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Pepper Mild Mottle Virus as an Indicator of Fecal Pollution along an Urban Stretch of the
Chattahoochee River in Atlanta, GA, 2014

By

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B.S., Tuskegee University, 2012

Thesis Submitted to the Graduate Faculty of Georgia State University in Partial
Fulfillment of the Requirements for the Degree

MASTER OF PUBLIC HEALTH

ATLANTA, GEORGIA
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Abstract

The Chattahoochee River is an essential surface water source as it provides over 70 percent of Metro Atlanta's drinking water, amounting to over 300 million gallons. In addition to serving as Metro Atlanta's primary source of drinking water, the Chattahoochee River serves as a major point of discharge for industrial and municipal waste as well as urban runoff.

The primary goal of this study was to assess the presence of Pepper Mild Mottle Virus in the Chattahoochee River. During a five-month period in 2014, water samples were collected at fifteen sample sites and two outfall sites in the Chattahoochee River. PMMoV was tested for in 6 out of 17 samples. A one-way ANOVA analysis ($p < 0.05$), of concentrations across sampling locations resulted in a p-value of 0.044. As a result, it can be determined that the location of the sampling sites does result in a statistically significant difference in the PMMoV values observed. Furthermore, a one-way ANOVA analysis ($p < 0.05$), of concentrations across sampling dates resulted in a p-value of 0.063. Therefore, it is determined that the dates on which sampling took place did not result in a statistically significant difference in the PMMoV values observed across time. Furthermore, the MS2 virus was also detected in these samples. Through a paired t-test ($p < 0.05$), between the sample concentrations with and without MS2 presence, it was determined that there was no statistical difference in concentration of PMMoV when MS2 is present since $p = 0.0740$

The results indicate that PMMoV was present in the Chattahoochee River due to the detection of PMMoV in the samples collected. However, additional investigations, using a larger sample size, are needed to assess PMMoV as a viable indicator of fecal contamination of ambient surface waters and recreational waters.

Index Words: PMMoV, Chattahoochee River, MS2

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Darian Morgan

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Approval Page

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Chapter I INTRODUCTION

1.1 Background

The Chattahoochee River originates from the Blue Ridge Mountains of Georgia and flows southward into Florida, forming a natural border between Georgia and Alabama. The Chattahoochee River is the most heavily used water resource in Georgia (EPD, 1997). Furthermore, the Chattahoochee River is an essential surface water source for the metro Atlanta area as it provides over 70 percent of the city's drinking water, amounting to over 300 million gallons (EPD, 1997). In addition to serving as Metro Atlanta's primary source of drinking water, the Chattahoochee River acts as a major point of discharge for industrial and municipal waste as well as urban runoff. Due to Metropolitan Atlanta's dependence on the Chattahoochee River as both a source of drinking water and a site for waste discharge, both monitoring and maintaining the integrity of the river's surface water is of vital importance.

The city of Atlanta has undergone a steady rise in population beginning in the early 1990s and continuing through the 2000s. Recently, The City of Atlanta has averaged an annual growth of approximately 37,283 new residents since 2010. This recent trend is slower than the growth the city of Atlanta experienced in the 1990s and early 2000s. Overall, the region averaged more than 77,000 new residents each year between 1990 and

2010. With a consistently increasing stream of new residents each year, the amount of sewage flowing into the city and surrounding areas sewage systems began to grow as well. Despite the increase in economic vitality as a result of the city's population growth, the need for environmental overhauls on the Atlanta sewage system has grown more urgent. In the late 1880s, sanitary sewers were built for residents of the city but as expected have started to break and crack. An improved system was designed and installed to carry storm water and household waste. However, that system has degraded due to population growth. The sanitary sewer system becomes overburdened, leading to sanitary sewer overflow (SSO) events. During these events, a mixture of groundwater, untreated sewage, and stormwater overflow can find its way into streams and creeks through dilapidated pipes and manholes. (History, 2010; Perkins, 2014; and Smith, 2015). According to Clean Water Atlanta, in 1998 Atlanta entered into a Consent Decree. The consent decree included a directive to evaluate and implement short and long-term plans for eliminating water quality violations for the city of Atlanta. Currently, the City of Atlanta is under two consent decrees resulting from lawsuits against the City filed by several constituents including the Upper Chattahoochee Riverkeeper and later joined by EPA and EPD. Today the city is under a combined sewer overflow (CSO) remediation plan. (History, 2010)

As a result of multiple issues including sewage overflows, bacterial pollution of the waterways, and inadequate collection capacity, the city of Atlanta was sued for violating the Clean Water Act in 1995 (EPA, 1999). In response to the violations, the Federal

Clean Water Act, Safe Drinking Water Act, and the State Water Quality Control Act were enforced to protect the Chattahoochee River's water quality. This policy implemented the defining and monitoring of definitive water quality standards for the health of the public (EPD, 1997). The City of Atlanta and the Federal Government agreed to a monetary settlement and to take corrective action to bring the sewer system into compliance with the Clean Water Act and the Georgia Water Quality Control Act. A Consent Decree created the objective of eliminating future water quality violations from sanitary sewer overflows. This Consent Decree encompassed improving the cities' Water Reclamation Centers (WRCs), investigating sewer pipe conditions, as well as ending water quality violations resulting from CSOs. Specifically, one of the primary goals of the Consent Decree was to improve the current water quality conditions in the receiving waters downstream of the city, mainly the Chattahoochee River, by improving the water quality of the effluent discharging from the WRCs located around the city (Overview, 2010).

