1-1-2009

# Determining the distribution of antibiotic resistant and fecal indicator bacteria in the Ohio River

Caitlin Nicole Swecker
swecker5@marshall.edu

# *Determining the distribution of antibiotic resistant and fecal indicator bacteria in the Ohio River*

Thesis submitted to
The Graduate College
of Marshall University

---

In partial fulfillment of
the requirements for the degree of
Master of Science
Biological Sciences

**By: Caitlin Nicole Swecker**

Approved by
Charles C. Somerville, Committee Chair
Thomas G. Jones, Committee Member
Wendy Trzyna, Committee Member

**MARSHALL UNIVERSITY**

**August 21, 2009**

# ACKNOWLEDGEMENTS

First and foremost I would like to thank the Lord above for giving me faith and blessing me with the support I have from my family, my friends, my professors, and my colleagues throughout my entire life and more specifically my college career at Marshall University. Without such love, encouragement, and support from some very special people in my life, I would not be writing this acknowledgement.

To Dr Chuck Somerville, my committee chair: I write with my dearest thanks and am grateful and honored that I was able to work and study under you throughout my graduate years at Marshall University. You are an outstanding professor, advisor, and role model to everyone you meet, and especially to me. As the Chair of Biology and professor you were always willing to find the time to advise me and guide me throughout this project. Your time spent in the field and in the lab with me truly shows your dedication to your graduate students, and I appreciate every minute of your help. It has been a privilege getting to know you, learn from you, work by your side and most of all, have your support in not only my educational career at Marshall but in my future endeavors that lie ahead.

To Dr Tom Jones, my committee member: You are truly an inspirational person. You have provided not only the support and the knowledge I have needed to get through, but your encouragement along the way and passion towards each one of your students has showed me what an outstanding professor is all about. You have given myself and other students opportunities to not only learn about some the world's most marvelous wonders through your teachings, but because of you we were able to experience these wonders first hand. From diving the coral reefs of Bonaire to exploring some of the deepest and darkest caves in Kentucky with your guidance, we learned so much more than what any lecture could do. You have played a significant role in my educational career as well as my life. You are a dear friend to my family and I know they appreciate you as much as I do. Without you and Dr. Somerville, I would have nothing to write and no one to thank. I sincerely thank you for your guidance and support, as well as all of your help in making this project possible. Without your time, financial support, equipment, and lab crew, there would have been no project. I can't thank you and your lab members enough for the hard work and assistance you all provided to make this a success. With that I'd like to extend my thanks to the following people for their help throughout this project: Keith Donahue, Geoffrey Smith, Brad Musser, Sean Reese, Sean Collins, Brian Bridgewater, Matt Kinsey, Tim Dotson, Derek McKinney and Ursula Husted.

To Dr Wendy Trzyna, my committee member: From being taught by you in your classroom to teaching under you as a lab instructor, you have helped me become a better teacher myself. You gave me guidance while allowing me to take on the responsibilities of running my own classroom. I cannot tell you how that experience alone has helped me become a more

confident person, public speaker, and instructor. You show so much passion for microbiology and it shows in your work, teachings, and your students. Thank you for being such a great role model and for all of your help and guidance along the way.

I would also like to extend my appreciation to Dr O'keefe and Dr Evans for all of your help and guidance throughout this project.

To my friends and family: You have all made these past 6 years at college an experience I'll never forget. Without your love and support I couldn't have made it this long. Each and every one of you has made a special impression in my life and I will never forget the lifelong memories we've made, they will be cherished forever, and I look forward to sharing more with all of you. A special thanks goes to my roommate of 5 years, Leslie, for encouraging me to keep writing when I felt like quitting, for helping me even though she had no clue what we were doing, but most of all, for always being a true friend. You are an amazing person Les and I know that you will go far in life. We have accomplished so much and celebrated many milestones these past few years together. I am so proud of you and I couldn't have done this without you. Thank You! Mike: You have made this past year and half the best year and half of my life. You have supported me in every way possible, and beyond. I can't imagine my life without you and I thank you for all of the love and support you have given me and for being my rock. I love you with all of my heart! To my grandparents and family: Thank you for your thoughts and prayers. They have carried me through this journey. Your love and support will always be felt and cherished. I love you all!

And a special acknowledgement goes to my biggest supporters ever: My Mom, Dad & Brother…

To my one and only sibling, Casey: Although you are my older brother you have well exceeded the duties of being my big brother. You helped get me started, you've helped me all along the way, and you've helped me finish. You have been there for me throughout this entire process. This would not have been possible without all of the love, support, and guidance you have given to me. Marshall would not have been the same without you, and I'm not just speaking for myself. You made the "Swecker" name known by your talents and hard work alone. And although I hope that I made my own impression, I am so proud to still be known as "Casey's little sister." You deserve every bit of recognition and I thank you so much for being an inspiration to me. I love you!

To my parents, Mark and Sandy: Thank you for all of your love and support throughout my entire life. You have helped make me the person I am today and for that I can't express how grateful and how thankful I am. You have both shown me what it means to be successful, and without a doubt I feel like I have succeeded thus far in my life and will continue to strive for my true hopes and dreams. You have taught me what love is and what love means, and your unconditional love has helped get me to where I am today. You have made it possible for me to experience life to its fullest and have believed in me every step of the way. I love you both with all of my heart and appreciate every sacrifice you have made to get me to this point in my life. You have supported and encouraged me when I've needed it most and for that ***I'd like to dedicate this work to you!***

# Table of Contents

# LIST OF FIGURES

# ABSTRACT


## *Determining the distribution of antibiotic resistant and fecal indicator bacteria in the Ohio River*

**Caitlin Swecker, Department of Biological Sciences, Marshall University, One John Marshall Drive, Huntington, WV 25755**

The Ohio River extends 981 miles from Pittsburgh, PA to Cairo, Ill providing drinking water to over three million people, a natural habitat for aquatic life, a public recreation resource, a major transportation route, and a source of water for industry. The Guyandotte River is a highly impacted tributary emptying into the Ohio River in Huntington, WV. The objectives of this study were to determine if sediment load is correlated to the number of antibiotic resistant bacteria (ARB) and determine if a single surface sample is a sufficient representative measurement of ARB populations in a large river. In 2007, subsurface and bottom water samples from the Ohio River were analyzed for total coliforms, *E. coli*, as well as tetracycline resistant and ciprofloxacin resistant coliforms and *E. coli.* In 2008, samples were collected from the mouth of the Guyandotte River and the mainstem Ohio River via SCUBA. Samples were analyzed for coliforms and *E. coli* using IDEXX Quanti-Tray/2000©. The R2A plate count method was used to determine antibiotic resistance to tetracycline, ciprofloxacin, and virginiamycin. Water chemistry and sediment data were also collected. SigmaStat 3.5 and Statistica 8.0 were used to analyze and detect significant differences between sites and samples. Results show that depth and sediment were not contributing factors; however, location was a factor due to the direct input of water and bacteria from the tributary. Intense sampling may be needed in order to detect the impact of a source as well as in determining whether water quality standards are met at that location.

# CHAPTER I

## *THE OHIO RIVER*

The Ohio River's glacial birth dates back millions of years, its European settlement history began in the early 1600's, and it became an important commercial route for transportation during the 1800's (Ohio Historical Society, 2005). Today, the Ohio River extends 981 miles beginning at the confluence of the Monongahela and Allegheny Rivers in Pittsburgh, Pennsylvania, meandering southwesterly along the borders of West Virginia, Ohio, Kentucky, and Indiana, until reaching Cairo, Illinois, where it then empties into the Mississippi River. Twenty one major tributaries contribute to the Ohio River drainage basin (Benke & Cushing, 2005) within 15 states, while 20 locks and dams maintain year round navigation. The Ohio River basin is home to over 25 million people and provides drinking water to approximately five million. It is an active and important route for industry, transporting coal, oil, petroleum and other resources by barge (ORSANCO, 2006). It provides a water resource for the generation of power, and for major manufacturers; including 67 power plants, 28 major petroleum facilities, 12 grain elevators and terminals, and 29 chemical plants (Benke & Cushing, 2005).

The Ohio River is termed a high order stream. There are many discrepancies on the exact order; therefore many just use the term "large river" to describe its hierarchy. This large river provides recreational opportunities, as well as a significant habitat for a diversity of aquatic fauna and wildlife. Due to the various uses of this watershed there are a multitude of negative impacts occurring directly, as well as indirectly. According to the Ohio River Valley Water

Sanitation Commission's (ORSANCO) 2007 Ohio River Fact Sheet, nonpoint source pollution from urban runoff, agricultural activities, and abandoned mines are the major causes of water pollution in the Ohio River. The US EPA survey indicates that 1,200 combined sewer outflows (CSOs) are located in cities along the mainstem of the Ohio River (ORSANCO, 1997). These CSOs empty a combination of storm water overflow and untreated human and industrial waste directly into the river during rain events. The city of Huntington, WV has a combined sewer system including 23 CSO discharge points with 15 discharging directly into the Ohio River, while the remaining eight discharge into tributaries of the Ohio River (James, 1994). In 2005, only 55% of West Virginians were connected to a public sewer system, leaving the other 45% using onsite home systems such as septic systems or direct discharge pipes leading straight into their local river or stream. In 2002, the United States Environmental Protection Agency (USEPA) estimated that 60% of onsite home systems were failing resulting in contamination to surface and groundwater (West Virginia Rivers Coalition, 2005). Statistics like these show how important it is to understand the impacts on major water resources like the Ohio River.

