	75 ppb		71.4	64.9	68.5
	150 ppb		151.8	154.9	153.2
Live Media	T1 B	3	7.5	19.8	206.0
	T2 B	5	6.1	13.1	197.2
	T3 B	7	6.6	20.0	210.7
	T4 B	9	5.8	21.5	212.2
	T5 B	11	6.4	22.3	208.9
	T6 B	13	6.4	26.5	206.5
Live Lactate	T1 Y	3	5.9	7.6	213.9
	T2 Y	5	7.5	37.8	214.8
	T3 Y	7	6.2	8.1	216.9
	T4 Y	9	6.8	7.2	219.6
	T5 Y	11	6.4	9.0	210.3
	T6 Y	13	5.6	7.7	216.6
Killed Lactate	T1 KL	3	5.2	8.3	207.5
	T2 KL	5	5.7	37.6	216.6
	T3 KL	7	Did not run		
	T4 KL	9	4.6	9.0	212.6
	T5 KL	11	4.8	10.4	218.3
	T6 KL	13	5.3	8.5	216.0

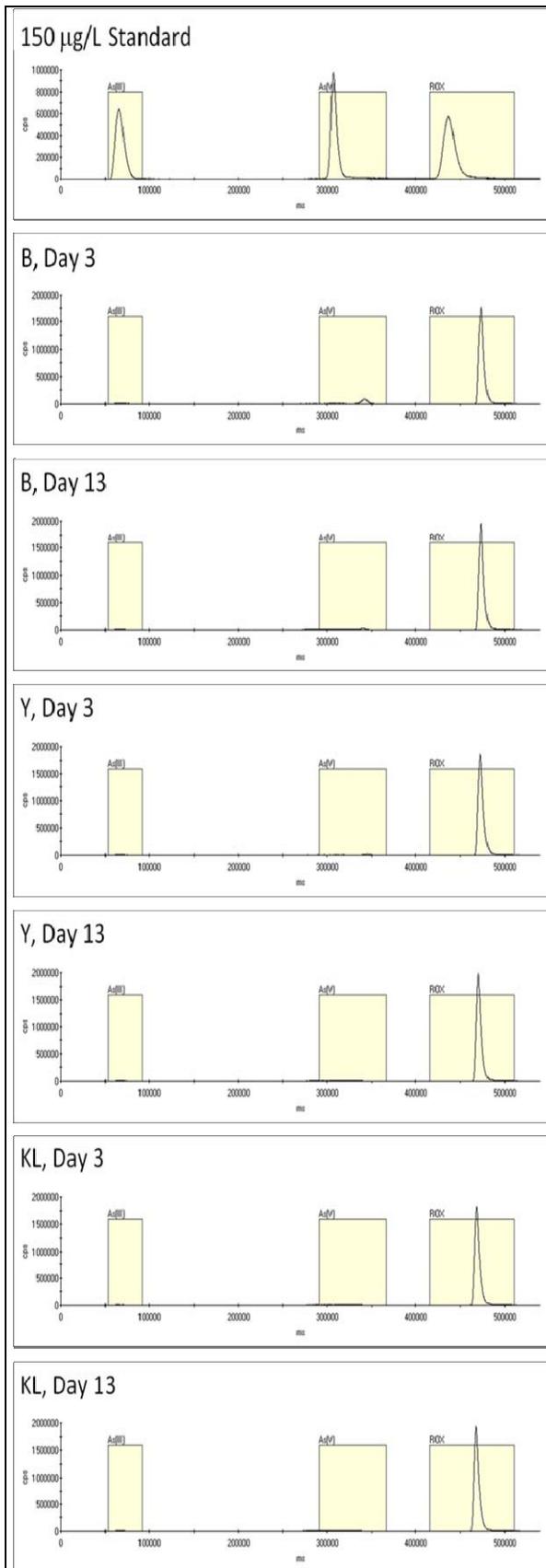


Figure 5. HPLC-ICPMS speciation chromatographs of experiments analyzed on July 11, 2008 showing elution pattern 2. Horizontal axis: milliseconds (ms); Vertical axis: counts per second (cps)

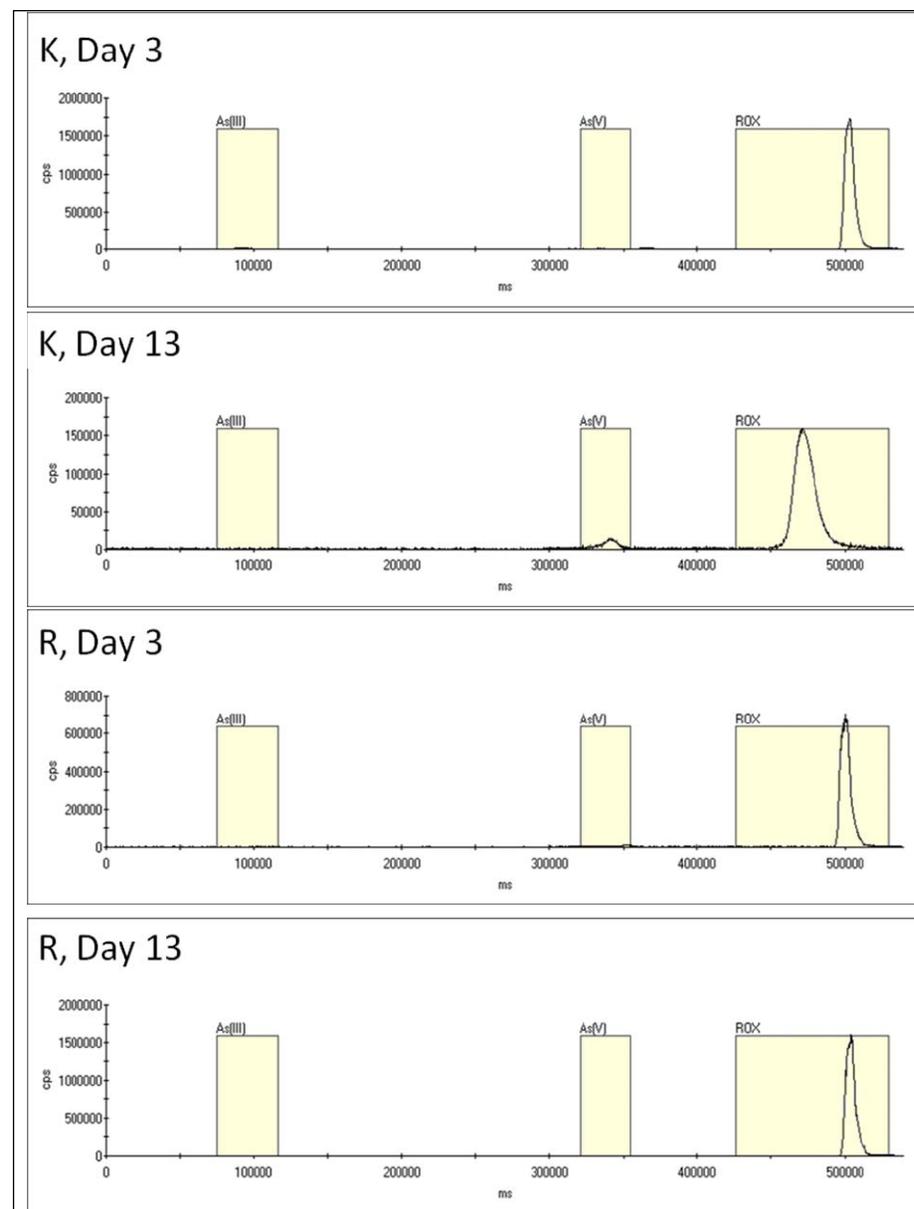
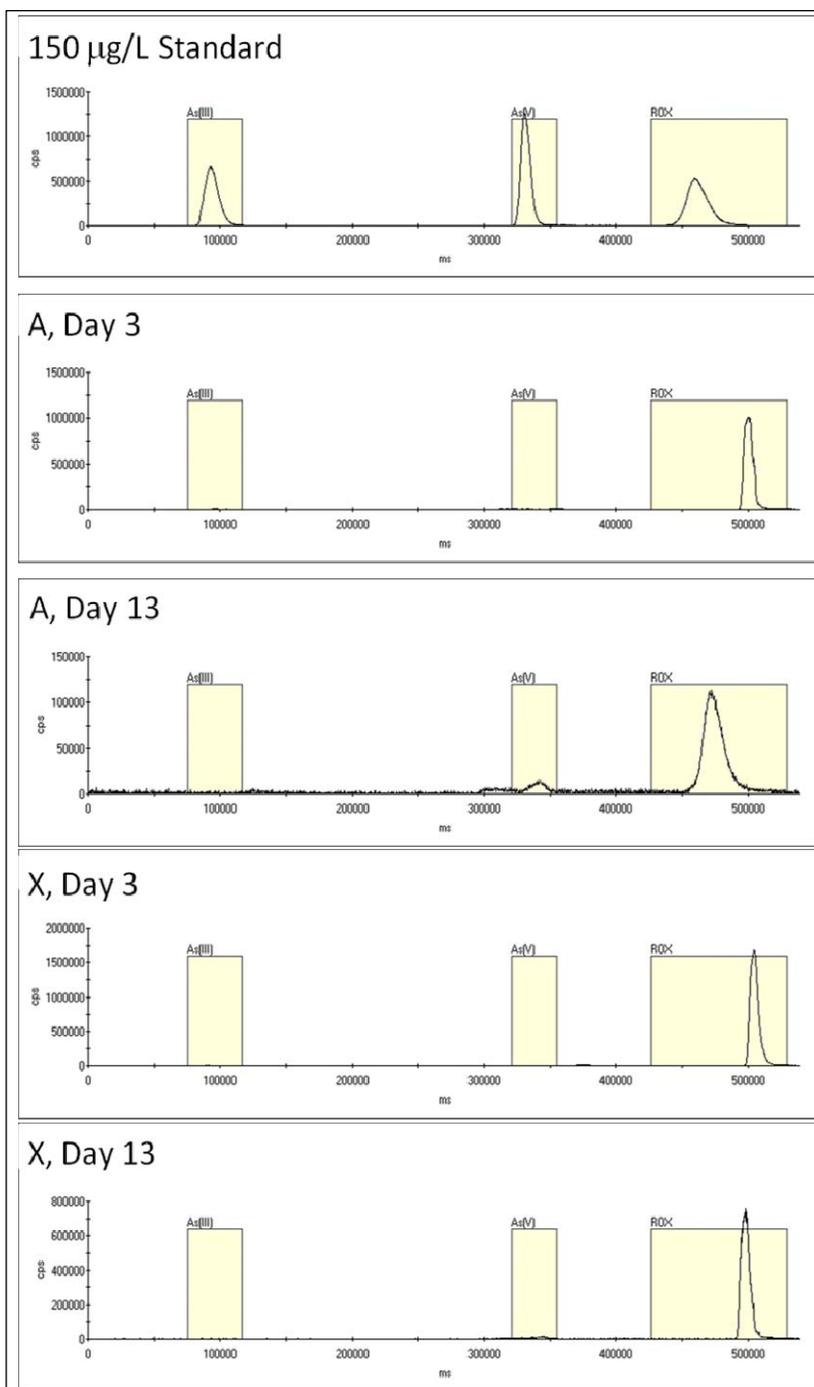


Figure 6. (Above and left) HPLC-ICPMS speciation chromatographs analyzed on July 2, 2008 showing both elution pattern 1 and elution pattern 2. Horizontal axis: milliseconds (ms); Vertical axis: counts per second (cps)

Impacts of Hypolimnetic Oxygenation Systems in a Drinking Water Reservoir

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ABSTRACT

Anoxic conditions occur in water bodies when the oxygen demands of the environment are unable to be met. Hypolimnetic anoxia is especially common in eutrophic reservoirs subject to thermal stratification. Raw water received from such reservoirs is frequently of poor quality and requires costly treatments down the line. Hypolimnetic oxygenation systems are used to remediate this problem by replenishing dissolved oxygen (DO) without disrupting the natural stratification of the reservoir. An elevated flux of DO to the sediment, termed the sediment oxygen demand (SOD), occurs due to increased DO and turbulence in the water column. In order to properly design these systems, this induced SOD must be quantified. DO profiling was conducted in a temperature-controlled atmosphere on sediment core samples extracted from Carvin's Cove Reservoir, Virginia. Fick's Law was applied to these profiles to calculate the SOD. SOD was also estimated by monitoring DO depletion in the core water column for 24 hours, following extraction. A mini-diffuser was then employed to simulate an oxygenation system in a sediment core. One effect of anoxia is elevated levels of soluble metals, like manganese, in the water column as they flux out of the sediment. Oxygenation systems do not directly reduce these levels. Manganese, known to have a slow kinetic rate, is strongly controlled by microbial processes. Bacterial colonies from Carvin's Cove benthic zone sediment were analyzed to investigate this microbial effect and were verified to be capable of manganese oxidation.

Key Words: Anoxia, Oxygenation, Sediment oxygen demand, Manganese oxidation

Introduction

Anoxic conditions occur in a water body when the dissolved oxygen (DO) demand is greater than the DO content. Lakes are subject to the biological and chemical oxygen demands of algal and aquatic respiration, aerobic decomposition of organic matter, and oxidation of metals (Gantzer and Little 2008). DO depletion is common in eutrophic lakes as a result of the high nutrient content and, thus, high primary productivity (McGinnis *et al.* 2004). In an anoxic, reducing environment, reduced forms of manganese (Mn), iron (Fe), hydrogen sulfide, ammonia, and phosphorus can be produced. Metals persist in soluble form and are released from the sediments into the water column. Phosphorus may lead to further productivity, and thus, additional increases in oxygen demand. Hydrogen sulfide, Mn, Fe, and other metals can cause poor water clarity, odor, and taste (Singleton and Little 2006). Raw water received from anoxic reservoirs can thus require substantial treatment prior to distribution to a community.

Hypolimnetic oxygenation systems remediate anoxia by pumping DO directly into the lower layer of a stratified lake, where DO-depleted conditions tend to exist. Low flow rates are maintained to ensure the natural thermal stratification of the reservoir is not disturbed (Singleton and Little 2006). Oxygenation is currently used in many lakes; however, the impact of these systems on the natural environment still needs to be fully quantified so that these systems can be optimized to increase raw water quality and minimize treatment costs.

DO consumed by sediment processes, often termed sediment oxygen demand (SOD), can be modeled according to Fick's Law:

$$SOD = J_{O_2} = -\phi D_m \frac{dC}{dz} \quad (1)$$

, where J_{O_2} is the flux of DO across the sediment water interface (SWI), ϕ is sediment porosity, D_{O_2} is the diffusion coefficient of oxygen in water, and dC/dz is the DO concentration gradient through the diffusive boundary layer (DBL). Lorke *et al.* (2003) found porosities typically greater than 0.95 in the uppermost sediment layers (2003). Furthermore, sediment cores used in this study had extremely high water contents in the uppermost sediment; thus, a porosity of 1.0 (that of water) was used as a simplifying assumption for this work.

The first objective of this work was to estimate SOD in Carvin's Cove. Immediately upon extraction, sediment cores were taken to a laboratory where estimates of the DO flux into the sediment were conducted using two methods. Method 1 applies Fick's Law to DO profiles across the SWI (Berg *et al.* 2001). DO profiles were taken at the beginning and end of the 24 hour period following extraction. Initial and final SOD calculations were averaged to approximate the rate of DO flux to the sediments during this 24 hour period. Method 2 is based on DO depletion from the core water, following Walker and Snodgrass (1986). DO concentration in the water column overlying the SWI was monitored for the 24 hour period and the overall depletion was used to calculate SOD, according to Equation (2),

$$SOD = \frac{-V}{A_s} \frac{d[O_2]}{dt} \quad (2)$$

, where V is the volume of water overlaying the SWI, A_s is the sediment area across which oxygen is transferred, and $d[O_2]/dt$, is the rate of DO depletion.

SOD is controlled by transport of DO through the diffusive boundary layer (DBL), the region above the SWI in which DO rapidly changes, and by consumption processes in the sediments. Increasing the DO above the DBL by oxygenation increases the concentration gradient and, therefore, increases the flux of oxygen to the sediment, as exhibited by Equation (1). Turbulence (mixing) induced by oxygenation also increases the transport of DO into the sediment by decreasing the DBL thickness. This combined effect is known as the oxygenator-induced SOD (Higashino *et al.* 2003).

In the past, oxygenation systems have been under-designed due to the lack of knowledge of this additional DO demand. A second objective of this project is to measure DO profiles during the transient

stages between saturated and anoxic steady-state conditions to study the SOD and the sediment oxic region as a function of oxygenation.

A second issue with oxygenation systems is the unpredictability of soluble Mn. Since soluble forms of both Fe and Mn are favored in reduced, anoxic environments, it would be reasonable to assume both will return to insoluble forms when oxygen is replenished. This is the case with Fe, which has rapid redox kinetics; however, there is a significant lag-time before Mn levels decrease following DO replenishment due to its slow redox kinetics (Gantzer and Little 2008). One hypothesis is that Mn oxidation in this environment is not easily chemically oxidized and is strongly influenced by microbial redox processes (Gounot 2006). Pure bacterial colonies were isolated from Carvin's Cove sediment and tested for Mn-oxidizing ability. The sediment samples tested positive for the presence of Mn-oxidizing bacteria, thus supporting this hypothesis. This preliminary verification will later be expanded to explain the soluble metal flux data from Carvin's Cove.

Research Methods

Study Site

Carvin's Cove Reservoir is a man-made, monomictic, eutrophic water supply located in Western Virginia. During the thermally-stratified summer months, raw water quality is typically poor due to hypolimnetic anoxia. Two 625 m linear bubble-plume diffusers are installed in the deepest trenches of the lake, nearest the dam. The location and schematic of Carvin's Cove are pictured in Figure 1.

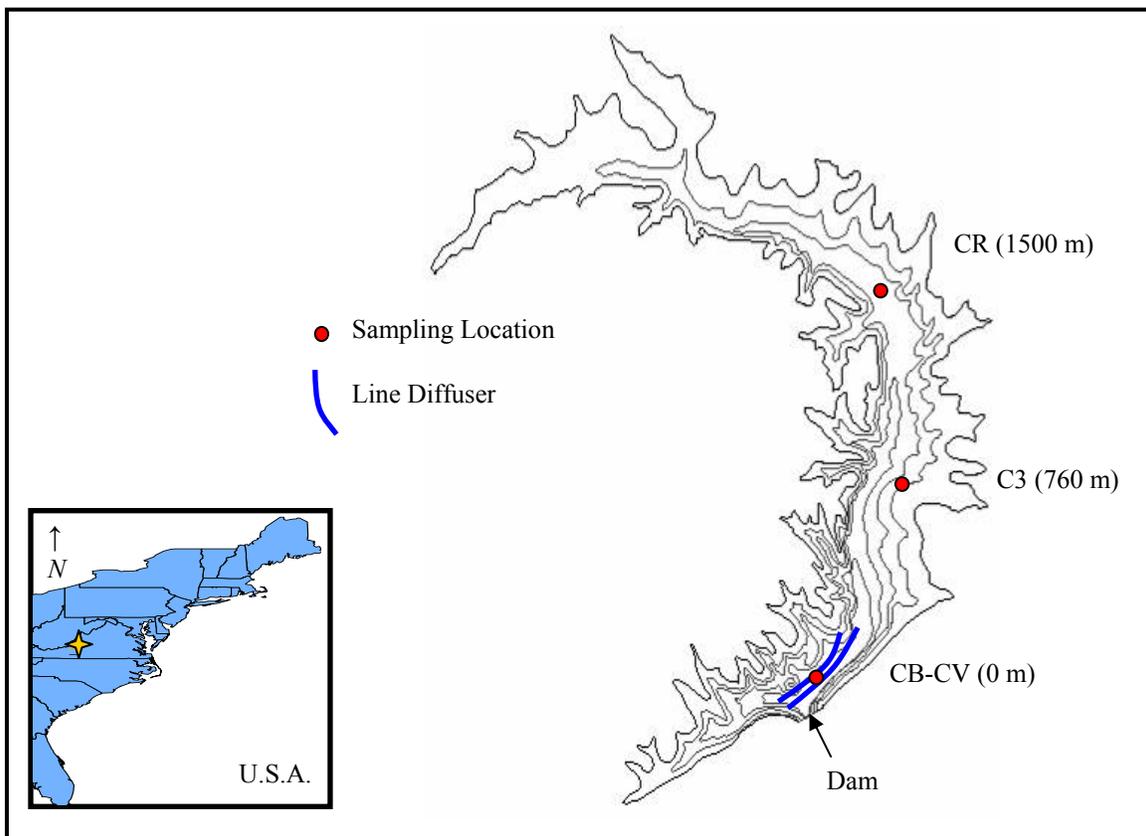


Figure 1. Carvin's Cove Reservoir, Roanoke County, Virginia

Two line diffusers, 625 m each, are installed near the dam. Notice the three sampling locations: CB-CV between the diffusers, C3 about 760 m upstream, and CR about 1500 m upstream.

Field Methods

Sediment cores were extracted from Carvin's Cove at three locations (Figure 1) in 9 cm diameter plexiglass tubes. A Uwitec ball corer was used to collect samples and a Uwitec core cutter was used to slice the cores. Cut cores had 10 cm of water column overlying the sediment. Only cores with undisturbed sediment surfaces were retained for testing. Cores were covered to block light and stored on ice to prevent photosynthetic respiration and to reasonably maintain in-situ reservoir temperatures. Sediment samples for microbial testing were taken from the uppermost 2 cm of the benthic zone.



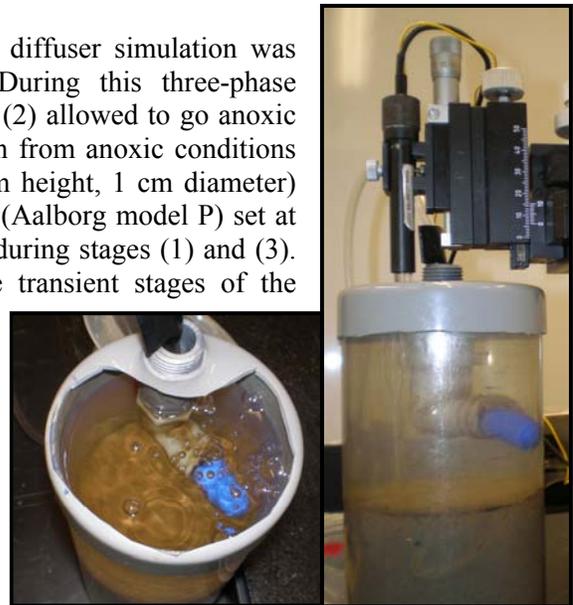
Photograph of Core Extraction

SOD Measurements

Sediment cores were transported to an 18°C, insulated laboratory. For a 24 hour period following extraction, DO measurements were taken at 5 cm above the SWI (Method 2). During this period, the core waters were slightly disturbed by a stirring device to mimic in-situ conditions. DO profiles were taken at the beginning and end of this period (Method 1). DO was measured with a 100 μm diameter tip, glass DO microsensor (Unisense OX-100) connected to a picoammeter (Unisense PA2000). The measurements were recorded using a microprofiling computer program (Unisense Profix) and calibrated daily using Winkler titrations. The method employed was similar to that in other studies quantifying DO distribution within extracted sediments (House 2003; Rasmussen and Jorgensen 1992; Revsbech *et al.* 1986; Wiltshire *et al.* 1996). To obtain profiles, the sensor was manually lowered at 1 mm intervals into the water column and sediment using a micromanipulator attached to a magnetic stand.

Diffuser Simulation

To study the effect of oxygenation on SOD, a diffuser simulation was conducted on cores extracted from Location C3. During this three-phase simulation, the cores were (1) brought to DO-saturation, (2) allowed to go anoxic from previously established DO-saturation, and (3) taken from anoxic conditions back to DO-saturation. A sandstone mini-diffuser (2 cm height, 1 cm diameter) was placed 2 cm above the SWI. A low-flow rotameter (Aalborg model P) set at 50 ml/min controlled air-flow through the mini-diffuser during stages (1) and (3). DO microprofiles were taken intermittently during the transient stages of the simulation. The sediment was able to reach anoxia due to the complete depletion of DO in the diffusive boundary layer (Revsbech *et al.* 1980). Steady-state oxic conditions were indicated by unchanging DO gradients across the SWI, implying DO was diffusing to the sediment at the same rate that it was being consumed by benthic chemical and biological reactions. This approach is similar to that of Beutel *et al.* 2007).



Photographs of Diffuser Simulation Set-Up

Sediment cores were incubated in the dark except for short periods when profiles were obtained. During the transition period from DO-saturation to anoxia, efforts were made to keep the cores as quiescent as possible to avoid transport of DO into the DBL. Water column temperature was monitored to insure that the desired temperature was maintained.