"The First Amended Consent Decree (FACD) authorizes the review of building permit applications that propose adding new flows into the sewer system, utilizes technology such as closed-circuit televisions to inspect and monitor the condition of drains, and controls and revises plans to operate the collection system more efficiently" (Overview, 2010). This program includes the division of the Greensferry and McDaniel CSO Basins and the Stockade Sub-basin (Custer CSO Basin). Dividing these basins will expand the

city's total separated area from 85% to about 90% while eliminating two CSO facilities. The FACD also calls for the construction of deep-rock tunnel storage and treatment systems that will capture and store combined stormwater. This stormwater will be treated at two CSO facilities, before being discharged into the Chattahoochee River. The city of Atlanta hopes the number of overflows is reduced from 60+ per year to only 4 per year at the four facilities that will remain after the changes have been implemented. Any remaining overflows will be screened, disinfected, and dechlorinated before being discharged to a receiving stream. These changes should allow for the water quality standards to be met.

The water quality of the Chattahoochee River varies from season to season, but currently, there is no National Park Service health advisory in effect. However, there are a few areas along the Chattahoochee that have been designated for monitoring or as a health advisory area, such as Chattahoochee River at Atlanta (Paces Ferry Rd) (Perlman., 2014). Consequently, the determination of what factors affect water quality, specifically in the Chattahoochee River, is vital to maintaining regulations and providing a clean environment. The previous management of the river placed great emphasis on point sources from municipal or industrial water pollution control facilities, but presently nonpoint sources of pollution through stormwater the Chattahoochee River (Smith, 2015). The continued rise in the population of Atlanta and the subsequent development of the watershed may lead to more stormwater runoff and nonpoint source loading as more

impervious surfaces prevent rainfall from infiltrating the ground, resulting in increased stormwater runoff, flooding, and stream bank erosion. Due to the importance of the Chattahoochee River, effective methods for monitoring the quality of river water are needed.

1.2 Purpose of the Study

Pathogenic plant viruses have been responsible for the lack of crop production around the world. The pepper mild mottle virus (PMMoV) is a plant pathogenic virus that has been found worldwide and grows specifically on species of field grown bell, hot, and ornamental pepper species. Previous studies have investigated the PMMoV's viability as an indicator of fecal contamination (Rosario, et al., 2009). Presently, the current bacterial indicators used for water monitoring does not always necessarily correlate with the presence of pathogens. The primary objective of this investigation was to assess the presence of PMMoV, a potential indicator of possible human fecal pollution in the Chattahoochee River. Studies on the presence of PMMoV in surface waters, such as rivers, and also as a fecal indicator are limited as of now. In previous investigations, PMMoV's presence has been detected in other marine environments such as surface seawater ponds, water from irrigated farmlands and rivers (Rosario et. al., 2009) (Kuroda et al., 2015). Environments, where PMMoV is found to be present, may indicate sources of pollution, such as wastewater discharges, in the Chattahoochee River, as well as reflect the impact of urbanization on the river and surface water. Although the Chattahoochee

River is a designated local, state, and federal waterway of interest, along with being a recreational waterway, no studies have been conducted to assess the presence of PMMoV and evaluate its contribution to the water quality of the Chattahoochee River and other surface waters.

1.3 Research Questions:

Are there any spatial or temporal variations in concentration of PMMoV along the Chattahoochee River?

Is there any correlation between the presence of bacteriophage MS2 and PMMoV concentrations?

Does the discharge of effluent from the Camp Creek Outfall and the Douglas County Outfall into the Chattahoochee River affect the concentration of PMMoV downstream?

Chapter II Review of Literature

2.1 Urbanization

The surface water of an area is essential to the life and vitality of its surrounding communities. Due to this fact, numerous studies have been conducted globally to examine the effects of urbanization on surface waters, and also to identify and develop trends that can be used in predicting contaminant concentration in that body of water. Water reaches human consumption through various pathways, usually beginning with collection from ground or surface water source, and then treated through several filtration methods at municipal treatment plants .After treatment, that water is supplied to the public for public consumption.

Several Studies have investigated the effects of urbanization on the water quality in a municipality. These studies have shown that urbanization has resulted in above average fecal coliform levels, non-point and point pollution, runoff, discharges and several other factors that alter the composition of water bodies (Oiste, 2014; Peters, 2009, Smith, 2015) Rivers are essential for agricultural production for numerous countries and municipalities. Additionally, rivers are the most vulnerable bodies of water to contamination due to domestic, industrial, and agricultural discharges. (Boyacioglu, 2010) Particularly in urban areas, it is quite difficult to monitor and to hold accountable

those who are responsible for making illegal discharges into the rivers. Without consistent monitoring, surface waters in high population density areas face a grim outlook. The Dianchi Lake Basin, located in Kunming City, China, is an area with a dense population and developed economy supported by an assortment of intensive human activities. With the predominantly high usage of water resources and minimal inflow of clean water, Dianchi Lake is presently facing a potential water crisis due to pollution. Several initiatives since 1986 focused on pollution control of the Dianchi Lake basin, however due to the heavy population burden and pollution loading these efforts have been unsuccessful. (Liu et al. 2015)