While there are many different parameters necessary to understanding the quality of a large river system, this paper will focus on bacteria, specifically the effects and distribution of antibiotic resistant bacteria (ARB) in the Ohio River, and whether current methods of detection are reliable in identifying the potential risks ARB have on the aquatic environment and public health.

## *WHY STUDY BACTERIA?*

Bacteria play a vital role in maintaining life on earth. Bacteria are essential to the environment and necessary for the production of atmospheric oxygen, the recycling of nutrients, decomposition, and the fixation of nitrogen into its usable form. Without bacteria, life on earth could not exist. A small fraction of bacteria are responsible for causing diseases in plants and animals, and those pathogenic bacteria are often transmitted via surface waters. Human practices contribute to the distribution of potentially dangerous bacteria, including antibiotic resistant bacteria.

## *THE THREAT OF ANTIBIOTIC RESISTANCE*

Research has shown that antibiotic resistant bacteria are common in surface waters, specifically within the Ohio River (Somerville, Smith, Loughman, & Johnson, 2004); (Smith & Somerville, 2003); (Somerville, Saunders, & Van Meter, May 2002). The threat that these microbes may cause treatable diseases to become untreatable has become a cause for further research and preventive actions against this threat. The warning of antibiotic resistance dates back to 1945, when Alexander Fleming, discoverer of penicillin, interviewed with the *New York Times*, stating that "the misuse of penicillin could lead to the selection and propagation of the mutant forms of bacteria resistant to the drug" (Levy, 2002). Single drug resistant strains are not as much as a threat; however, the emergence of multi-drug resistant strains of bacteria and other pathogens are cause for concern all around the world. The main causes of multi-drug resistance (MDR) are the overuse and misuse of antibiotics in human medicine, veterinary medicine, agriculture, and aquaculture (McManus & Stockwell, 2001). The Center for Disease Control

(CDC) estimates that of the 50 million pounds of antibiotics produced in the United States a year, half is consumed by humans, 40% is used in animal feed to prevent disease and promote growth, while the remaining 10% is used to treat infections of fish in aquaculture industry and prevent bacterial diseases in plants (McManus & Stockwell, 2001); (Murray, 1997). The large amount of antibiotics used annually around the world is only the cause; however, it's the effects of such use that has medical and veterinary researchers, pharmaceutical companies, federal agencies for disease control, and environmentalists concerned.

## *Antibiotics in the Environment*

Pharmaceuticals, specifically antibiotics, may enter the environment by several pathways. Some common routes of contamination include; waste or loss during production, excretion from humans and animals, improper disposal, dispersion on fruits and plants, fertilization of cropland with manure or sewage sludge, and direct input from sewage treatment plants (STPs), combined sewer outflows (CSOs) or straight pipelines into local lakes, rivers, or streams. Whether the antibiotics have been metabolized by humans or animals or filtered through STPs, many still have the ability to remain active or stable within the environment (Kummerer, 2001). These pathways eventually will lead to the contamination of soils, sediments, surface water, groundwater, and even drinking water. The widespread use and persistence of antimicrobial agents in the environment have led to the emergence of antibiotic resistant bacteria.

## *An Early Assumption*

Most research on antibiotic resistance begins with the assumption that ARB originate within the intestinal gut flora of warm blooded animals, such as humans and animals after

prolonged exposure to antibiotics. While normal resident bacteria of the gut are capable of being resistant, only a small percentage of gut flora are capable of being studied due to their strict anaerobic nature. The overuse and misuse of antibiotics in human medicine and agriculture allows undigested antibiotics, as well as antibiotic resistant bacteria to be excreted into the environment through feces. Although fecal bacteria are likely to be resistant to antibiotics, many other bacterial species including those not of fecal origin have been found to be resistant as well.

Many government and local environmental agencies use coliform bacteria as biological indicators of food and water quality. Coliform bacteria comprise a common group of gram negative, rod-shaped, lactose fermenting bacteria, and non spore forming bacteria which includes the genera, *Citrobacter, Enterobacter, Klebsiella* and *Escherichia.* Coliforms are commonly found in the environment and thermotolerant coliforms capable of growth at 44.5°C are predominately found in the gut and feces of humans and animals. *Escherichia coli* is primarily of fecal origin and therefore has been effectively used to test for fecal contamination in food and water. It has been commonly assumed that environmental ARB are primarily distributed by fecal contamination; however, current research findings do not support this assumption. Previous studies on the Ohio River show significant differences in the distributions of *E. coli* and coliforms versus antibiotic resistant bacteria from surface samples. Analyses also show that antibiotic resistant bacteria are present in much greater numbers than *E. coli* and coliforms; thus concluding that ARB are not a subset of fecal indicator bacteria (Smith & Somerville, 2003) (Somerville, Saunders, & Van Meter, May 2002) (Somerville, Smith, Loughman, & Johnson, 2004). If environmental ARB are not primarily derived from fecal contamination then further work is required to determine the factors that determine or predict their distributions.

*MONITORING BACTERIA LEVELS IN THE OHIO RIVER*

It is important to continue monitoring bacteria and fecal contamination for managing water quality and protecting public health. The Ohio River Valley Sanitation Commission (ORSANCO) routinely monitors fecal indicator bacteria levels to assess public water supply use and contact recreation use of the Ohio River. The 2008 Biennial Assessment of Ohio River Water Quality Conditions summarizes the rivers conditions based on data collected from 2006 - 2007. According to their assessment, due to the exceedingly high counts of the fecal coliform bacteria, approximately one-tenth of the river is classified as partially supporting public water supply use. Data from 2003 – 2007 show approximately 484 miles (50%) of the river being classified as impaired for contact recreation use (ORSANCO, 2006). According to the data, the Ohio River often exceeds the bacteria criterion for recreation use, therefore, posing a threat to those who come into contact with the water. The fecal coliform standard states that "the monthly geometric mean is not to exceed 200 fecal coliforms per 100 ml, nor any sample exceed 400/100 ml" and the "*E. coli* monthly geometric mean is not to exceed 130/100 ml, nor any sample exceed 240/100 ml (ORSANCO River Facts/Conditions)." The risk associated with exposure to ARB is unclear because neither the pathogenicity of these cells nor the likelihood of their transferring resistance to pathogenic bacteria is known. If a credible risk does exist, it cannot be managed by monitoring for fecal indicator bacteria because the two populations are distinct, as previously described.

### *ANTIBIOTIC RESISTANT BACTERIA IN TREATED WATER*

Recent studies also show ARB present in treated water and drinking water (Armstrong, Shigeno, Calomiris, & Seidler, 1981); (Silvia, 2006); (Somerville, 2008). Silvia and his colleagues along with many other researchers have recognized that wastewater treatment plants harbor ARB and provide the perfect place for resistance gene transfer among microorganisms. Studies have shown significant increases among resistant strains of coliform bacteria in treated water versus untreated water (Armstrong, Shigeno, Calomiris, & Seidler, 1981) (Agerse, 2007). Armstrong and his colleagues investigated the occurrence of multi-drug resistant (MDR) bacteria in drinking water and found that 33% of the bacteria enumerated from several communities were resistant to two or more antibiotics. Their results showed an enrichment of MDR bacteria from standard plate count bacteria in complex treated water as compared to raw river water, 86.1% to 27.1% respectively (Armstrong, Shigeno, Calomiris, & Seidler, 1981). A 2008 study by Somerville and colleagues at Marshall University found ARB present in Huntington's drinking water supply, which comes from the Ohio River. Ampicillin resistant bacteria were found present in high numbers (~70,000 CFU/ml) in the first 100 ml of water coming from a laboratory tap. After the first 100 ml of water ran from the tap the numbers dropped by approximately 50%; however ARB levels still remained detectable in some samples even after 10 minutes of running the water. These results suggest that the resistant bacteria are resident inside the pipelines or within the faucets. Further research is being done on ARB found in drinking water supplied by the Ohio River (Somerville, 2008). With this current evidence, the following questions should be addressed: 1. Are current monitoring methodologies sufficient for

determining water quality? and 2. should ARB be a factor in determining water quality and public health standards?

### *SAMPLING LARGE RIVERS*

The current methods for taking bacteria samples within a river or stream usually consist of one to three surface grab samples at a designated sampling location. Sterile bottles are used, and samples are most often collected at the surface only. A detailed description of this technique is discussed in the Materials and Methods section, Chapter III. While the single surface sample technique is used by most governmental and local agencies assessing water quality, this study compares surface samples to those taken at different depths and at the bottom of the river to determine whether there were significant differences between samples as a function of depth. This approach addresses an important question whether single surface samples provide representative measurements of coliform bacteria, *E. coli*, and ARB in large rivers. The working hypothesis is that bacterial numbers will be greater in samples at the bottom of the river and in samples with greater amounts of sediment, thereby, casting doubt on the accuracy of the current detection methods.