Manganese Oxidation

Sediment samples taken from the uppermost 2 cm of the benthic zone at Carvin's Cove were plated on agar plates of the Mn-containing media described by Stein *et al.* (2001). Probable Mn-oxidizing colonies – those of brown or dark orange hues – were selected and streaked in order to obtain isolates. Fourteen isolate colonies were then inoculated in Stein's Mn-broth. Samples were reacted in a 1:5 ratio with Leukoberbelin Blue (LBB) – a chemical indicator which reacts with oxidized Mn to produce a color change – initially and following a 28 day incubation period. Absorption measurements of each reacted sample were taken with a Hitachi Digital Spectrophotometer at 620 nm. A potassium permanganate calibration curve was employed to relate absorption to oxidized-Mn concentration. This method of verification followed that published in the *Manual of Environmental Microbiology* (Tebo *et al.* 2007).

Results and Discussion

SOD Measurements

SOD estimates according to both Methods 1 and 2 were found to be in agreement, with a maximum percent difference of 5% (Table 1). The largest flux of DO to the sediments occurred in the core extracted at Location C3 and the smallest at Location CR, with Location CB-CV falling in between.

Table 1. 24 Hour SOD Measurements

Location	Date	SOD (mg/m ² -d)		Percent Difference (%)
		Method 1	Method 2	
CR	17-Jun	72	68	5
C3	19-Jun	230	240	4
CB-CV	19-Jun	129	132	3

This progression can be explained by the nature of the bubble-plume created by the oxygenation system. These plumes tend to cause advection of oxygenated waters far upstream. These advection processes most directly impact Location C3 and do not extend to CR. Since Location CR tends to remain anoxic, negligible DO reaches the sediment to be consumed; hence low SOD is expected. Though Location CB-CV lies between the diffuser lines and does experience elevated DO at the SWI, a significant amount of the oxygenated water is forced away from the system by the plume, rather than directly down to the sediment immediately near the diffuser lines (McGinnis *et al.* 2004)

Diffuser Simulation

Transient profiles obtained during this study depict the changing SOD and oxic sediment zone depth over the course of oxygenation. During the transient phase when the core was taken from saturated to anoxic conditions, the SOD decreased from approximately 350 to 25 mg/m²-d over the course of 72 hours. The DO penetration zone, in which the sediment is oxic, decreased from 5 mm below the SWI at saturation to negligible (~0 mm) at oxic, steady-state conditions (Figure 2a). During the second transient phase from anoxic to saturated conditions, SOD increased rapidly from 0 to about 360 mg/m²-d within 24 hours. The penetration zone returned to 5 mm below the SWI by the point of saturation (Figures 2b). The magnitude of increase in SOD with oxygenation emphasizes the need to design systems that account for the additional DO demand.

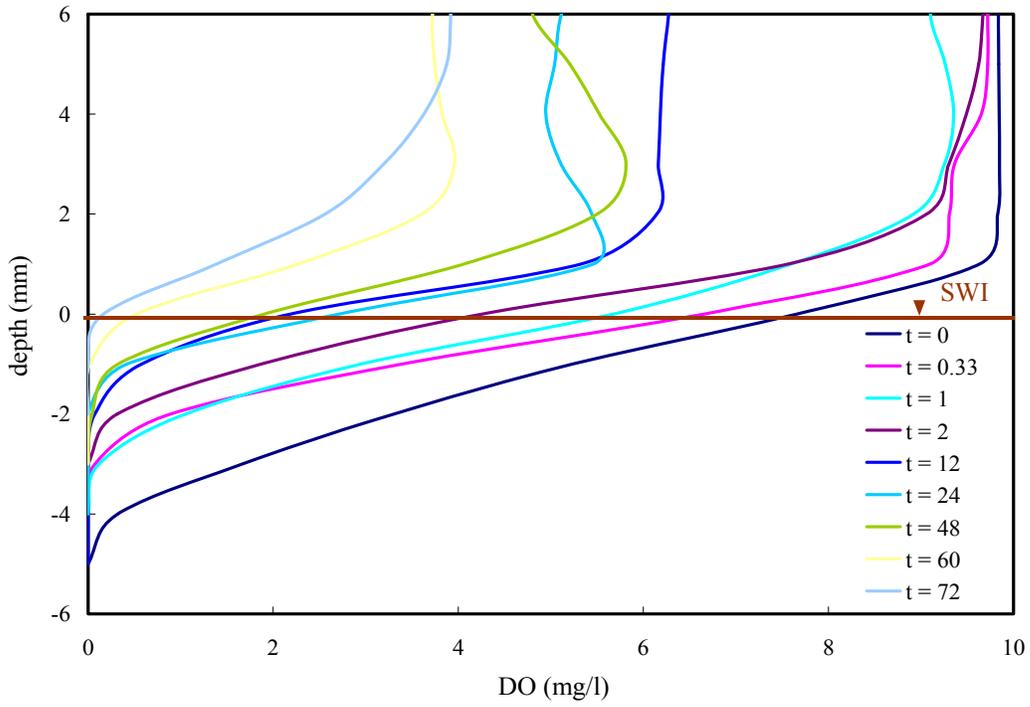


Figure 2a. Diffuse Simulation: Oxidic to Anoxic DO Profiles

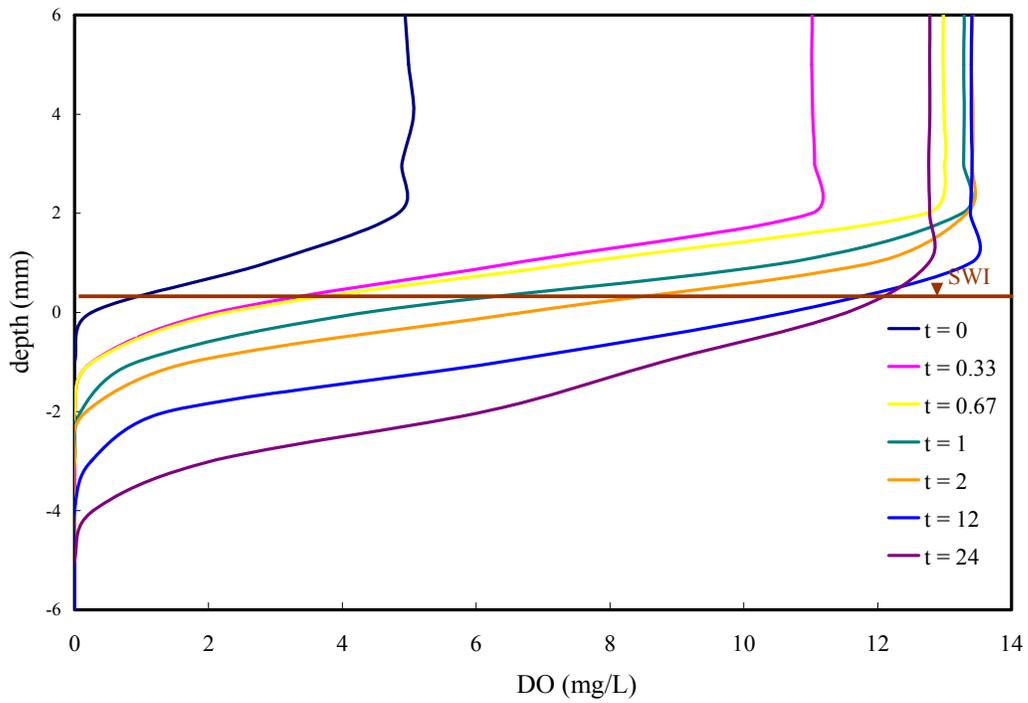
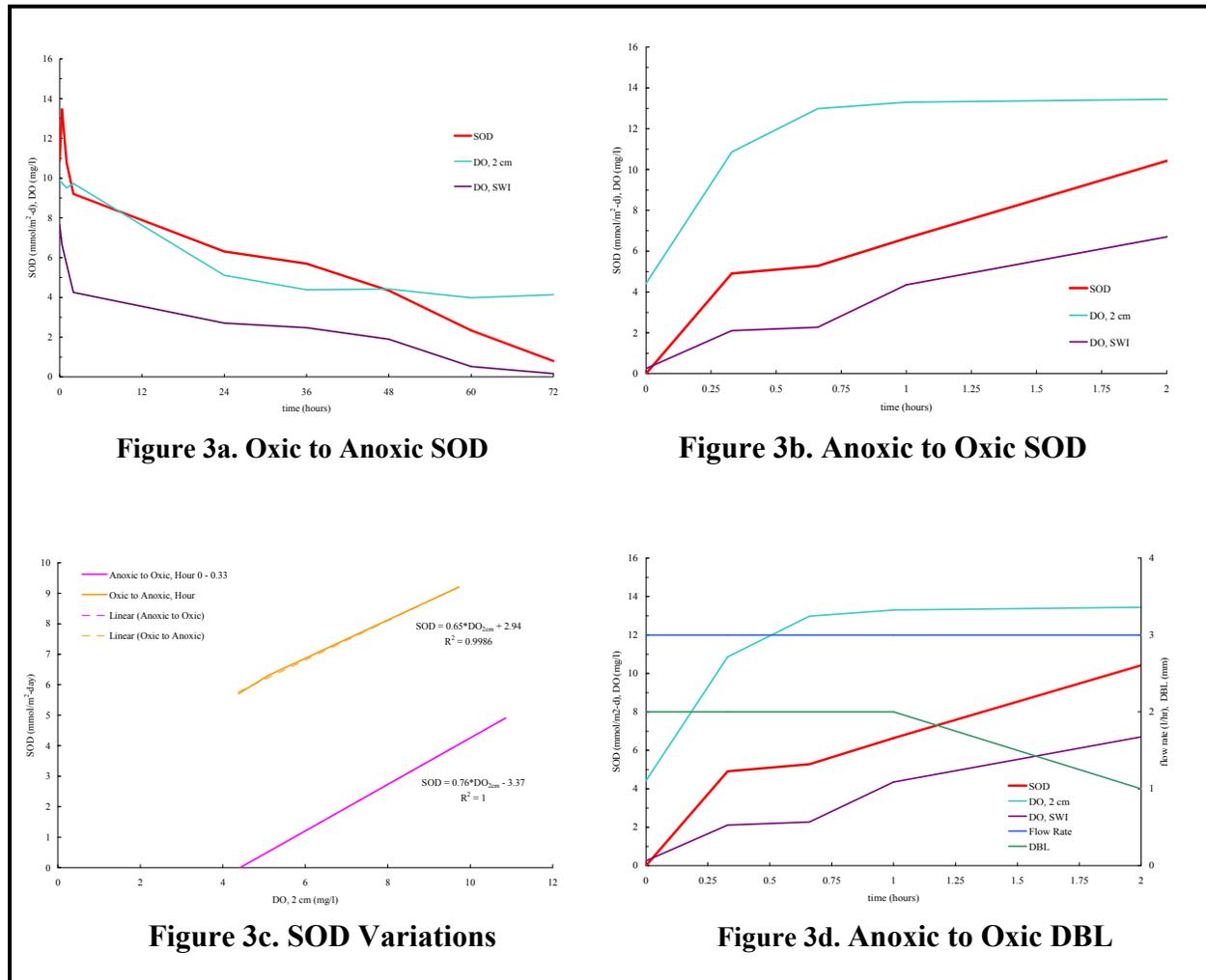


Figure 2b. Diffuse Simulation: Anoxic to Oxidic DO Profiles

SOD does not appear to be solely dependent on DO content in the bottom boundary layer (BBL) of the water column (Figures 3a, b); however, in the range from 4 to 10 mg/l, SOD is directly proportional to DO concentrations in the BBL (Figure 3c). SOD is highly dependent on DO at the SWI, indicating that the diffusive process here is transport-limited. DO transported to the SWI is readily consumed by the microbial and biological processes occurring within the sediment. Induced turbulence during the oxygenation period has a strong effect on SOD. While DO concentrations do increase in the BBL as a result of oxygenation, it is the mixing that drives the high-DO water down to the SWI. The DBL degrades from about 2 to 1 mm during the anoxic to oxic period, as turbulent mixing within the water column compresses the DBL thickness (Figure 3d). As this occurs, DO in the BBL can diffuse more directly through this thinner layer into the sediment and SOD is more directly impacted by water column DO concentrations (e.g., the direct correlation between SOD and DO during the peak oxygenation (DO ~4-10 mg/L) phase).



Manganese Oxidation

Seven of the fourteen selected colonies were verified as Mn-oxidizers by the LBB method. These samples contained between two and five times the background levels of oxidized Mn after the 4 week incubation period (Figure 4). Two controls (C1, C2) of pure Mn-broth and one of LBB solution were incubated in the same environment as the samples to ensure that the indicated oxidation was biological, rather than chemical. DI water was used as the spectrophotometer standard.

Each of the colonies which oxidized Mn appeared as an irregularly-shaped orange or brown mass on the initial agar plates. The presence of these bacterial colonies supports the hypothesis that sediment microbes have a significant impact on Mn redox processes in the sediment benthic zone. Further investigations must be undertaken to understand the impact of oxygenation on this microbial activity so that the use of these systems to control soluble Mn levels in reservoirs can be optimized.

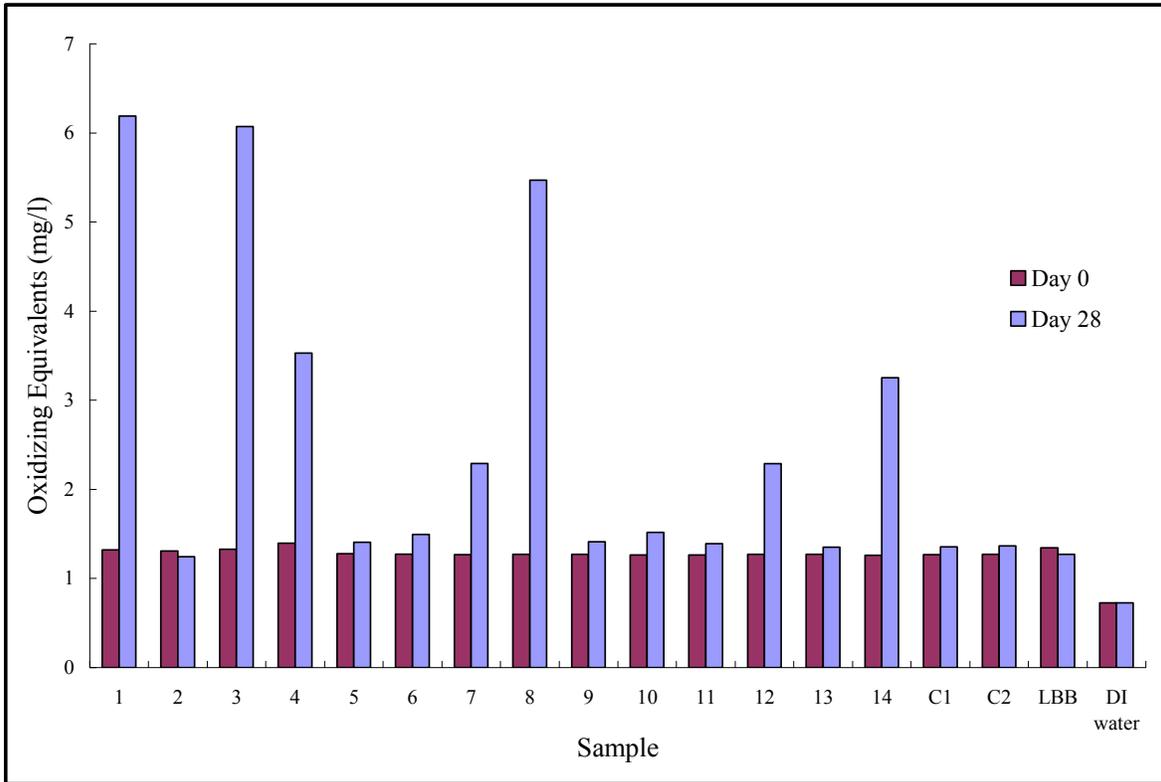


Figure 4. Manganese-Oxidizing Capability of Carvins Cove Benthic Zone Bacteria
 Seven samples exhibited definite increases in oxidized manganese levels, suggesting the ability of these bacterial communities to oxidize manganese.

Conclusion

Hypolimnetic oxygenation systems are highly effective means to alleviate anoxia in drinking water reservoirs. Furthermore, these systems deliver DO without mixing the bottom and surface waters, thus maintaining the natural thermal stratification of the lake. In this way, oxygenation systems are beneficial in that they do not tend to disturb the aquatic system.

Oxygenation systems in the past have been widely under-designed, due to the lack of knowledge on oxygenation-induced SOD. Understanding the transient nature of DO fluxes across the SWI will allow this phenomenon to be better understood so that future systems can be optimally designed. Systems need to be designed to minimize induced SOD while still maintaining a sediment oxic zone that adequately supports Mn-oxidation processes (chemical and biological), thus suppressing Mn (and other soluble metal) fluxes out of the sediment and ultimately improving water quality. Further investigation of the bacteria responsible for controlling Mn redox processes in freshwater sediment environments will provide deeper understanding of Mn kinetics and will ultimately allow for better control of soluble Mn in drinking water.

Acknowledgements

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Persistence of Fecal Indicator Bacteria (*Enterococcus*) in Fairview Beach Sands and Sediments

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ABSTRACT

Fairview beach sands and sediments from Stafford County, VA were analyzed for the presence-absence of fecal indicator bacteria. Biolog and Antibiotic Resistance Analysis (ARA) were performed in order to create a clear distinction of *Enterococcus* between human and non-human sources of fecal bacteria. These methods allow for accurate Microbial Source Tracking (MST) and speciation of *Enterococcus* in sand, water, and sediment samples.

Keywords: Antibiotic Resistance Analysis (ARA), Biolog, Fairview Beach, Fecal Indicator Bacteria

Introduction

Fecal contamination by *Enterococcus* at public beaches is a major cause of concern for human health and sanitation. This microorganism indicates the presence of pathogens, viruses, and parasites which can eventually manifest in infection and disease. Scientists are most interested in determining whether the fecal bacteria are of human or non-human origin. If the source of fecal matter is determined to be human, the public is put at a higher risk for contracting enteric viruses and diseases and appropriate corrective measures must be taken (Hartz, 2007).

Microbial Source Tracking (MST) aims to find the sources of non-point source fecal pollution within a specific watershed. In using MST, scientists can determine if fecal bacteria are of human or non-human origin and use the knowledge to implement abatement technologies (Wade, 2007). In this study, two methodologies of MST were used; Antibiotic Resistance Analysis (ARA) was used in combination with Biolog to ascertain the sources of pollution using fecal indicator organisms. Both methods are used in this study as a means of quality control in order to ensure accuracy in reporting results and drawing conclusions.

Fairview beach in Stafford County, Virginia was a principle site of interest for research in MST. Over the past several years, the beach has been noted for multiple closures due to a high incidence of bacterial contamination. Environmental health professionals regularly sample at public beaches during the swimming season (weekly, May-September). If *Enterococcus* counts exceed 104 colony forming units (cfu)/ 100 mL of water, a swimming advisory is posted (Virginia Dept. of Health, 2008). Bacterial counts of this magnitude pose a serious threat for gastrointestinal disease and infection. In 2004, there were 47 no-swimming advisories at 16 of Virginia's beaches. Out of these advisories, 11 were at Fairview Beach. In 2007, there were 12 advisories, 9 of which occurred at Fairview Beach. Currently, during the 2008 swimming season there have been 8 advisories, 6 of which were at Fairview (Table 1). This high incidence of bacterial contamination needs careful monitoring by scientists and concerned citizens' alike

(Hagedorn, 2007). The reduction in closures at beaches other than Fairview Beach (36 in 2004, 3 in 2007, 2 to date in 2008) is a result of successfully finding sources of fecal pollution and correcting the problems at the other beaches, leaving only Fairview as the remaining public beach with persistent pollution issues.

Table 1: Virginia Department of Health Monitoring Data (cfu/100mL) for 2008.

Beach Name	Sampling Date	Sampling Location 1	Sampling Location 2	Sampling Location 3	Average	Swimming Status
FAIRVIEW BEACH	5/20/2008	320	320	320	320	Swimming Advisory
	5/22/2008	240	200	270	237	Swimming Advisory
	5/27/2008	24	24	22	23	Open
	6/3/2008	320	320	320	320	Swimming Advisory
	6/4/2008	320	320	320	320	Swimming Advisory
	6/9/2008	30	<10	84	38	Open
	6/16/2008	54	22	8	28	Open
	6/23/2008	120	68	130	106	Swimming Advisory
	6/25/2008	16	6	20	14	Open
	6/30/2008	30	10	<10	14	Open
	7/7/2008	32	38	60	43	Open
	7/14/2008	321	321	321	321	Swimming Advisory
	7/16/2008	36	18	44	33	Open
	7/21/2008	110	30	110	83	Open

(Virginia Department of Health, Summer 2008)

In order to use evaluate data from Fairview Beach, ARA and Biolog testing must be completed to find the sources of pollution. In order for laboratory analysis to proceed, several steps must first be taken. Known fecal samples from cows, birds, dogs, chickens, humans, and other wildlife must be taken from the surrounding areas in order to compare diverse source samples (Wade, 2007). These samples are then added to a “source library” to use for later comparison of collected data. Scientists must use sterile equipment while collecting water and sediment samples from contaminated sites in order to ensure no contamination will interfere with sample analysis. After the samples have been collected, membrane filtration is used to plate the fecal bacteria samples on Membrane-Enterococcus Indoxyl-β-D-Glucoside (mEI) media. After the samples of bacterium have been plated, the plates are inverted and incubated in warm, moist environments (41°C for *Enterococcus*) in order for bacterial growth to occur. Subsequently, following incubation period, colonies are picked from the media plates with sterile toothpicks and placed in microwell plates with enterococcal broth, a medium that allows for the selective growth of *Enterococcus*. After a second period of incubation, growth on the microwell plates is recorded. A binary numerical system is used to distinguish between presence-absence of black wells. Wells that have turned black indicate the isolate has hydrolyzed the esculin protein; this is a positive identification that the isolate is a species of *Enterococcus*. The bacteria from the microwell plates are then replica-plated using a 48-prong replicator on Trypticase Soy Agar (TSA) that has been inoculated with varying concentrations of antibiotics. Due to horizontal gene transfer between different bacteria there is reasonable evidence that some bacteria will show resistance to the antibiotics that is source related. The plates are then incubated to allow time for sufficient growth to occur. After the incubation period growth patterns on the plates are observed and recorded. Since the original sources of bacteria were known bacterium samples, ARA has allowed the creation of a “source library” in Dr. Hagedorn’s lab over the past few years for analysis and comparison of data at Virginia’s beaches.

Identical procedures are utilized during the collection of unknown samples. In this way, growth patterns of unknown samples can be documented and compared with the library to find similarities. The samples are then run through a statistical program that ascertains the probability that isolates from a given sample are from a particular source.

After ARA analysis, a Biolog application is utilized as a mechanism for quality control (Biolog, Inc). This machine uses liquid inoculum in conjunction with 96 carbon sources for bacteria to metabolize. The software is able to accurately detect carbon usage patterns by microbes based on the reaction of tetrazolium redox dye. Using these patterns, the Biolog program is able to perform speciation matches on the bacteria. With this information, researchers can determine specific sources of fecal contamination and propose corrective measures.

Research Methods

Sample Collection

The source tracking team visited Fairview Beach on July, 2, 2008 in order to collect samples for laboratory analysis. Sterile plastic bottles were used to ensure no contamination would affect the results of the study. Four kinds of samples were taken from each Virginia Department of Health (VDH) sampling sites: dry beach sand (high tide line), wet beach sand and water (low tide line), water plus sediment, and water. On the beach near VDH site 1 a long concrete pipe stretches through the beach and dumps effluent directly in the beach water. Samples of water and sediment were taken in several places surrounding the pipeline. In addition, the team surveyed the surrounding land for possible septic system failure as many trailers and mobile homes surrounded the vicinity. Two samples were taken at separate sites indicating possible septic failure. The samples taken were put in an ice cooler and taken back to the laboratory.



VDH Site 1



VDH Site 2



VDH Site 3



Beach Drain Pipe



Beach Drain Pipe Hole

Fluorometry Testing

During the research conducted a Turner AU-10 fluorometer was used in order to determine the levels of optical brighteners in a particular sample. Optical brighteners are highly substituted, aromatic compounds that are typically found in commercial laundry detergents and dishwashing detergents (Hagedorn et. al, 2005). In addition, these compounds have many double bonds that are excited by ultraviolet light. Optical brighteners absorb light in the near-ultraviolet range (360-365 nanometers) and re-emit light in the blue region (400-440 nm) (Hagedorn et. al, 2005). In order to detect this absorption, a fluorometer is used for detection. A high reading of optical brighteners in a sample suggests the presence of sewage waste effluent in the sample source. A fluorometer signal unit greater than 100 typically indicates that the sample has come from a human sewage source. This in turn, implies that the specific sample is likely to have higher fecal indicator bacteria counts. This method allows for rapid indicator testing for the presence-absence of fecal bacteria (Wade, 2007).