A study in 2003 focused on the effects of urbanization on stream water quality in the Metro Atlanta area. (Peters, 2009) The study concluded that urban development can change the natural flow and pathways of water bodies. Urbanization due to economic and industrial growth increases the potential for several adverse outcomes such as environmental land insecurity, poor air & water quality, noise pollution, and waste disposal difficulties (Uttara et al, 2012). Water quality especially deteriorates due to eutrophication and pollution, subsequently resulting in a loss of biodiversity and biotic homogenization. Climatic change, itself is affected by urbanization as temperatures increase due to the lack of cooling because of the increased construction of impervious surfaces (Yu, S. 2012, Pauchard, 2006, Tayan and Toros, 1997, Uttara, 2015). Despite the potentially harmful results of urbanization and industrial growth, many in the local

population benefit from the increased industrial opportunities. Industrial development provides such benefits as a potentially improved quality of life, transportation convenience through the building of highway infrastructure, new career opportunities, and access to resources that are not as easily attained by those living in rural communities (Sallis, 2009)

Urban stormwater systems that collect and convey runoff from impervious surfaces serve as a passageway for sewage originating from breaks in sanitary infrastructure. (Sauer, 2011). Storm water flow from an impervious surface can result in several adverse outcomes such as stream & habitat degradation; low base flows and increased toxic loadings from several nonpoint sources. (Thurston, 2003). This issue is attributed to the amount of impervious surfaces that do not allow water to seep into the ground, subsequently being filtered throughout layers of soil. Instead of sifting through the earth to become groundwater, precipitation instead becomes runoff eventually flowing down the watershed to the lowest point, making its way into a river via streams and creeks. (Smith, 2015) Contamination via the discharging of sewage into surface waters is a major human health concern. Additionally, proper protection of urban watersheds is even more vital as human population expands. According to a study published in 2009 by DiDonato et al., microorganisms were sampled from creeks representing, forested, suburban, and urban watersheds for indicators of water quality. The investigators, found these microorganisms to have the highest concentrations in stream headwaters and more

developed watersheds. (DiDonato et al., 2009; Perkins, 2014). This result displays the strong correlation for increased contamination among urban watersheds in comparison to other bodies of water due to impervious surfaces characteristic of urban environments. Impervious surface coverage has been considered a quantifiable land-use indicator that correlates closely with polluted runoff. (Arnold & Gibbons, 1996) Furthermore, due to the results of the study, there is also the potential to forecast indicator concentrations under land use change scenarios.

2.2 Weather Pattern Trends and Stormwater Runoff

"There is widespread recognition of the degrading influence of urban stormwater runoff on stream ecosystems and of the need to mitigate these impacts using stormwater control measures." (Fletcher, 2014). According to Smith 2015, both combined sewer systems and separate sewer systems have a tendency to overflow during rain events resulting in large volumes of wastewater and storm water being discharged into the watersheds. (Lee and Bang, 2000, Balmforth, 1990, Lee et al. 1996, Smith 2015). CSOs are usually held responsible for the deterioration in water quality of receiving waters, as more pollutants are likely to enter the receiving waters from their discharge locations. According to Suárez and Puertas, long-term poor quality in a watershed is due to the failed maintenance and control of the CSOs, especially during rain events. Rivers and lakes are highly affected by contaminants discharged into them as a result of CSOs, especially when they are not controlled. (Suárez and Puertas, 2005). Discharges from combined

sewer systems, are especially relevant due to them containing a mixture of contaminants such as industrial wastewater, urban surface runoff, domestic wastewater and sewer deposits. Discharges from separate systems include mainly the runoff from urban surfaces, resulting in fewer pollutants (Suárez and Puertas 2005). This finding explains why CSOs receive the majority of the blame for damaging water quality in receiving waters.

Although CSOs receive a majority of the criticism for the deterioration in water quality because of the pollutant-filled discharges, storm runoff is also responsible for low standard water quality. Nonpoint source pollution is one of the causes of poor quality of receiving waters. Nonpoint pollution, originating primarily from agriculture and urban and industrial activity is a primary source of phosphorus and nitrogen to surface waters of the United States. (Carpenter, 1998). Urban non-point pollution can contain various pollutants from toxic chemicals stemming from motor vehicles to pesticides from lawn and gardens treatment tools. Furthermore, nonpoint pollution can also contain viruses, bacteria, and nutrients from pet waste, underperforming septic systems, and heavy metals. One study examined different sources of nonpoint pollution. These sources ranged from building siding and roofs; automobile brakes, tires, and oil leakage; to wet and dry atmospheric deposition. Atmospheric deposition is a major source of metals such as cadmium, copper, and lead (Davis, 2001). The study found that building siding was the biggest contributor of metals ranking as the highest source of lead and zinc and the second largest for copper and cadmium. Atmospheric deposition had a major contributing

role in cadmium loading but was a minor factor in contributing cadmium and iron. Automobiles were also found to be a source of heavy metals to the environment. The study found that emissions from the wear of brakes contained copper while tire wear contributed zinc. Oil leakage from automobiles added a minor amount of all the four metals. (Davis, 2001).

