FECAL BACTERIA INDICATOR TO DETERMINE POINT-SOURCE POLLUTION
UPSTREAM OF THE CITY OF PITTSBURGH, WESTERN PENNSYLVANIA, USA

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A Thesis
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Committee:
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Enrique Gomezdelcampo
Ray Larsen
ABSTRACT

Sheila Roberts, Advisor

Fecal bacteria loading in rivers surrounding Pittsburgh, Pennsylvania is a major concern. Fecal bacteria loading was thought to come from the city’s approximate 290 combined sewer overflows during heavy precipitation events, which overload the waste water infrastructure. However, water samples taken upstream of the city of Pittsburgh have high levels of fecal coliform, suggesting that fecal bacteria loading is occurring upstream of Pittsburgh. Twenty-four water samples were taken from the Allegheny River at Parker, Pennsylvania by the United States Geological Survey; nine exceeded the USEPA limit of 126 CFU / 100 mL of fecal coliform. Fourteen additional water samples were taken upstream and downstream of Oil City, Pennsylvania on the Allegheny River. The Southside Marina was chosen for the downstream sample site, while the public boat ramp in President Township was chosen for the upstream sampling site. These fourteen samples were analyzed for *Escherichia coli* instead of fecal coliform following USEPA protocol. The fourteen samples were collected during a time span of seven weeks, and represent dry, mixed, and wet weather conditions. Nine of these samples violated the USEPA suggested standard of 126 CFU/100 mL for a single sample. Many samples that exceeded the USEPA limit correlated with heavy precipitation events either the day before or the day of the sampling, though not all samples that violated the USEPA limit correlated with heavy precipitation events. The President Township sampling demonstrates the importance to sample upstream of sites thought to be the source of bacteria to verify that the site is indeed the only source of pollution.

Keywords: *Escherichia coli*, Colilert-18, Fecal Coliform, CSOs, Allegheny River.
“A little knowledge is a dangerous thing. So is a lot.”

-- Albert Einstein
This thesis is dedicated to my fiancée Martina, who has always supported my intellectual and spiritual growth for the past three years. Furthermore, I would also like to dedicate this thesis to my family who supported my academic trials and tribulations throughout my life.
ACKNOWLEDGEMENTS

This thesis was spurred and supported by Ted Buckwalter of the United States Geological Survey. I am grateful for the technical and moral support Ted has given during the process of this research. Additionally, I would like to thank Don Williams, Jaime McCoy, Ray Siwicki, and the rest of the Pittsburgh Project Office. Without their training and expertise this thesis would never have been started, nor would my interest in hydrology have been sparked.

I am also grateful to my committee, Drs. Sheila Roberts, Enrique Gomezdelcampo, and Ray Larsen for their tedious work they put into making this thesis. I would also like to thank Bowling Green State University and the Geological Society of America for their financial support through the Katzner and Graduate Research Grants respectively. Furthermore, I would like to thank my peers for their input and comments through the thesis process and to all of those that I did not mention thank you from the bottom of my heart.
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INTRODUCTION

Many rivers and tributaries in the United States and around the world are polluted by fecal bacteria loading. The United States Environmental Protection Agency (USEPA) has standards limiting the amount of fecal bacteria that can be present in rivers. Chicago for example was the first American city to experience drainage challenges, including problems with sanitation. In 1834, the city took the first of many steps to address the growing sanitation problem by digging a ditch down State Street which emptied into the Chicago River. In 1885, a large rainstorm caused sewage to inundate the city, and contaminated the Chicago River with raw sewage (Cain, 1978). Since that time, many rivers in the United States have had problems with bacterial pollution from sewage overflows.

In recent decades the federal funding for sewer systems has declined; however, the demand on these systems has increased. The USEPA estimates the current combined sewer overflows discharge approximately 1.2 trillion gallons of untreated sewage and stormwater each year throughout the United States. This problem arises because about 42 million people in 900 communities use the old combine sewer infrastructure in their wastewater municipalities. From 1999 through 2000 the city of San Diego had approximately 34 million gallons of raw sewage spilled from its municipal sewers. On a smaller scale Fort Pierce, Florida had roughly 8 million gallons of raw sewage released untreated, which affected the city’s source of drinking water (Whitman, 2000).

On Wednesday March 7, 2007 the House of Representatives approved a 1.7 billion dollar clean-water bill that would distribute the money to municipalities over a time span of five years. This bill is designed to modernize wastewater systems and
control sewage overflows due to aging infrastructure, which includes municipalities that still use the combined sewers. Rep. Dave Camp of Michigan stated that he supports the bill because in 2005 more than 1,000 sewer overflows occurred in the state, spilling more than 20 billion gallons of sewage and wastewater to the surrounding areas (AP, 2007).

In Detroit, the Jacobs Engineering Group was contracted by the city to build a structure intended to fix the combined sewer overflow problem by capturing the discharge from the 30 outfalls before they discharge to the Rouge River. This quick fix solution cost the city 73.5 million dollars (Landers, 2006). On a national level the estimated cost to fixing the municipal wastewater problems would cost the taxpayers an additional 12 billion dollars a year for a period of twenty years (Whitman, 2000).

It is important to note that before an expensive repair or infrastructure change is considered, sampling should occur upstream of the proposed infrastructure failure to ensure that the problem would be solved. The results from upstream sampling will verify if more than one site of point-source loading exists and if more than one infrastructure fix is required. This will ensure that all aging infrastructure repairs will be addressed to allow the river to meet USEPA standards.

The Allegheny River (Figure 1) begins in the upper Appalachian Mountains in northern Pennsylvania as a spring in a farm field, located just off of State Route 49 (USACE, 2005). The Allegheny River is the principal tributary of the Ohio River, and is approximately 325 miles long -- stretching through parts of New York and Western Pennsylvania. The Allegheny River flows southward across Pennsylvania passing southwestwardly by the cities of Warren, Oil City, and Franklin. South of Franklin, the river changes to a southeast flow in Clarion County (Rand McNally, 2006). Originally
the Allegheny River originated at the headwaters of what is now the Clarion River, but
due to glaciation the Allegheny River shifted into the Ohio-Mississippi system (Way,
1942).