Membrane Filtration

Samples collected from the Fairview beach site were filtered the following day using a sterile technique called membrane filtration. In this method, a sterile filter pad is placed in the device and a specific amount of sample is added using a pipette. The filtration method works by using a principle of exclusion. The device draws water down through the filter by using a pressurized vacuum force effectively eliminating the water but plating the bacteria on the filter membrane. In using this mechanism, filters can be aseptically moved to mEI media for incubation of the sample. This is an EPA-approved method (Method 1600 <http://www.epa.gov/nerlcwww/online.htm#recwater>).

Antibiotic Resistance Analysis (ARA)

This method utilizes varying concentrations of eight different antibiotics: amoxicillin, cephalothin, chlortetracycline hydrochloride, erythromycin, neomycin, streptomycin sulfate, tetracycline, and vancomycin due to their usage in humans and in the agricultural industry. After using membrane filtration and incubating a water sample, colonies of *Enterococcus* should be evident on mEI media. Next, 96-micro well plates must be prepared aseptically. An 8-channel pipettor is used to add 0.2 mL of enterococcal broth (used for cultivation of *Enterococcus*) in each microwell. Sterile toothpicks are used to transfer 24 isolates from source sample plates to individual microwells from growth. After the incubation period, microwell plates with enterococcal broth are checked for black or dark brown color. If no color change has occurred, the isolate is not considered for future analysis. Trypticase Soy Agar (TSA) is autoclaved at 115°C for 15 minutes and then placed in a 44°C water bath. After the agar has had time to cool, known concentrations of antibiotics are added and mixed. The TSA solution is then poured on to sterile 100x15mm Petri dishes and allowed to solidify. *Enterococcus* is then transferred from the microwell plates to the Petri dishes using a 48 prong replica-platter. The plates are then incubated 48 hours at 41°C to ensure log growth. After the incubation period, the plates are analyzed using a binary number system. A “1” indicated that the sample showed bacterial resistance, while “0” indicated that no bacterial growth was present.

Statistical Analysis of ARA

After ascertaining bacterial growth from ARA and recording results, the samples are statistically analyzed using SAS-JMP (v. 5.0.1, SAS Inst., Cary, NC). This program contains a library of ARA data from known sources of fecal pollution. The known sources of contamination are analyzed through ARA, and the presence-absence of growth is recorded. This program utilizes discriminant analysis; a statistical classification algorithm that compares isolates of unknown sources (from water samples) to the library of source isolates (Dickerson, 2008). In this way, a scientist can determine unknown sources of fecal bacteria by comparing it to the source library contained within the program (Wade, 2007). The data generated by this program also allows the researcher to find the Rate of Correct Classification (RCC) for all of the possible sources. RCC refers to the rate at which isolates within the library are correctly

classified using the models generated from those same isolates (Dickerson et. al, 2008). This statistic is significant when considering error percentage while analyzing data.

Biolog Assessment

A Biolog assay is also done on the samples as a secondary quality control assessment on the fecal bacteria. After the incubation period for *Enterococcus* is complete, a Biolog assessment is done on the sample to ascertain the specific speciation of the collected bacteria. Testing begins by streaking samples of *Enterococcus* on Biolog Universal Growth (BUG) agar to obtain isolates. The agar plates are incubated for ~24 hours at 35°C. Subsequently, a liquid inoculum is prepared based on specific characteristics of the microorganism. For example, the inoculum solution will be different if a microorganism is gram positive or gram negative. Bacterial cells are removed from the BUG plates and aseptically added to the suspension. The suspension produced is compared to a standardized Biolog solution with approximately 20% transmittance. Subsequent adjustments are made to render turbidity equivalent. The inoculum solution is then added to a MicroPlate using an 8-channel pipettor. This Microplate contains 96 different sources of carbohydrate, 30 of which *Enterococci* species metabolize. The Biolog machine measures the carbon source utilization of a microorganism to produce a pattern of resource use or a “fingerprint” (Solit, 1999). As microorganisms use carbon sources from the MicroPlate wells, they begin to respire. This respiration process reduces tetrazolium redox dye and the wells turn a bright shade of purple. The end result produces MicroPlate wells with patterns of purple wells that are characteristic of a particular microorganism. In this respect, the machine can read the plate, assess the results, find matches within the database, and produces an identification of the bacteria. The machine uses extensive algorithms to compare the observed well patterns and compare it to the database. In compiling this information, MicroLog software ranks the closest match to the particular microbe’s purple patterns. This type of carbon pattern matching is known as Progressive ID (PID) (Solit, 1999). Typically microorganisms use their preferred carbon source most rapidly, resulting in dark purple wells that quickly materialize. The less-preferred carbon sources are consumed very gradually by the microbes resulting in light purple wells. In using this method, the program is able to accurately identify species patterns by considering the sequences in which purple wells are formed. (Solit, 1999)

Results and Discussion

After obtaining samples from several Fairview Beach sites, laboratory analysis was done to isolate colonies of *Enterococcus*.

Trial 1

Fresh water samples from Fairview Beach were taken out of the refrigerator and membrane filtrated at volumes of 5 mL and 20 mL. Sediment samples were saturated with autoclaved water, and were filtered at the same volume. The filtered samples were put of mEI plates and incubated for ~48 hours at 41°C. *Enterococcus* growth on these plates was not sufficient enough to continue bacterial analysis. This procedure was repeated again at higher volumes without success. Some samples were filtered at higher volumes due to low quantities of sediment in the sample (Table 2). High amounts of sediment on filter paper can interfere with the growth of microorganisms by blocking the access of nutrients from a specific media.

Table 2: Fairview Beach *Enterococcus* counts.

Fairview Beach Filtered Colony Counts (mEI plates)			
Sample	Filtrated Volumes		
	5mL	20mL	10mL
Stream Up 30yds	8	0	-
Next to Reeds	0	0	-
Beach Drain Pipe	2	5	-
Pipe Sediment End	0	-	1
Drain Pipe Hole Sediment	1	-	0
Trailer Leachate by #1	2	2	
Below the Reeds	3	-	16
Water VDH 1	0	0	-
Wet Beach Sand VDH 1	1	-	2
Sediment VDH 1	2	-	4
Dry Beach Sand VDH 1	11	19	-
Water VDH 2	0	0	-
Wet Beach Sand VDH 2	8	-	8
Sediment VDH 2	1	-	0
Dry Beach Sand VDH 2	0	1	-
Water VDH 3	1	1	-
Wet Beach Sand VDH 3	1	-	0
Sediment VDH 3	0	-	4
Dry Beach Sand VDH 3	0	2	-

Fluorometric Analysis

Samples from Fairview Beach were tested for optical brighteners using fluorometric analysis. The machine was first calibrated with known standards of optical brighteners before testing selected samples (Table 3).

Table 3: Fluorometric Analysis.

Fairview Beach Fluorometric Analysis 7-2-08	
Sample	Fluorometer Signal Unit
Calibration 0ppb	-0.7
Calibration 20ppb	335.0
Calibration 30ppb	480.0
VDH water 1	25.4
VDH water 2	26.7
VDH water 3	26.2
VDH 1 Sediment	28.6
VDH 2 Sediment	26.3
VDH 3 Sediment	52.0
VDH 1 Beach Sand	26.2
VDH 2 Beach Sand	26.0
VDH 3 Beach Sand	25.0
Drain Pipe Sediment End	43.8
Drain Pipe Hole Sediment	94.5
Beach Drain Pipe	102.0
Stream 30 yds up, next to #1	86.8
Trailer Leachate, next to #1	68.7
Below Reeds	52.6

The results in Table 3 show typical fluorometer signal units found along the Potomac. These smaller numbers are likely due to the numerous waste water treatment plants releasing treated effluent into the river. Optical brighteners readily decay in the natural environment due to photodegradation by sunlight. However, the beach drain pipe represents a data site of interest due to a high fluorometer signal of 102 (positive). This high reading could possibly be due to a sewage pipe leak from a nearby apartment building, or perhaps an illegal or unknown sewer line connection leading into the drain pipe.

Trial 2

Due to poor growth on mEI plates, a new technique to cultivate *Enterococcus* was employed. Using a 96-well plate and an 8-channel pipettor, 0.2mL of enterococcal broth was added to all microwells. In addition, 0.1mL of a single Fairview beach sample solution was added to 16 of these wells for analysis. This procedure was repeated on the rest of the microwell plate, and continued on to other microwell plates for all collected samples. The microwell plates were incubated for approximately 48 hours at 41°C. After the incubation period all of the wells had turned black, a positive indication that the solution hydrolyzes esculin. As the color change also confirms presence of enterococcus, the samples were streaked with sterile inoculating loops on *mEnterococcus* agar plates (these plates are selective for enterococcus colonies). After incubating the *mEnterococcus* plates for 48 hours at 41°C insignificant growth was detected, and further analysis could not continue. This procedure was repeated again without success.

Trial 3

As growing *Enterococcus* colonies had failed on two separate mediums, a new approach using previous samples was implemented. Samples at Fairview Beach were taken on 6/10/08 at the same VDH sampling locations visited on 7/2/08. At this time, extremely high counts of enterococcus on mEI growth mediums were procured. These colonies were transferred to 96-well plates with enterococcal broth and allowed to incubate for ~48 hours. After the incubation period, the bacteria from individual microwells were streaked on *mEnterococcus* plates in order to obtain isolated colonies. These plates were incubated at 41°C for 48 hours. After incubation, these plates were stored in the refrigerator at 4°C for later examination. As there was a tremendous time lag between this initial streaking and the reexamination of this data, 24 colonies of each sample were transferred from *mEnterococcus* plates and put in microwell plates with an enterococcal broth. After incubation, TSA plates were streaked using 16 of the 24 microwells. These plates were incubated for ~48 and set aside for later analysis using Biolog software.

Using the inoculated microwell plate, ARA was done to ascertain the specific sources of fecal bacteria at Fairview Beach. The plates were allowed to incubate for 48 hours and the growth from the samples was recorded. This data was then placed into the JMP-SAS program to analyze the data. The results are shown in the table below. The percentages shown below represent how many isolates of the 24 (24 isolates of each sample were replica plated on the ARA plates) match a particular source category (Table 4).

Table 4: Statistical Results from JMP-SAS.

Fairview Beach Source Tracking Results (Percent)				
Sample Locations	Birds	Humans	Pets	Wildlife
VDH 1 Water	75.0	12.50	0.0	12.50
VDH 1 Sediment	58.3	0.0	12.50	29.1
VDH 2 Water	62.5	4.1	29.1	4.1
VDH 2 Sediment	75.0	16.6	4.1	4.1
VDH 3 Water	66.7	25.0	4.1	4.1
VDH 3 Sediment	25.0	16.6	37.5	20.8
FB Sediment SW	75.0	8.3	16.6	0.0
FB SW	58.3	29.1	12.5	0.0
FB SW Discharge	66.6	25.0	4.1	4.1

Table 5: Average Percentage of Each Source at Fairview Beach.

Fairview Beach Source Tracking Results (Total Counts and Average Percent)					
	Birds	Humans	Pets	Wildlife	Total
VDH 1 Water	18	3	0	3	24
VDH 1 Sediment	14	0	3	7	24
VDH 2 Water	15	1	7	1	24
VDH 2 Sediment	18	4	1	1	24
VDH 3 Water	16	6	1	1	24
VDH 3 Sediment	6	4	9	5	24
FB Sediment SW	18	2	4	0	24
FB SW	14	7	3	0	24
FB SW Discharge	16	6	1	1	24
Total	135	33	29	19	216
Average Percentage of Each Source at Fairview	62.50%	15.28%	13.43%	8.80%	100%

For samples taken on 6/10/08, the major sources of *Enterococcus* are from bird origin (62.5 %). The secondary sources are human, pet, and wildlife (15.28%, 13.43%, and 8.80% respectively, Table 5). In addition, the rate of correct classification (RCC) for all samples was greater than 80% for all of the samples under study, indicating robust statistical analysis with limited error. These results agree with the results from Dr. Hagedorn's lab for previous years at Fairview Beach. The high percentage from birds at a river beach is typical, especially on weekends when there are dozens of boats and people present and lots of opportunities for scavenger-type shorebirds (see image below). It is the human and pet (dog) contamination that is of the greatest concern as these sources can be controlled and should be eliminated. The health risk is always greater where human-origin pollution is detected due to the possible presence of human enteric viruses.



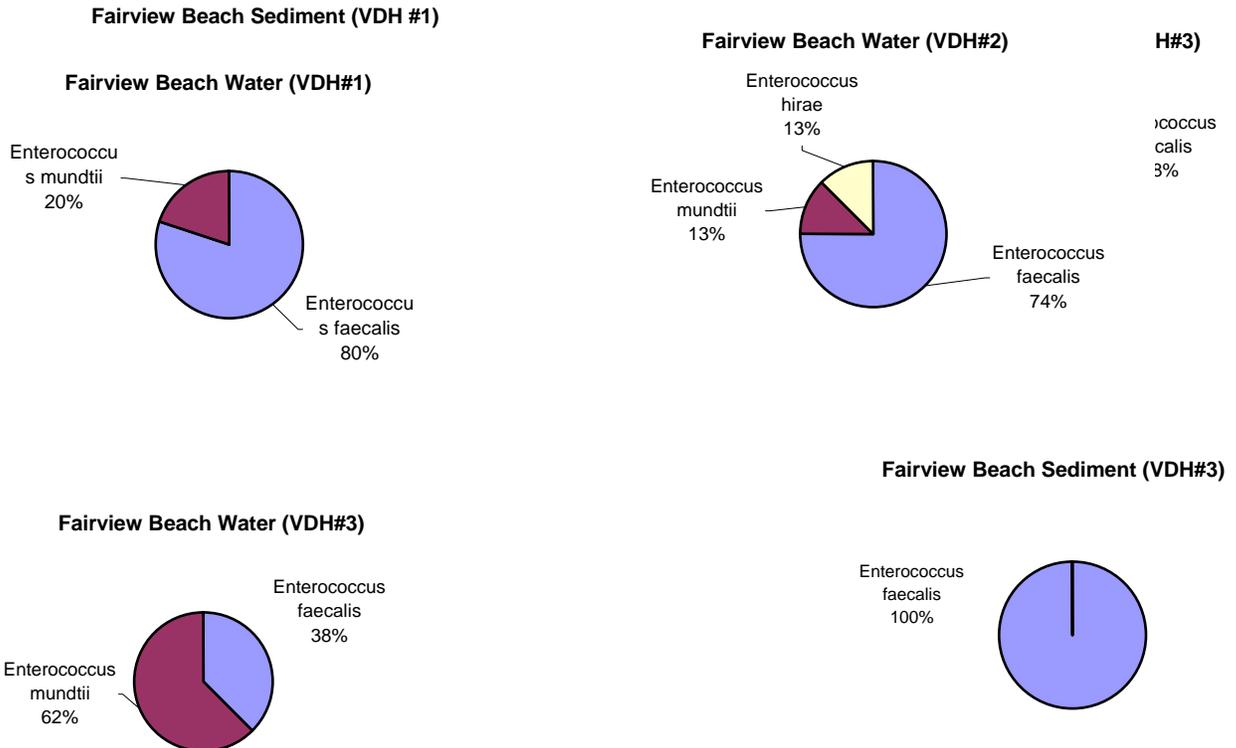
Boats at Fairview Beach on the 2007 Labor Day Weekend.

In addition to the ARA data obtained, fluorometry testing was also performed on all samples (Table 6). The machine was first calibrated with known standards (Tinopal CBS-X) of optical brighteners before testing selected samples.

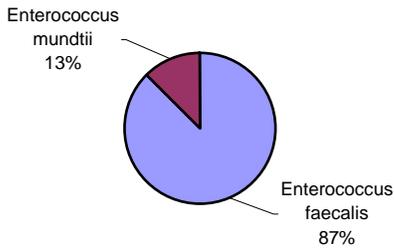
Table 6: Fluorometric Analysis.

Fairview Beach Fluorometry Analysis (6-10-08)	
Sample	Fluorometer Signal Unit (FSU)
Calibration 0 ppb	-0.5
Calibration 20 ppb	333.0
Calibration 30 ppb	548.0
VDH 1 Water	49.2
VDH 1 Sediment	52.2
VDH 2 Water	48.2
VDH 2 Sediment	55.8
VDH 3 Water	51.7
VDH 3 Sediment	49.3
FB Sediment SW	101.0
FB SW	101.0
FB SW Discharge	70.7

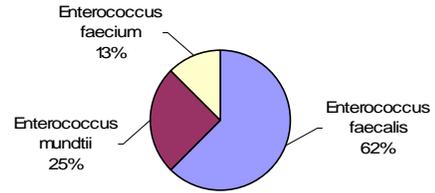
A Biolog assay was performed as a quality control parameter for this study. Analysis was performed on MicroPlates using MicroLog 3 4.20. Data obtained from the program is graphically represented in the pie charts below in percentage form. These results are formulated from the number of samples used in analysis. Several of the samples produced results that were outside of the *Enterococcus* genus and were not included in this data set. *Enterococcus* species *faecalis*, and *mundtii* are typical of shorebirds, humans, and dogs. Other species such as *hirae* and *columbae* are typical of wildlife such as deer and raccoons, while species such as *suis*, *bovis*, and *equis* are typical of livestock. The species *faecium* is spread over all source groups and is not useful for source tracking purposes.



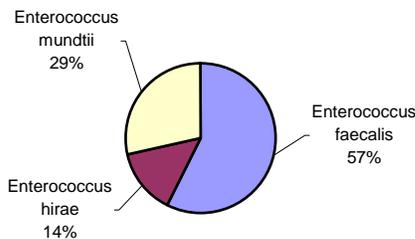
Fairview Beach Sediment (Beach Drain Pipe)



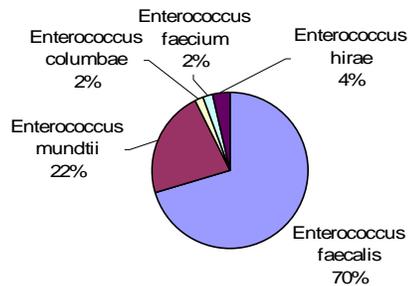
Fairview Beach Storm Water Discharge (Beach Drain Pipe)



Fairview Beach Storm Water (Drain Pipe)



Average Speciation of Fairview Beach Bacteria



Conclusions

Due to the high occurrence of optical brighteners in the beach pipeline, it is probable that there is a leak or a pipe from the apartment complex that is connected to the pipeline. In addition, there is a constant flow of water from this pipeline going directly into the Potomac. However, since that time there has been some remedial work done on this beach due to bacterial counts and beach advisories. This could account for the low readings on 7/2/08 (see Table 2). The last beach advisory occurred on 7/14/2008 (See Table 1). There is reasonable evidence that this advisory was weather related as Stafford, VA received heavy amounts of rain that stirred up sand and sediments. On the next sampling date, 7/16/2008, average *Enterococci* counts from the sampling sites were significantly under the advisory limit of 104 colony forming units/100mL water.

After completing ARA on the Fairview Beach samples from 6/10/08, it is evident that human sources of fecal bacteria are secondary to birds. This statistic was unexpected given the high readings of optical brighteners recorded on the beach pipeline sample. However, the results obtained can be explained by the magnitude of birds observed during busy periods on holidays and weekends. Nonetheless, given past data results indicating high human-caused *Enterococcus*, the beach will be continually monitored for fecal coliforms in the near future.

In completing a Biolog assay, it became evident that *Enterococcus faecalis* and *Enterococcus mundtii* (representing 70% and 22% of the total sample respectively) were the most prevalent species at Fairview Beach. These *Enterococcus* species are representative of shorebirds, humans, and dogs (Hagedorn et. al, 2007); the pie charts above show speciation profiles that are very characteristic of beach environments.

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Water Quality Assessment of a Mixed Land Use Watershed

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ABSTRACT

The goal of this study is to understand water quality issues in a mixed land use watershed. Specific objectives of this study are:

1. Water quality assessment through field measurement, water sampling and lab analysis
2. Evaluating land use impacts on water quality through stream corridor survey
3. Potential uses of water quality data for advancing real-time monitoring and data transfer

Study site for this research is the Stoubles Creek watershed that encompasses Virginia Tech campus in Blacksburg, Virginia. A YSI Sonde instrument was used in the field to measure pH, conductivity, dissolved oxygen, dissolved oxygen percent saturation, and water temperature at six sites on the watershed on different days. In addition, water samples were taken for laboratory analysis at the same sites and analyzed in the laboratory for nutrients, heavy metals and bacteria. A stream corridor survey was conducted to evaluate potential land use impacts on stream water quality. This paper documents observed water quality problems in the watershed and potential causes. It also provides a brief discussion of water quality data for advancing real-time monitoring which are detailed in the paper by Kenny et al. in these proceedings of research.

Keywords: Mixed Land Use Watershed, Water Quality, Real Time Measurement

Introduction

Stoubles Creek, a tributary of New River, is located in Montgomery County Virginia. It is a unique stream in that its two major headwaters are natural springs located in an urban area. The two headwater streams are the Webb and the Central Branches that come together to form the main branch of Stoubles Creek at the Virginia Tech Duck Pond. Once the stream passes the urban area it flows through an agricultural farm and a forested area before merging with the New River. A typical stream usually flows in the opposite order, beginning with the headwaters in a forested area. The Stoubles Creek watershed land use is 46.6% urban, 25.7% agricultural, and 27.7% forested. Future growth trends are leading towards more of the land being converted into urban. The urban area is the small college town of Blacksburg, Virginia. The agricultural area is mostly surrounded by the university (Virginia Tech) farm. Since early 1900s, Stoubles Creek is gradually forced to flow underground as the town of Blacksburg grew above it. More of the creek was forced underground when the Drillfield and the Duck Pond were constructed on the Virginia Tech campus in 1930s. By closing the stream, its natural functions such as supporting aquatic life, storing rainfall, filtering pollutants, and moving sediment are decreased (Buchholz et al. 2007).

Literature Review

Many of the water problems in urban areas are due, mostly, to pollutants that are washed off land by storms. The stormwater runoff from urbanized lands can change the health of water bodies, impact aquatic habitats, and cause algae to grow uncontrollably (Bakri et al. 2008). Urbanization dramatically alters the hydrologic response of streams and rivers to rainfall, resulting in higher runoff volume and peak discharge (Pellein et al. 2007). Cities install storm sewer systems that quickly channel this runoff from roads and other impervious surfaces. Runoff gathers speed once it enters the storm sewer system. When it leaves the system and empties into a stream, large volumes of quickly flowing runoff erode streambanks, damage streamside vegetation, and widen stream channels. In turn, this will result in lower water depths during non-storm periods, higher than normal water levels during wet weather periods, increased sediment loads, and higher water temperatures. Native fish and other aquatic life cannot survive in urban streams severely impacted by urban runoff. Urbanization also increases the variety and amount of pollutants transported to receiving waters. Sediment from development and new construction; oil, grease, and toxic chemicals from automobiles; nutrients and pesticides from turf management and gardening; viruses and bacteria from failing septic systems; road salts; and heavy metals (such as: Be, Mg, Al, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Sb, Ba, Pb and V) are examples of pollutants generated in urban areas. Sediments constitute the largest volume of pollutant loads to receiving waters in urban areas. When runoff enters storm drains, it carries many of these pollutants with it. In older cities, this polluted runoff is often released directly into the water without any treatment. Increased pollutant loads can harm fish and wildlife populations, kill native vegetation, foul drinking water supplies, and make recreational areas unsafe (Murakami et al. 2008).

Agricultural regions also have the ability to negatively affect water quality by adding chemicals. Runoff from agricultural fields introduces pesticides, soil, organic matter, manure, and fertilizer into small streams, increasing the volume of stream discharge and changing water quality. Runoff is not the only source as ground water discharge may also be a factor. The pervious soil can allow these chemicals to infiltrate into the drinking water or seep into the stream. Nutrient enrichment is one of the biggest problems in managing the river basins of the world. Nitrogen enrichment is a major cause. The Mississippi River Basin has seen a 600% increase in nitrogen use since the 1950's (Domagalski et al. 2007). Nitrogen is lost to surface water as ammonium-nitrogen or as nitrate through leaching. Increasing nitrate content in surface and ground water has been a national trend in recent years. Phosphorus is recognized as the primary factor for the eutrophication of surface water bodies which results in adverse impacts such as fish kills, reduced biodiversity, objectionable tastes and odors, increased drinking water treatment costs and the growth of toxic organisms. Although it has been estimated that most phosphorus is lost via overland, the movement of phosphorus through the soil profile can be significant for soils that have very low sorption capacity (i.e. soils low in clay, iron and aluminum oxides, and carbonates) (Singh et al. 2007).