Both separate sewer overflows (SSOs) and CSOs are dependent on rainfall. With this being said, the monitoring of local weather pattern trends is critical for the surveillance of a watershed. It can be assumed from previous research that more precipitation will result in more runoff, and thus more contamination in the watersheds. Rainfall events can result in CSOs that introduce multiple sewage-borne contaminants into the local aquatic environments, subsequently compromising the quality of that watershed and negatively impacting the public health of the local area. (Eriksson et. al., 2007; Rajal et al., 2007; and Gasperi et. al., 2008). A recent investigation found observed higher concentrations of various strains of viruses such as enteric adenoviruses and GII-noroviruses, due to the rainfall events (Hata et. al., 2014). Furthermore, the study found that concentrations of indicator microorganisms such as *E. coli*, TCS, and F-phages in the samples were higher during wet weather than during dry weather supporting the idea that rainfall events increase microorganism concentrations in watersheds. (Hata et. al., 2014). Fecal contamination was found to be more common during the wet season by another study. (Kostyle, 2015) This finding was applicable across several categories such as fecal

indicator bacteria, measurement methods, and population setting, Kostyla, 2015). A study of the Newport River Estuary yielded similar results. Despite seasonal variations, the data revealed a significant increase in fecal coliform concentrations after measured rainfall amounts of 2.54 cm (Coulliette & Noble, 2008).

Several studies have concluded that wastewater discharges are the most probable source of fecal contamination of surface waters. A study found that wastewater treatment plants with secondary treatment were an important source of potentially harmful bacteria such as *E. coli*, norovirus, *Giardia* and *Cryptosporidium*. The rainy season can cause comparably higher microbial loads in sewer overflows (Astrom et al., 2009) As aforementioned, discharges from sewer systems, specifically CSOs and SSOs during wet weather conditions, implicate high loads of indicator organisms and pathogens. Special emergency circumstances where untreated wastewater is discharged represent a significant pathogen source as well (Astrom et. al., 2009). Other variables such as flow intensity are a factor in relation to bacterial and pathogenic contamination. According to a study conducted by Bougeard et. al., the peaks where high of *E. coli* concentration occurred correlated with increases in river flow (Bougeard et al., 2011). Furthermore, McCarthy et al. also found that at two sample sites *E. coli* densities were highly correlated with the average flow intensity. (McCarthy et al., 2012). However, there have been studies that have contrasted these findings. For example, according to Chase et al., their investigation found that greater concentrations of fecal coliforms and *E. coli* were observed under no-flow conditions, and that there was actually a significant negative

correlation found between the flow rate and the levels of fecal coliforms in the water. Moreover, fecal concentrations were determined to be less under flowing conditions in comparison to fecal concentration levels under nonflowing conditions (Chase et. al., 2012)

Previously mentioned studies agree that nonpoint pollution and urban surface runoff are primary contributors to the decline in the water quality of urban water bodies. In a particular, study by Wang et. Al, 2015, findings indicated that 80% of the overall water pollution in the Nansi Lake Basin mainly came from nonpoint source pollution.. Agricultural fertilizers and pesticides both contribute more than 85% of the overall nonpoint source, coupled with livestock and aquaculture (Wang et. Al 2015). Routine and improved monitoring methods of the waterways are critical to determining what factors are influencing water quality degradation. The United States Clean Water Act does not directly regulate nonpoint source water pollution; however, it does provide mechanisms that urge states to address and correct nonpoint source water quality problems within their borders. States are being called on to legislate and enforce laws to limit pollution and maintain the water quality set forth by the Clean Water Act within their borders. Despite there being both shallow and in depth scientific knowledge available about the effects of nonpoint source pollution, willingness from both state and federal governments to address nonpoint source pollution has been rooted and connected instead to the cultural, economic, and political prominence of perceived nonpoint source pollution problems, especially in regards to agricultural components (Kundis et. al., 2015).

2.3 Fecal Contamination:

Surface freshwater is a widely used source of drinking water for communities across the world. The majority of the world's drinking water uses surface water as drinking water as its source for the human population (Hörman et. al., 2004). Public health protection requires standards and regulations in the water quality of surface waters in the United States. These rules and regulations are enforced under the Clean Water Act. The sampling and analysis of drinking water for the presence of indicator microorganisms is an essential process for determining the microbiological quality of local water sources and to the assessment of any possible threat to the public health of the community. Despite advances in medicine and the prevention of water-borne illnesses, drinking water-related outbreaks are still occurring worldwide. Moreover, there is not a global standard method of testing drinking water for safety as different indicator microorganisms are being used worldwide as a tool for the microbiological examination of drinking water. The presence of indicator microorganisms (IMs) can imply possible fecal contamination of drinking water, which may contain harmful pathogens and reflect the overlying problem of water quality deterioration (Saxena, 2015). Indicator microorganisms are not considered to be pathogenic to humans (Verhille, 2013). The foundation for the protection of public health from waterborne diseases (WBDs) was based on this very principle (Saxena, 2015). The most widely used IMs are coliforms

(total coliforms (TCs)), fecal or thermotolerant coliforms, *Escherichia coli*, enterococci (fecal streptococci or intestinal enterococci) and bacteriophages.

Sources of fecal contamination can vary from location to location. Fecal contamination is a serious concern for managers of water resources, due to the easy accessibility of pathogens from the urban environment entering watersheds through various pathways. For examples, some of the pathways include the discharge of inadequately treated sewage or wastewater effluent, storm water runoff, CSOs, and SSOs (Arnone, 2007). Furthermore, the processes implemented at wastewater treatment facilities to remediate wastewater are not entirely capable of eliminating the pathogenic organisms found in wastewater, allowing for the discharging of microorganisms to into the surface water. Additionally, the discharge of any domestic sewage can lead to the contamination of groundwater, causing public health concern.