The Allegheny River has historically been an important navigable river for
transportation of goods. However, recently it has also been heavily used for recreational
purposes. In 2005 the city of Pittsburgh, and the three rivers (Allegheny, Monongahela,
and Ohio Rivers) played host to the 2005 Bassmaster Classic. The Bassmaster Classic
alone brought approximately 80,000 people to the waters of the Allegheny River (ESPN,
2005). The city of Pittsburgh and the three rivers also play host to the annual regatta,
which serves as the city’s annual 4th of July celebration. The regatta also attracts a large
recreational crowd to the river’s water for fun, family events.
Figure 1. Major cities with known combined sewer overflows on the Allegheny River.
The summer in Western Pennsylvania also brings thunderstorms and heavy precipitation. Much of this region also has aging wastewater infrastructure in the form of combined sewers. These combined sewers are sewer systems that carry both sewage and stormwater runoff (NRC, 2005). Under normal conditions these combined sewers are able to handle the flow of wastewater and transport it to the wastewater treatment plant (WWTP) for treatment prior to release. However, under wet weather conditions, 0.30 inches of precipitation in six hours time duration as defined by the Ohio River Valley Water Sanitation Commission (ORSANCO), the combined sewers fail (Fulton, 2004). This failure is designed in the infrastructure in order to protect the WWTP, and instead of transporting the wastewater and stormwater to the WWTP, wastewater is released directly into a waterway, river, or stream, untreated. This failure is known as a combined sewer overflow (CSO) and occurs during heavy storms (Figure 2).

Figure 2. An example of a combined sewer overflow flapper that is leaking untreated wastewater into Sawmill Run at Duquesne Heights.
The CSO is a type of point source pollution in which pollutants are discharged from a concentrated and recognizable source, in this case, the combined sewer outlet (Taebi, 2004). Additionally, separated sewers from a sanitary sewer system can overflow much like a combined sewer known as a sanitary sewer overflow (SSO) at any location upstream of a sewage treatment plant. According to the USEPA at least 40,000 SSO events occur each year in addition to the CSOs events (Sier, 2005).

The CSOs in Western Pennsylvania are currently releasing fecal bacteria into the Allegheny, Monongahela, and Ohio Rivers during wet weather events, which at times also play host to recreational users. Direct contact with fecal bacteria during recreational usage can cause gastrointestinal disease, and left untreated, even death (Viessman, 1985). Current studies are being conducted on the fecal bacteria indicators around the city of Pittsburgh. However, no current emphasis on studying fecal bacteria upstream of the city of Pittsburgh is being conducted. Preliminary data in Table 1, suggests that CSOs from cities upstream of Pittsburgh may also be contributing to the fecal bacterial load. This is due to the fact that fecal coliform levels exceeded the USEPA standard during some of the sampling times. Measurements of fecal coliform levels were done in colony forming units (CFU) per 100 mL. The reason that colony forming units are used instead of microscopic counts of all cells is due to the fact that CFUs measures viable or live cells that can grow into colonies. It is therefore important to study the Allegheny River above the city of Pittsburgh, PA and determine if CSOs outside of Pittsburgh, PA is contributing to the high level of fecal bacteria loading.
Table 1: WQN samples collected by the USGS on the Allegheny River at Parker with water quality parameters and fecal coliform data. The “e” before an instantaneous discharge represents an estimated value, because a true value could not be calculated. Fecal Coliform results with an asterisk show the days in which the sample had more than 200 CFU/100mL for fecal coliform (Siwicki, 2002, 2003, and 2004).

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<th>Instantaneous Discharge (CFS)</th>
<th>Fecal Coliform (CFU/100mL)</th>
<th>DO (mg/l)</th>
<th>pH</th>
<th>Specific Conductivity (µS/cm)</th>
<th>Temperature (°C)</th>
<th>BOD (mg/L)</th>
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</table>
BACKGROUND

The United States Geological Survey collected water samples from the Allegheny River at Parker, PA for the Water Quality Network (WQN) program. The WQN data was collected at this site from October 2001 through December 2004 on a monthly basis. During the sampling years 2002, 2003, and 2004, water samples were collected and tested for fecal coliform contamination. The USGS also has continuous stream gage records dating back to October 1932 for this location. The collected WQN fecal coliform data high level bacteria days (i.e. > 200 CFU/100mL) were compared to precipitation data from Clarion, Crawford, and Venango Counties. This was done using IFLOWS rain gage data in these counties, which would show if the fecal bacteria loading was related to a storm weather event. This comparison would show if a location above Pittsburgh, Pennsylvania could be contributing non-point or point source pollution of the bacteria loading during wet weather events. The comparison confirmed that either point source or non-point source pollution was occurring upstream of Pittsburgh, Pennsylvania.

The Allegheny County Sanitary Authority, ALCOSAN, is the wastewater treatment municipality for Allegheny County, which includes the city of Pittsburgh. ALCOSAN currently estimates a three billion dollar project to replace the aging 1950s infrastructure, which would address the current Combined Sewer Overflows (CSO) problem (Hutchinson, 2001). During normal operation the combined sewer system takes both raw sewage and stormwater runoff to a waste treatment facility. However, during a wet weather event the combined sewer system is often stressed due to the increased load of the stormwater runoff. In order to protect the waste treatment facility the municipality
either goes into bypass mode or the unprocessed mixture of domestic waste and stormwater is discharged directly into streams (National Research Council, 2005).

With approximately 290 CSOs, the city of Pittsburgh is thought to be the primary contributor of fecal coliform loading to the three rivers (Allegheny, Monongahela, and Ohio Rivers). However, samples collected on the Allegheny River at Parker, upstream of the city of Pittsburgh, show that fecal coliform loading is occurring above Pittsburgh, and additional sources of fecal coliform loading should be considered.

Fecal coliform assaying or testing has been around since 1904 when it was first used to detect the presence of fecal contamination in foods. Many states now use fecal coliform assays to test water quality of water in lakes, beaches, and streams to not only identify the presence of fecal contamination, but to measure the level or amount of fecal loading occurring using the most probable number (MPN) method. When conducting a fecal coliform assay the fecal coliform, a gram-negative bacillus, is fed a lactose rich medium. The fecal coliform ferments the lactose with acid, and a gas production results at a temperature of $44 \pm 0.5$°C in roughly 48 hours. Thus one would be able to count specific amounts of fecal coliform colonies through the use of a microscope and grid to assay how much fecal contamination is present. However, several other genera of bacteria could also be picked up in the fecal coliform assay (Paraska, 2000.) These other genera of bacteria are non-fecal in origin, and would give a false-positive reading in the fecal coliform assay. *Klebsiella, Enterobacter,* and *Citobacter* are all bacteria species that can grow in water, food, and waste but are not fecal in origin.