Both urban and agricultural areas are impacting water quality of the Stoubles Creek. The Virginia Department of Environmental Quality (DEQ) has designated Stoubles Creek as an impaired stream for biological degradation and bacterial contamination. Possible stressors are organic matter, nutrients, and sediment. Organic matter has a number of sources that include sewer overflow, urban runoff of grass clippings and oils. Perhaps major reasons for increased sediment and nutrients level in the creek are the lack of a vegetative riparian buffer and bank erosion. Another big factor is the man made alterations such as the road culverts that artificially widen the stream. The main source of sediment runoff is likely from construction sites during intense storms. The dirt road at the Virginia Tech farm is also a big contributor. The total maximum daily load (TMDL) report developed for the Stroubles Creek watershed identified the sediment as the prominent stressor, causing the benthic impairment in the creek (DEQ 2003). The TMDL implementation plan developed for watershed restoration includes upgrading the sewer system, limiting livestock access to the stream, and installing riparian buffer along the stream banks (Yagow et al. 2006).

Research Goals and Objectives

The goal of this study is to understand water quality issues in a mixed land use watershed. Specific objectives of this study are:

4. Water quality assessment through field measurement, water sampling and lab analysis
5. Evaluating land use impacts on water quality through stream corridor survey
6. Potential uses of water quality data for advancing real-time monitoring and data transfer

Research Methods

Study Site

The study site is the Stroubles Creek watershed. Figure 1 show locations of six sites where water quality was measured. The first site was behind the YMCA parking lot (the Webb Branch). The second site was behind the Blacksburg fire station (Central Branch). The third site was on campus at the bridge across from the Physics and Chemistry building (Central Branch). The fourth site was on campus at the bridge across from the Drillfield (Webb Branch). The two branches then merge at the Duck Pond and become Stroubles Creek below the Duck Pond. The fifth site was at the bridge just below the Duck Pond and the sixth site was located just below the Virginia Tech Farm. Sampling dates are recorded in Table 1.

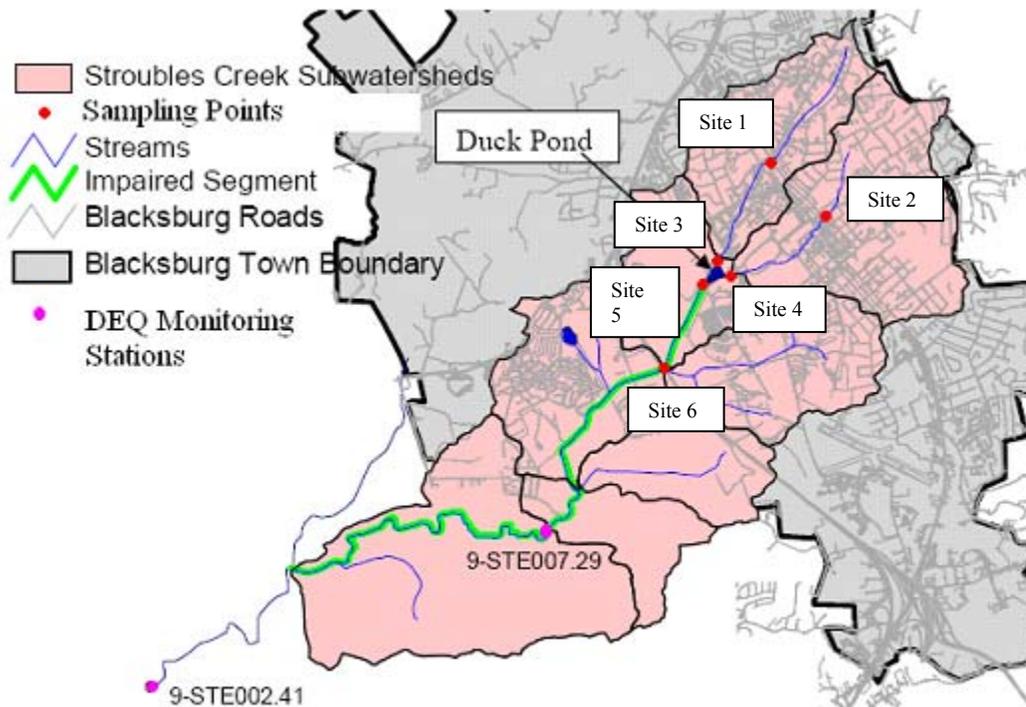


Figure 1. Stroubles Creek Watershed and Location of Sampling Sites

Table 1. Location and Sampling Dates

Site No.	Site Location	GPS Location	Sampling Dates
1	Webb Branch behind YMCA	N37 14.315; W80 25.164	6/2, 6/9, 6/19, 6/22
2	Central Branch behind Fire Station	N37 13.895; W80 24.818	6/2, 6/9, 6/19, 6/22
3	Central Branch above Duck Pond	N37 13.558; W80 25.526	6/2, 6/9, 6/19, 6/22
4	Webb Branch above Duck pond	N37 13.675; W80 25.638	6/2, 6/9, 6/19, 6/22
5	Stroubles Creek below Duck Pond	N37 13.440; W80 25.803	6/2, 6/9, 6/19, 6/22
6	Stroubles Creek at Plantation Rd.	N37 12.918; W80 26.339	6/2, 6/9, 6/19, 6/22

Field Measurements

A YSI Sonde instrument was used to measure dissolved oxygen, dissolved oxygen percent saturation pH, conductivity and water temperature.

Water Sampling and Laboratory Analysis

Samples were collected in 500 ml bottles for laboratory analysis. The samples were placed in ice and taken to the Water Resources Lab at Latham Hall. A portion of the samples were first run in the Bran and Lucbbe Auto Analyzer 3 (Figure 2) to test for nutrients such as nitrate, ammonium, and phosphorous. It was run using setting MT-15. Another portion was run in the VISTA-MPX (Figure 3) to test for heavy metals. The portion is divided up into fifteen to twenty tiny test tubes. These tubes are conveyed into the machine where each is hit with a different wavelength to test for each substance. The final portion of samples was run in the liquiTOC machine to determine total suspended solids.



Figure 2. Bran and Lucbbe Auto Analyzer 3



Figure 3. VISTA-MPX

Water samples were also analyzed for bacteria concentration. Microbial source tracking method (Williams 2008) was applied to determine the source of bacterial contamination in the Duck Pond.

Rainfall Data

Rainfall data for study period were obtained from the National Weather Station in Blacksburg and are plotted in Figure 3. There was an intense rainfall event on June 22.

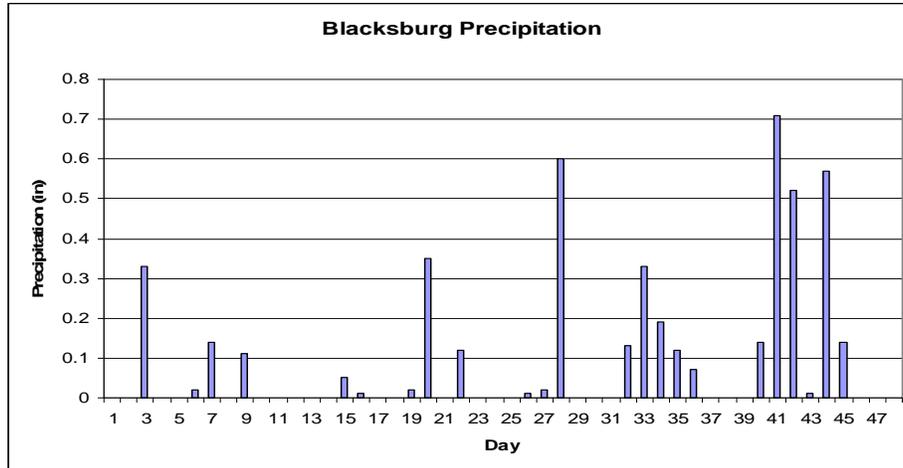


Figure 2. Precipitation for Blacksburg, VA from May 26 to July 12

Stream Corridor Assessment

The stream corridor assessment was conducted using the Stroubles Creek Corridor Assessment Protocol (de Leon et al. 2001). The researcher would then complete the assessment of many different factors that could lead to environmental problems using a rating system that looks at severity, correctability, and accessibility. Some environmental problems the researcher assesses include: erosion sites, channel alterations, trash dumping, construction near stream, inadequate buffers, and presence of animal wastes.

Results and Discussion

Table 2 show results for field measurements of water quality. One point of note in Table 2 is the big jump in temperature and drop in dissolved oxygen at site 5 below the Duck Pond. The Duck Pond is for the most part without shade so that is one probable cause. Site 5 also had two 6 inch pipes and one 72 inch pipes on the right bank discharging unknown water source into the stream within 50 feet upstream of the sampling site.

Table 2. Water Quality Measurements Taken with YSI Sonde

Site No.	Temperature		Specific Conductivity		Dissolved Oxygen		pH		DO % Saturation	
	(deg C)		(us)		(mg/L)					
	9-Jun	19-Jun	9-Jun	19-Jun	9-Jun	19-Jun	9-Jun	19-Jun	9-Jun	19-Jun
1	15.57	13.75	713	708	9.53	9.24	7.54	6.97	95.9	89.3
2	19.75	14.91	659	533	7.65	8.69	8.02	7.87	83.9	86.2
3	16.58	16.09	703	711	8.59	8.55	7.97	7.89	85.8	84.4
4	18.59	15.85	677	698	9.3	9.38	8.03	7.81	73.6	46.6
5	26.28	21.26	617	518	5.93	4.13	7.89	7.54	99.6	94.9
6	21.2	16.62	557	515	7.61	8.21	7.74	7.65	88.3	87.0

Table 3 shows laboratory results for nutrient concentrations and total suspended solids. The values for ammonium and phosphate are quite low during regular base flow. However, increase in ammonia and

phosphate concentration was observed during the intense storm of June 22. Elevated levels of nitrate and total suspended solids (TSS) were observed in most samples. Nitrate and TSS concentrations are also graphically displayed in Figure 5 and Figure 6, respectively.

Most sites have lawns either at the bank or in close proximity and there is inadequate buffer (shrubs and trees) throughout. It is speculated that nitrates values are high at site 3 because that section of the stream runs under the Drill Field and the fertilizer in runoff water is a possible reason. TSS values get off to a bad start at site 1. Within 100 feet on the left stream bank is construction of multi-family housing. The developer tried to stop runoff by putting a small fence at the edge of the property but the fence is not really adequate to handle storm runoff and a lot of sediment is getting through. A great portion of nutrient runoff could be decreased by increasing or creating a buffer along both sides of the stream bank. However, at some sites such as the fire station, where the stream runs in a rock channel with a link fence on the sides, the cost would be too uneconomical.

Table 3. Laboratory Results for Nutrients and Total Suspended Solids

	Flow Condition	Nitrate	Ammonium	Phosphate	Total Suspended Solids
Site #		(mg/L)	(mg/L)	(mg/L)	(mg/L)
1	Base Flow	1.931	<.008731	<.004034	3.962
	Intense Rainstorm	1.181	0.091	0.023	10.33
2	Base Flow	1.534	<.008731	0.005	4.929
	Intense Rainstorm	0.655	0.101	0.051	10.251
3	Base Flow	2.638	<.008731	0.005	4.092
	Intense Rainstorm	1.042	0.124	0.088	10.257
4	Base Flow	1.62	<.008731	0.006	4.106
	Intense Rainstorm	0.905	0.095	0.062	10.891
5	Base Flow	0.881	0.047	0.013	6.864
	Intense Rainstorm	0.727	0.012	0.021	8.233
6	Base Flow	1.518	<.008731	0.006	4.957
	Intense Rainstorm	1.212	<.008731	0.02	7.966

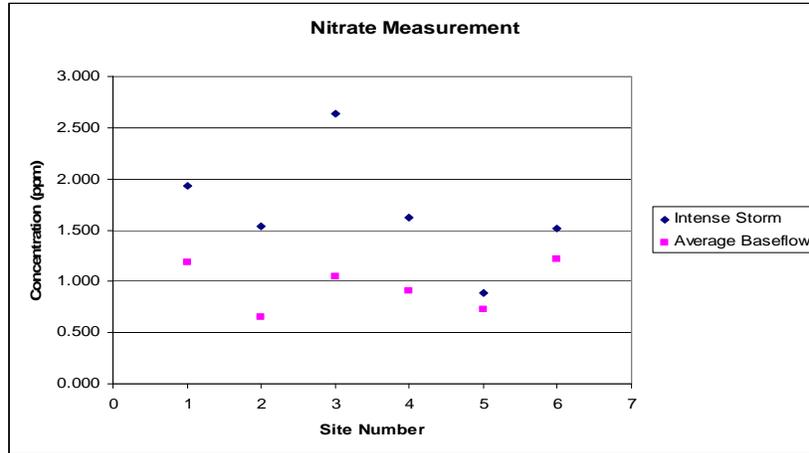


Figure 5. Nitrate Measured at Each Site

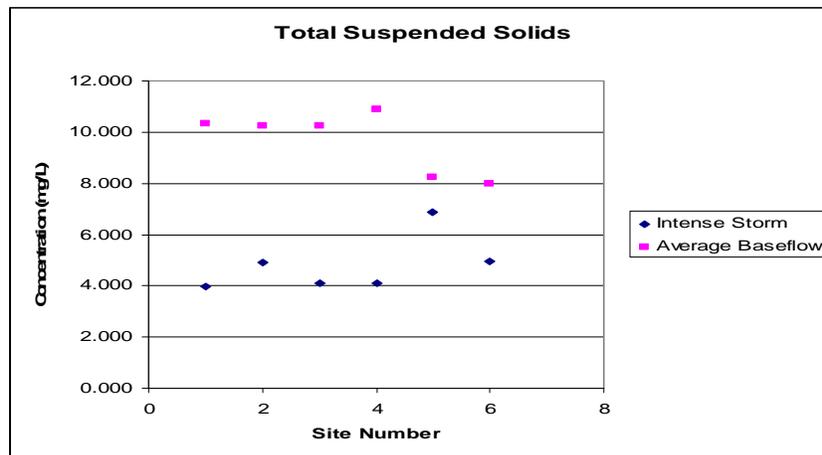


Figure 6. Total Suspended Solids Measured at Each Site

Table 4 shows laboratory results for metal concentrations. The only substance that was not below the detectable limits was Silicon. Silicon is present throughout the stream. One possible source is pesticides which are used on the farms and lawns of the watershed. Some pesticides contain Silicone which contains a few silicon atoms. There are also could be unknown sources of silicon for numerous unknown pipes that discharge water outlet into the stream.

Table 4. Heavy Metal Measurements at Each Site

		Cd	Cu	P	Pb	Si	Zn
Site #		mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
1	Base Flow	<0.018416	<0.126289	<0.013233	<0.02266	4.355	<0.02644
	Intense Rainstorm	<0.018416	<0.126289	<0.013233	<0.02266	1.562	<0.02644
2	Base Flow	<0.018416	<0.126289	<0.013233	<0.02266	3.124	<0.02644
	Intense Rainstorm	<0.018416	<0.126289	<0.013233	<0.02266	0.607	<0.02644
3	Base Flow	<0.018416	<0.126289	<0.013233	<0.02266	4.636	<0.02644
	Intense Rainstorm	<0.018416	<0.126289	<0.013233	<0.02266	0.643	<0.02644
4	Base Flow	<0.018416	<0.126289	<0.013233	<0.02266	3.812	<0.02644
	Intense Rainstorm	<0.018416	<0.126289	<0.013233	<0.02266	1.066	<0.02644
5	Base Flow	<0.018416	<0.126289	<0.013233	<0.02266	2.445	<0.02644
	Intense Rainstorm	<0.018416	<0.126289	<0.013233	<0.02266	2.937	<0.02644
6	Base Flow	<0.018416	<0.126289	<0.013233	<0.02266	3.504	<0.02644
	Intense Rainstorm	<0.018416	<0.126289	<0.013233	<0.02266	3.104	<0.02644

Bacteria Analysis

Samples were taken from three sites located around the Duck Pond and the Duck Pond itself for bacterial analysis. All samples tested positive for both E. Coli and Enterococcus. The results of the E. Coli test are shown in Table 5. The measured values are several times higher than the Virginia standard of 235 cfu/100mL for surface waters. The testing sites are located in the urban region of the watershed. The most likely cause is sewer overflow, pets and wildlife.

Table 5. E. coli Bacteria Concentrations

Site #	Concentration (cfu/100mL)
3	3650
4	1290
Duck Pond	2600
5	3750

A bacterial source tracking technique was applied to determine potential sources of bacterial contamination. Methodology for this technique is described in the paper by Williams (2008). Results are shown in Figures 7 through 10.

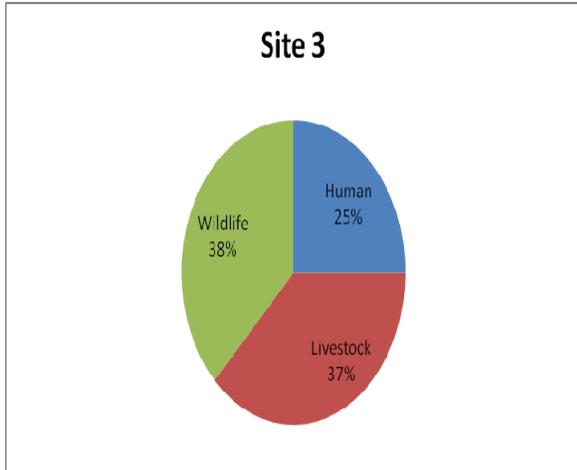


Figure 7. E. coli source at site 3

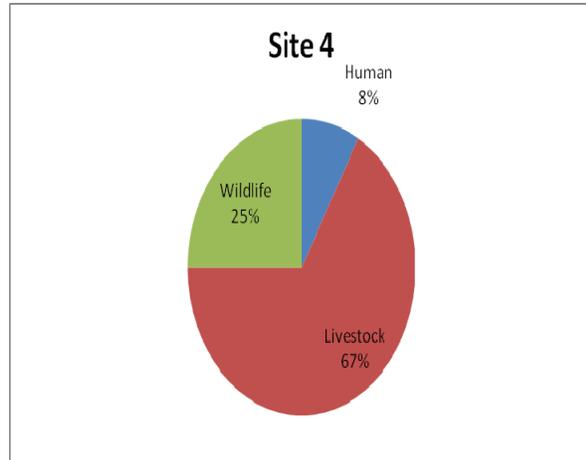


Figure 8. E. coli source at site 4

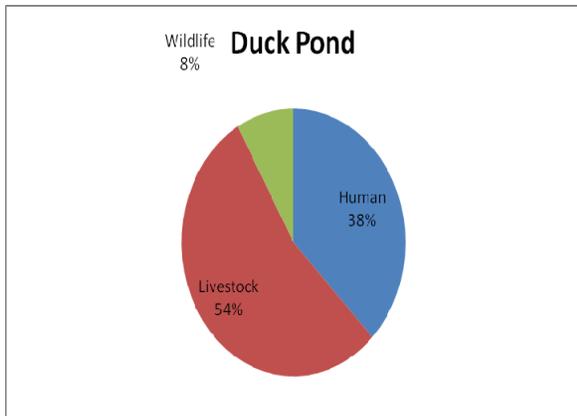


Figure 9. E. coli source at Duck Pond

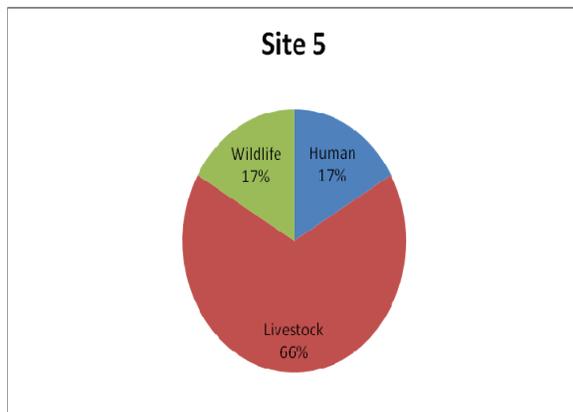


Figure 10. E. coli source at site 5

Conclusion

Stoubles Creek currently has a benthic impairment due to sediment and nutrients. Another problem for the stream is the very high numbers of E. coli bacteria found at locations in the Duck Pond area. Total suspended sediment was the streams biggest problem and should be addressed by taking better precautions at construction sites. Also the dirt road at the Virginia Tech farm should be addressed. Nitrate was also a key pollutant running off from lawns and farms. During intense storms the excess water is able to dilute the concentration, but as base flow returns to normal the problem worsens.

Stream corridor survey shed some light on our findings. Some common problems throughout the stream are a lack of adequate buffer and pipes that outflow into the stream. Site 1 had multi-family home construction on the left bank without an effective fence to keep the sediment from running off during an intense storm. Site 3 had erosion problems as huge chunks of Hokie Stone were carried downstream during major storms. Site 6 had the stream altered by a road culvert making it flow through a very wide area with a very low depth during base flow. It also receives sediment from the dirt road at the Virginia Tech Farm. At several of the sites there were also pipes which crossed the stream.

It is important to be able to get continuous measurements in monitoring water quality to be able to understand what is going on at Stoubles Creek. There is a great deal of uncertainty in taking a limited number of samples at certain times. There is a need for implementing sensor technologies and real-time

measurements of flow and water quality. The sensors would send the data electronically through the Compact DAQ. From there the data could either be sent wirelessly to a remote site or stored inside a terminal computer for periodic download. The real-time monitoring in conjunction with using LabView software is the second goal of this study described in the paper by Kenny et al. (2008). Labview is the software that is preferred because it is scalable, programmable, and gives a great deal of control over the process. For example, LabView has the ability to tell the sensors to increase their sampling rate if rainfall increases, go into sleep mode, or turn on and off. It can also take the data coming in and display it in a meaningful way such as in graphical form almost instantly. The LabView application block can be accessed over the web remotely and manipulated by an end user.

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Integration of LabVIEW into Stroubles Creek Watershed Assessment

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ABSTRACT

The use of LabVIEW as data acquisition (DAQ) software in a real-time remote monitoring (RTRM) system of the Stroubles Creek watershed, in Montgomery County, VA, is explored. By examining communication of existing software, HyperTerminal and Hydras 3 LT, a LabVIEW virtual instrument (VI) is developed to read, store, process, and display data from a HydroLab MiniSonde 4a (sonde), in real-time. In addition, a client PC is able to remotely control the developed VI through wireless internet. A RTRM system has the potential to vastly increase one's understanding of their watershed, expedite intervention, initiate automatic controls, and allow for better management.

Keywords: real-time remote monitoring, data acquisition, LabVIEW, watershed management

Introduction

Data collection is an essential part of managing one's water resources, and as water quality is being increasingly stressed by population growth, urbanization, and industrial pressures (Caraco, 1995; National Research Council, 2000; World Resources Institute, 2003; Boothe, 2005) it is critical to have an effective monitoring system that can provide an adequate supply of helpful data in a timely manner. In general, data is important so that one may determine the condition of their water resources, direct proper restoration efforts, and to determine the efficacy of the restoration efforts. Specifically, data in a timely manner allows one to react to developing environmental problems in order to limit their damage. Conventional methods of data gathering consist of infrequent field visits. The problem is that these excursions are labor- and time-intensive, they are done too infrequently to build up an important data set of water quality fluctuations and trends and they don't provide the temporal and spatial resolution needed to understand, alert, and address the factors that lead up to acute environmental problems.

Research Objective

The research objective was to develop, and integrate LabVIEW into, a real-time remote monitoring (RTRM) system of the Stroubles Creek watershed, in Montgomery County, VA. The sub-objectives of the paper were to communicate between LabVIEW and the sensor, HydroLab MiniSonde 4a; process the data with LabVIEW; and to remotely broadcast the data.

Development of Real-time Remote Watershed Monitoring System

This paper discusses development of a prototype real-time remote watershed monitoring system. Continuous real-time remote monitoring consists of a sensor and data acquisition (DAQ) software on site that collects, analyzes, and transmits data frequently. This data can be accessed remotely, in real-time. Continuous monitoring can be used to constantly monitor certain water quality indicators as well as follow trends by providing data with high temporal resolution. Real-time remote access allows managers, or the appropriate authorities, to get the needed information so they can respond immediately. RTRM speeds up the flow of data in the monitoring system (see Figure 1). In addition, a control feedback loop is easily incorporated into the set-up that allows automated responses such as a telephone call when standards are breached or increased sampling rates when it rains.