### *WHY SEDIMENT?*

The transfer and emergence of new resistant genes occur most frequently within "environmental compartments" with high bacterial densities (Murray, 1997). Examples of these compartments include soils, sediments, and sewage sludge (Kummerer, 2004). Many antibiotics are capable of entering the environment and persisting in an active state because they are resistant to biodegradation. This allows the concentrations within these "environmental

compartments" to be much greater due to the sorption of antibiotics onto solid surfaces. Soils, sediments, and sludge allow bacteria to form biofilms on their surfaces, creating a suitable environment for resistance and gene transfer to occur. It has been said that quinolones, sulphonamides, and tetracyclines (see Chapter II for descriptions) absorb and adsorb well to sediments. Kummerer (2004) investigated this concluding that "An increased antibacterial resistance in sedimentary bacteria is often the most sensitive environmental indicator of past antibacterial use." This study investigates whether sediments play an important role in the distribution of ARB within the Ohio River.

### *IMPACTS FROM THE GUYANDOTTE RIVER*

The Guyandotte River is a major tributary of the Ohio River; entering the mainstem from the south at Huntington, West Virginia. Under USA EPA Clean Water Act regulations, the Guyandotte watershed is considered an impaired system for the following: pH, aluminum, iron, manganese, selenium, fecal coliform bacteria and/or biological impairments (WV EPA, 2004). These impairments are mostly a result of poor wastewater treatment, sedimentation, abandoned mine drainage, and litter (Upper Guyandotte Water Association, 2006). The impacts that the Guyandotte River has on the Ohio River have never been examined extensively; therefore, the second part of this study compares samples taken in the Guyandotte River to samples taken in the mainstem Ohio River upstream and downstream of the Guyandotte. The objectives of this portion of the study are to determine how sampling location in the mainstem are effected by tributary input. Data for fecal coliform bacteria, *E. coli*, total cultivable bacteria, antibiotic resistant bacteria, as well as, temperature, pH, dissolved oxygen, and turbidity were collected to

investigate whether the Guyandotte River water has a detectable effect on the downstream portion of the Ohio River and how well individual sample points represent conditions along a sample transect. Sediment data were also analyzed to determine if sediment load from the Guyandotte River is detectable in the Ohio River and whether that has an effect on the distribution of ARB. Because the Guyandotte River is a local river, it is an important water source worth studying to the researchers of this project and the community of Huntington, WV.

# CHAPTER II

## *ORIGIN AND DISCOVERY OF ANTIBIOTICS*

An antibiotic was first defined as "a natural substance produced by one microorganism that selectively inhibits the growth of another microorganism" (MedicineNet, Inc, 1996-2009). The first natural antibiotic, pyocyanase, was discovered in 1888, and the first chemical drug, Salvarsin was produced in 1910. Although these discoveries may have led to the golden age of antibiotics, these early drugs were later found to be toxic and ineffective. It wasn't until 1928, when Alexander Fleming discovered the first "true antibiotic," penicillin, which was able to kill the pathogenic bacterium, *Staphylococcus aureus*. The discovery of Protonsil, the first man-made drug, led to the success of synthetic antimicrobials like sulfonamides and quinolones, two families of antibiotics still widely used today in modern medicine. Broad-spectrum antibiotics, such as tetracycline, began to appear in the late 1940s, and were used against both Gram positive and Gram negative bacteria (Levy, 2002). Antimicrobial discoveries have continued for years all over the world. While many of these early antibiotics are still in use, growing antibiotic resistance means that the search for new and effective antibiotics must continue.

## *INTRODUCTION OF ANTIBIOTICS: TETRACYCLINE, CIPROFLOXACIN, AND VIRGINIAMYCIN*

This study used three different antibiotics to test for resistant bacteria: tetracycline, ciprofloxacin, and virginiamycin. Part One of this research will only include tetracycline and ciprofloxacin resistant coliform bacteria and *E. coli*. Part Two of this research will include all

three antibiotics and analyze total cultivable resistant bacteria present for each antibiotic. The following section will introduce and summarize each antibiotic, including their uses in medicine, as well as their use in past research.

## TETRACYCLINE

Tetracycline [Fig 1] is a broad spectrum antibiotic, meaning that it is effective against both Gram positive and Gram negative bacteria. The tetracycline family of antibiotics ranks second to penicillin in worldwide production and use (Levy, 2002). Tetracycline inhibits protein synthesis by binding to the small subunit of the prokaryotic ribosome, thus preventing bacterial growth. It has been used in human medicine since 1950 for the treatment of several types of bacterial infections including urinary, respiratory, and skin infections, as well in the treatment against typhus, rickettsial infections, parasite infections, Lyme disease, cholera, anthrax, syphilis, and acne. Tetracycline is also widely used in animal husbandry as a growth promoter and in fisheries. Because tetracycline was one of the most commonly used antibiotics during the 1950s and 1960s, bacterial resistance to the drug and tetracycline like derivatives is widespread (Speer, Shoemaker, & Salyers, 1992). Researchers have identified approximately thirty-eight classes of tetracycline resistant genes found in bacteria (Harvey, Funk, Wittum, & Hoet, 2009). In 2001, Chee-Sanford and colleagues tested waste lagoons and groundwater near two swine farms for eight tetracycline resistant determinants. All eight determinants were found in total DNA extracted from the waters and were detected as far as 250 meters downstream of the lagoons (Chee-Sanford, Aminov, Krapac, Garrigues-Jeanjean, & Mackie, 2001). Tetracycline resistance has also been frequently detected among bacteria isolated from fish tissues and sediments. Resistance among *Aeromonas* species has become widespread due to their aquatic nature.

*Aeromonas spp*. are mostly found in freshwater environments and are highly associated with human gastroenteritis infections, as well as disease in freshwater fishes (DePaola, Flynn, McPhearson, & Levy, 1988); (Benke & Cushing, 2005). Many of these tetracycline resistant genes are found on horizontally transferable plasmids; therefore, making resistance easily spread among other bacterial species, such as *E. coli*, in aquatic environments (Agerse, 2007); (Jin Jun, et al., 2004).

## CIPROFLOXACIN

Ciprofloxacin [Fig 2] is a broad spectrum antibiotic used mostly in human medicine. It was introduced into human medicine in the 1980's and has not been FDA approved for use in veterinary medicine or agriculture. Ciprofloxacin belongs to the fluoroquinolone class of antibiotics and is given as prophylaxis for anthrax. It is also widely used in the United States to treat urinary tract infections and infectious diarrhea commonly caused by *E. coli*, as well as treatment for gonococcal infections (Livermore, 2002). Ciprofloxacin works by inhibiting the reproduction and repair mechanisms of bacterial DNA. Previous studies on flouroquinolone resistance have been done in clinical settings, testing patient stool and blood samples. Results from these clinical studies show flouroquinolone resistance most prevalent among bacteria within the family Enterobacteriaceae, especially among strains of *E .coli* (Cometta, Calandra, Bille, & Glauser) (Kern, Andriof, Oethinger, Kern, Hacker, & Marre, 1994) (Kern, Markus, & Andriof, 1994). In 2005, the United States Food and Drug Administration (US FDA) banned the use of enrofloxacin, a flouroquinilone used to treat bacterial infections in poultry, because scientific data indicated its use led to the emergence of flouroquinilone resistant *Campylobacter*, a bacterium which causes foodborne illnesses in humans (US FDA, 2000).

There is a limited amount of data associated with the occurrence of ciprofloxacin resistant bacteria in the environment; however, past studies on the Ohio River have shown ciprofloxacin- resistant bacteria to be present around mainly urban areas. In 2007, a thesis study on the Mud River, the last major tributary of the Guyandotte River before it enters the Ohio River showed consistently elevated counts of ciprofloxacin resistant bacteria at one site regardless of whether samples were taken during dry weather or after a rain event. The researcher concluded that there was a direct input of ARB into the river at that site (Dotson, 2008). Although survey data on ciprofloxacin resistant bacteria found in the Ohio River basin indicate median counts per ml at or below 500 CFU/ml, that still translates to billions or trillions of ciprofloxacin resistant bacteria flowing past a point in the river at any given time. The public health input of that population is not yet known. It is important to continue monitoring watersheds in and near urban areas, where the direct input of ARB and/or antibiotic containing contaminants (i.e. hospital wastewater effluent) have the potential to enter without treatment.