For this study, *Escherichia coli (E. coli)* was used as the fecal bacteria indicator as it is not affected by the other bacteria genera when assayed (Doyle, 2006). Other studies
found that expected values of *E. coli* in stormwater is between 1,000 and 100,000 CFU/100 mL (Marksalek, 2004). *E. coli* reside in the human intestinal tract and other warm-blooded animals, and are excreted in large numbers in feces. Typically the average feces contains approximately 50 million coliforms per gram, and these coliforms can contaminate waterways when loaded into the system. Wastewater that is untreated can contain more than 3 million CFU/100 mL, and can contain other bacteria and viruses, which can cause enteric diseases in humans. Thus water that has been contaminated by untreated wastewater is identified as being potentially dangerous (Viessman, 1985).

All indicator bacteria used to study fecal bacteria loading suffer from die-off rates. The die-off rates of these indicator bacteria are vital to understanding the survivability and use of these microorganisms when testing sewage polluted water. The indicator bacteria die-off rates can be affected by many factors such as depth, temperature, pH, conductivity, dissolved oxygen, and turbidity. In a study conducted by Easton, *et. al* (1999) in which the die-off rates of *E. coli*, fecal coliform, and Enterococci were measured, the die-off rate for *E. coli* was found to be roughly 0.331% each day. Although, it should be noted that the die-off rate is not a linear response and the dir-off for the first few days is substantially greater than subsequent days. *E. coli* concentrations for the experiment started out at 1.0E+08 cells/mL, and after twenty days concentrations of *E. coli* were 1.5E+03 cells/mL (Easton *et. al*, 1999).

**Previous Work**

Water samples were collected from the Allegheny River at Parker by the USGS as part of the WQN program. The bacteria samples collected for the WQN program from Parker were obtained using a DH-95 sampler in combination with a bridge crane. Each
sample was collected in a one liter Nalgene bottle that had been triple-rinsed with river water prior to sampling, according to WQN sampling protocol (Noonan, 2005). A single vertical isokinetic sample located in the approximate center of the river channel was used to collect the bacteria sample. The sample was then shipped overnight to be analyzed at the Pennsylvania Department of Environmental Protection (DEP) Harrisburg Lab. The average hold time before incubation of the bacteria was twenty-four hours (Noonan, 2005).

The fecal coliform data collected by the WQN program were not a true representation of fecal coliform concentration due to the fact that the samples were held for at least twenty-four hours before they were incubated. During this time, die off of the fecal coliform was probably occurring in the sample. Thus, the indicated fecal coliform numbers may actually be higher than the reported values on all of the collected samples (Fulton, 2004). To avoid the loss of true fecal coliform concentrations, the USEPA recommends a maximum holding time of six hours (USEPA, 1976). The bacteria samples collected on the Allegheny River at Parker can be seen in Table 1.

According to USEPA standards, fecal coliform bacteria must be under 200 CFU / 100 ml during the summer and under 2000 CFU/100 ml during winter months (USEPA, 1976). The United States Environmental Protection Agency holds the summer months to stricter standards because these are also the peak recreational months, and it is more likely an individual will come into contact with the bacteria. Out of the twenty-four times sampled between the years of 2001 and 2004, nine samples were above 200 CFU / 100 ml, and six of the nine samples violated USEPA standards (Table 1).
Samples can be collected during wet weather and dry weather events to determine background level of fecal bacteria (dry weather) and whether point source pollution is occurring (wet weather.) During wet weather sampling, a period of forty-eight hours of no precipitation must precede a storm that accumulates approximately a third of an inch of precipitation during a six hour time period. Dry weather sampling requires seventy-two hours of no precipitation preceding the sampling event (Fulton, 2004). The samples taken from the Allegheny River at Parker were for the WQN, and were not originally intended for the use of measuring fecal bacteria loading. Thus, when the samples were collected they did not follow the protocol for wet or dry weather sampling as established by ORSANCO.

In order to use the WQN data collected at Parker, the samples were correlated with precipitation data collected from the National Weather Service (National Weather Service, 2005). The precipitation data was collected using the Integrated Flood Observing and Warning System from the rain gages in Clarion, Crawford, and Venango counties (IFLOWS.) The precipitation data was averaged to compensate for any inaccuracies, such as a clogged rain gage.

Each county in the watersheds that drain into the Allegheny River upstream of Parker (Clarion, Crawford and Venango) had rain gages to collect precipitation data. Clarion County had eight rain gages, Crawford County had nine rain gages, and Venango County had eight rain gages. The precipitation from each of the gages were summed and a mean for the daily precipitation for each county was obtained. The results can be seen graphically in Figures 3, 4, and 5 for each county. They are listed as events, in which
event one represents the first instance of fecal coliform levels that violated USEPA standards, and continues numerically in a time sequential order.

The results show that most of the recorded high fecal coliform levels occurred during wet weather events. These violations of the USEPA standards for fecal bacteria along with the precipitation values over the required 0.3 inches either the day of or day before the WQN water sample suggests a link between wet weather events and fecal bacteria loading of the Allegheny River. Events for Clarion County such as event one and event three had high precipitation values recorded after that of the sampling, and most likely do not represent point source pollution triggered by wet weather events.
Figure 3. Precipitation data for Clarion County showing precipitation values two days previous, and two days after each WQN sampling event (National Weather Service, 2005.)
Figure 4. Precipitation data for Crawford County showing precipitation values two days previous, and two days after each WQN sampling event (National Weather Service, 2005.)
Figure 5. Precipitation data for Venango County showing precipitation values two days previous, and two days after each WQN sampling event (National Weather Service, 2005.)
The precipitation data shows that accumulation of rainfall for most events were substantial enough to trigger a wet weather event, which could have caused CSOs and lead to the fecal bacteria loading in the Allegheny River. Looking at the fecal coliform results, it is apparent that either non-point source loading, point source loading, or both exist when triggered by wet weather events. However, the severity of the loading is not apparent. This is due to the fact that fecal coliform data had a holding time of 24 hours, which would allow for bacteria die-off and lower the actual count of fecal coliform. Though it is apparent that the wet weather events in Clarion, Crawford, and Venango counties correspond with high fecal coliform concentrations
SITES

Two sampling sites were selected for this study to target point-source pollution of
gastrointestinal bacteria in the Allegheny River. Upstream and downstream sampling sites for the
suspected source are needed to confine the area where point-source pollution can be
detected. By limiting the distance between the two sampling sites the amount of non-
point source pollution detected is reduced. Thus Oil City was selected as the downstream
site, while President Township was selected as the upstream sampling site.