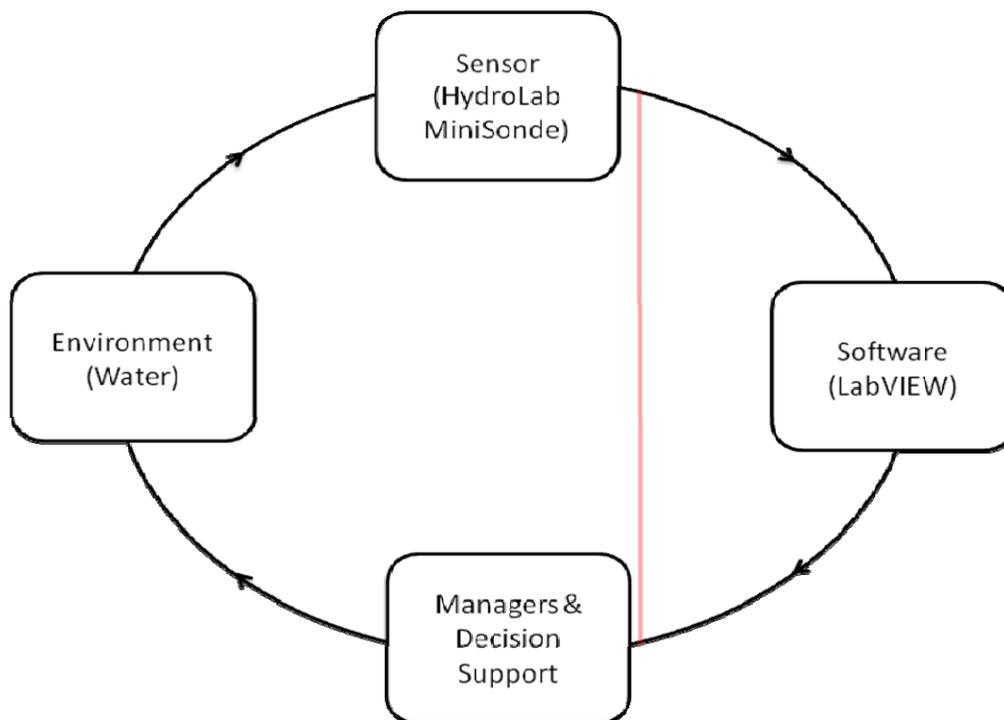


Figure 1. Flow of Data through a Monitoring System. The Highlighted Section is the Focus of the Paper

The intention is to develop a RTRM system for Stroubles Creek, Montgomery County, VA. The portion of the project presented in this paper is to design, test, and develop certain components of the RTRM system in the laboratory, while keeping in mind the end-use of field deployment. Latter parts of the project will finish what's presented in this paper and deploy the system to the field, which includes powering it, providing access to wireless communication, and using field sturdy equipment. Given a sensor, HydroLab MiniSonde 4a, that performs Data Acquisition (DAQ), the focus of this paper is in developing the DAQ software and on how the resulting information can be communicated to remote users or managers (Figure 1).

Data Acquisition Software can be the most critical factor in obtaining reliable, high performance operation and it transforms one's PC and DAQ hardware into a complete DAQ, analysis, and

display system (Gateway Engineering Education Coalition, 2003). It is desirable for DAQ software in a RTRM system to be headless (does not require a user), programmable and scalable. Headless operation allows the system to fully support the benefits of a RTRM system, in that no user is needed; the data can be gathered processed and relayed behind the scenes. Programmability allows the system to be adapted to include interesting features such as controlling the sensor; see Figure 2 for ways the process loop can be completed to fashion a control. Two useful examples of controls are: a sensor is set to read the water quality every 15 minutes, but its software is alerted by weather data when it is raining, the software changes the sensor's sampling frequency from 15 minutes to 1 minute to capture the interesting water quality data that comes with rain storm events; similarly, during a rainstorm an actuator may be triggered to grab bottles of water to be tested in the laboratory for further analysis. It is important to note that the DAQ software must be able to both receive data and send data to the sensor for full potential of a RTRM system. LabVIEW is being used in this project because it satisfies the requirements of DAQ software in a RTRM system. LabVIEW has the additional benefit of a program used in the education system to create virtual labs (Auer, 2003; Malki, 2002). A schematic showing benchmarks of the project is shown in Figure 3.

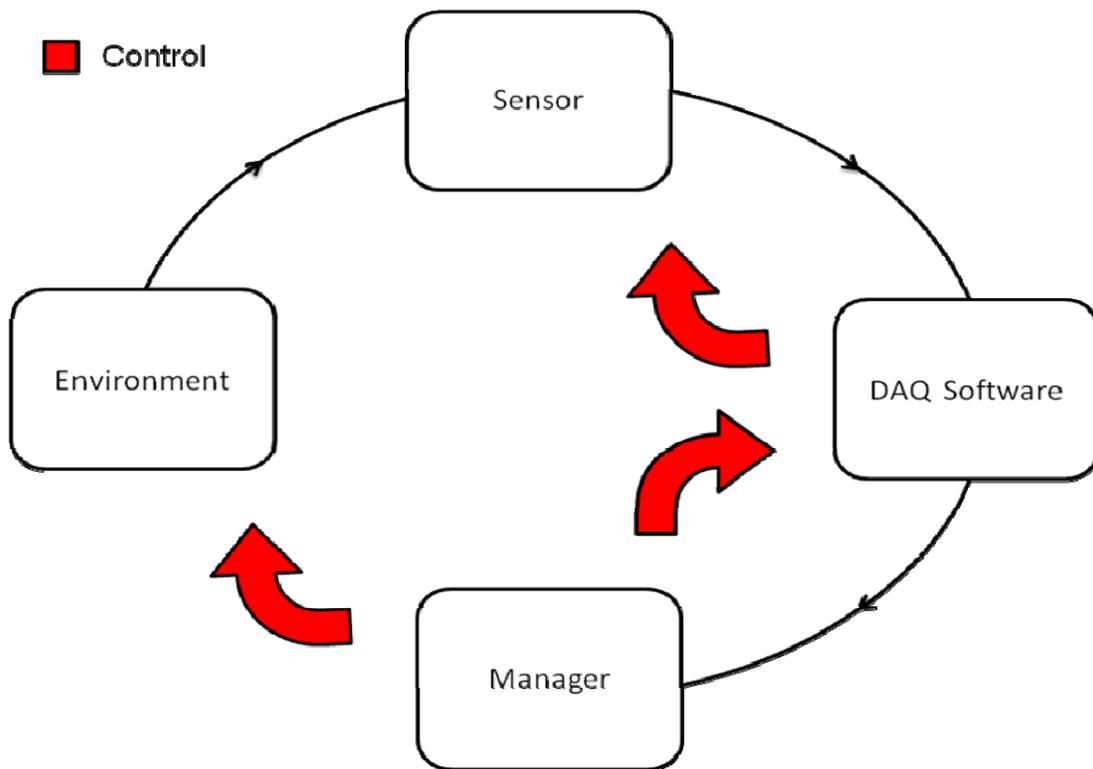


Figure 2. Process Flow with Possible Controls Shown in Red

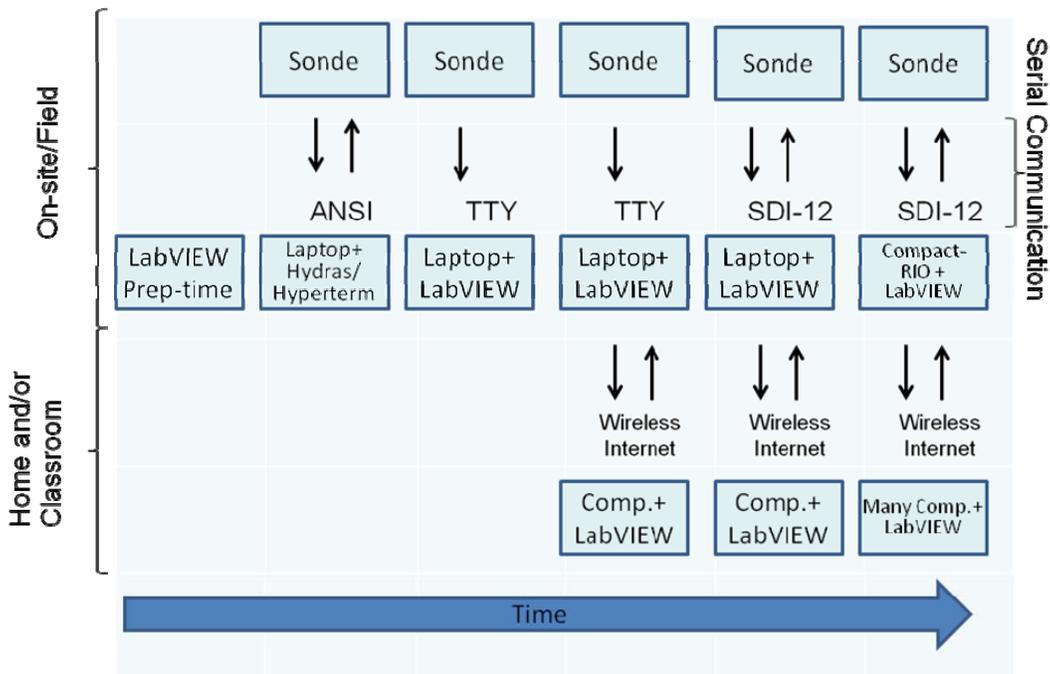


Figure 3. Schematic of Schedule for Development of Real-time Remote Watershed Monitoring System

LabVIEW

LabVIEW is a graphical programming language, developed by National Instruments, that can perform the role of DAQ software, see Figure 1, by acquiring, analyzing, and displaying the data. LabVIEW programs, called virtual instruments (VIs), consist of a front panel which contains the User Graphical Interface (GUI) and a block diagram which contains the ‘code’ (the real script is behind the scenes) for the VI, see Figure 4. As a graphical program, the ‘code’ consists of wires and icons, where the wires represent data flow and the icons are smaller VIs, called subVIs, from the LabVIEW library, or user generated, that perform functions. Dataflow programming allows the programmer to focus on manipulation and processing of the data, this, LabVIEW’s large data base of customizable functions, and its graphical programming makes LabVIEW an easy programming ‘language’ to learn and makes it productive for scientists and engineers.

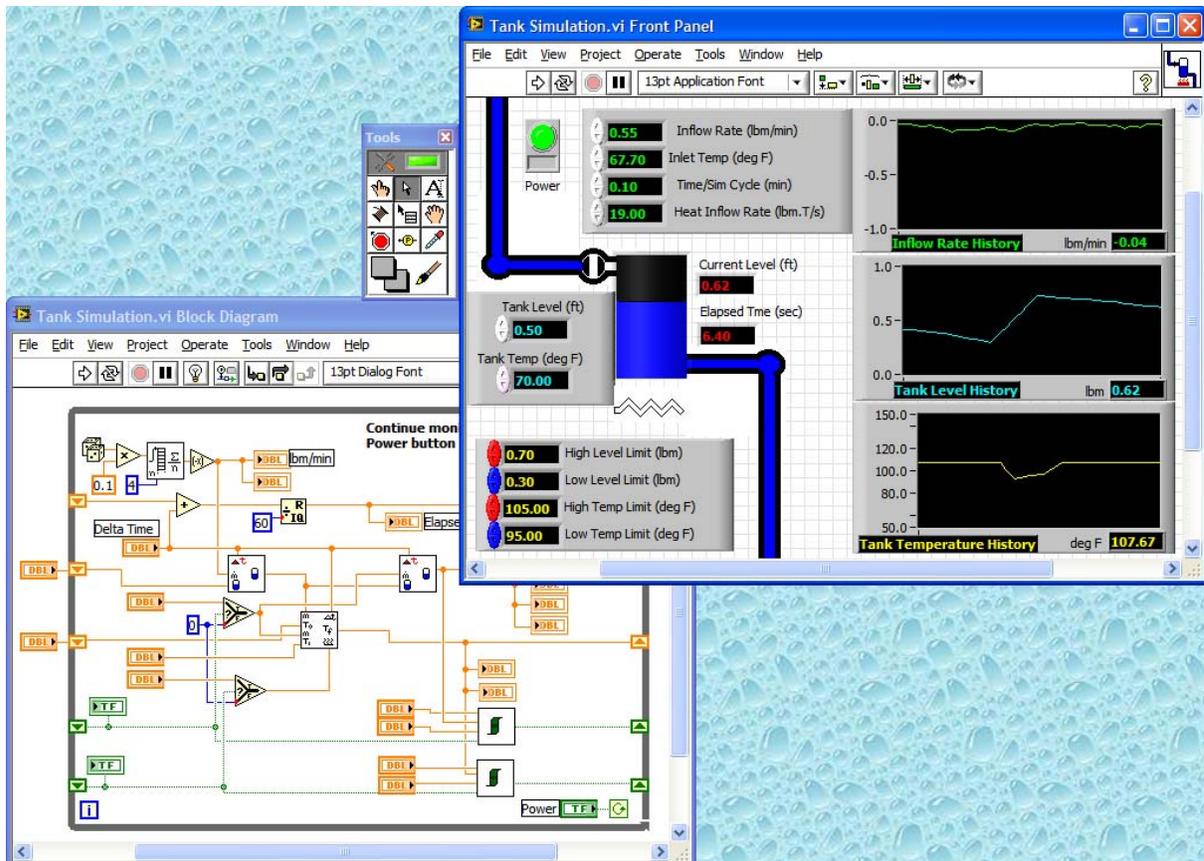


Figure 4. Example LabVIEW Virtual Instrument: Front Panel and Block Diagram

LabVIEW can be programmed, meaning it can run without a user (headless), which makes it a valuable part of a RTRM system. The other software that is used in this study to communicate with the sonde (Hydras 3 LT and HyperTerminal) both requires a user at the controls. LabVIEW's programmability also means that it can be a part of and enact control functions in the process loop (Figure 2).

Description of Real-time Remote Watershed Monitoring Site

The Stroubles Creek watershed, located in Montgomery County, VA, is a unique watershed in that its mixed land use is in the opposite direction of most streams, that is, the head waters are in the urban town of Blacksburg, then the stream runs through agricultural fields, and then forests. The stream was 303(d) listed as impaired in 1996, 1998, and 2002 (DEQ, 2003). The stream is benthically impaired for 4.98 miles starting at the outfall of the "Duck Pond", see Figure 5. Stroubles Creek's stressors are considered to be sediment, organic matter, nutrients and bacteria. The Duck Pond is a potential location for a RTRM system because it is at the beginning of the impaired section and it is a high profile location. See Gronwald, 2008, et al, for more detail on a watershed assessment of Stroubles Creek.

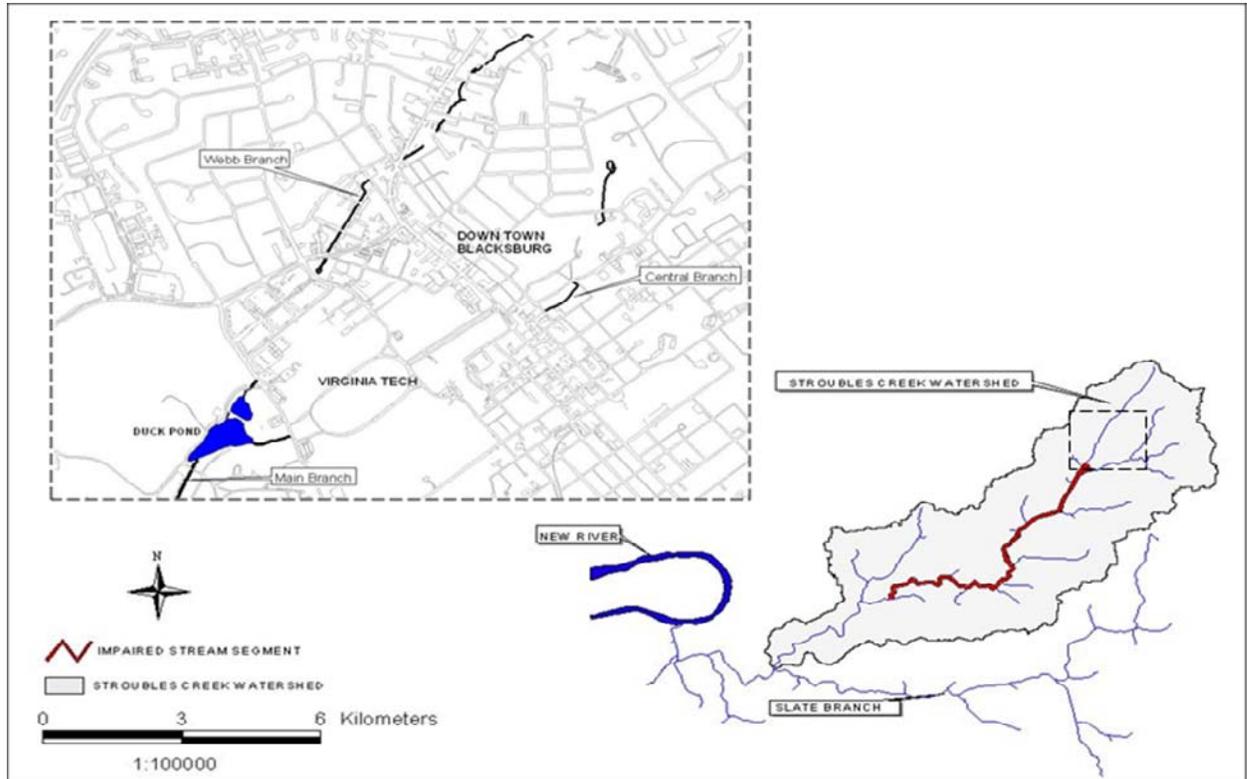


Figure 5. Stroubles Creek Watershed (impaired stream segment is highlighted in red)

Methods

The laboratory set-up consisted of a HydroLab MiniSonde 4a (sonde), connected via an USB-to-Serial (RS-232) converter to a Fujitsu T4215 Tablet with LabVIEW 8.5 full version installed. The sonde was equipped with temperature, pH, DO, and conductivity sensor, and a circulator. The sonde's storage cup was kept $\frac{3}{4}$ full and left on the sonde during experimentation and storage. Picture of the laboratory set-up is shown in Figure 6.

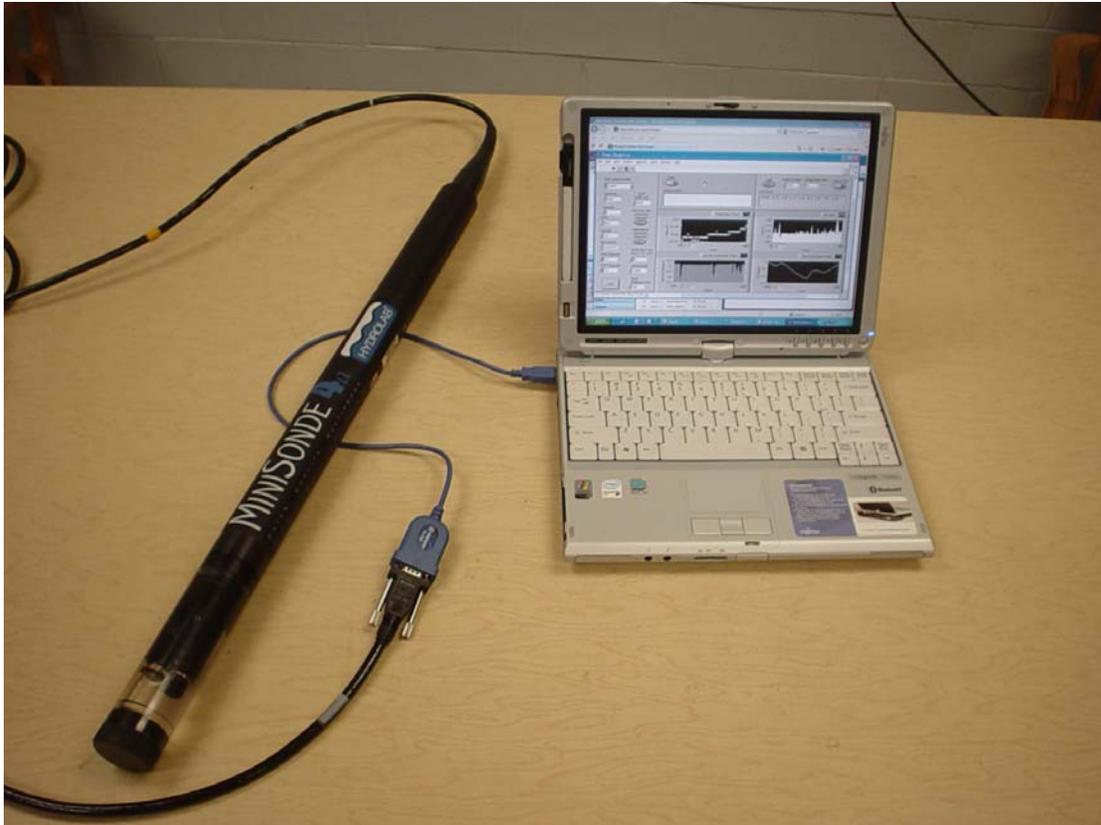


Figure 6. Laboratory Set-up

Software used to communicate with the sonde was LabVIEW, Hydras 3 LT, and HyperTerminal, where Hydras 3 LT was developed to communicate with HydroLab sondes. Communication was done at 9600 bps, 8 bits, no parity, 1 stop bit and XON/XOFF flow control. The sonde is capable of ANSI, TTY, Modbus, and SDI-12 mode of operation. A host of settings, functions, programs, and methods were explored, using all the software, in order to determine how LabVIEW could send and receive data to and from the sonde and to determine what format it would be received in and what format it should be sent in; the underlying code that HyperTerminal and Hydras 3 LT use to communicate with the sonde was sought after. A VI was then developed to acquire, analyze and display data read in from the sonde. Next, different options for remotely controlling the VI were explored, viz. LabVIEW's web publishing tool and creating a virtual port. The Duck Pond was patrolled with the Tablet PC for points of wireless internet access.

Results

The sonde was in ANSI mode by default. HyperTerminal and Hydras were both used to communicate with the sonde in ANSI mode. Each showed an extensive menu system with commands to the sonde and changes in software settings. HyperTerminal's menu was command based, while Hydras 3 LT's was graphical based. See Figure 7 for HyperTerminal's ANSI mode of operation. HyperTerminal and Hydras 3 LT can change the sonde's mode to TTY and enable SDI-12. ANSI and TTY mode display the data from the sensors line by line about every second. TTY mode has a limited set of commands, viz. go to menu; exit TTY mode ' q' (note the space is needed prior to the letter to enter in commands), display heading ' h'; and display measurements ' m'. See Figure 8 for HyperTerminal's TTY mode of operation.