## VIRGINIAMYCIN

Virginiamycin [Fig 3] belongs to the class of antibiotics known as streptogramins, and has been used in the United States for over twenty six years. It is primarily used as a food additive in veterinary medicine for growth promotion and to prevent and control diseases in food-producing animals such as chickens, turkeys, swine, and cattle (US FDA, 2004). Virginiamycin inhibits protein synthesis by binding to the ribosomal RNA 23S subunit of the 50S subunit therefore halting translation and bacterial reproduction (Yonath, 2005). Many studies on virginiamycin resistance have focused on the potential effects streptogramin use in food-producing animals has on streptogramin resistance in human medicine. The use of

virginiamycin in food animals has been banned in Denmark since 1998 and Europe since 1999 due to the spread of streptogramin resistant strains of *Enterococcus faecium* in humans (Aarestrup, Seyfarth, Hanne-Dorthe, Pedersen, Hendriksen, & Bager, 2001) (Mehnam, Beighton, Philpott-howard, & Woodford, 2000). Enterococcal infections are the leading cause of urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis. *E. faecium* has become a widespread nosocomial infection affecting patients worldwide. In 1999, the US FDA approved Synercid, a streptogramin antibiotic, for the treatment of vancomycin resistant *E. faecium* infections. Emerging resistance among Synercid using patients has encouraged the FDA to perform a risk assessment to determine the relationship between the use of virginiamycin in food producing animals and the development of streptrogramin (Synercid) resistant *E. faecium* in human medicine (US FDA, 2000).

Since virginiamycin is still being used today in veterinary and agricultural applications in the United States, the spread of virginiamycin resistant bacteria is likely. Agriculture remains an economically important activity in the Ohio River Valley, and a large percentage of land within the basin is used for farming. According to the EPA, agriculture is the second leading source of pollution to the Ohio River and Tennessee River basins due to pastureland, animal holding, and feedlots. As a result, 40% of the rivers and streams are impaired, not achieving full support for aquatic life (WV EPA & ORSANCO, 1994).

**FIGURE 1: CHEMICAL STRUCTURE OF TETRACYCLINE**

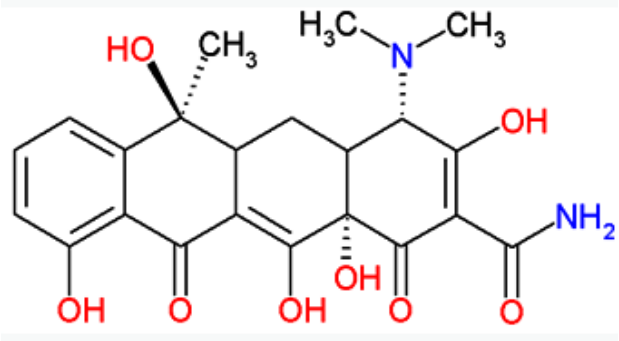



**FIGURE 2: CHEMICAL STRUCTURE OF CIPROFLOXACIN**

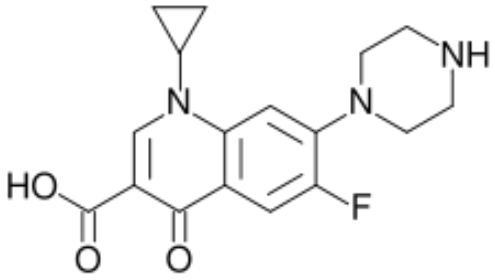
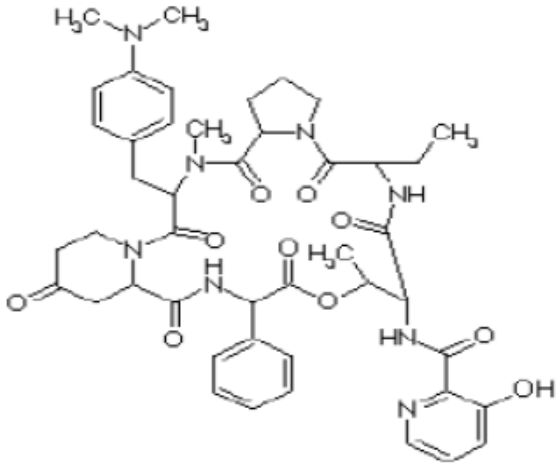