Oil City is located in Venango County in the western portion of Pennsylvania, and
had a population of 11,504 people in the year 2000 (Census, 2000). Only 4,762 homes
are occupied in Oil City, with an average household size of 2.37 people and a median
household income of 29,060 dollars (U.S. Census, 2000). Oil City was founded in 1860,
and was previously known as Cornplanter, in honor of an old Seneca Indian chief (Way,
1942).

President Township is also located in Venango County, and has a population of
543 people. The average household size of President Township is 2.25 people with a
median income of 26,172 dollars, well below the national average of 41,994 dollars
(Census, 2000). President Township also has no known combined sewer outlets making
sure that all point-source pollution would be coming from Oil City’s combined sewer
oulets.

Water samples were collected from the right bank of the Allegheny River at both Oil City
and President Township. Sampling locations are marked for both locations (Figure 6).
The first sample collected was the sample downstream of Oil City, which was collected
at the Southside Marina. The Southside Marina locality was recorded using GPS, and its
coordinates were N41.42327° and W079.72427° using the NAD83 datum. The
Southside Marina contains one boat ramp for public recreational purposes. The river
substrate in the area consists of clay and silt sized mud with cobble sized rocks
sporadically covering the riverbed.

The second water sample was collected upstream of Oil City in President
Township. The recorded GPS locality of this site was N41.45578° and W079.55886°
again using the NAD83 datum. This site was also a boat ramp provided by the city to
allow access to the Allegheny River for public recreational purpose. The river substrate
off the President Township boat ramp consists of gravel to cobble sized gravel. The boat
ramp here provided access to the right bank of the Allegheny River.

The Allegheny River has two ice control structures located between the sampling
sites of Oil City and President Township, which were installed in 1983 by the Army
Corps of Engineers. The control structures are 20 feet long steel boxes, which transects
the river to help control the ice buildup of frozen ice at the confluences of Oil Creek and
the Allegheny River. These structures only affect ice buildup in the river, and did not
affect any results in this study.
Figure 6. A contour map made from the USGS seamless dataset of the surrounding area of Oil City with both the upstream and downstream sites labeled. The downstream site is labeled as Oil City and the upstream site is labeled as President Township for the representative cities where they were collected (USGS, 2006).
The land use for the Oil City and President Township study area varies from high intensity developed regions to barren land. The land use for this area can be seen in Figure 7. Of all the land uses in the surrounding area the type of most interest was that classified as pasture/hay. The pasture/hay classification is areas of grasses, legumes, or grass-legume mixtures planted for livestock grazing or the production of seed or hay crops.
Figure 7. Land use dataset for the Oil City and President Township area, in which the amount of pasture/hay area to ensure non-point source pollution from livestock was not casing elevated levels of \textit{E. coli} (USEPA, 2006).
METHODOLOGY

Sample Collection

Two sample sites were selected to determine Oil City’s contribution of fecal bacteria loading due to combined sewer overflows (CSOs) from wet weather events. The downstream site was selected by determining accessibility to the Allegheny River, ability to monitor the effects of the CSOs, and placement above the Waste Water Treatment Plant (WWTP) to ensure fecal loading from any bypass events were not occurring. The upstream sampling site was selected to be far away from the CSOs of Oil City but in the same drainage basin to experience the same wet weather events.

The sampling conducted during this study followed USEPA protocol, and USGS sampling methods. All samples were collected using a DH-81a sampler, with sterilized 1 liter Nalgene bottles. Both the upstream and downstream samples were taken on the right bank of the Allegheny River, and were equal depth integrated (EDI) samples (Figure 8). An EDI sample collects an equal amount of water from the complete water column to ensure a representative profile of the river. Each EDI sample collected was taken at least five feet from the bank in the stream current in order to have a well-mixed water sample. Furthermore, the downstream sample was collected first in order to not positively skew the possible effects of the CSOs of Oil City. After collecting a water sampler the nozzle to the DH-81a sampler could not be sterilized in the field, which would allow for bacteria to contaminate the next water sample. By collecting the upstream sample last it would allow for the sample to be positively skewed if any additional bacteria on the nozzle raised the amount present in the second sample. The nozzle allows for water to flow into
the 1 liter Nalgene sample bottles, and thus would allow the collected bacteria into the water sample.

After the sample were collected it was immediately transferred to a sterilized 120 mL sample bottle for storage and laboratory analysis. Upon completion of sampling the DH-81a sampler and bottles were cleaned using an antibacterial soap rinse, and a 30 minute soak in dilute bleach. This is done to kill any fecal bacteria prior to starting the next sampling collection. Additionally, the sampler and sample bottles were rinsed three times with water to remove any bleach from the sampler and collection bottles so the fecal bacteria were not killed during the collection of succeeding samples from the residual bleach.

Figure 8. Collection of a downstream water sample with a DH-81a sampler to obtain an equal depth integrated (EDI) sample during a low flow, dry weather period at the Southside Marina in Oil City, PA.
Lab Analysis

After both the Oil City and President Township samples were collected they were transported to Free-Col Laboratories in Meadville, PA. The samples were held for a maximum of two hours, which more than adequately fulfills the USEPA’s six hour holding time limit. When Free-Col laboratories took the samples they were immediately put into a sample holder, which is held at a constant temperature of 4°C until they were analyzed. All samples were analyzed after a maximum of seven hours after collection using the Colilert-18 test.

The Colilert-18 test is a chromogenic substrate test, and is currently the fastest USEPA-approved test for both total coliforms and \textit{E. coli}. It is a commercially developed liquid medium by IDEXX laboratories Inc. allowing for the simultaneous detection of total coliform and \textit{E. coli} (Cole, 2005). The Colilert-18 test can detect \textit{E. coli} in 18-22 hours through an optimized nutrient formula (MUG), and a water bath that is initially used to bring the water samples rapidly to 35°C, which is the incubation temperature. The water samples were transported in a sterile 120 mL sample bottle containing 10 mg of sodium thiosulfate. The thiosulfate neutralizes any residual chlorine from the cleaning of the DH-81a sampling bottles, and staves off the continuation of bactericidal action during the transportation from the sampling sites to the laboratory.

The additional water above the 100 mL line in the sample container is pipetted out into a small beaker prior to analysis of \textit{E. coli}. This water is then tested for residual chlorine using 0.16 grams of 4,5-Dihydroxy-2,3-pentanedione (DPD) free chlorine reagent. If this water turns red to pink within one minute after adding the DPD free reagent then the whole sample is discarded as the chlorine will have killed off some or all
the *E. coli* making the sample biased (Paraska, 2000). If the chlorine test comes back negative then 5 mg of 4-methylumbelliferyl-β-D-glucuronide is added to the 100 mL water sample and the water sample is sealed and shaken until the reagent powder is dissolved (BAM, 1998). The sample is then incubated in a water bath, which is regulated at 35±0.5°C for twenty minutes. Following the water bath incubation, the sample bottle is dried off, and the sample/reagent mixture is placed into a Quanti-tray 2000. The tray filled with the sample/reagent solution is then sealed, and placed in a regular incubator at 35±0.5°C for 18 hours.