The Clean Water Act of 1972 contains certain requirements governing U.S. bodies of water, in hopes of maintaining chemical, physical, and biological integrity. A microbial water quality standard consists of a measure or some indicator of a bacterial indicator organism. However, developing a total maximum daily load (TMDL) for a supplementary indicator or pathogen is an additional also requirement if any impairment such as a water disease outbreak were to occur, This would be needed even after the water body is in compliance with the standard. This occurs because indicator organisms

do not reflect the presence of pathogen contamination with complete certainty (Arnone, 2007),

Historically, the presence of total coliforms and fecal coliforms such as *E. coli*, have been the indicator microorganisms used to assess water quality. The need for another indicator has driven recent research into the PMMoV as a viable fecal indicator. Presently, the bacterial indicators regularly used to detect fecal contamination, such as fecal coliforms and enterococci, often do not correlate with the presence of viruses and other pathogens associated with fecal matter (Rosario et. al., 2009). A research study was designed to assess the utility of the PMMoV as an indicator of fecal pollution in the coastal marine environment. The investigators used Quantitative PCR to determine the abundance of PMMoV in a variety of samples that included: raw sewage, treated wastewater, seawater exposed to wastewater, and fecal samples from various animals (Rosario et. al., 2009). The study's results indicated that PMMoV was present in all wastewater samples at high concentrations of raw sewage. The study's researchers concluded that PMMoV is a promising indicator of fecal pollution in marine environments (Rosario et. al., 2009). Another study tested the viability of the PMMoV as a fecal indicator in the Ruhr and Rhine rivers of Germany. In addition to testing PMMoV as a possible fecal indicator, the researchers assessed whether the human picobirnaviruses (hPBV) and Torque teno virus (TTV) were suitable indicators of fecal contamination in river water as well. These viruses were of interest since they are detected at substantial levels in human fecal matter

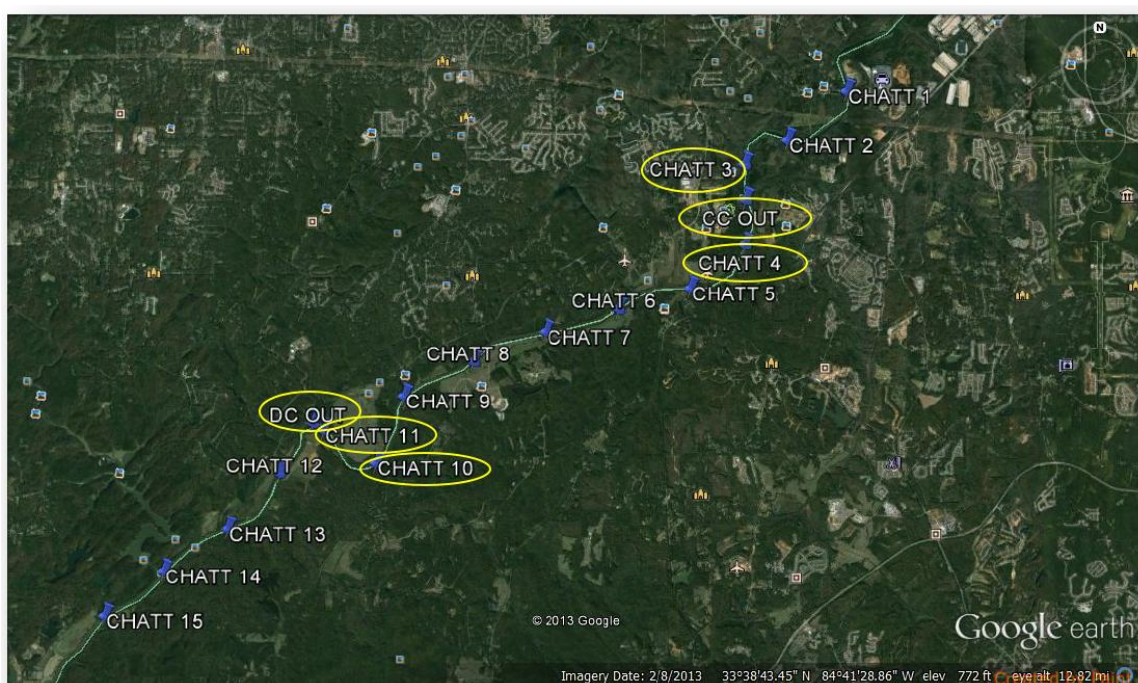
(Hamza et. al., 2011). The procedure utilized quantitative PCR to determine the abundance of PMMoV, hPBV, and TTV and compared the results to the concentration of human adenoviruses (HAdV) and human polyomaviruses (HPyV). The investigation's results found that PMMOV was detected in all samples. The researchers concluded that PMMoV showed promising potential as an indicator of fecal pollution in surface waters similar to the Ruhr and Rhine Rivers.

CHAPTER III METHODS

3.1 River Sample Site Description

Fifteen locations were designated as water sample collection sites along fourteen-miles of the Chattahoochee River with each collection site approximately one mile apart.