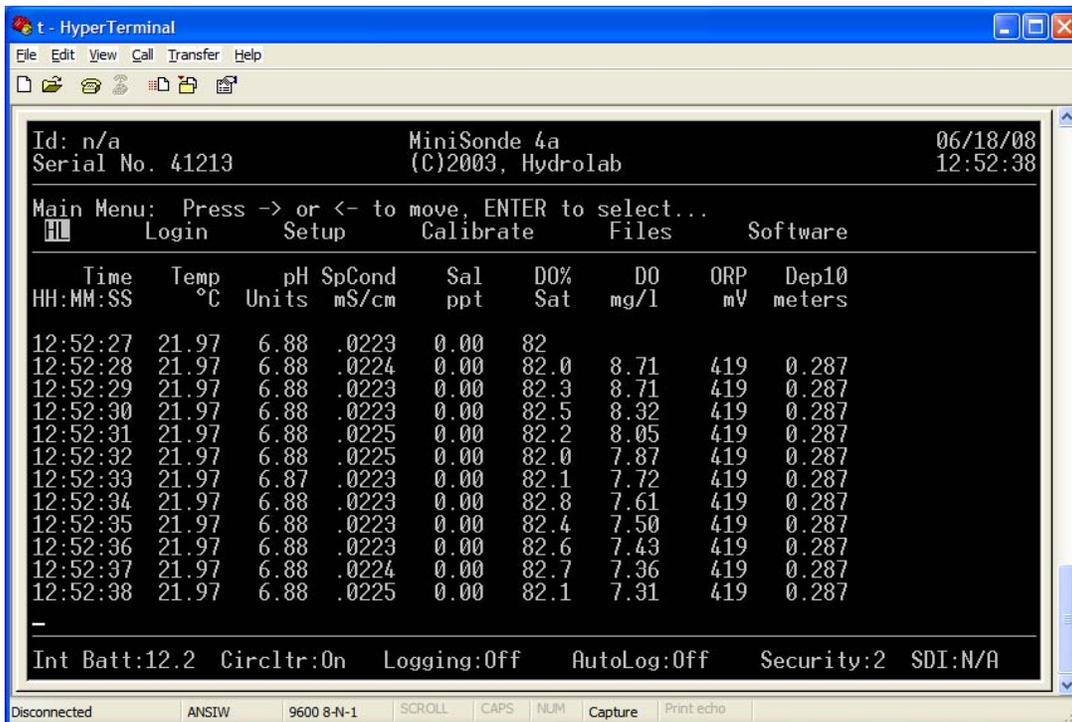


Figure 7. HyperTerminal communicating with HydroLab MiniSonde 4a in ANSI Mode of Operation, and reading in an Example Set of Data

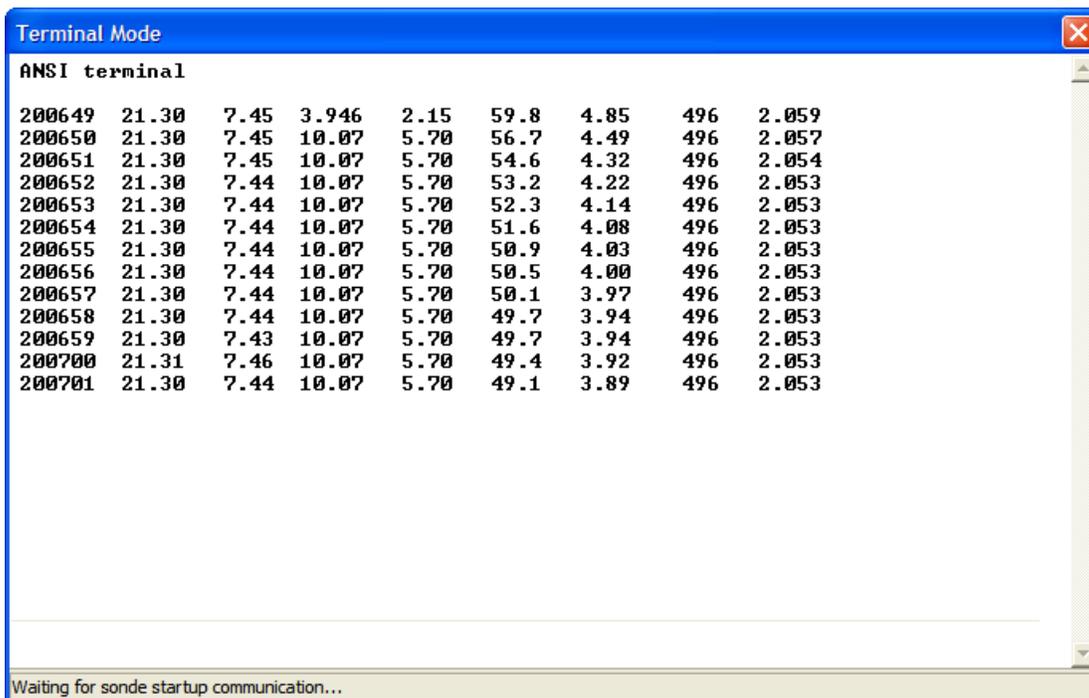


Figure 8. HyperTerminal communicating with HydroLab MiniSonde 4a in TTY Mode of Operation, and reading in an Example Set of Data

TTY mode was used to communicate with LabVIEW. A modified version of the ‘Advanced Serial Write and Read’.vi was used to read in lines of data (strings), use the limited TTY command set (write strings), and process the data. Some modifications were the added ability to append the data to an existing file/create a new file, break the incoming string into substrings and convert to numbers, and display selected parameters on graphs. The VI’s front panel and the processing part of the block diagram can be seen in Figure 9 and Figure 10, respectively. The wider command set, beyond the limited TTY command set, could not be realized in LabVIEW.

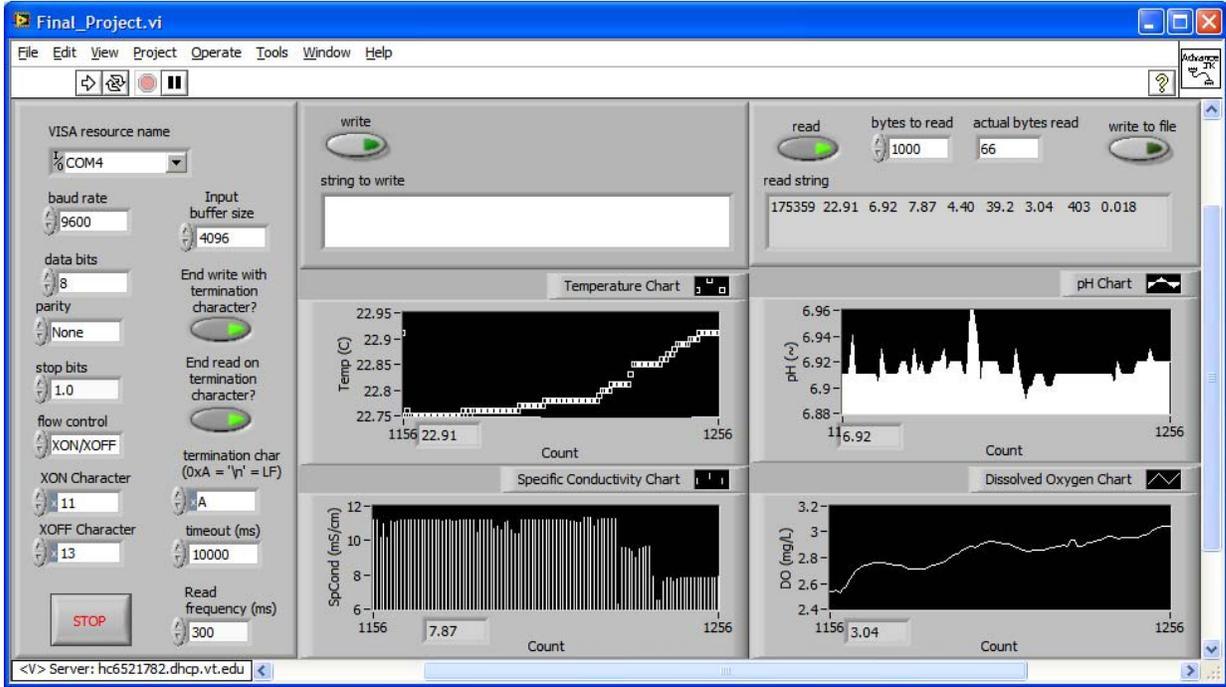


Figure 9. Developed Virtual Instrument Front Panel showing Data that was read in while Sonde was attached in the Laboratory.

The Tablet PC was used as a server and the VI was published to the web. Remote users (clients) accessed the VI in real-time, by going to the published website. Note that an internet connection is needed for both the server and client computer and that LabVIEW or the free LabVIEW runtime engine must be installed on the client computers. The client computers had the ability to manipulate the GUI and couldn’t view the block diagram. As an alternative remote system, a virtual port was created, but communication between the server and client computer could not be established.

Around the Duck Pond wireless access was found with Tablet PC where the Central branch becomes daylighted after the Drillfield. The connection was weak, but the Tablet PC is not built to detect weak signals.

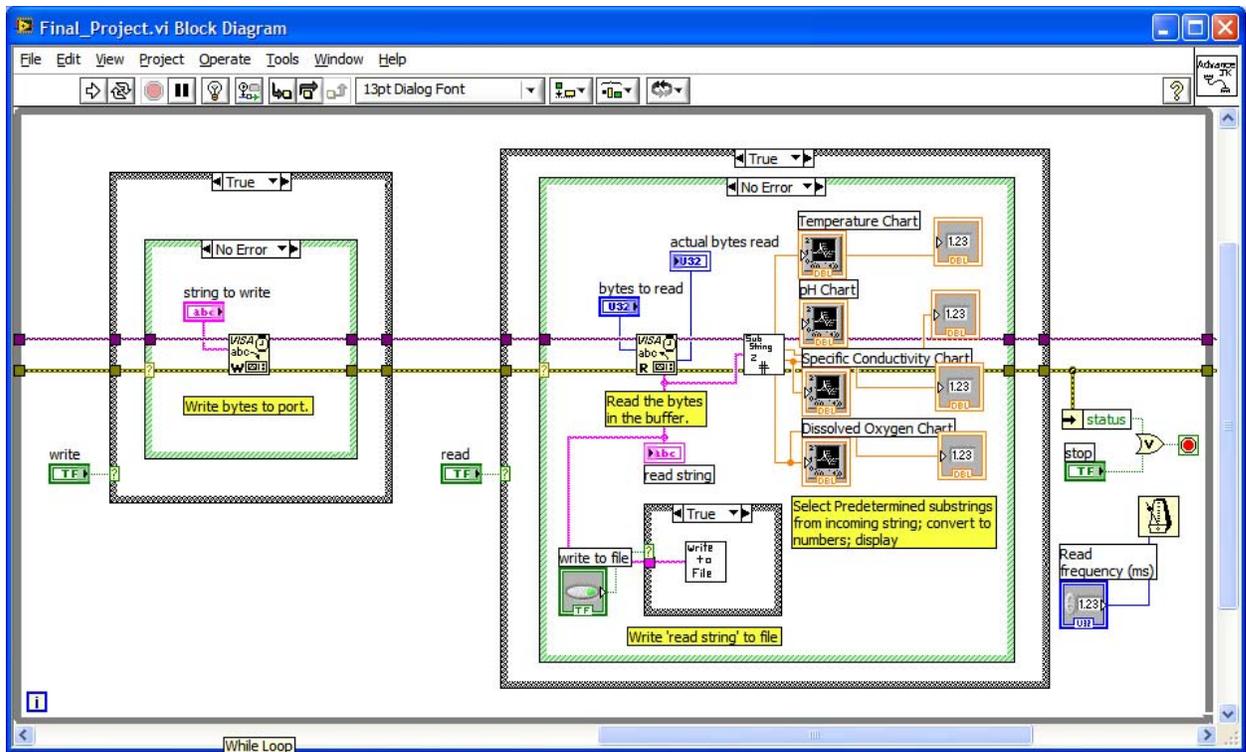


Figure 10. Part of VI Block Diagram where processing takes place

Discussion

The HydroLab MiniSonde 4a performs DAQ, which is to say that its transducers read in analog signals and the sonde filters, conditions, amplifies and digitizes the data. It is believed that the sonde does not have a micro-processor to perform DAQ and other functions, but rather an application specific integrated circuit (ASIC) with non-volatile memory. It is important to note that when the sonde is put into TTY mode, and/or the circulator is turned off/on, those positions will be the same upon powering up even when the sonde's batteries are removed.

LabVIEW could read from the sonde when the sonde was in ANSI mode, but most of the characters were unrecognizable and the output was nonsensical and random. TTY mode displays the data line by line consistently in the same format, which is needed to process it and extract values. HyperTerminal/Hydras 3 LT was needed to originally put the sonde in TTY mode.

A modified 'Advanced Serial Write and Read'.vi was used over the 'Basic Serial Write and Read'.vi because the advanced read write contains a buffer, and termination character, both of which fix timing issue that chopped the incoming data when using the modified 'Basic Serial Write and Read'.vi

The wide command set that was available through HyperTerminal and Hydras 3 LT could not be realized through LabVIEW. HyperTerminal and Hydras 3 LT use code that lies underneath their GUI to manipulate the sonde with their wide command set. Thus the code and the method exist, but is unknown to the author. The sonde is SDI-12 compatible. SDI-12 has a large set of standard commands. It is hypothesized that the underlying code uses SDI-12 commands to avoid redundant engineering. Repeated attempts to obtain this information from the hardware (Hach)

and software (OTT) manufacturer were made with little success. An alternative method to communicate with the sonde would be to use a SDI-12/RS-232 converter and command the sonde through SDI-12 communication protocol (NI Developer Zone, 2008; Postolache, 2007).

Creating a virtual port and communicating through it is commonly done. It is unknown why communication could not be established in this project with the virtual port, although it may be blocked by the internet service provider (ISP). Virtual ports were explored here as an inferior alternative to commanding the sonde through code. The virtual port would allow a user to remotely use HyperTerminal or Hydras 3 LT to change parameters, but these actions are not programmable, nor automatic, and thus do not fulfill the desired DAQ software properties.

Wireless access around the Duck Pond can be expanded in a number of ways to provide locations suitable for deployment of the RTRM system. Installing a high gain antenna amplifies any existing signal and is likely to be the most effective measure; a wireless repeater can be used to extend the wireless range into areas of low access; or the Virginia Tech Communication Network Services (CNS) can expand their network to include the Duck Pond.

Miscellaneous

During the project access was made available to unique hardware and software technologies, here is commentary on some used. The unique feature of the Tablet PC is its inking ability. The reduction of paper, the gained organization of going digital, freedom of expression and document review are good benefits but the true advantage of the inking ability is realized not when one uses ink by their self, but rather when it is used to communicate ideas to others. Different collaboration software like Skype, DyKnow, NetMeeting and OneNote were explored and all offer an enhanced ability to understand when one is able to use a pen. The reason is that text is sequential and it is difficult to reference anything, but inking adds another dimension to communication, where one can refer to parts of documents or other parts of the discussion. The drawback to this is that if one does not see the inking take place and therefore does not know the order of the strokes, or one later reviews an inking, it may be difficult to follow precisely because it is not sequential. A primary desire is for software to be able to share a document with other users, but give control to one of the users so that the viewers see the part of the document that the user in control sees, while allowing all of them to ink on the document. This could only be achieved in DyKnow, which is not free. NetMeeting does not allow the viewers to ink. OneNote does not allow a user to have control of what is viewed by other users. Skype is voice communication software that does not claim to do this, but rather aides in the endeavor.

Conclusion and Future Work

Using existing software, HyperTerminal and Hydras 3 LT which can successfully communicate with the HydroLab MiniSonde 4a, development of a LabVIEW VI that could communicate with the sonde was made possible. A VI was developed that could read in data from the sonde, store it to a file, process it, and display it, which is a key part of a RTRM system. Another key part of a RTRM system is being able for one's DAQ software to be able to send commands to the sensor, and despite knowledge of serial communication, protocols, and frequent attempted communication with the manufactures of the sonde hardware and software, commanding the sonde from LabVIEW could not be realized. But it should be noted that this feat is definitely possible, just not within the time frame given. Alternatively, an SDI-12/RS-232 converter can be used in conjunction with SDI-12 Serial-digital Interface Standard to send commands.

Using LabVIEW's web publishing tool, the VI, was remotely controlled by a client computer with access to the internet. The implication is that the sonde can be hooked up to a computer with

the VI, on site, and the sonde's data and the VI's GUI can be accessed by students, researchers, or managers in their office. Thus key steps in the RTRM system were achieved. A RTRM system could be set up with all the information present, except that its full potential would not be realized without being able to send commands from LabVIEW to the sonde, which would allow for more controls in the RTRM system. A fully developed RTRM system has the potential to vastly increase one's data and understanding of their watershed, as well as expedite data/status delivery and thus allow quicker and new responses by watershed managers. LabVIEW provides an easy tool in developing a RTRM system, as well as possibilities for educational uses after its deployment.

Due to limitations in time and the scope of the paper, there still exist many different aspects or continuations of this project to be explored, researched and documented. This project is part of a larger goal to deploy a RTRM system on Stroubles Creek. More work still needs to be done here including: the ability to send commands from LabVIEW to the sonde, replacing the Tablet PC server with a CompactRIO (field capable device), establishing wireless internet access, powering the remote system, and integrating this into Blacksburg's water resource management and monitoring plan, research and educational uses. The potential for actuators and additional sensors, the sampling frequency, and the final location all need to be explored. A branch of this continuing study should focus on how a RTRM system is adopted to Stroubles creek, in general terms, so other locals can use a similar method to build up their RTRM systems in a similar fashion but based on their constraints. Further study needs to be done on water quality indicators that can be measured by sensors and how those relate to environmental impairments, especially in Stroubles Creek.

Finally, one could explore new management and intervention options that open up to water resource managers when they have access to lots of analyzed data in real-time as opposed to the convention where a manager receives little data and finds out that their stream is impaired after the fact.

Acknowledgments

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Decentralized and Small-Scale Drinking Water Treatment Systems: Evaluation of Case Studies in Puebla and Querétaro, Mexico

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ABSTRACT

Drinking water scarcity is a prevalent problem in many corners of the world. With increasing population, this problem can only be expected to increase in the coming years and it is imperative that clean, potable water is accessible to all people. Implementing centralized and conventional drinking water infrastructure is cost prohibitive particularly in rural environments of developing countries. Decentralized and small-scale drinking water treatment systems provide an alternative and potential solution to this problem. The goal of this research is to study the feasibility of decentralized drinking water systems. The research team visited several operating decentralized drinking water systems in Puebla and Querétaro, Mexico (installed by Portagua, US) to gather information on system operation and efficiency, maintenance, cost and community impacts. This report discusses the decentralized water purification drinking and bottling systems experience in Puebla and Querétaro, Mexico and provides information on decentralized system costs, efficiency and potential benefits to small communities where these systems are implemented. In addition, the report provides insight on possible uses of small-scale purification drinking water systems for commercial purposes and disaster area needs. The report is concluded with recommendations for future research.

Keywords: decentralized systems, rural community, purification, pipeless, bottling plant, energy efficiency, potable water treatment, Mexico

Introduction

Providing water to those who need it has been an issue surrounding humanity for centuries. With the human populations growing at a rapid rate and water use increasing just as fast, global communities are forced to find solutions to this problem. Since 1900, there has been a six-fold increase in water use for only a two-fold increase in population size (<http://ag.arizona.edu/AZWATER/awr/dec99/Feature2.htm>). The United Nations has estimated that by 2025, 1.8 billion people will be without clean water, and another two-thirds of the world will be living in water stressed regions (FAO 2006). According to the UNICEF, 1.6 million children die every year due to the lack of clean water or the ingestion of contaminants in water. In 2005, when the United Nations started promoting the “International Water for Life Decade”, 1.1 billion people did not have access to clean drinking water. Most of the un-served populations (around 84%) live in rural areas (WHO/UNICEF 2006a). Most of the urban population has access to purified water due to government policies which have given priority to urban areas, leaving behind the rural population. The U.N. has pledged to halve the un-served population by 2015 (WHO/UNICEF 2006b). Therefore, to achieve this goal, it is imperative to mobilize resources and intelligence along with innovations.

In Latin America, over seventy-eight million residents are without treated water. In Mexico specifically, only 61% of rural dwellers have a form of treated water supply (WHO/UNICEF 2000). While this has improved since 1995, there is still a need to insure clean water is provided to all residents. Many cities in Mexico have water trucks that transport water in tanks from neighborhood to neighborhood, filling up residents' household tanks with drinking water. The public water distribution system does not reach most residents that live outside of the urban areas due to high infrastructure cost, thus the need for water trucks to deliver drinking water. In addition, most of the natural water sources are not safe to drink without proper treatment (GHEF 2008).

Centralized water treatment and distribution systems are commonly used in the United States and around the world, especially in urban areas. Commonly, a water source is treated and transported via pipes to the residents in the city. Ideally, a water storage tank for treated water is located at the highest point in service area and the water is distributed by gravity flow to households and other buildings. The energy used for transporting this water to customers is not well documented. In the United States 4% of electricity use is attributed to water treatment and delivery from which about 80% is used for water distribution. This amounts to approximately 75 billion kWh a year that cost about four billion dollars (Ghimire 2007). It is also widely noted that the largest contributing factor to rising energy use in the water industry is increasing urban sprawl that requires more pipes to be placed to reach those customers. Energy use is especially high when moving water from a lower elevation to a higher elevation; additional pumping must be installed in order to counteract gravity.

Decentralized water systems refer to small-scale water supply systems that use local water resources and serve small communities or low population areas. In general, these systems provide purified water to customers in bottled form (bottled is defined as any size container). In the U.S. it is considered an inconvenience to purchase bottled drinking water for home consumption on a daily or weekly basis. However, in most developing countries residents would often view the idea of purchasing bottled water a convenience. The idea of safe, affordable, ready to drink water is attractive to citizens of many nations.

Research Goal and Objectives

The overall goal of this project is to study the efficiency of small-scale decentralized drinking water systems as compared to centralized systems in rural environments of developing countries. Specific objectives of the project are:

1. Evaluate case studies of small-scale decentralized purification drinking water systems in Mexico
2. Visit selected case study sites in Mexico to observe system operation and gather information
3. Show examples of successful implementation of decentralized systems which could serve as a blueprint for other rural areas in developing countries
4. Perform a comparative analysis of energy and water delivery efficiency of decentralized versus centralized systems.

This report will address objectives 1, 2 and 3. It was rather difficult to address objective 4 due to a lack of readily available data in case study sites. However, some conclusions related to objective 4 are derived from current literature.

Research Approach

The research approach consisted of literature review and visiting several decentralized small-scale drinking and bottling water systems in Puebla and Querétaro, Mexico to collect data and interview owners and operators of these systems. This study focuses on small-scale purification drinking water treatment systems manufactured by Portaqua, LLC (www.portaqua.us). Their system provides nearly 40 water processing units to private investors, communities and governmental agencies in Puebla and Querétaro, Mexico. In June 2008, the research team visited five Portaqua drinking water processing plants, three plants in Puebla (Puebla, Chinantla, Chiautla de Tapia) and two plants in Querétaro (State Water Commission in Santiago de Querétaro and nearby Peñuelas) (Figure 1). The research team also visited a decentralized rainwater harvesting system in Xacxamayo near Puebla.

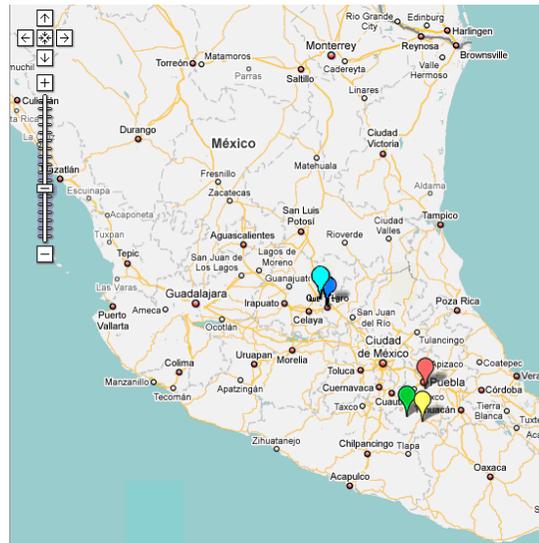


Figure 1. Location of Portaqua case study sites in Mexico.

Note: Portaqua, LLC (www.portaqua.us) has offices located in both the United States and Mexico. The U.S. operation is located at Virginia Tech Corporate Research Center in Blacksburg, Virginia.

Results and Discussion

Portaqua Small-Scale Drinking Water Processing and Bottling Systems

Portaqua is a lightweight drinking water processing system designed for easy setup and operations. The system provides multi-process purification of water designed to remove a broad range of contaminants including arsenic, pesticides and metal contaminants. This removes a barrier that traditional filtration and chlorination does not provide. According to Portaqua manufacturers, system installation can be completed in about two to seven hours. The system is about four feet wide, three feet deep, and stands about seven feet high and can fit in a small room. Figure 2 shows a typical Portaqua water processing and bottling system. Most systems are equipped with a programmable logic control (PLC) component which facilitates automated operation of the system. The automated system allows for easy operator training and enables them to run the system without supervision. Minimal educational background is required to operate the system. There are four Portaqua water treatment models that evolved over time that serve various purposes:

- The Bottling Plant System (BPS) is a water processing system with full capability of a bottling plant that can handle all types of plastic water bottles – mostly privately owned systems.
- Commercial Plant System (CPS) is a water processing system that provides drinking quality water to manufacturing plants, hotels, hospitals and other similar facilities.
- Micro 50 Plant System (MPS) provides water in five gallon containers to small communities of 1,000 to 12,000 people.
- Emergency Plant System (EPS) is designed to be set up in about two hours. It can provide drinking water on a daily basis to 3,000 to 12,000 people in disaster areas. EPS water purification plant is transportable to disaster areas by truck, helicopter and airplane.



Figure 2. Portaqua water treatment and bottling system

Tables 1 and Table 2 show water treatment capabilities, capacity, energy consumption, dimension, weight and other characteristics of Portaqua water processing systems. Portaqua systems are mostly operated by plugging in to local electricity (see Table 1 for energy requirement) but can be connected to a generator. Approximately, 1.7 gallons of gasoline is needed to treat 1,000 gallons of water.