**FIGURE 3: CHEMICAL STRUCTURE OF VIRGINIAMYCIN**



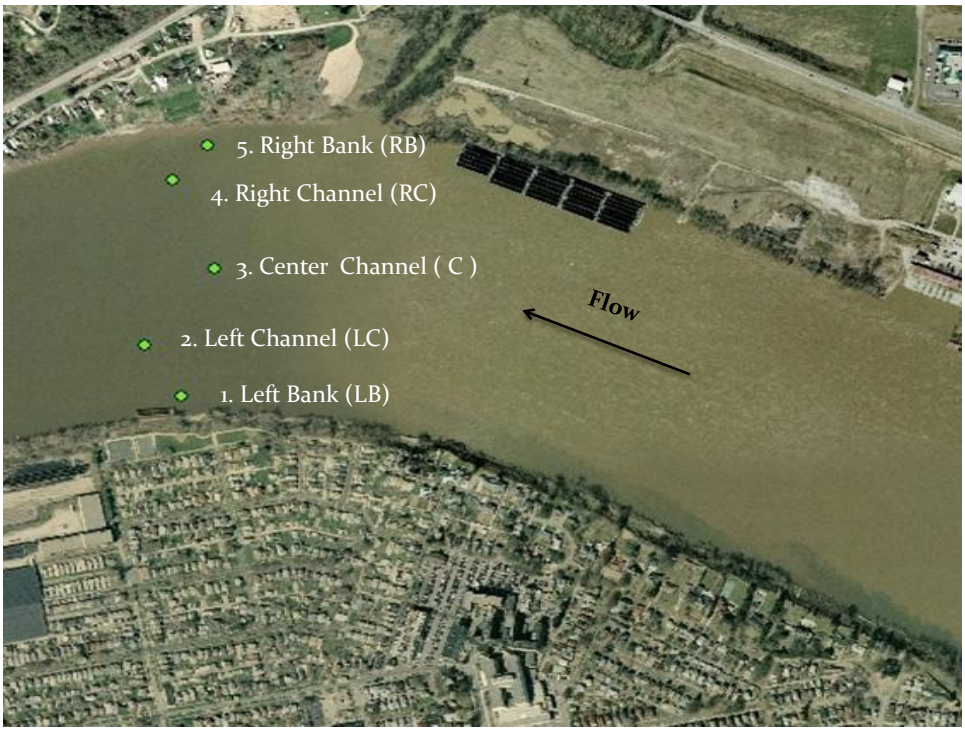
# CHAPTER III: MATERIALS & METHODS

## *PART 1: OHIO RIVER STUDY, 2007*

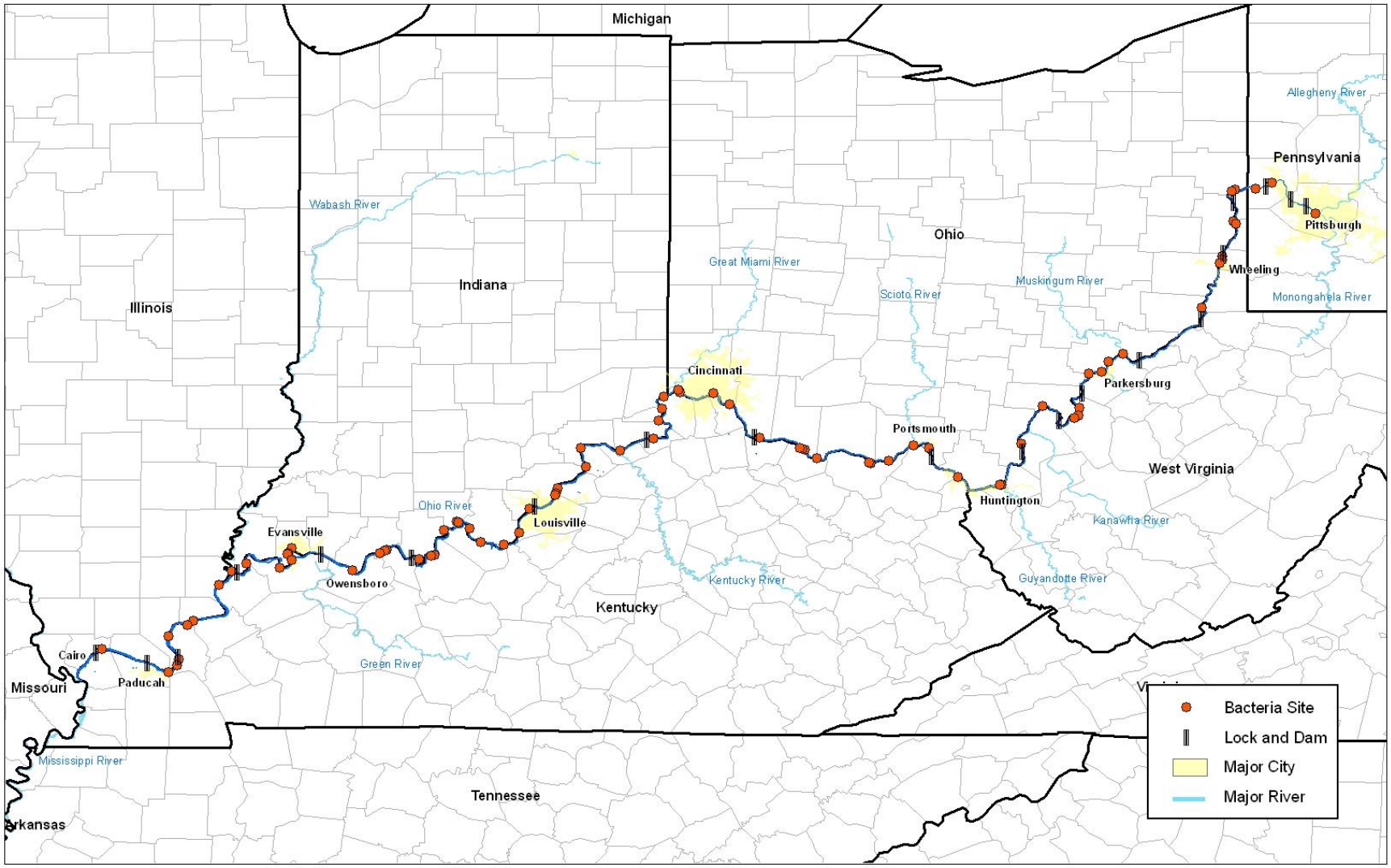
### OVERVIEW

During the summer of 2007, 77 sites along the mainstem of the Ohio River were sampled for bacteria. All sites were randomly chosen by one-tenth river mile using Excel's random number generator. The location across the width of the river at each river mile was also randomly chosen, designated by; 1. Left Bank (LB), 2. Left Channel (LC), 3. Center/Navigational Channel (C), 4. Right Channel (RC), and 5. Right Bank (RB) [Fig 4]. All sites were located by boat, with the aid of the Ohio River navigational charts and a Garmin 480C GPS unit. GPS waypoints were also recorded to mark the precise location of the boat at each site along the river [Fig 5]. A total of six 100 ml water samples were aseptically taken at each site, including three subsurface (≤ 1 ft below surface tension layer) samples and three bottom samples. The bottom samples were collected via SCUBA. For each set of samples, surface and bottom, one water sample was used as the control (no antibiotic added), and the other two samples were used to test for tetracycline resistant coliforms and *E. coli*, and ciprofloxacin resistant coliforms and *E. coli*.

**FIGURE 4: AERIAL PHOTO DEPICTING RIVER QUADRANTS**



## FIGURE 5: OHIO RIVER RUN 2007, SITE MAP



## WATER SAMPLE COLLECTION

Sterile 100 ml collection bottles were used to collect three subsurface water samples and three samples collected from the bottom of the river. Dates and times were noted for each sample site. The subsurface samples were collected from the boat, and the bottom samples were collected by a SCUBA diver. The bottom sampling method included using a hand "wafting" motion at the bottom of the river to stir up the substrate just before taking the sample. Neutral buoyancy was maintained to avoid mixing and stirring up of the water in any other form than the "wafting" motion. The three sterile bottles were then opened by the diver and tightly closed before bringing the sample to the surface. All water samples were placed on ice and processed for total coliforms, *E. coli*, and antibiotic resistant coliforms and *E. coli* using the IDEXX Colilert® Quanti Tray/® 2000 method, with and without antibiotic addition, within six hours of each collection.

## ENUMERATION OF TOTAL AND RESISTANT COLIFORM BACTERIA AND *E. COLI*

Coliforms and *E. coli* were enumerated as well as tetracycline and ciprofloxacin resistant coliforms and *E. coli* using the IDEXX Colilert® QuantiTray® 2000/ method. Excess water was poured off of each collection bottle until the water meniscus reached the 100 ml mark. One pre-measured Colilert® reagent packet was added to each bottle of sample water. The bottles were vigorously shaken until the Colilert® reagent was completely dissolved. The control samples were poured into the IDEXX 97 well QuantiTray®/2000, heat sealed, and incubated at 35°C for 24 hours. Total coliforms and *E. coli* MPN's were analyzed for each of the control samples as described below.

To select for antibiotic resistant coliform and *E. coli* from each sample set, antibiotics were aseptically added to the sample bottles after the Colilert® reagent had dissolved. Tetracycline hydrochloride was added at a final concentration of 12.5 µg/ml to one sample and ciprofloxacin was added at a final concentration of 4.7 µg/ml to a separate sample for each set. The samples were then shaken again to thoroughly mix and transferred to an IDEXX 97 well QuantiTray®/2000, heat sealed and incubated at 35°C for 24 hours.

After incubation, the QuantiTrays were removed from the incubator and analyzed for the presence of coliform bacteria and *E. coli*. Yellow wells indicated a positive reaction for coliform bacteria as a result of the hydrolysis of ortho-Nitrophenyl-β-galactoside (ONPG) by the bacterial enzyme β-galactosidase, while fluorescence under UV light indicated a positive reaction for *E. coli* as a result of the hydrolysis of 4-methylumbelliferyl-βD-glucuronide (MUG) by β-glucuronidase. Colorless wells indicated the absence of coliforms and *E. coli*. All positive wells were counted and the most probable number (MPN) per 100 ml sample was determined for total coliforms, *E. coli*, tetracycline resistant coliforms, tetracycline resistant *E .coli*, ciprofloxacin resistant coliforms, and ciprofloxacin resistant *E. coli* using the IDEXX Colilert ® QuantiTray® 2000 Most Probable Number Table (Appendix C).

## *PART 2: OHIO RIVER/GUYANDOTTE RIVER STUDY, 2008*

### SITE DESCRIPTION

On July 3, 2008, a detailed study of a small section of the Ohio River was done at the mouth of the Guyandotte River. This study was done to determine how well a single grab sample represented bacteriological conditions at other horizontal and vertical positions along a perpendicular river transect. The Guyandotte River was chosen because it is known to be highly impacted by abandoned acid mine drainage, pollution, poor wastewater treatment, and sedimentation. Land use and the current conditions of the Guyandotte River make it a known source for bacterial loading into the Ohio River (Somerville, Saunders, & Van Meter, May 2002). Previous studies on the Mud River, the last tributary of the Guyandotte River before it empties into the Ohio River, has also been studied in some detail, revealing it too is a possible source for *E. coli*, and antibiotic resistant bacteria loading into the Ohio River via flow from the Guyandotte River (Dotson, 2008). Water chemistry and sediment data were also collected to look for relationships between these variables and bacterial counts.

Sets of samples were taken within the Guyandotte River, in the Ohio River above the Guyandotte at river mile 305, and just below the mouth of the Guyandotte River. A total of 13 sites were sampled. Samples were taken along a horizontal transect, perpendicular to flow, to include sites on the left bank (LB), center channel (C), and right bank (RB) of the Guyandotte River. Ohio River samples were taken at the left bank (LB), left channel (LC), center navigational channel (C), right channel (RC), and right bank (RB). At each site samples were collected at every five feet in depth via SCUBA.

To collect samples at every five feet, a SCUBA diver followed an anchored rope marked in five feet increments from the surface taking two 100 ml samples at each stopping point until reaching the bottom of the river. The maximum depth reached was 20 feet in the Ohio River. After each collection, the water samples were placed on ice and processed in the laboratory within six hours of collection.

Both rivers were navigated by boat and GPS waypoints were marked at each site using a Garmin 480C GPS unit. [Figures 6 & 7]. A total of 43 water samples were collected and enumerated for *E. coli* and total coliforms (see IDEXX Colilert® Quanti Tray®/2000 Method from part 1), as well as total cultivable bacteria, and antibiotic resistant bacteria using the R2A plate count method.

## Enumeration of Total Cultivable and Antibiotic Resistant Bacteria

To enumerate total cultivable bacteria, 100 µl (0.1 ml) of river water from one of the sample bottles for each site and depth was added to 9.9 ml of sterile water to dilute the sample. 100 µl aliquots of the diluted samples were aseptically transferred onto prepared Difco R2A agar plates containing fungizone (375 ng/ml). Five, 5 mm sterile glass beads were added to each plate and the plates were shaken horizontally to evenly spread the diluted water sample on the surface of the agar. Once the plate was inoculated the beads were discarded into a beaker containing 95% ethanol. This step was performed in triplicate to calculate the average colony forming units of bacteria per milliliter (CFU/ml) of sample water. Each set of three plates was wrapped with parafilm to keep them from drying out and incubated at 25°C for seven days.

To enumerate antibiotic resistant bacteria, R2A agar plates were prepared containing fungizone plus the appropriate concentration of a single antibiotic. The specified concentrations used for the antibiotics ciprofloxacin, tetracycline hydrochloride, and virginiamycin were 4.7 µg/ml, 12.5 µg/ml, and 16 µg/ml, respectively. Triplicate plates for each antibiotic were prepared by aseptically transferring 100 µl of undiluted river water for each sample onto the R2A plates (containing fungizone plus antibiotic). Five, 5 mm sterile glass beads were used to evenly spread the solution across the surface of the agar and then discarded as previously described. Upon inoculation, each set of plates was wrapped in parafilm and incubated at 25°C for seven days.

After seven days, all plates were removed from the incubator and the numbers of colonies per plate were counted. Triplicate plate counts were averaged and the number of CFU per ml in the original sample was determined by multiplying the average number of colonies counted by the dilution factor; $10^3$ for total cultivable bacteria, and $10^1$ for ARB. This raw data is summarized in Tables 3 - 7 of Appendix B.

See Appendix C for detailed protocols summarizing the preparation and enumeration of total cultivable and ARB using the R2A spread plate method.