Map 1: Chattahoochee River Sample Sites



Both the Camp Creek and Douglas County wastewater treatment plants have effluent outfalls along the fourteen-mile stretch in which the sample collection sites were located. The Camp Creek Outfall is positioned between sites Chatt 3 and Chatt 4 and the Douglas County Outfall is between sites Chatt 11 and Chatt 12

3.2 Sample Collection: Chattahoochee River

The Chattahoochee River was sampled via boat on 5/12/14, 6/19/14, 7/10/14, 8/5/2014, and 9/11/14. Using the grab sample method, one liter of river water was collected in sterilized bottles at each of the water sample sites and at the two water treatment outfall sites. Only six of the sites where the samples were collected were tested for PMMoV. Furthermore, effluent was collected directly from the outfall pipeline at the Camp Creek Outfall only if the wastewater plant was releasing effluent at the time of sample collection. Douglas County Outfall samples were not collected directly from the pipeline but within close proximity if the outfall was unreachable by boat. In addition to water samples, the date, time, geographic location (latitude and longitude), dissolved oxygen (DO), and pH were recorded at each sample site on each sampling round. All liter bottles containing samples were stored in coolers filled with ice to preserve the samples while being transported from the Chattahoochee River to the Georgia State University (GSU) School of Public Health (SPH) lab. Samples remained in coolers on ice until processed, which was no longer than six hours. As previously mentioned, sampling and testing for PMMoV focused on the outfalls and the sites immediately upstream and downstream of these outfalls. Therefore, sample sites Chatt 3 and Chatt 4 and Chatt 11 and Chatt 12 and CC Out and DC Out were sampled for PMMoV. Each site was 1 mile upstream and 1 mile downstream from the outfall. One-liter grab samples were stored at 4C and shipped overnight to the University of Arizona for Polymerase Chain Reaction (PCR) analysis for PMMoV.

3.3 Detection of PMMoV

Detection of PMMoV was performed at the University of Arizona according to the method of Quantitative Polymerase Chain Reaction analysis using TaqMan-based qPCR assays for viruses were performed with a LightCycler® 480 Real-Time PCR Instrument II (Kitajima, 2014).

3.4 Statistical Analyses

All original data was organized and stored in Microsoft Excel 2008 prior to statistical Analyses. Graphs were created using GraphPad Prism version 5 & 6. Statistical Analyses of the data was performed with GraphPad Prism as well. These statistical analyses included paired-t-test as well as a one-way ANOVA to determine any statistically significant differences. For all statistical analyses, the level of significance was reported as $p < 0.05$.

CHAPTER IV RESULTS

4.1 Chattahoochee River

As shown in Table 1, the sampling results for the investigations varied across sampling sites and dates. A one-way ANOVA analysis ($p < 0.05$), as shown in Table 2, of concentrations across sampling locations, resulted in a p-value of 0.044. This resulting p-value ($p = 0.044$) was not higher than the test value of $p < 0.05$. As a result, it can be determined that the location of the sampling sites does result in a statistically significant difference in the PMMoV values observed. Furthermore, a one-way ANOVA analysis ($p < 0.05$), of concentrations across sampling dates, led to a p-value of 0.063 (Table 3). The resulting p-value of 0.06313 was higher than the test value of $p < 0.05$ therefore, indicating that the dates on which sampling took place did not result in a statistically significant difference in the PMMoV values observed across time.

As Shown in Figure 1, the PMMoV concentrations were not similar across sample sites. Chatt #3 showed the lowest concentration of PMMoV of all the sites where PMMoV was determined to be present. CC Out (#16), which is an outfall located between Chatt #3 and Chatt #4, showed the highest concentration of PMMoV across all the sites where PMMoV was determined to be present. Furthermore, DC out (#17), another outfall located between Chatt #11 and Chatt #12, however, did not show a similarly high concentration of PMMoV. This result could have occurred due to sampling method as DC outfall was harder to access. Samples were frequently taken from the water

surrounding the outfall and not directly from the outfall itself, possibly explaining the lower concentrations. It is notable that PMMoV was found both upstream and downstream of each outfall. The average PMMoV concentration was higher downstream of the outfall than upstream.

Table 1: PMMoV Sampling Results from the Chattahoochee River by Site and Date, Atlanta Georgia, 2013

	Chatt 3	CC out	Chatt 4	Chatt 11	DC out	Chatt 12
5/12/14	824000	98700000	71000	149000	632000	0*
6/19/14	919000	81300000	1810000	1730000	2000000	1660000
7/10/14	1430000	44500000	1810000	4750000	4260000	7660000
8/5/14	86400	20600	35400	62000	53600	40500
9/11/14	205000	205000	7470	37800	7470	33500

*Concentration values in copies/L

* Sample was non-detectable

Figure 1. Chattahoochee River Sampling Results PMMoV Concentration vs. Site Location

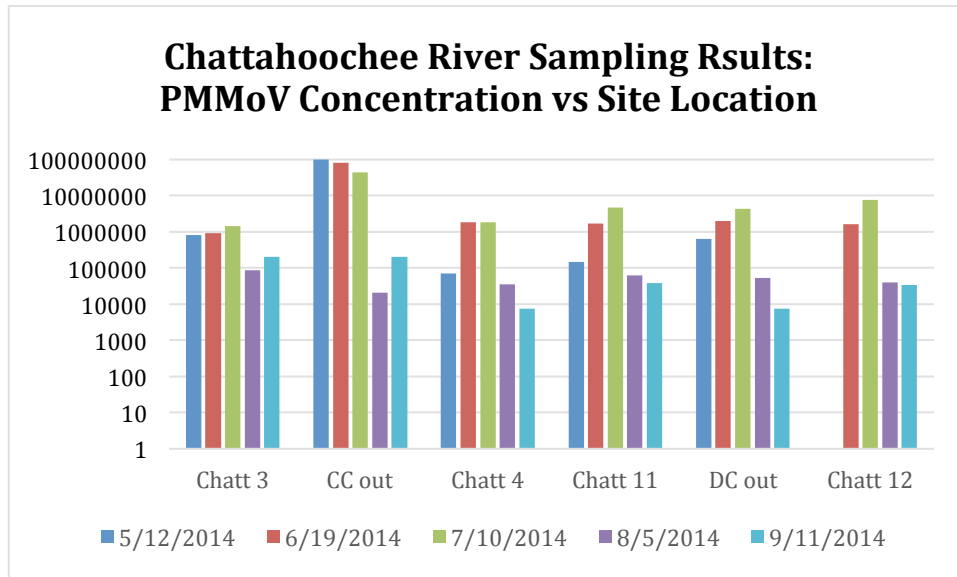


Table 2: One-Way ANOVA Analysis of selected Water quality variables from the Chattahoochee River by site Atlanta, Georgia, 2013