The Quanti-tray 2000 has wells in which the sample/reagent mixture is placed. After the 18 hours in the incubator, the wells in the Quanti-tray 2000 that test positive for *E. coli* are counted. A positive for *E. coli* is a yellow-colored, fluorescent well (Table 2).

<table>
<thead>
<tr>
<th>Quanti-tray 2000 Appearance</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorless to Slight Yellow</td>
<td>Negative for Total Coliforms</td>
</tr>
<tr>
<td>Yellow Color and No Fluorescence</td>
<td>Total Coliform Positive and <em>E. coli</em> Negative</td>
</tr>
<tr>
<td>Yellow Color and Fluorescence</td>
<td>Total Coliform Positive and <em>E. coli</em> Positive</td>
</tr>
</tbody>
</table>

The most probable number (MPN) to determine the amount of *E. coli* present in the water sample is calculated by counting the wells that are positive and referring to the reference card provided with the Quanti-tray 2000. A blank control is also processed at the same time of the water sample. The blank control is a 100 mL sample of sterile reagent water, to assure quality control of the Colilert-18 test (Paraska, 2000).

The MPN method is a statistical model in which a serial dilution is applied. The minimum amount of dilutions required for the MPN method is three with 3, 5, or 10 tubes per dilution. Since the MPN method is used for the detection of bacteria all the dilutions are incubated, and after the end of incubation the pattern of positive and negatives for the
tubes are recorded. The observed pattern is then compared to a standardized MPN table used to generate the MPN of bacteria in the original water sample.

Using the IDEXX Quanti-Tray 2000 the same MPN method can be conducted; however, instead of the traditional 15-tube serial dilutions at: 1:1, 1:10, and 1:100 sample to dilutent, no dilution is necessary. This is due to the fact that the Quanti-Tray 2000 is equipped with wells divided into 97 wells of two different volumes. These wells allow for the same statistical process of the MPN 15-tube serial dilution method, and have a 95% confidence limit (IDEXX, 2000).

**Precipitation Analysis**

To ensure that the bacteria samples were correlated to wet weather events past precipitation values they were analyzed spatially using archived IFLOWS precipitation data and ArcGIS v9.1. The IFLOWS archive stores tipping-bucket rain gages data at known fixed locations (Figure 9). Each tipping-bucket rain gage transmits the amount of precipitation collected at hourly intervals to the National Weather Service. Data is displayed in a webpage format, and can be copied and pasted into Microsoft Notepad where unwanted information can be deleted, such as precipitation for time periods not desired.
Figure 9. Locations of tipping-bucket rain gages in the IFLOWS network used to reconstruct precipitation values for sampling events.
The Notepad document is then saved using the date as the filename, and imported into a Microsoft Excel® spreadsheet. All cells are then sorted by site ID number, and copied into another spreadsheet that contains the site ID with location values latitude and longitude, for each site. For this study, tipping-bucket rain gage data with non reporting information, represented by a hyphen, was deleted from the Excel spreadsheet to ensure that the null value did not affect interpolation of the data in ArcGIS. Once the completed Excel spreadsheet is created for a date it is saved as a Microsoft Dbase IV file.

ArcCatalog was used to create the shapefile used in the precipitation analysis by using the counties shapefile. After both shapefiles were imported into the ArcGIS project the precipitation database was imported into the project, and then displayed as an (X,Y) dataset. That dataset was then selected and the 3D Analyst tool was used to interpolate the data to raster format using the Inverse Distance Weighted (IDW) method. The IDW method was used for precipitation reconstruction to attenuate the influence of distant rain gages. This would allow for a better reconstruction of the storm intensity over a particular area.

The settings for the IDW method were the same for each date analyzed in which the input points were the precipitation databases from IFLOWS, the Z value was the precipitation value, the power was 2, and the search radius was set to 12 points. After processing, the IDW raster was then classified under symbology to separate the precipitation ranges using jenks (natural breaks). Jenks were used because they best represent the strength of intensity of the past precipitation events in localized areas, which allowed for the best determination of the past precipitation in the small sampling area.
Finally, the two sampling locations were plotted using the collected GPS points to show the area of interest, affected by the precipitation. The result of this analysis led to classification of the samples as dry, wet, or mixed weather events, based upon precipitation values 48 to 72 hours prior to sampling and during sampling. Additionally, through the comparison of the storm intensity at the two sampling sites one can distinguish if non-point source pollution is occurring in the upstream sample. If rain intensity was drastically higher in the upstream sampling site than the downstream sampling site, and *E. coli* counts were higher upstream this could be explained as non-point source pollution. Alternatively, the increased precipitation could flush fecal bacteria of local wildlife from the upstream area, while leaving the downstream sampling site unaffected.

**Statistical Analysis**

Minitab 14.1 was used to run regression analysis on precipitation and *E. coli* for both Oil City and President Township. Regression analysis was run on the wet weather samples to show correlation between wet weather events (precipitation) and *E. coli* counts. The regression analysis was first ran with the September 5, 2006 value as reported by Free-Col Laboratories, and then ran with the September 5, 2006 values switched. The last regression model ran through Minitab excluded the September 5, 2006 data. The three regression models created were used to help determine the correlation between the precipitation and *E. coli* for each sampling event.
RESULTS

Using the afore-mentioned methodology, fourteen EDI water samples from the Allegheny River were collected and analyzed (Table 3). The results were reported by Free-Col Laboratory within one week of sampling prior to the next sampling date (Figure 10). All Free-Col Laboratory reports can be viewed in Appendix A.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Client's Sample ID</th>
<th>Allegheny River or City</th>
<th>Date Sampled:</th>
<th>Time Sampled:</th>
<th>Analyte</th>
<th>Result (cfu/100ml)</th>
<th>Date Analyzed</th>
<th>Start Time</th>
<th>Analyst</th>
<th>Method Source</th>
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</thead>
<tbody>
<tr>
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<td></td>
<td>Allegheny River or Oil City</td>
<td>8/8/2006</td>
<td>11:02</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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</table>

Figure 10. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site.
The *E. coli* was reported using the MPN method, and counted using colony forming units (CFU) per 100 milliliters of water sampled, which can be seen for each sample in Table 3.