Table 1. Capabilities of Portagua Water Processing Systems (Source: Portagua Product Technical Data)

System Type	Treatment Functions	Capacity (gallons/day)	Energy Consumption (kWh/1000 gallon)
The Bottling Plant System (BPS)	Pre-chlorination, particle filtration, carbon filter, reverse osmosis, bio-control (ozone/UV/mixed oxidant) one or combination of bio-control and PLC.	12K – 60K	11
Commercial Plant System (CPS)	Pre-chlorination, particle and organic using either (particle filtration & carbon filter or ultra-filtration membrane), reverse osmosis, bio-control (ozone/UV/mixed oxidant) one or combination of bio-control and PLC.	12K – 60K	11
Micro 50 Plant System (MPS)	Pre-chlorination, particle filtration, organic carbon filter, reverse osmosis, bio-control (ozone/UV/mixed oxidant) one or combination of bio-control and PLC.	2K – 12K	11
Emergency Plant System (EPS)	Pre-chlorination, UF membrane organic and particle filtration, reverse osmosis, mixed oxidant biocontrol	10K – 20K	13.2* * The ultrafiltration (UF) process in EPS system requires additional energy

Drinking water quality for the installed systems is tested monthly for bacteria and every three month for chemical and physical parameters using established protocols. A report is issued to regulatory governmental agencies upon request. An example of water testing report is documented in Appendix 1. The Portagua water purification system can treat freshwater, brackish water and sea water. Salt removal capability of the Portagua systems is shown in Table 2. For all systems, salinity of water to be treated should not exceed 5,000 µmhos/cm without a company review.

Table 2. Portaqua Systems Specifications (Source: Portaqua Product Technical Data)

System Type	Dimension (inch)	Area Requirement (ft)	Weight (lbs)	Electric Requirement	Pretreatment Storage Tank (inch)
Bottling Plant(R)	75 x 53 x 79	16 x 16	800	30A @ 220VAC 60 Hz.	48 x 40 x 40
Bottling Plant(NR)	75 x 53 x 79	16 x 16	750	20 A @ 220 VAC 60 Hz	48 x 40 x 40
Commercial Plant (R)	62 x 40 x 79	12 x 12	600	25A @ 220 VAC 60 Hz.	48 x 40 x 40
Commercial Plant (NR)	62 x 40 x 79	12 x 12	610	15 A @ 220 VAC 60 Hz	48 x 40 x 40
Emergency Plant (W,R)	75 x 53 x 79	16 x 16	800	40A @ 220 VAC 60 Hz	48 x 40 x 40
Emergency Plant (S ,R)	62 x 40 x 79	12 x 12	680	25 A @ 220 VAC 60 Hz	48 x 40 x 40
Emergency Plant (W , NR)	75 x 53 x 79	16 x 16	750	20 A @ 220 VAC 60 Hz	48 x 40 x 40
Emergency Plant (S , NR)	62 x 40 x 79	12 x 12	610	15 A @ 220 VAC 60 Hz	48 x 40 x 40
Micro 50 Plant System	54 x 40 x 79	12 x 12	540	25A @ 220 VAC 60 Hz	48 x 40 x 40

*W Waterbag Filler Attached; *S Spigot Attached; *R Salt Removal; **NR No Salt Removal

Status of Drinking Water Problems in Puebla

The state of Puebla in central Mexico is about 80 miles south of Mexico City. The state area is 13,096 square- miles. As of 2000, the state’s population was a little over five million. The capital, also called Puebla, is a city of close to three million residents. It is a valley in the midst of mountains and volcanoes with an average temperature of around 21 to 27 degrees Celsius. The main industry is manufacturing. About 24% of the state’s economy and another 18% can be attributed to service sector businesses. The average household has four people and earns an income of 2.46 USD per hour (Nations Encyclopedia 2008). Most homes in rural areas of the state do not have running water. Groundwater is an abundant resource in most localities. However, available groundwater is usually contaminated and it is not recommended for drinking purposes.

The public water system in Puebla does not reach all of the residents. Providing drinking water to residents of Puebla is a challenge because of the area’s mountainous geography and infrastructure costs. Within the city, where residents do not receive water via pipes, drinking water is usually delivered through trucks which fill storage tanks that sit under the house (cistern) and is later pumped to the roof of houses. Most residents buy five gallon bottled water from a local store. With a limited number of residents served by the public water system, it is important to reach the rural areas through some other means. Piping water is not practical, as many villages are on mountainsides outside of any feasible range for piping water in a cost efficient manner. In one case as described later, the government has attempted to place a mountaintop rainwater capture

system to deliver water to the town below but the system has not been successful. Another alternative water delivery approach is using donkeys. Additionally, some residents, with governmental assistance, have created their own household rainwater collection systems.



Figure 3. Women washing clothes in stream used for potable water and water delivery by donkey

To meet water needs, the Puebla government has developed programs and incentives to relocate rural populations to nearby urban areas. Such incentives include monetary compensation to entice residents to move to cities. The government has speculated that relocating rural people to urbanized areas will cost less in the long run for the government than providing water through a water delivery infrastructure. In addition, the state of Puebla has permitted private ownership and operation of utilities (water and sanitation). Several small communities surrounding Puebla and Queretaro's capitals have purchased small drinking water systems. Portaqua has installed several small water processing systems in residential neighborhoods within proximity of the city of Puebla. The research team visited a few systems in June 2008.

Puebla vicinity water processing and bottling system

The first case study site (Purificadora "Cenral") which the research team visited is located in the vicinity of Puebla. This is a privately owned system where the owner converted an inside garden area of his house to install a Portaqua Bottling Plant System (PBS) (see Table 1). The source of raw water is a local well. Water is trucked 3,000 gallons at a time and brought for treatment. After processing, five gallon bottles are filled and seals are placed over the lids. The unit is state certified and produces 1000-five gallon bottles of water a month. Customers of this plant are mainly households near the water processing facility. The owner and his family are trained to clean used bottles and run the processing and bottling system. The bottled water from this plant is sold at less than half the price of bottled water at the local convenience store.

In most cases, electricity use for water processing is not metered independent of other uses and it is difficult to estimate direct energy costs. For this water processing system, the electricity bill was available separate from other household electricity uses and enabled the research team to estimate energy cost of the system. The energy requirement for the plant is 11 kWh/1,000 gal of treated water. The electricity bill for this system was 400 to 500 pesos a month (around 40-50 USD). To conservatively estimate, this means that the system spends about seven cents per five gallon bottle (a little over one penny per gallon of clean water distributed to the public). Other costs incurred are health inspection fees (25 USD), purchasing and delivery of raw water from local well (30 USD for every 3,000 gallons). Table 3 shows cost breakdown for this plant. Cost to owner is approximately .16 USD for every five gallon bottle produced. It should be noted that electricity cost in Mexico is regulated by the government.

Table 3. Cost estimate for water processing and bottling 5 gallons bottled water

	Monthly Production	Variable costs:	Costs in MXP		cost/bottle
Local Income:		Plastic bottle	\$ 15.00	0.02	\$ 0.25
Electricity (not prod):	\$ 300.00	Label for			
Telephone:	\$ -	carboys	\$ 0.20	0.33	\$ 0.07
Manpower and		Top	\$ 0.15	1.05	\$ 0.16
Employees *:	\$ 2,000.00	Stamp			
Fees:	\$ -	guarantee	\$ 0.15	1.05	\$ 0.16
Stationery:	\$ 200.00	Raw Water pipe			
Advertising:	\$ -	10m3	\$ 200.00	0.002	\$ 0.48
Mtto newspaper plant:	\$ 350.00	Electricity (P)	\$ -	0.07	\$ -
Analysis periodic		Detergent wash	\$ 120.00	0.0025	\$ 0.30
water:	\$ 330.00	Cartridges	\$ 350.00	0.0003	0.09
Uniforms /		Several	\$ 0.10	1.00	0.10
eq.seguridad:	\$ 100.00	Taxes	\$ -	1.00	-
Permits / licenses	\$ -	other	\$ -	1.00	-
other	\$ -	other	\$ -	1.00	-
Total (ex-work)	\$ 3,280.00				\$ 1.60
					(0.16USD)
	\$/mes	Variable costs:	Cost in		Cost/bottle
Fixed costs:	\$ 1,000.00	Driver	MXP	Factor	
Income estate *	\$ -	Commission:	\$ 0.20	1	\$ 0.20
Salary Driver and	\$ 500.00	Other. Pick:	\$ 0.59	1	\$ 0.59
helper:					
Other not mechanical	\$ 100.00				
Permits, plates,	\$ -				
licenses:					
Uniforms					
Total (cost)	\$ 1,600.00				\$ 0.79
					(0.08 USD)

Note: Exchange rate (August 2008) is approximately 10 Mexican Pesos per 1 US Dollar

Chinantla water processing and bottling system

Chinantla is a small city in the southeastern part of Puebla with a population (2005 census) of 2264 inhabitants (<http://www.chinantla.com/>). A convenience store owner has purchased and operates a Portaqua Bottling Plant System (BPS) (Figure 4). The source of raw water is a nearby dug well about 100 ft from the plant from which water is piped to the processing plant. Treated water is sold as 20 oz bottled water in the owner's convenience store as well as in 5-gallon containers that are delivered by truck to several households in the community.



Figure 4. Water Processing and Bottling System in Chinantla, Puebla

Chiautla de Tapia water treatment and bottling system

Chiautla de Tapia is a city in the mountainous area of southern Puebla and relies mainly on trucked water. A plant that uses hibiscuses flowers to produce liquors and jams is located in this area (Figure 5). A Portaqua Commercial Plant System (CPS) has been in operation in the plant for two years. The CPS provides clean water for producing liquors and jam. In addition, the water processing system supplies water to a community of about 20,000 residents. The decentralized water system is considered a positive addition to the community health and its economy.



Figure 5. Water Processing and Bottling System in Chiautla de Tapia

The Xacxamayo decentralized rainwater harvesting system

Xacxamayo is a small town of approximately 200 families near Puebla. The town is separated from Puebla by the lake Valsequillo. In order to get to the town, one must cross the lake via a ferry and then drive approximately twenty miles into the mountains. Due to the mountainous and remote location of the town, establishing water utilities infrastructure is difficult and expensive. Thus, the government has offered cash incentives to convince town residents to move to more populated areas without much success. As an alternative to conventional water systems, the government has constructed a rainwater collection reservoir at the top of the mountain above the town. Concrete channels direct rainwater from mountaintop watershed to the collection reservoir (Figure 6). The water from the reservoir is directed to nearby two sequenced carbon filter containers that are over eight feet high. The treated water from the second carbon filter is moved to a concrete storage container and delivered by pipe and gravity flow to town below.



Figure 6. Mountaintop rainwater capture and delivery in Xacxamayo

Water leakage from the reservoir and absence of electric power on the mountain top to treat water properly are major technical issues. Interviews of town residents indicated that the mountaintop rainwater harvesting has not been effective and does not meet its goal of providing water to town residents. Several households have developed individual rooftop rainwater capture systems which are much more effective than the government's system (Figure 7). The government provides subsidies to households (around thirty homes are selected a year) to retrofit rooftops rainwater collection and purchase other needed material. The households are expected to provide the labor for installing the system. This approach has proved to be feasible, because most of the town residents have been involved in construction projects and are knowledgeable about how to make concrete water storage structures. The alternative to rainwater capture from the mountain above is water delivery by donkeys in 5-gallon containers from across the lake. The state government is planning to explore alternative water treatment and energy use techniques for implementing mountaintop rainwater harvesting and drinking water systems. Portaqua is looking into possibilities for water treatment and delivery system in Xacxamayo.



Figure 7. Rooftop rainwater collection and water delivery in Xacxamayo

Status of Drinking Water Problems in Querétaro

The State of Querétaro is a mountainous region located about 160 miles to the northeast of Mexico City. The 2005 census shows the state population as 1,598,139 residents and 34% of population live in rural areas. The capital city of Santiago de Querétaro is the largest municipality in the state with a population of 734,139. Querétaro has experienced chronic water shortage and lack of drinking water. The National Water Commission (CNA or Conagua) has identified Querétaro as a high priority area (ranking of "5") to receive assistance from the federal government for water infrastructure. As a result, the federal government provides incentives for

water projects that reduces energy and/or increases water access for Querétaro residents. The high priority ranking allows the state of Querétaro to consider innovative opportunities for finding practical solutions to water problems such as implementing decentralized water processing systems. Querétaro has collaborated with Portaqua, LLC to establish several Portaqua systems around the state. Querétaro is also on track to help set “best practice” meetings and workshops in place for small water system owners.

Mobile emergency unit

The state of Querétaro has purchased an Emergency Plant System (EPS) from Portaqua for fast response to anticipated disasters (Figure 8). This lightweight mobile unit is readily mounted on a truck and can be quickly moved to an area in crisis and it can even be lifted by helicopter if needed. The state is using the same Portaqua system when not in emergency use to supply its own bottled needs in their regional state offices. This has reduced the governmental bottle water cost as it is less expensive than outside suppliers.



Figure 8. Emergency water processing unit in Querétaro

Peñuelas community decentralized water system

The small community of Peñuelas (population about 10,000) in Querétaro is a unique example of a community’s attempt to have a system that is owned and operated by the public (Figure 9). There is no profit associated with this water system and it is a favorite concept of the research team. The community submitted a request to the Inter-American Bank (IAB) and received funds to purchase a Portaqua decentralized water processing and bottling system instead of building a conventional water treatment facility that would have costs in the millions of dollars. The community has installed and is operating a Portaqua Micro 50 Plant System (MPS). The cost of five gallons water to community residents is about half of the cost of what private owners would provide to the community.

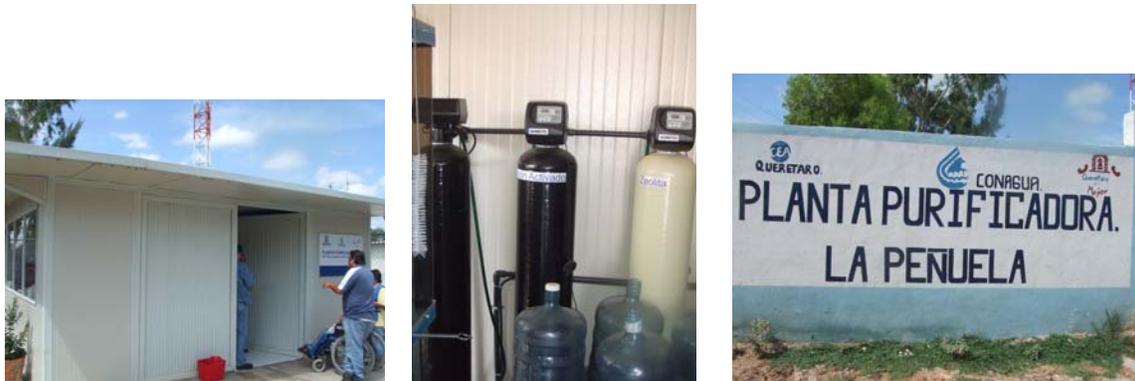


Figure 9. Peñuelas community decentralized water system

Cost and Energy Efficiency of Decentralized Systems

There are obviously some political disadvantages to giving up public control of water systems to private organization and companies that cause many developing countries to be hesitant about embracing decentralized systems. However, after observing, interviewing, and documenting the environment, it seems clear that there are many benefits to using decentralized independent systems, especially in small communities that are far from a conventional centralized (piped) system in urban areas.

The study show the low cost of water treatment and bottling systems which is beneficial for community health and development (see Table 2). In addition, decentralized and small-scale systems eliminate the need for pipeline infrastructure which is responsible for a major portion of the system initial cost. Cost information related to pipeline infrastructure was not available for the study areas in Mexico. However, a recent study in Montgomery County, Virginia estimated that it will cost \$100 to \$125 per foot to lay a new water line (Grady and Younos 2008). This cost may be much lower in developing countries. However, as the geographic condition in Xacxamayo (described above) indicates, the initial infrastructure cost can be a detrimental factor in funding and implementing drinking water projects particularly in mountainous area. Operating cost is a second factor that needs to be considered. A typical water distribution system, where water is transferred from a location away from where water is used, consumes about 2/3rd of operating cost because of energy needed to pump water (other 1/3rd is attributed to water treatment) (Chen et al. 2007). In mountainous areas energy cost can be much higher and implementing conventional piped water distribution system can be cost prohibitive. Conventional centralized systems cannot be afforded by small communities unless extensively subsidized by the government. In contrast, small-scale decentralized systems depend on energy only for water treatment and are affordable by small communities.

The main component of energy usage in decentralized systems such as Portaqua is attributed to membrane that is installed in the system. The type and number of membranes determine the amount of energy required for water treatment. The energy usage for Portaqua systems is 11 kWh/1,000 gal to 13.2 kWh/1,000 gal of treated water (see Table 1). Previous research shows that for reverse osmosis (RO) membranes used for desalination of brackish and sea water, the mechanical energy used to operate the water treatment pump is 15 kWh/1,000 gal to 38 kWh/1,000 gal (Younos and Tulou 2005). Further advance in membrane technologies is expected to increase energy efficiency of water treatment systems.

Conclusions

The Portaqua systems that were visited in Puebla and Querétaro provided much insight into the uses of small-scale drinking water systems. Some of the benefits of these systems are described below.

Operation: The completely automated system allows easy operation. One-day operator training can provide sufficient knowledge to an operator with minimal education to run the system effectively. With one button operation, a used bottle is sanitized, while at the same time the treatment system is activated and a clean bottle filled with drinking water for the customer. Energy use and efficiency of these systems are comparable to large water treatment systems.

Cost to community: As observed in Peñuelas, Querétaro, in the community owned system, the water is provided at minimal cost to the residents of the community. Even at the sites where they were privately owned and used for profit, the costs were still significantly less than the bottled water sold at stores from larger suppliers.

Return on investment: The initial cost of one of these systems (\$13,000 to \$30,000) can be a large investment for a private owner. However, with a significant customer base and some form of marketing, it is possible for the owner to see returns on their investment within two to four years. In addition, because water is a necessity, customers will most likely be constant purchasers every month. This provides a consistent income flow to the owners.

Job creation: Small-scale decentralized systems create jobs in small communities where drinking water is produced locally and can contribute to a higher standard of living.

Prospect for commercial use: As seen in the Jam Factory (Chiautla de Tapia), it is possible for these small-scale systems to support commercial plants as well. This industry could be expanded to provide water to hospitals, clinics, public areas, and other places. The clean water can be used in processed goods and also used to help patients at a hospital.

Emergency use: With the emergency Portaqua system, a government could have ready-to-go equipment in case of a disaster that cuts off potable water to residents. These systems can be flown or driven into the location where the emergency conditions exist. The emergency system can be used in the U.S. as well to meet water needs in the aftermath of natural disasters.

Some areas of concern are noted below:

Maintaining high water quality: Regular water quality reports and system inspection are desired to ensure these systems work properly and are providing at least the minimum water quality to their customers. A huge increase in the amount of small-scale systems would lead to a need for more regulatory requirements and increased number of inspectors. However, with good planning these costs can be offset and justified by the cost savings incurred through energy and infrastructure and improved community health.

Improved certification program: There is a need to develop an improved framework for operator certification to ensure system maintenance and public health.

Recommendations for Future Research

1. Lack of data and time prevented a comprehensive analysis of the cost feasibility and the sustainability of small-scale systems as compared to a conventional system in a real-world situation. There is a need to perform this type of analysis on a regional basis to better justify large scale implementation of decentralized systems in rural and sub-urban environments.
2. There is a need to measure and document energy demand and consumption by small-scale decentralized systems under different operational conditions.
3. In many locations in developing countries the operation of decentralized systems will depend on electricity and other fuels which may not be available. There is a need to research to the potential of using renewable (solar, wind, etc.) energy resources for operating these systems.
4. Using small-scale decentralized systems can have potential benefits in rural and isolated communities of the U.S. as well. This potential needs to be explored.

Acknowledgments

This research was supported by the NSF-REU program (Grant No. 0649070) awarded to Virginia Tech. Travel support to Mexico to visit study site was provided by Portaqua, LLC. Special Acknowledgments are due to Mr. Max Junghanns and Mr. Rafael Gonzalez for hosting the research team, their guidance through various locations in Mexico and providing the data for case study sites documented in this report. Monica Licher and Caitlin Grady assisted with editing the final manuscript.

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Appendix

Dirección de Planeación Estratégica
Gerencia de Aseguramiento de la Calidad
Coordinación de Laboratorio
Laboratorio Central de Calidad del Agua
Av. 5 de Febrero No. 35, Col. Las Campanas, C.P. 76010, Querétaro, Qro.



Informe de Resultados de Pruebas en Agua Potable

Folio: Nov-470

Datos del Servicio		Fecha de Muestreo	30-Nov-05
Nombre del Cliente	Administración Colón-Tolimán-Peñamiller	Hora de Muestreo	9:00 Hrs.
Atención	C. Héctor Rodríguez Varela	Fecha de Recepción	30-Nov-05
Lugar de Muestreo	Pozo Aposentos Peñamiller, Qro.	Fecha de Fin de Pruebas	08-Dic-05
Nombre del Muestreador	Antonio Femuzca	Tipo(s) de Recipiente(s)	P, V
		Cantidad de Muestra	2.25 L Aprox.

Prueba	Resultado	Permisible	Unidad	Norma	Prueba	Resultado	Permisible	Unidad	Norma
Bacteriológicas					Manganoso				
Coliformes Totales	0	Ausencia	UFC/100 mL	NOM-41-SSA1-93, Ap.B		0.15	mg/L	NOM-117-SSA1-94	
Coliformes Fecales	0	Ausencia	UFC/100 mL	NOM-41-SSA1-93, Ap.B	Mercurio	0.001	mg/L	NOM-117-SSA1-94	
Organolépticas					Plomo	0.01	mg/L	NOM-117-SSA1-94	
* Sabor		Agradable	No Aplica	SM-2190	Sodio	200.00	mg/L	NOM-117-SSA1-94	
Clor	Agradable	Agradable	No Aplica	NMX-AA-053-02	Zinc	5.00	mg/L	NOM-117-SSA1-94	
FÍSICAS					Cromatográficas				
Color	10.0	20.0	Unidades	NOM-41-SSA1-93, Ap.A1	* Aldrín	0.03	µg/L	NOM-41-SSA1-93, Ap.A20	
Sólidos Disueltos Totales	1120.0	1000.0	mg/L	NOM-41-SSA1-93, Ap.A17	* Benceno	10.00	µg/L	SM-6200	
Turbiedad	0.71	5.0	UTN	NOM-41-SSA1-93, Ap.A2	* Clordano (Total isómeros)	0.20	µg/L	NOM-41-SSA1-93, Ap.A20	
Químicas					* D.D.T. (Total isómeros)	1.00	µg/L	NOM-41-SSA1-93, Ap.A20	
Cianuros (como CN ⁻)		0.07	mg/L	NOM-41-SSA1-93, Ap.A6	* Dieldrín	0.03	µg/L	NOM-41-SSA1-93, Ap.A20	
Cloro Residual Libre		0.20 - 1.50	mg/L	KIT	* Etilbenceno	300.00	µg/L	SM-6200	
Cloruros (como Cl ⁻)		250.0	mg/L	NOM-41-SSA1-93, Ap.A8	* Gamma-HCH (Lindano)	2.00	µg/L	NOM-41-SSA1-93, Ap.A20	
Dureza Total (como CaCO ₃)	606.1	500.0	mg/L	NOM-41-SSA1-93, Ap.A9	* Hexaclorobenceno	1.00	µg/L	SM-6410	
* Fenoles		0.30	mg/L	NOM-41-SSA1-93, Ap.A10	* Heptacloro (y su epóxido)	0.03	µg/L	NOM-41-SSA1-93, Ap.A20	
Fluoruros (como F ⁻)	0.517	1.50	mg/L	NOM-41-SSA1-93, Ap.A11	* Metoxicloro	20.00	µg/L	NOM-41-SSA1-93, Ap.A20	
Nitratos (como N)	0.338	10.00	mg/L	NOM-41-SSA1-93, Ap.A12	* Tolueno	700.00	µg/L	SM-6200	
Nitritos (como N)		1.00	mg/L	NOM-41-SSA1-93, Ap.A13	* Trihalometanos Totales	0.20	µg/L	SM-6202	
Nitrógeno Amoniacal (N)	0.426	0.50	mg/L	SM-4502-NH ₄	* Xileno (Tres isómeros)	500.00	µg/L	SM-6200	
pH (Potencial de Hidrógeno)	7.34	6.5 - 8.5	Unidades	NOM-41-SSA1-93, Ap.A3	* 2,4-D	30.00	µg/L	SM-6640	
* S.A.A.M.		0.50	mg/L	NOM-41-SSA1-93, Ap.A19	Radiactivas				
Sulfatos (como SO ₄ ²⁻)	914.440	400.00	mg/L	NOM-41-SSA1-93, Ap.A18	** Radiactividad Alfa Global	0.56	Bq/L	SM-7110	
* Yodo Residual Libre		0.2 - 0.5	mg/L	SM-4904	** Radiactividad Beta Global	1.85	Bq/L	SM-7110	
Absorción Atómica					Otras Características (No descritas en NOM-127-SSA1-1994)				
Aluminio		0.20	mg/L	NOM-117-SSA1-94	Alcalinidad Total		No Aplica	mg/L	NOM-41-SSA1-93, Ap.A4
Arsénico		0.025	mg/L	NOM-117-SSA1-94	Calcio		No Aplica	mg/L	NMX-AA-072-SCFI-01
Bario		0.70	mg/L	NOM-117-SSA1-94	Conductividad Eléctrica	2120.00	No Aplica	µmhos/cm	NMX-AA-093-SCFI-00
Cadmio		0.005	mg/L	NOM-117-SSA1-94	Magnesio		No Aplica	mg/L	NMX-AA-072-SCFI-01
Cobre		2.00	mg/L	NOM-117-SSA1-94	Sólidos Totales		No Aplica	mg/L	NMX-AA-034-SCFI-01
Cromo Total		0.05	mg/L	NOM-117-SSA1-94	Temperatura	-	No Aplica	° C	NMX-AA-007-SCFI-00
Hierro		0.30	mg/L	NOM-117-SSA1-94	Índice de Langelier (Is)		No Aplica	Unidades	ASTM D3739-83
					Comportamiento del Agua		No Aplica	No Aplica	ASTM D3739-83
Observaciones:					Representante Autorizado				
El agua se considera muy dura (Más de 300 mg/L)					Q.M. Juan Ciro Rivera Solís				
Analista Físicoquímicos	Analista Bacteriológicos			Analista Abs. Atómica	Revisión				
Juana López Rivera	Ma. Isabel Vigil Montero			TLC. Israel A. Sánchez R.	Q. en A. José Alfredo Quintanar Hernández				

* Prueba en desarrollo

** Prueba subcontratada

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Green Building Design: A Case Study Application to Car Dealerships and Implications for Water and Energy Conservation

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ABSTRACT

There is a national trend to design and implement green and sustainable buildings. Rainwater harvesting is an important element of a sustainable building for water and energy conservation and is recognized in the Leadership in Energy and Environmental Design (LEED) rating system. A major advantage of a rainwater harvesting system is less dependency on potable water for non-potable uses, and therefore reduced level of water distribution and energy input into the building. This paper discusses the implications of installing a rainwater harvesting system for a car dealership in terms of potable water and energy savings.