PMMoV Concentrations By Site						* P-Value
Chatt 3	CC out	Chatt 4	Chatt 11	DC out	Chatt 12	=0.0044
824000	98700000	71000	149000	632000	0	
919000	81300000	1810000	1730000	2000000	1660000	
1430000	44500000	1810000	4750000	4260000	7660000	
86400	20600	35400	62000	53600	40500	
205000	205000	7470	37800	7470	33500	
*692880	*44945120	*746774	*1345760	*1390614	*692880	

* level of significance reported as p<.05

*Concentration values in copies/L

* Average concentrations per site

Table 3: One-Way ANOVA Analysis of selected Water quality variables from the Chattahoochee River by Date, Atlanta, Georgia, 2013

PMMoV Concentrations By Date					*P-Value
5/12/14	6/19/14	7/10/14	8/5/14	9/11/14	=0.6313
824000	919000	1430000	86400	205000	
98700000	81300000	44500000	20600	205000	
71000	1810000	1810000	35400	7470	
149000	1730000	4750000	62000	37800	
632000	2000000	4260000	53600	7470	
0	1660000	7660000	40500	33500	

* level of significance reported as $p < .05$

*Concentration values in copies/L

Bacteriophage MS2 is a potential indicator of the presence of human viruses in water, As shown in Table 4, MS2 was found in 19 out of 30 sample collections from the aforementioned sites. No statistical significance could be drawn from just those results. However, via a paired t-test,($p < 0.05$), between the sample concentrations with and without MS2 presence, it was determined that there was no statistical difference in concentration of PMMoV when MS2 is present since $p = 0.0740$.

Table 4 MS2 Sampling Results for Chattahoochee River, Atlanta, Georgia 2014

*Presence MS2 by Site	5/12/14	6/19/14	7/10/14	8/5/14	9/11/14	*P-Value
Chatt #3	+	-	+	+	+	P=0.740
Chatt #4	+	-	+	+	+	
Chatt #11	+	-	-	+	+	
Chatt#12	+	-	+	+	+	
CC Out (#16)	-	-	-	+	+	
DC Out (#17)	-	-	-	+	+	

* t-test with level of significance reported as $p < 0.05$

* +/- = Presence of MS2 in Sample for Site

As previously mentioned, PMMoV concentrations varied across sampling sites As displayed in Table 5, through paired t-test ($p < 0.05$) it was determined that there were no statistically significant differences between the concentrations of PMMoV found upstream (Chatt 3) the Camp Creek Outfall or downstream (Chatt 4) the Camp Creek Outfall. Furthermore, as shown in Table 6 through paired t-test ($p < 0.05$) it was determined that there were no statistically significant differences between the concentrations of PMMoV found upstream. Through paired t-test (Table 5) it was

determined that there were no statistically significant differences between the concentrations of PMMoV found upstream (Chatt 3) the Camp Creek Outfall or downstream (Chatt 4) the Camp Creek Outfall. Furthermore, through paired t-test (Table 6) it was determined that there were no statistically significant differences between the concentrations of PMMoV found upstream (Chatt 11) the Douglas County Outfall or downstream (Chatt 12) the Douglas County Outfall.

Table 5. Paired T-Test Analysis of selected Water Quality Variables from the Chattahoochee River by site, Atlanta, GA, 2013

PMMoV Concentrations By Site				
Chatt 3	CC Outfall	Chatt 4	*P-Value (Chatt 3 vs. CC Out)	*P-Value (Chatt 4 vs. CC Out)
824000.	98,700,000	71000	= 0.0928	=0.0931
919000.	81,300,000	1810000		
1430000.	44,500,000	1810000		
86400.	20600	35400		
205000.	205000	747		

*t-test with level of significance reported as p<.05
 *Concentration values in copies/L

Table 6. Paired T-Test Analysis of selected Water Quality Variables from the Chattahoochee River by site, Atlanta, GA, 2013

PMMoV Concentrations By Site				
Chatt 11	DC Outfall	Chatt 12	*P-Value (Chatt 11 vs. DC Out)	*P-Value (Chatt 12 vs. DC Out)
149000.	632000	0	= 0.7979	=0.5443
1730000.	2000000	1660000		
4750000.	4260000	7660000		
62000.	53600	40500		
37800.	7470	33500		

* t-test with level of significance reported as p<.05
 *Concentration values in copies/L