Table 3 shows the most probable number (MPN) in CFU/100 mL, as well as the average daily discharge for each sample. A graphical representation of the MPN of *E. coli* for both Oil City and President Township can be seen in Figure 11. Additionally, a graphical representation of the MPN of *E. coli* for both Oil City and President Township with respect to discharge can be seen in Figure 10 to show how *E. coli* counts respond to changes in discharge. Using both the MPN and discharge data, one can find the estimated *E. coli* load for the Allegheny River at both Oil City and President Township using the following equation:

\[
X_{\text{CFU Day}} = \frac{\text{MPN (E. Coli)}}{100 \text{ mL}} \times \frac{1000 \text{ mL}}{1 \text{ Liter}} \times \frac{28.32 \text{ Liters}}{1 \text{ ft}^3} \times \frac{\text{Discharge (ft}^3/\text{s})}{1 \text{ second}} \times \frac{86,400 \text{ seconds}}{1 \text{ Day}}
\]

The results for the estimated *E. coli* load or discharge for each sampling date can also be seen in Table 3.
Table 3. *E. coli* MPN counts for both the upstream and downstream sampling sites along with the daily discharge of the Allegheny River taken at Parker, PA. Additionally the predicted daily load of *E. coli* for the Allegheny River.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Date</th>
<th>Sample Time</th>
<th>Location</th>
<th>E. Coli (CFU/100 mL)</th>
<th>Discharge (cfs)</th>
<th>Daily Load of <em>E. coli</em> (CFU)</th>
</tr>
</thead>
<tbody>
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<td>8/8/2006</td>
<td>11:02 AM</td>
<td>Oil City, PA</td>
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<td>6040</td>
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</tr>
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<td>11:46 AM</td>
<td>President Township, PA</td>
<td>34</td>
<td>6040</td>
<td>5.0E+14</td>
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<td>Oil City, PA</td>
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<td>3740</td>
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<td>President Township, PA</td>
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<td>3740</td>
<td>1.8E+15</td>
</tr>
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<td>8/22/2006</td>
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<td>Oil City, PA</td>
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<td>3.0E+16</td>
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<td>10:51 AM</td>
<td>President Township, PA</td>
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<td>9.7E+15</td>
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<td>9/12/2006</td>
<td>10:25 AM</td>
<td>President Township, PA</td>
<td>33</td>
<td>10400</td>
<td>8.4E+14</td>
</tr>
<tr>
<td>13</td>
<td>9/19/2006</td>
<td>8:58 AM</td>
<td>Oil City, PA</td>
<td>435</td>
<td>11800</td>
<td>1.3E+16</td>
</tr>
<tr>
<td>14</td>
<td>9/19/2006</td>
<td>9:25 AM</td>
<td>President Township, PA</td>
<td>93</td>
<td>11800</td>
<td>2.7E+15</td>
</tr>
</tbody>
</table>
Figure 11. Relative levels of *E. coli* for both the upstream (President Township) and downstream (Oil City) sampling sites with respect to discharge for each sampling event. Note that the chart is interpolating both discharge and *E. coli* results for the time period between the sampling dates.
Additionally, the *E. coli* load or discharge for each sampling date was compared against precipitation using the above mentioned methodology in ArcGIS to verify if the sample collected was dry, mixed, or wet weather sample. Figure 12 shows the precipitation of the two sample sites along with the surrounding area of Western Pennsylvania. All of the ArcGIS precipitation maps generated can be seen in Appendix B.
Figure 12. An example of reconstructed precipitation values in inches created using IDW interpolation of precipitation values recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 29, 2006.
The three regression models ran through Minitab showed produced regression lines, which demonstrates the response of *E. coli* counts to precipitation. Each the response can be seen in the regression line in which Model 1 has the September 5, 2006 data as reported, Model 2 switches the September 5, 2006 data, and Model 3 excludes the datapoint, Table 4. The plots of the regression line for each model can be seen in Appendix C.

Table 4. The response of *E. coli* for each sampling site as a function of precipitation in which the regression model used the least square regression method.

<table>
<thead>
<tr>
<th>Regression</th>
<th>Location</th>
<th>Regression Line</th>
<th>Squared Correlation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>Oil City</td>
<td><em>E. coli</em>=80+833*(in.)</td>
<td>68.3</td>
</tr>
<tr>
<td>Model 1</td>
<td>President Township</td>
<td><em>E. coli</em>=388-99.9*(in.)</td>
<td>3.9</td>
</tr>
<tr>
<td>Model 2</td>
<td>Oil City</td>
<td><em>E. coli</em>=355+669*(in.)</td>
<td>41.9</td>
</tr>
<tr>
<td>Model 2</td>
<td>President Township</td>
<td><em>E. coli</em>=124+35.6*(in.)</td>
<td>5.6</td>
</tr>
<tr>
<td>Model 3</td>
<td>Oil City</td>
<td><em>E. coli</em>=22+868*(in.)</td>
<td>68.1</td>
</tr>
<tr>
<td>Model 3</td>
<td>President Township</td>
<td><em>E. coli</em>=67.4+64.8*(in.)</td>
<td>21.2</td>
</tr>
</tbody>
</table>
DISCUSSION

The ArcGIS precipitation maps were compared to field notes recorded during sampling to determine if the sample collected was under dry, mixed, or wet weather conditions. In doing so the only discrepancy was on the September 5, 2006 sample date. The precipitation maps show precipitation below the sample area, but not in Oil City or President Township. Observations during the sampling event recorded a steady light rainfall for more than one hour during the sampling and transportation phases between the two sample sites. Additionally, the ArcGIS maps of the reconstructed precipitation events for most sampling dates show localized anomalies in Butler County. The precipitation recorded at Butler County shows a strong repeating rainfall of approximately one inch in the southeastern corner of the county. These localized anomalies may have been caused by a faulty tipping-bucket rain gage or gages in that region.

Precipitation data collected in Butler County should not affect the reconstructed precipitation values for Oil City and President Township as it is significantly far enough away to be unaffected by the rain gage data for the interpolation would not use values near Butler County. However, for the purpose of this study I believe the definitions for dry, mixed, and wet weather events as defined by the Ohio River Valley Water Sanitation Commission (ORSANCO) fall short of precipitation values that can trigger CSOs in Oil City. Using the ORSANCO definitions for the reconstructed precipitation classifies events as mixed weather events either not causing a flush of the CSOs in Oil City, or a light flush based on $E.\ coli$ MPN results from sampling for this study. This can be seen in the August 8$^{th}$, 15$^{th}$, and 22$^{nd}$ sampling dates, in which August 8$^{th}$ and 22$^{nd}$ should be
classified as dry weather samples. The ORSANCO definitions would call these first three samples mixed weather conditions, when observations recorded no precipitation for the August 8\textsuperscript{th} or 22\textsuperscript{nd} dates, but light steady rain for the August 15\textsuperscript{th} sampling date.