**FIGURE 6: AERIAL PHOTO DEPICTING 2008 STUDY SITES IN THE OHIO RIVER AND GUYANDOTTE RIVER**

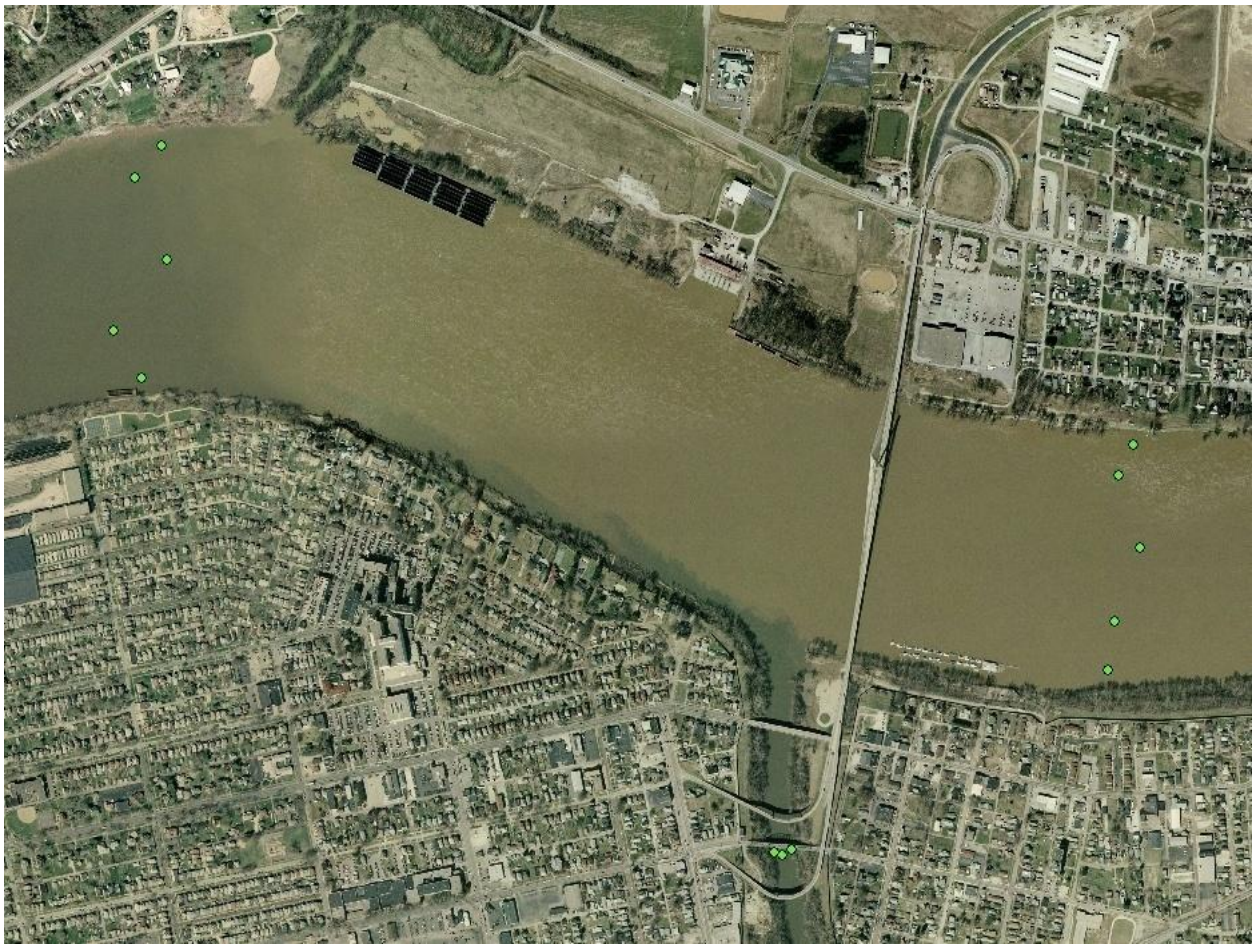


**FIGURE 7: AERIAL PHOTO DEPICTING 2008 STUDY SITES IN THE GUYANDOTTE RIVER (ZOOMED IN)**

## WATER CHEMISTRY

Water chemistry data was collected at the surface and at every five feet in depth, including temperature, pH, percent dissolved oxygen (%DO), and turbidity using a YSI. The diver descended with the YSI as the results were read from the handheld base on the boat. The diver wore a full face mask and used Ocean Reef dive Com gear to allow communication between the diver and boat at all times. Water chemistry data are summarized in Table 2 of Appendix B.

### Sediment Analysis

To determine the amount of sediment present in each sample, a simple vacuum filtration method was used. Each sample was vigorously shaken prior to filtering to equally suspend the amount of the sediment throughout the 100 ml collection bottle. A sub-sample (25 ml) of each sample was filtered through a pre-weighed 0.45 micron membrane filter. Upon filtration, each filter membrane with sediment was placed in a drying oven for 24 hour. The filters were then weighed and the amount of sediment in grams per 25 ml of water was calculated. This calculation was then converted to mg/ml of sediment [Table 8 of Appendix B].

## *DATA ANALYSIS*

The objectives of part 1 of this study were to analyze the distribution of total *E. coli*, total coliforms, as well as tetracycline resistant *E. coli* and coliforms, and ciprofloxacin resistant *E. coli* and coliforms along the length of the Ohio River. Subsurface and bottom samples were compared to determine whether there were any significant differences between samples taken at

the surface and samples taken at the bottom of the river which were manipulated to include sediment. Statistica 8.0 and SigmaStat 3.5 statistical analysis programs were used to analyze the data. Nonparametric statistics including Spearman's rho correlations and Mann-Whitney rank sum tests were used based on the assumption that the population tested was not normally distributed. This assumption was verified when the data set failed to pass the Kolmogorov-Smirnov normality test [Results shown in Appendix A]. Bacteria were plotted by river mile to determine bacterial distribution patterns, as well as locate sites where bacterial populations "spiked" along the river. These sites were further examined to determine possible inputs of bacterial loading, such as tributaries, outfalls, and sewer outputs.

Part 2 of this study further investigated how *E. coli* and ARB are distributed throughout the water column and tested the hypothesis that sediments are critical to their distribution. Analysis of both studies were used to determine whether a single surface sample provides a representative measurement of bacteria for the entire river at a specific location, and decide whether ARB should be used in assessing water quality and meeting public health standards. To explore these questions, once again, statistical analysis was performed using Statistica 8.0 and SigmaStat 3.5. All data variables including river quadrant, depth, pH, percent dissolved oxygen (%DO), turbidity, *E. coli*, total cultivable bacteria (TCB), ciprofloxacin resistant bacteria (CipR), tetracycline resistant bacteria (TetR), virginiamycin resistant bacteria (VirR), and amount of sediment were compared. Pearson's product-moment correlation coefficient (PMCC) was used to compare all variables with each other to determine the correlation coefficient, designated Pearson's r, as well as significant *P*-values for each correlation. All significant correlations were graphed and visualized by scatter plots. Regression analysis was used to analyze and plot

bacterial data and sediment data, without any regard to the location or depth at which the sample was taken to determine whether the amount of sediment in a sample could predict the amount of bacteria in the sample. PMCC was used to analyze all variables within the Guyandotte River, in the Ohio River upstream of the Guyandotte River, and in the Ohio River downstream of the Guyandotte River separately. These results were used to determine whether depth and location across the river (quadrant) had an effect on the amount of *E. coli*, TCB, and ARB present at that location. Further analysis by ANOVA was used to establish whether or not the Guyandotte River is a source for total bacteria, *E. coli*, and/or antibiotic resistant bacteria into the Ohio River. Water chemistry data for each location was also compared for similarities and dissimilarities between the Guyandotte River and the Ohio River.

# CHAPTER IV: RESULTS

## *PART 1 : OHIO RIVER STUDY, 2007*

### COLIFORM BACTERIA DISTRIBUTIONS

All subsurface bacterial counts for total coliforms, tetracycline resistant coliforms and ciprofloxacin resistant coliforms were plotted and compared to bottom bacterial counts by river mile [Figs. 8 – 10]. These scatter plots show spikes in specific bacterial counts at different river locations. The majority of total coliform subsurface and bottom counts were above the MPN >2419.6 enumeration limit for the QuantiTray assay [Fig. 8]. These counts were recorded as 2500 MPN for graphing purposes but the actual MPN for these samples is not known.

Subsurface tetracycline resistant (TetR) coliform counts showed spikes at four specific locations along the river as compared to the remaining sites. These four locations include river miles, 36.1, 324.2, 528.4, and 793.0 [Fig. 9]. Several more bottom sample sites than top sample sites showed spikes in TetR coliforms. These river miles included 0.2, 36.1, 87.7, 414.8, 623.7, and 740.0 [Fig. 9]. There was an overall lower number of ciprofloxacin resistant (CipR) coliforms present across all sites, subsurface and bottom, as compared to TetR coliforms. The Mann-Whitney Rank Sum Test verified that the differences between subsurface and bottom coliform populations were significant. Many of the samples and sites had zero CipR bacterial cells present. Two spikes are seen in the subsurface samples in Figure 10 at river miles 324.2 and 369.2, with only 39.3 CipR coliform cells (MPN) and 18.3 CipR coliform cells (MPN)

respectively. Figure 10 also shows three spikes in CipR coliforms in samples taken from the bottom of the river at river miles 0.2, 36.1, and 87.7, with CipR cell counts of 103.9, 123.9, and 248.9, respectively. When comparing bottom CipR and TetR coliforms, river miles 0.2, 36.1, and 87.7 were found to have increased numbers of coliforms that were resistant to both antibiotics. See Figure 49 for descriptions of each of these river mile sites in Chapter V: Discussion.