CHAPTER V DISCUSSION

5.1 Importance of Study

Due to Atlanta and its surrounding areas' dependence on the Chattahoochee River, the importance of monitoring and maintaining the integrity of these surface waters is of high magnitude. With a steadily growing population, there will be more sanitary sewage in the system, eventually making its way to the Chattahoochee River. Previously the city of Atlanta had to overcome the poor water quality and install measures that protect the rivers water quality. Additional changes were made to ensure that wastewater was sufficiently appropriately discharged into the Chattahoochee River. Methods of testing are required to maintain the proper water quality of the River, Accurate indicators of fecal pollution are needed to minimize public health risks associated with wastewater contamination in recreational waters like the Chattahoochee River. Unfortunately, many times the bacterial indicators presently utilized to assess and monitor water quality do not necessarily correlate or accurately reflect the presence of pathogens. (Rosario, et al. 2009). The PMMoV is abundant in wastewater from the United States, suggesting its use as an indicator of human fecal pollution (Rosario, et al. 2009). The advantage of using PMMoV instead of human enteric viruses to indicate fecal pollution is that the presence of PMMoV in wastewater is independent of active human infection. This is important

since other viral indicators depend on the degree of infection in the population and the release of the virus into the wastewater system at any given time (Rosario, et al. 2009). Therefore there can be some variability and inconsistency when using a human enteric virus. There is a lack of published investigations on the use of the PMMoV as a viable indicator of fecal contamination in surface waters. This research also opens the opportunity for investigation into whether there is any correlation to the presence of MS2 and PMMoV in surface waters.

5.2 Major Findings

The primary goal of this study was to assess the presence of PMMoV in the Chattahoochee River. The study found concentrations of the PMMoV in the samples taken from the Chattahoochee River between the dates 5/12/14 through 9/11/14. Overall, there were few similarities in concentrations found across sample dates. Camp Creek Outfall (CC Out), which is located upstream between Chatt #3 & Chatt #4., contained the highest concentration of the PMMoV of all the sampling points. However, in comparison, Douglas County Outfall (DC out), which is located between Chatt #11 and Chatt #12, did not have high concentrations of PMMoV detected. Additionally, there were several instances where the concentration found was higher upstream of the outfall than downstream of the outfall. This could possibly be due to various factors such as pollution and stormwater runoff. This investigation was the first to look for the presence of PMMoV in the Chattahoochee River, however there have been other studies as

previously mentioned conducted in other areas around the region and country.. Further investigation would be needed to explain the high concentration at this specific sampling point on all sample dates, relative to all the other sampling points.

The findings in this investigation are consistent with those in previously published literature. In Germany, PMMoV has been positive in all the samples of river waters containing WWTP effluent (Hamza et. al., 2011). In Japan, PMMoV has been detected in 76% of surface water samples used as drinking water sources (Haramoto et al., 2013). In this study 97% of the samples that were tested for the presence of PMMoV were shown to be positive for the presence of PMMoV. Additionally, the detection of PMMoV in all samples at the outfalls is consistent with the study conducted by Rosario et. al., (2009) in which samples exposed to wastewater or sewage was found to contain PMMoV. Although this investigation corresponds with previously published literature, there are several differences in this study that exist in comparison to the other studies conducted on PMMoV in surface waters. One of the more noticeable differences is the sample size taken in this study. For our investigation a small sample size was taken over 5 months. In comparison, the investigation by Rosario and others had multiples samples taken over a longer period of time. Another difference is that the samples in this investigation were taken from one source, the Chattahoochee River, while in the study conducted by Rosario and others included samples that were taken from several different sources (raw sewage, wastewater, and seawater). The presence of PMMoV upstream of the outfall does suggest there are other sources of contamination other than the outfall. These sources of

contamination could range from human recreational pollution to the antiquated sewer system used by the City of Atlanta for CSOs, which contaminate this waterway. There could be other possible non-point sources of pollution. However, since PMMoV is found in fecal contamination, the CSOs may be the leading factor in finding PMMoV upstream of the outfalls. PMMoV is considered very stable in the environment but more information on its persistence is needed,

Furthermore, the results show that it cannot be concluded that there is specific correlation between the presence of MS2 and PMMoV in surface water. The results varied with there not being any statistical significance toward a correlation between MS2 and PMMoV. There are no other studies that investigate the presence of MS2 and PMMoV in surface waters with which to compare this investigation. Therefore, monitoring for MS2 presence may or may not indicate that PMMoV is present in surface waters, such as the Chattahoochee River. This finding correlates with a study conducted by Luther et al., who concluded that monitoring for fecal indicator bacteria, such as MS2 may not adequately detect viral contamination (Luther & Fujioka, 2004). Additionally, seasonal variability was not examined in this study. However, since PMMoV is based on dietary behavior and is not dependent on active human infection, no large seasonal variations are expected. Before PMMoV can be used as a viable fecal indicator in other parts of the world with different dietary preferences, studies will need to determine the prevalence of PMMoV in sewage from each geographic region, as well as the baseline presence of PMMoV in local recreational waters (Rosario et. al., 2009).

5.3 Strengths and Limitations

Strengths

To date, there are no studies investigating the current water quality, the presence of PMMoV, and the correlation between the presence of MS2 and PMMoV in the Chattahoochee River.

Limitations

This research is comprised of a tiny sample size from the Chattahoochee River, making it difficult to make assumptions and apply them to a larger scale. PMMoV was only present in 6 of 17 samples taken from the Chattahoochee River from May to September. Sampling methods also varied due to several factors.

5.4 Future Research

The Chattahoochee River must comply with Federal and State standards for water quality. Continuous monitoring, as well as additional varied sampling across the state, will improve the amount of statistically significant results. Future investigations should investigate the sources of contamination both from point sources and collect stormwater runoff to assess where nonpoint pollution is higher in concentration. Future studies should also investigate the presence of PMMoV in a larger sample size taken along the Chattahoochee River and other ambient surface waters to assess its prevalence and eventual viability as a fecal indicator.

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