The reconstructed precipitation maps aided in defining the sample type collected (dry, mixed, or wet weather) as well as gauging storm intensity. This ensured that precipitation was approximately equal for both the upstream and downstream sampling sites. Through the comparison of the storm intensity at the two sampling sites one can distinguish if non-point source pollution is occurring in the upstream sample. If rain intensity was drastically higher in the upstream sampling site than the downstream sampling site, and \textit{E. coli} counts were higher upstream this could be explained as non-point source pollution. Alternatively, the increased precipitation could flush fecal bacteria of local wildlife from the upstream area, while leaving the downstream sampling site unaffected.

September 5, 2006 is the only sampling date in which the President Township \textit{E. coli} sample was greater than that of Oil City’s. The reconstructed precipitation data for September 5, 2006 shows that both President Township and Oil City both received the same amount of light rain precipitation of 0.0-0.06 inches of rain on this day. Therefore, President Township’s high \textit{E. coli} counts could not represent non-point source pollution from local wildlife. Additionally, for September 4, 2006 the reconstructed precipitation values also show that President Township and Oil City had the same precipitation values of 0.0-0.17 inches of rain.

The first regression model used all wet weather events, and used the reconstructed precipitation values for both Oil City and President Township along with the measured \textit{E.
coli concentrations to determine if their was a correlation between precipitation and E. coli counts. The two sampling sites were ran independently to see if Oil City showed more of a correlation between wet weather events and E. coli counts than that of President Township. The regression line for Oil City in the first model was: \( E. coli = 80 + 833 \times (\text{inches of precipitation}) \) with a squared correlation of 68.3%, which explains 68.3% of the variation in the E. coli counts. The regression line for President Township was: \( E. coli = 388 - 100 \times (\text{inches of precipitation}) \) with a squared correlation of only 3.9%. This implies that there is a base of 388 CFU/100 mL in the Allegheny River at President Village, and that as precipitation falls the amount of E. coli decreases by 100 CFU/100 mL for every inch of precipitation. This negative association is unexpected for either non-point source or point-source fecal bacteria loading.

The second regression model switched the E. coli counts for Oil City and President Township collected on September 5, 2006, which changed the regression lines. This model showed the correlation between precipitation and E. coli counts if the reported values by Free-col Laboratories were switched by mistake, and to help explain more of the variation in the E. coli counts. The regression line for this model for Oil City became: \( E. coli = 355 + 699 \times (\text{inches of precipitation}) \) with a squared correlation of 41.9%. The base increased, and the association decreased, and the regression line explains only 41.9% of the variation in the E. coli counts, a decrease from model 1. President Township’s new regression line became: \( E. coli = 124 + 35.6 \times (\text{inches of precipitation}) \) with a squared correlation of 5.6%. The base count decreased and the association is now positive; however, the regression line does not explain much of the variation in the E. coli counts at only 5.6%.
If the data collected on September 5, 2006 is a statistical outlier, a computational run without the data would prove that September 5, 2006 is an outlier or erroneous point. The regression line for Oil City in this model became: \( E. coli = 22 + 868 \times \text{(inches of precipitation)} \) with a squared correlation of 68.1%. This squared correlation is not drastically different from the first regression model with the statistical outlier. The regression line for President Township became: \( E. coli = 67.4 + 64.8 \times \text{(inches of precipitation)} \) with a squared correlation of 21.2%. This regression line is the best fit out of all the models ran in Minitab for the variation of \( E. coli \) caused by precipitation in President Township.

Omitting the September 5, 2006 data gives the best representation of the association of precipitation and \( E. coli \). When comparing the two regression lines in which the data is excluded one can see that Oil City has a greater response (increase in \( E. coli \)) for every additional inch of precipitation.

The sampling methodology shows a strong correlation between wet weather events and fecal bacteria loading in the Allegheny River. For the sampling dates August 15, August 29, September 12, and September 19, 2006 precipitation values were all over 0.33 inches the definition of a wet weather event. For each one of these sampling dates the sample taken violated USEPA standards of 200 CFU/100 mL. Additionally, President Township was consistently lower than Oil City except for the September 5, 2006 sample. With the exception of one data set out of seven, President Township was consistently lower than that of Oil City. The only other problem in the sampling was on August 15 when both samples exceeded the maximum value of \( E. Coli \) colonies able to be counted because dilution was conducted on these samples. August 15 represented the
first sample collected where precipitation was observed. All other wet weather samples were analyzed after a one-tenth dilution was conducted on the sample. The mixed weather event exceeded the maximum MPN count (>200 CFU/100 mL) without dilution.

Of the fourteen samples taken on the Allegheny River, nine clearly violated the USEPA limit. The samples that did not violate the USEPA limit were either dry weather samples or samples from President Township during wet weather events. It is important to note that samples collected at Oil City during wet weather events always were in violation of the set USEPA limits. This leads to two possible explanations:

1. Wet weather events triggered the release of untreated wastewater via combined sewer overflows present in Oil City causing fecal contamination at higher levels than that of President Township in the Allegheny River.

2. Higher levels of *E. coli* measured in the Allegheny River at Oil City are the result of non-point source loading from wildlife in the area, and since there is a seventeen mile distance between the two sample locations fecal concentration is naturally going to increase.

Wildlife non-point source pollution was limited by having two close sampling locations, which were only 17.25 miles apart. This distance would restrict the amount of non-point source pollution, and not cause an average *E. coli* count twice that of President Township due to wildlife non-point source pollution. Additionally, the regression analysis for President Township showed no correlation between wet weather events and *E. coli* counts. The best explanation for the dramatic increase in fecal bacteria levels is that wet weather events triggered CSOs draining untreated wastewater into the Allegheny River.
The USGS conducted similar studies on the Allegheny River in the city of
Pittsburgh, PA. The USGS had two sample sites located around and within the city of
Pittsburgh, PA. The first sample site was located at Oakmont, while the second sampling
location was at the 9th Street Bridge. For each site the USGS measured concentrations of
fecal coliform, \textit{E. coli}, and Enterococci. The first sampling site, Oakmont, is the
upstream sampling site outside the city limits of Pittsburgh, while the 9th Street Bridge is
the downstream sampling site located approximately in the center of Pittsburgh.