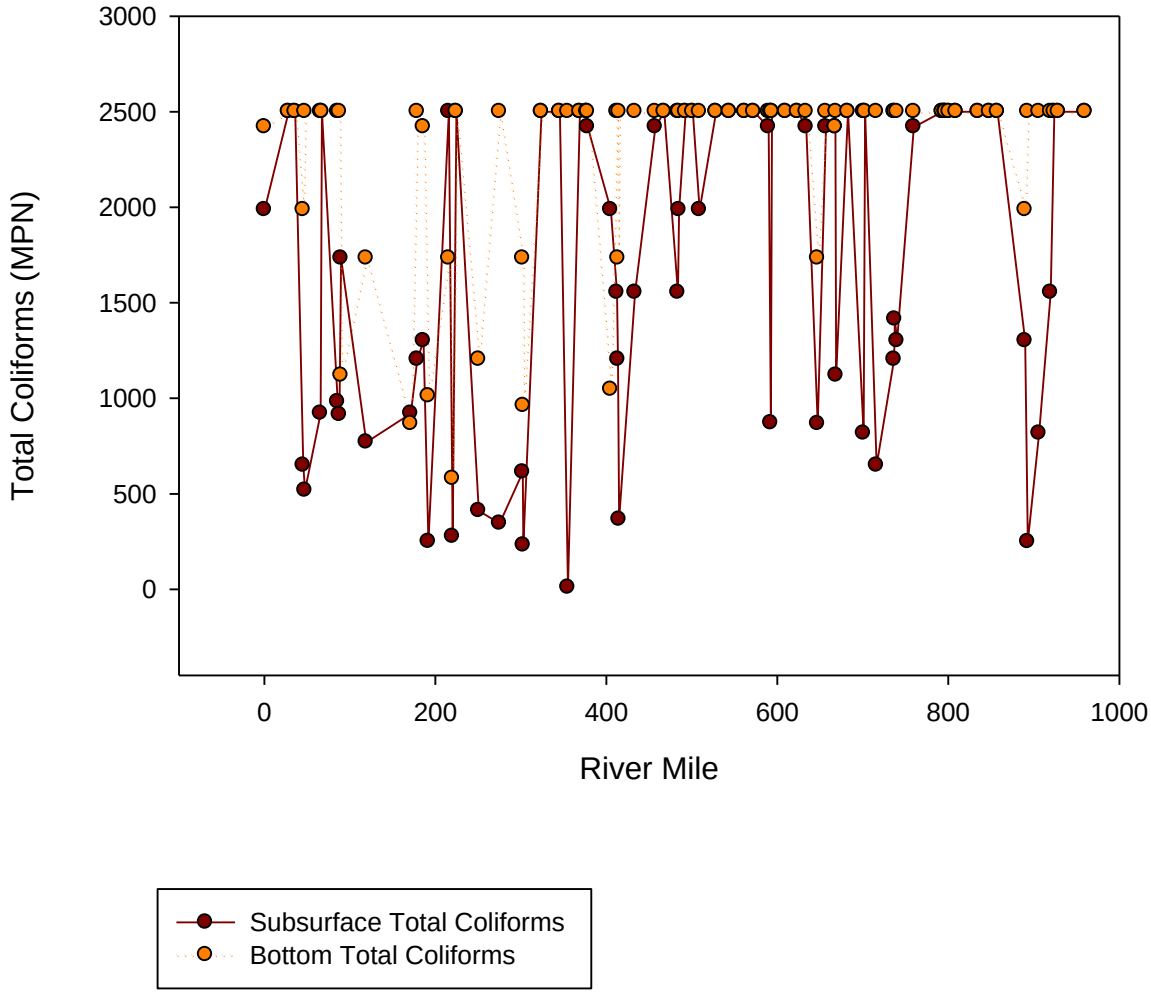



**FIGURE 8:** Ohio River Study 2007, Subsurface vs Bottom Total Coliform Bacteria Counts by River Mile.

Sites with > 2419.6 MPN of coliform bacteria are graphed as 2500 MPN, due to the maximum limitability of the IDEXX QuantiTray 2000 Method.

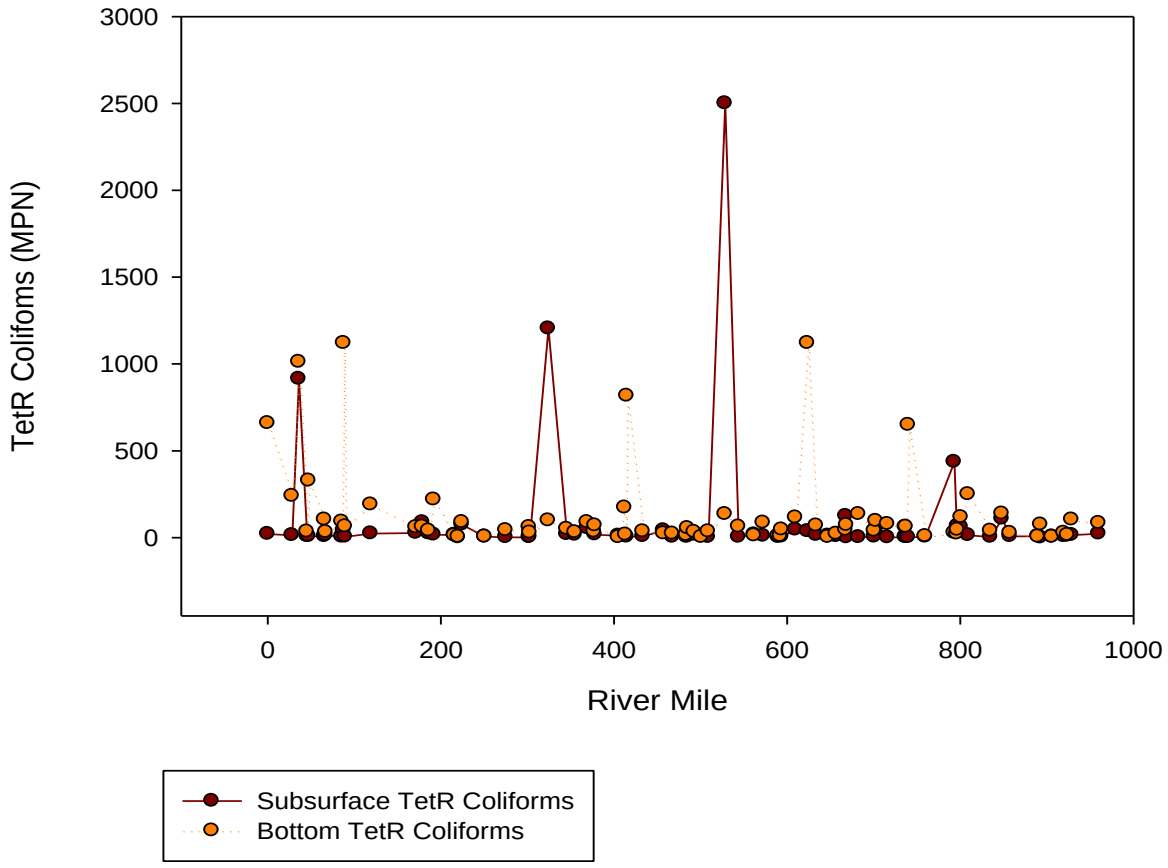



**FIGURE 9:** Ohio River Study 2007, Subsurface vs Bottom Tetracycline Resistant (TetR) Coliform Bacteria Counts by River Mile.

Sites with >2419.6 MPN of TetR coliform bacteria are graphed as 2500 MPN, due to the maximum limitability of the IDEXX QuantiTray 2000 Method. Spikes in subsurface TetR coliforms are shown at river miles, 36.1 (MPN 913.9), 324.2 (MPN 1203.3), 528.4 (MPN >2419.6), and 793.0 (MPN 435.2). Spikes in bottom TetR coliforms are shown at river miles, 0.2 (MPN 658.6), 36.1 (MPN 1011.2), 87.7 (MPN 1119.9), 414.8 (MPN 816.0), 623.7 (MPN 1119.9) and 740.0 (MPN 648.8).