Keyword: green buildings, rainwater harvesting, energy conservation, car dealerships

Introduction

Green engineering is a relatively new field. The goals of the Green Engineering Program are to incorporate “green” or environmentally conscious thinking and approaches in the academic and industrial communities and to minimize generation of pollution at the source and risk to human health and environment (EPA 2004, EPA 2008a). While the basis of green engineering is to protect the population and the environment, it has also begun to drive industrial growth. The practice of green engineering has expanded to include governmental institutions such as the United States Military and huge corporations such as General Motors (Nelson 2008).

The concept of green engineering is also extended to building construction. Green building is the practice of creating structures and using processes that are environmentally responsible and resource-efficient throughout a building’s life-cycle (EPA 2008b). Efficient use water, energy, construction materials and other natural resources are major elements of a green building. Green buildings are flourishing across the United States. San Francisco has 27 branches of public green libraries. Its new Main Library utilizes natural lighting and is among 26 other public libraries in the area that is entirely air conditioning-free (Fialkoff 2008). The Bank of America Tower

located in Midtown Manhattan, New York, is one of the most innovative buildings in the United States in terms of environmental goals (Figure 1). It has a grey-water system installed to capture and filter rainwater and wastewater, and a green roof to prevent heat from entering the building. Additionally, daylight dimming and LED lights are other features of the building that reduce energy consumption dramatically (Burke 2004).



Figure 11. Bank of America Tower, the first Platinum LEED certified skyscraper

Source: www.archiseek.com

In 1998, the U.S. Green Building Council (USGBC) developed a new Green Building Rating System, Leadership in Energy and Environmental Design, also known as LEED. The LEED mission is to “encourage and accelerate global adoption of sustainable green building development practices through the creation and implementation of universally understood and accepted standards, tools and performance criteria.”

As shown in Figure 2, at present, the majority (75%) of buildings in the U.S. do not follow green standards. The LEED certification system aims to challenge innovative designers and engineers to develop new techniques to promote sustainability. A point system is used to determine which of the four levels of certification is to be awarded: 26-32 points for Certified, 33-38 points for Silver, 39-51 points for Gold, and 52-69 points for Platinum (UGBC 2006). While the initial construction costs of a Green Building may be higher, benefits can be experienced indirectly by society as a whole. Additionally, the overall energy efficiency of the building quickly pays off high initial costs. According to a recent study done by the New Buildings Institute, LEED certified buildings consume on an average of 25 to 30% less energy, whereas Gold and Platinum LEED certified buildings consume approximately 50% less energy. Rental space in LEED certified buildings are also at a higher demand and even have higher occupancy (Sustainable Facility 2008).

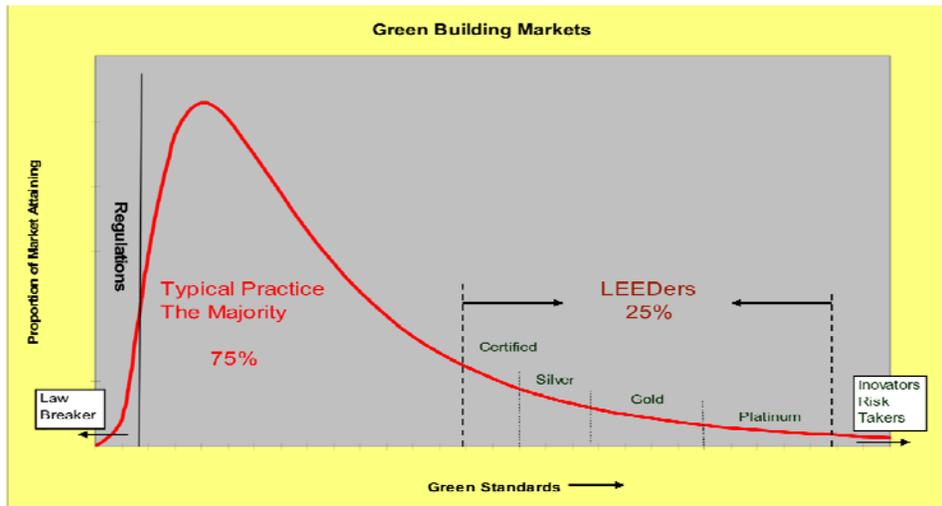


Figure 2. LEED Certification Green Building Markets

Source: LEED Policy Manual (USGBC 2008)

Research Objectives

The objective of this research was to extend the application of green building design to car dealerships with implementing a rainwater harvesting system. Specific objectives were:

1. Review the concept of rainwater harvesting and system design
2. Determine water use in car dealerships
3. Determine water availability (rainwater harvesting) water use balance
4. Discuss the water and energy conservation aspects of rainwater harvesting

Research Methods

Research methods consisted of a review of literature pertinent to design of rainwater harvesting systems, preliminary design of a rainwater harvesting system for selected car dealership, site visit, data collection and data analysis.

Rainwater Harvesting

Water and energy use efficiency of buildings are major elements of a sustainable “green” building. Figure 3 shows major components of a rooftop rainwater harvesting system: catchment area (rooftop), conveyance (pipes that take water from rooftop surface to storage tank), storage and distribution (system that delivers rainwater), and filter. The catchment area allows rainwater to be temporarily collected; collected water then travels down the conveyance and proceeds into the storage tank. A pump is used to distribute water for indoor and outdoor (landscape irrigation) uses. Since rainwater harvesting systems are built on individual buildings, its implementation will reduce the demand for city-treated water and decreases the amount of water needed to be treated at water treatment plants (Kinkade-Leavrio 2007).

Indoor and outdoor use of captured rainwater reduces overall water usage from the main city line. Energy conservation can be realized at two levels: reduced energy use at city water treatment plant as less water needs to be treated and reduced energy use due to decreased water distribution

through pipe and pumping system. The captured water can be used without extensive energy-consuming treatment that would be otherwise lost to runoff and evaporation.

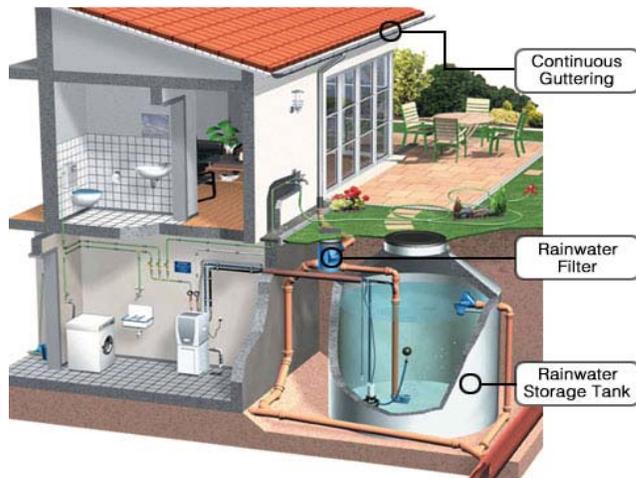


Figure 3. Rainwater Capture System

Source: www.rainwater-solutions.com

Rainwater Harvesting Case Studies

Numerous rainwater harvesting systems are implemented in buildings across the U.S. and other countries, where the water is used for a variety of activities (Gowland and Younos 2008). The Club Casitas De Las Campanas golf course in Santa Fe, New Mexico collects 250,053 gallons of water yearly to irrigate its landscape (Kinkade-Leavrio 2007). Eggleston Laundry, a laundry facility in Norfolk, Virginia, collects 897,600 gallons of water to run its washing machines (Gowland and Younos 2008). Additionally, the Toyota Logistics Service Vehicle Distribution Center in Portland, Oregon uses its 757,699 gallons of yearly collected water for indoor plumbing (Kinkade-Leavrio 2007). Rainwater capture systems are even being implemented at universities. Portland State University's Epler Hall is a certified LEED Silver building and collects 230,000 gallons of water annually that is used for indoor plumbing and landscape irrigation. It reduces the building's demand for city-treated water by 110,000 gallons and saves approximately \$1,000/year in wastewater expenses (Portland State University 2008).



Figure 4. Portland State University's Stephen Epler Hall

Source: <http://www.interfaceengineering.com/>

Description of Study Site

The Nissan Car Dealership located in Christiansburg, Virginia is a 25,400 square rooftop area building that has undergone a recent façade and interior renovation (Figure 5). For the aesthetic value and protection of the deterioration of the paint, the car maintenance staff washes each car parked on the lot of the New River Nissan dealership once every couple months. Kim Martin, an associate at the dealership, says there are approximately 350 cars on their lot, and during the summer months of May-August when the car selling is at its low, the staff washes 10-12 cars a week, and an estimated 15-18 cars per week when car sales pick up.



Figure 5. New River Nissan in Christiansburg, VA

Data Collection

Water use data

The monthly water usage and bimonthly water bills from 2003-2007 were obtained from the New River Nissan Dealership. Additionally, a weekly log given to the car maintenance staff recorded the number of cars washed per week.

Rainfall Data

The average rainfall data for Christiansburg, Virginia was obtained from the National Climactic Data Center’s website (<http://www.ncdc.noaa.gov/oa/ncdc.html>) Figure 6 shows the inches of rainfall from 2002-2007, and the average monthly rainfall trend.

Rainfall in Christiansburg, VA

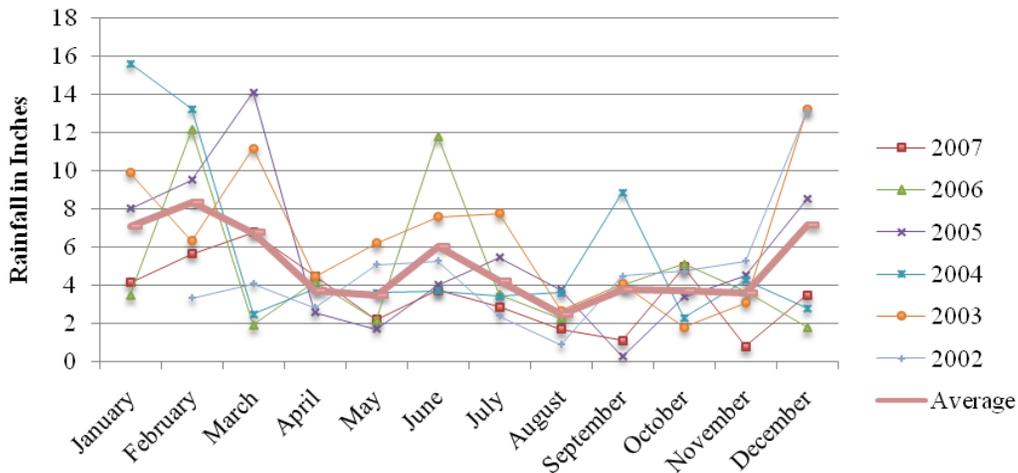


Figure 6. Monthly Rainfall Data from 2002-2007, Christiansburg, Virginia

Estimation of Harvestable Rainwater

Harvestable rainwater was estimated from rooftop area and monthly average rainfall. An equation show below that is suggested in Virginia Rainwater Harvesting Manual for rainwater harvesting

system design was used to make the estimate (<http://www.rainwatermanagement.com/>). The procedure compensates for potential losses due to splash and evaporation.

$$\text{Rooftop area} \quad * \quad \text{Average Monthly} \quad * \quad 0.62 * 0.95 = \quad \text{Gallons} \\ \text{Square-ft} \quad \quad \quad \text{Rainfall (in)} \quad \quad \quad \quad \quad \quad \quad \quad \text{Collected}$$

Multiplying by 0.62 is a conversion factor, which allows the amount to be shown in gallons, and multiplying by 0.95 implies 95 percent efficiency from the system. Rainwater harvesting systems typically have 90-95% efficiency.

A Java program was written to perform calculations in a manner so that the inputs are variables of the above equation.

System Recommendations

The tank height cannot exceed the height of the building due to gravity flow. The height of New River Nissan is approximately 20 feet. An above ground CorGal Tank, model 1502-WT-SAP-CFT-25, with a diameter of 15' and a height of 7'-3" has a volume capacity of 8,900 gallons (Water Storage Tanks, Inc. <http://www.corgaltanks.com/>). While the building's total monthly water use is 12,575 gallons, it is unnecessary to have a tank the size of the monthly use due to the rate at which rainfall is subtracted by the rate at which water is used from the tank. The most cost-efficient method of pumping water is simply by allowing gravity to apply pressure; having an above ground tank would allow the water to flow out without much additional energy. A 1-hp pump is expected to provide adequate water pressure for car washing and other indoor uses of water.

Results and Discussion

Water Usage and Available Rainfall

New River Nissan spends an average of \$53.14 on 16,251.336 gallons of water per billing term, which is bimonthly. According to these water use trends and assuming the dealership washes approximately 15 cars per week, they wash a total of 65.22 cars, which rounds up to 66 cars per month. The 8,125.67 gallons of water spent each month on washing the 66 cars equates to 142.56 gallons of water per car. Figure 7 shows that the monthly water usage is consistent, while the available monthly rainfall fluctuates with the different seasons. Since Christiansburg, VA gets 5.022 inches of rainfall monthly, New River Nissan's 25,400 square foot roof would be capable of capturing an average of 78,240.92 gallons of water per month. As shown in Figure 8, the average harvestable water is significantly higher every month of the year, and is approximately higher each month by an average of 8.62 times.

Water Used in Gallons for Car Washing

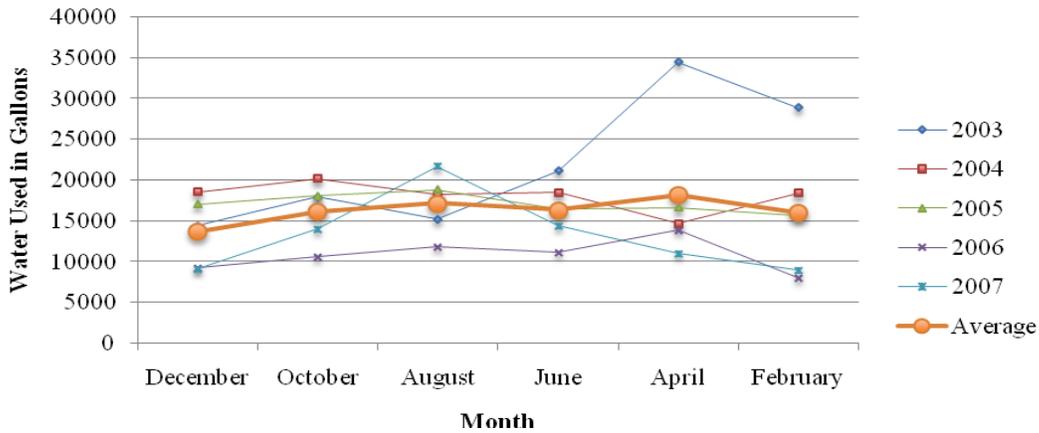


Figure 7. New River Nissan's water use for car washing.

Average Water Used vs. Average Harvestable Water

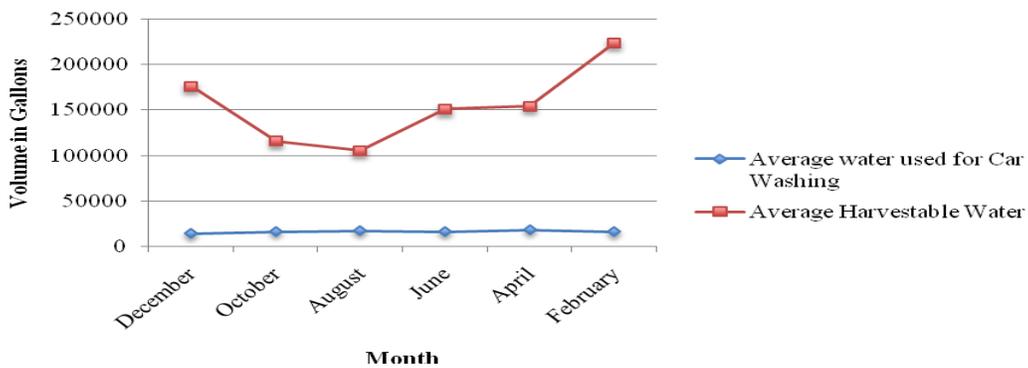


Figure 8. New River Nissan's average water used for car washing versus average harvestable water for Christiansburg, VA.

Potential Water and Energy Savings

Local Energy Savings

Since the monthly rainfall exceeds that of the amount of water used for car washing in New River Nissan, it would be practical to extend the rainwater use to include indoor use for flushing toilets. Recent data from January to April 2008 water bills shows the average monthly total use of water in the Nissan Building is 12,575 gallons. While it may not be entirely accurate to extrapolate this information to cover the entire year but it can be used as an estimate to show that the monthly harvestable rainfall that can adequately meet the water need for the entire building.

Average Water Used vs. Average Harvestable Water

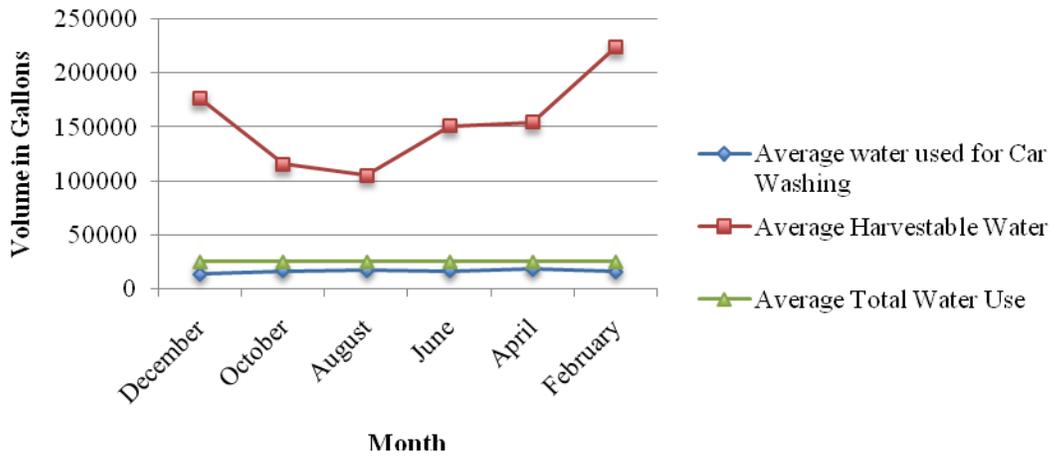


Figure 9. New River Nissan’s total water use, water use for car washing, and harvestable water in Christiansburg, VA.

According to previous research at Virginia Tech the energy consumption for water treatment and distribution for the Water Authority that serves Blacksburg and Christiansburg was 0.44 kWh/m³ or 0.00167 kWh/gal (Chen et al. 2007). The average monthly use of water for car wash in the New River Nissan is 8,125.67 gallons. This will translate to 162.84 kW/h energy saving for car wash and a cumulative energy saving of 814.19 kWh in five years (Figure 10). If total water use (12,575 gallons) in the building is considered then five year energy saving will increase to 1257 kWh. As of May 2008, according to the American Electric Power, the energy provider for New River Nissan, the average energy cost per kWh is \$0.13. If the entire Nissan Building were to be independent of public water system, the energy cost saving for five year period will be only \$163.4.

The implications of these energy savings are significant, due to the potential in expanding this practice to the additional 35 car dealerships in the immediate Christiansburg and nearby areas (Figure 11). Assuming the average water usage and number of cars on each of the 36 car dealership lots are the same, in one year if each dealership were to be independent of public water system, the energy saving would be 5,862.24 kWh and \$762.09. In five years, if all of the car dealerships were to be independent of city water for washing cars, there will be cumulative savings of 29,311.2 kWh and \$3,810.46 in monetary savings.

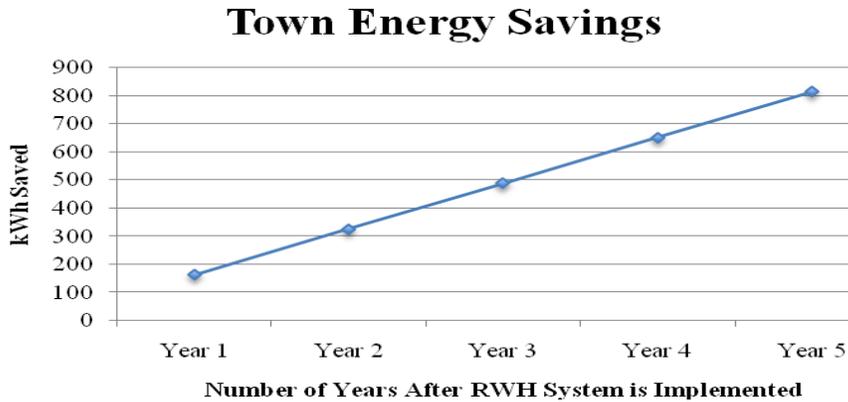


Figure 10. The linear trend of projected town energy savings in five years.

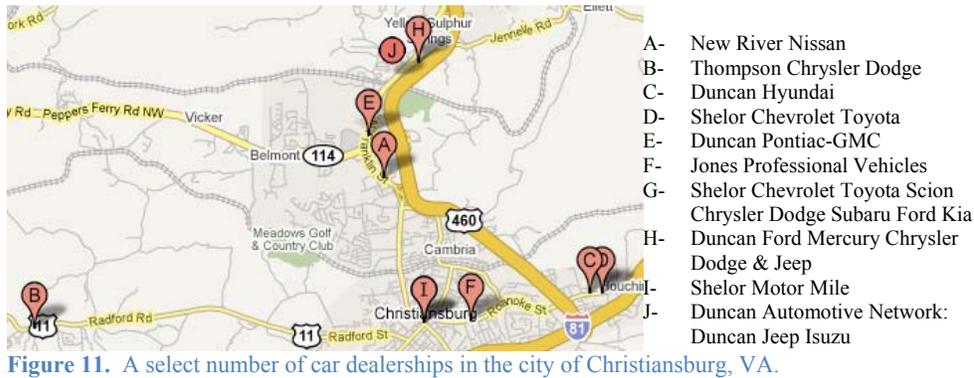


Figure 11. A select number of car dealerships in the city of Christiansburg, VA.

Source: Yellowpages <http://www.yellowpages.com> (2008)

Conclusions

Overall, installing a rainwater harvesting system for New River Nissan can only benefit the building and its users in the long run. Since the building is located on a hill, it takes more energy to pump water to the building. This dealership could be the role model for the additional nine dealerships in the immediate Christiansburg, Virginia area. For New River Nissan’s current monthly usage of 8,125.668 gallons of water for washing cars and a total usage of 12,575 gallons of water, the building would only need an 8,900 gallon tank. Additionally, to have even higher energy savings, New River Nissan could look into the possibility of installing solar panels on the roof to offset increasing electricity prices. The implications of these energy savings are significant, due to the potential in expanding this practice to the additional 35 car dealerships in the immediate Christiansburg and nearby areas.

Acknowledgements

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