For the Allegheny River at Oakmont 135 samples were tested for fecal coliform
while 136 samples were tested for \textit{E. coli} and Enterococci. The maximum values for
fecal coliform, \textit{E. coli}, and Enterococci were 5,570, 1,100, and 690 CFU/100 mL
respectfully. The median values for the fecal indicator bacteria (fecal coliform, \textit{E. coli},
and Enterococci) were 95, 58, and 15 CFU/100 mL. Additionally, if using \textit{E. coli} as a
standard in this study 13\% of the 136 samples violated the water quality standard of 126
CFU/100 mL.

The Allegheny River at the 9th Street Bridge sampling site, located in the center of
Pittsburgh, was also tested for the same three fecal bacteria indicators. The numbers of
samples were 143 samples for fecal coliform and Enterococci, and 141 samples for \textit{E. coli}. The maximum values for fecal coliform, \textit{E. coli}, and Enterococci were 22,000
7,900, and 995 CFU/100 mL, while the median values were 300, 150, and 30 respectively
(Buckwalter, 2006).

By comparing these two locations to that of Oil City one can see that even though
the maximum \textit{E. Coli} counts may have been greater in Pittsburgh (7,900 CFU/100 mL)
than that of Oil City (>2420 CFU/100 mL) the affects of the fecal bacteria loading in Oil City should not be overlooked.
CONCLUSION

This research demonstrated that even if the combined sewers were replaced in the city of Pittsburgh at an estimated cost of three billion dollars, the Allegheny River would still violate USEPA standards of *E. coli* during the summer months due to point source fecal loading upstream.

Although high coliform values are detected in Pittsburgh, Pennsylvania they are not the only source of contamination leading to the fecal bacteria loading of the Allegheny River. Fecal bacteria indicators collected above the city of Pittsburgh, PA show that during wet weather events fecal loading is occurring upstream of Pittsburgh in the Allegheny River. Samples taken at Oil City and President Township show that the fecal bacteria loading is mainly attributed to the point-source pollution from Oil City’s CSOs as the samples collected at Oil City were at least twice as high as those collected at President Township. Since President Township is consistently lower than that of Oil City it can be inferred that point-source pollution from CSOs starts at Oil City, while the lower levels of fecal bacteria upstream is likely from non-point source loading from local wildlife. This is strengthened with the comparison of *E. coli* counts versus discharge. In Figure 11, one can visually see that with an increase of discharge the *E. coli* counts follow the general increasing trend more closely than that of President Township.

Additionally, when regression analysis was conducted on *E. coli* as a function of precipitation it showed that a stronger correlation was observed for Oil City than that of President Township. The regression analysis was the strongest when the September 5, 2006 sample was not included in the dataset, showing that this was an erroneous data point. This was strongly seen when the data was included resulted in President Township
having a negative association between *E. coli* and precipitation. This would not be the case for either non-point source loading or point-source loading of fecal bacteria.

Similar studies should be warranted and conducted in other cities that contain combined sewers upstream of Pittsburgh as they might be contributing as well. This study also demonstrates the importance of sampling upstream of known point source pollution sites to ensure that it is the sole contributor of a problem at any location. This will allow for the best possible solution design as it will encompass the whole point-source pollution problem. Additionally, it will allow for the best cost to benefit solution to be designed and implemented for the municipality’s service area.
FUTURE INVESTIGATIONS

In order to determine if any other CSOs on the Allegheny River are contributing to the loading of fecal bacteria more studies should be conducted in a similar manner to this study. Cities such as Warren, Franklin, Parker, and Titusville have known CSOs, which can attribute to point source fecal loading of the Allegheny River during wet weather events.

A similar study could be conducted to establish if point source pollution is occurring at these locations. Furthermore, similar studies would show the magnitude of CSOs loading from wet weather events, and that will allow comparison between the cities. By comparing the cities one can establish which systems need to be treated or corrected first in order for the Allegheny River to meet USEPA standards for *E. coli* during wet weather events.

Additionally, the whole Allegheny River should be studied for a singular wet weather event to see the combined effects of the CSOs from each city. This could be done by either using multiple sampling teams or through the use of an automated sampler with a portable lab for *E. coli* analysis.
REFERENCES


USEPA. 2006. “Multi-Resolution Land Characteristics Consortium: National Land
Cover Data.” Washington, DC.


Appendix A
Figure 1. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site for the August 8, 2006 sampling.
Figure 2. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site for the August 15, 2006 sampling.
Figure 3. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site for the August 22, 2006 sampling.
Figure 4. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site for the August 29, 2006 sampling.
Figure 5. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site for the September 5, 2006 sampling.
Figure 6. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site for the September 12, 2006 sampling.
Figure 7. A summary report generated by Free-Col Laboratories showing results of *E. coli* using the MPN method for each sampling site for the September 19, 2006 sampling.
Appendix B
Figure 1. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 6, 2006.
Figure 2. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 7, 2006.
Figure 3. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 8, 2006.
Figure 4. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 14, 2006.
Figure 5. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 15, 2006.
Figure 6. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 20, 2006.
Figure 7. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 21, 2006.
Figure 8. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 22, 2006.
Figure 9. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for August 28, 2006.
Figure 10. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFOWS tipping-bucket rain gages in Western Pennsylvania for August 29, 2006.
Figure 11. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for September 4, 2006.
Figure 12. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for September 5, 2006.
Figure 13. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for September 11, 2006
Figure 14. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for September 12, 2006
Figure 15. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for September 18, 2006
Figure 16. Reconstructed precipitation values created using IDW interpolation of precipitation values (in.) recorded by the IFLOWS tipping-bucket rain gages in Western Pennsylvania for September 19, 2006.
Appendix C
Figure 1. The first regression model analyzed for correlation between precipitation and *E. coli* counts for Oil City, Pennsylvania.

Figure 2. The first regression model analyzed for correlation between precipitation and *E. coli* counts for President Township, Pennsylvania.
Figure 3. The second regression model analyzed for correlation between precipitation and *E. coli* counts for Oil City, Pennsylvania.

Figure 4. The second regression model analyzed for correlation between precipitation and *E. coli* counts for President Township, Pennsylvania.
Figure 5. The third regression model analyzed for correlation between precipitation and *E. coli* counts for Oil City, Pennsylvania.

Figure 6. The third regression model analyzed for correlation between precipitation and *E. coli* counts for President Township, Pennsylvania.