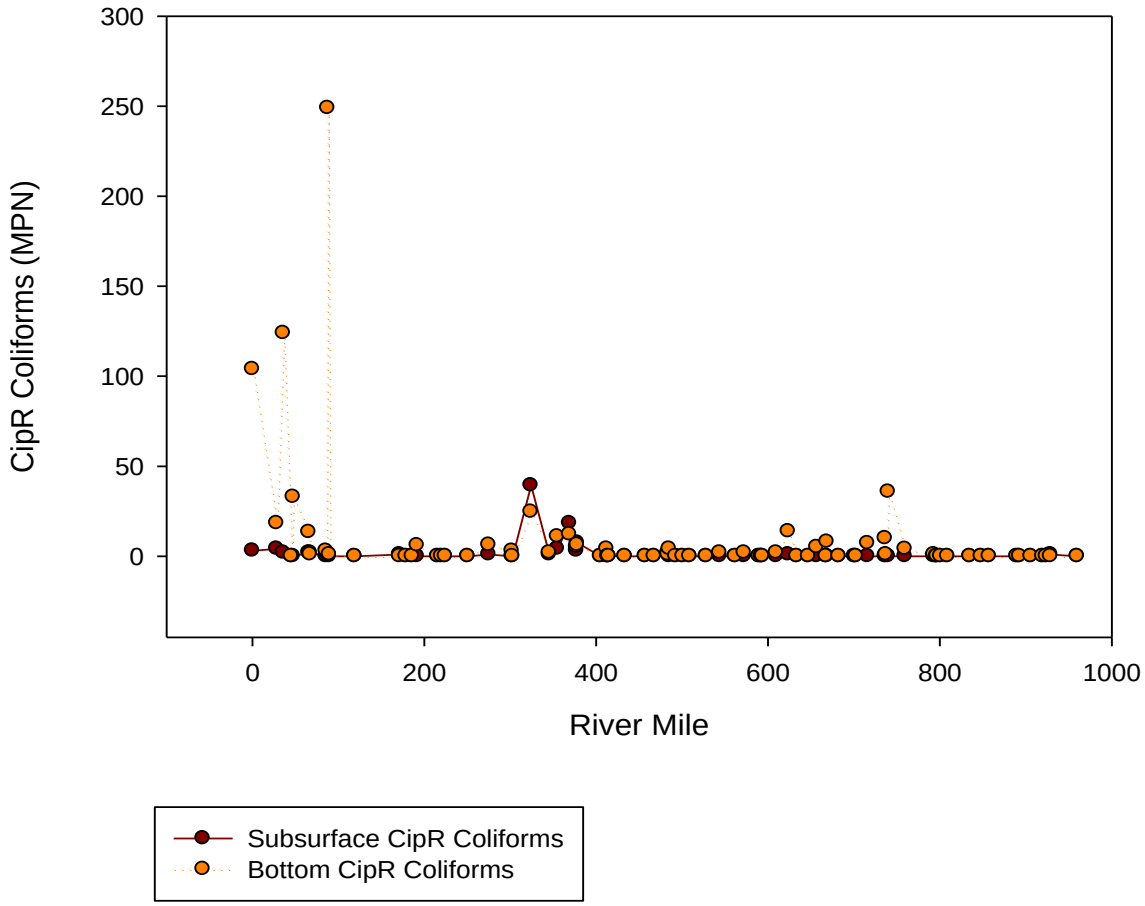



**FIGURE 10:** Ohio River Study 2007, Subsurface vs Bottom Ciprofloxacin Resistant (CipR) Coliform Bacteria Counts by River Mile

Spikes in subsurface CipR coliform bacteria are shown at river miles 324.2 (MPN 39.3) and 369.2 (MPN 18.3). Spikes in bottom CipR coliform bacteria are shown at river miles 0.2 (103.9 MPN), 36.1 (123.9 MPN), and 87.7 (248.9 MPN).

## *E. COLI* DISTRIBUTIONS

All subsurface and bottom bacterial counts for total *E. coli*, tetracycline resistant *E. coli* (TetR*Ec*), and ciprofloxacin resistant *E. coli* (CipR*Ec*) were plotted by river mile [Figs. 11-13]. Like the coliform scatter plots, these plots also reveal "spikes" in *E. coli* contamination throughout the Ohio River at specific river mile locations. Subsurface total *E. coli* samples showed increased counts at river miles 89.7, 324.2, and 302.2 [Fig. 11]. River miles 89.7 and 324.2 exceed the recreational water use criteria, which states no single 100 ml sample can exceed 240 MPN of *E. coli* (ORSANCO River Facts/Conditions). Although total bottom *E. coli* counts did not exceed the criteria Figure 11 also shows an increase in *E. coli* taken from the bottom of the river at river miles 0.2 and 186.2. River mile 324.2, furthermore, showed an increase in subsurface and bottom TetR*Ec* [Fig. 12]. At river mile 793.0, the subsurface sample contained 272.3 cells (MPN) of TetR*Ec*, once again exceeding the criteria, as well as being resistant [Fig. 12]. The majority of samples taken from the bottom of the river showed < 10 MPN of TetR*Ec* with all samples containing MPN <60 for TetR*Ec* [Fig. 12]. CipR*Ec* counts were lower than TetR*Ec* counts throughout all of the samples with the majority having zero cells and the rest having < 30 MPN [Figs 13]. Figure 49 lists the descriptions of each of these sites in Chapter V: Discussion.

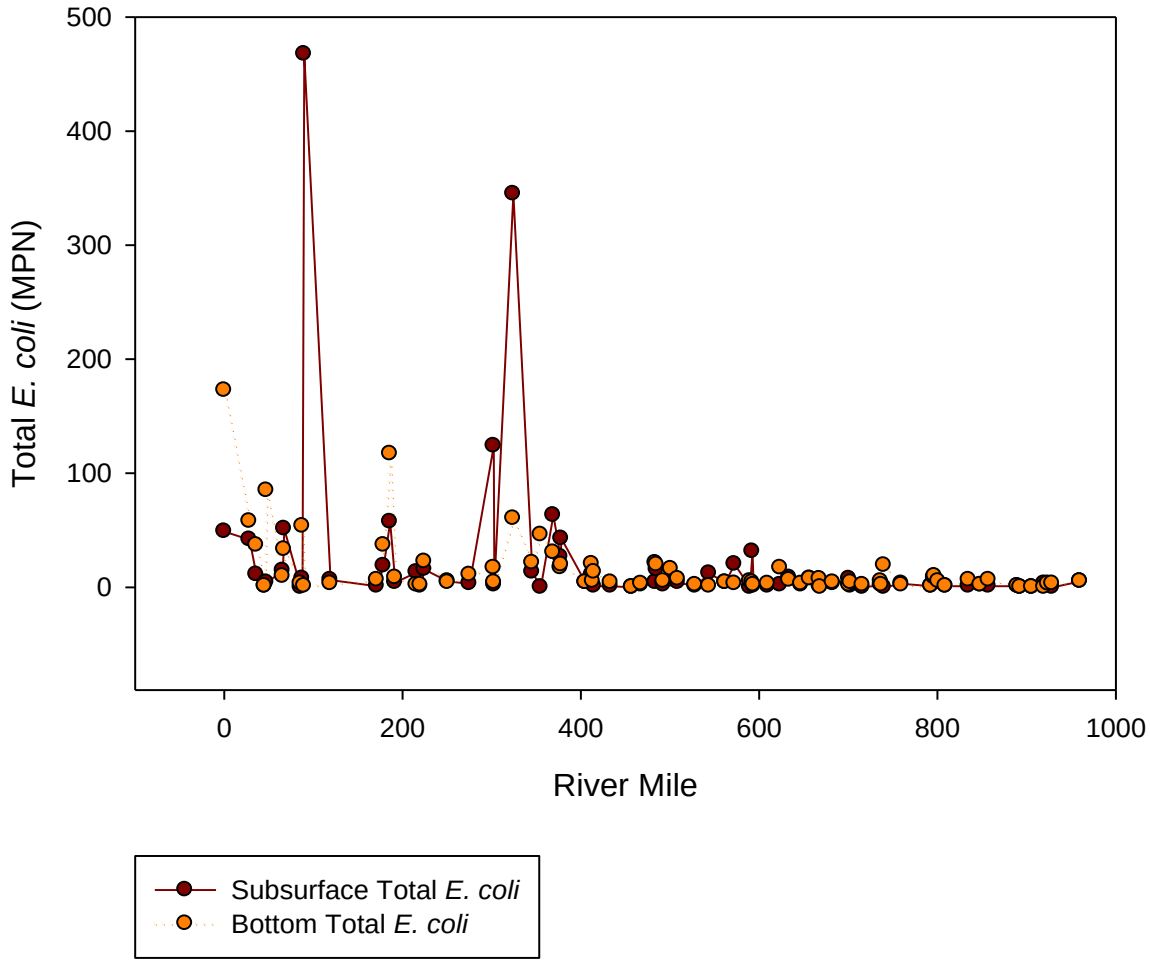



**FIGURE 11:** Ohio River Study 2007, Subsurface vs Bottom Total *E. coli* Bacteria Counts by River Mile

Spikes in subsurface total *E. coli* bacteria counts are shown at river miles, 89.7 (MPN 467.4), 302.2 (MPN 123.9) and 324.2 (MPN 344.8). Spikes in bottom total *E. coli* bacteria counts are shown at river miles, 0.2 (MPN 172.6), 47.7 (MPN 84.7) and 186.2 (MPN 116.9)

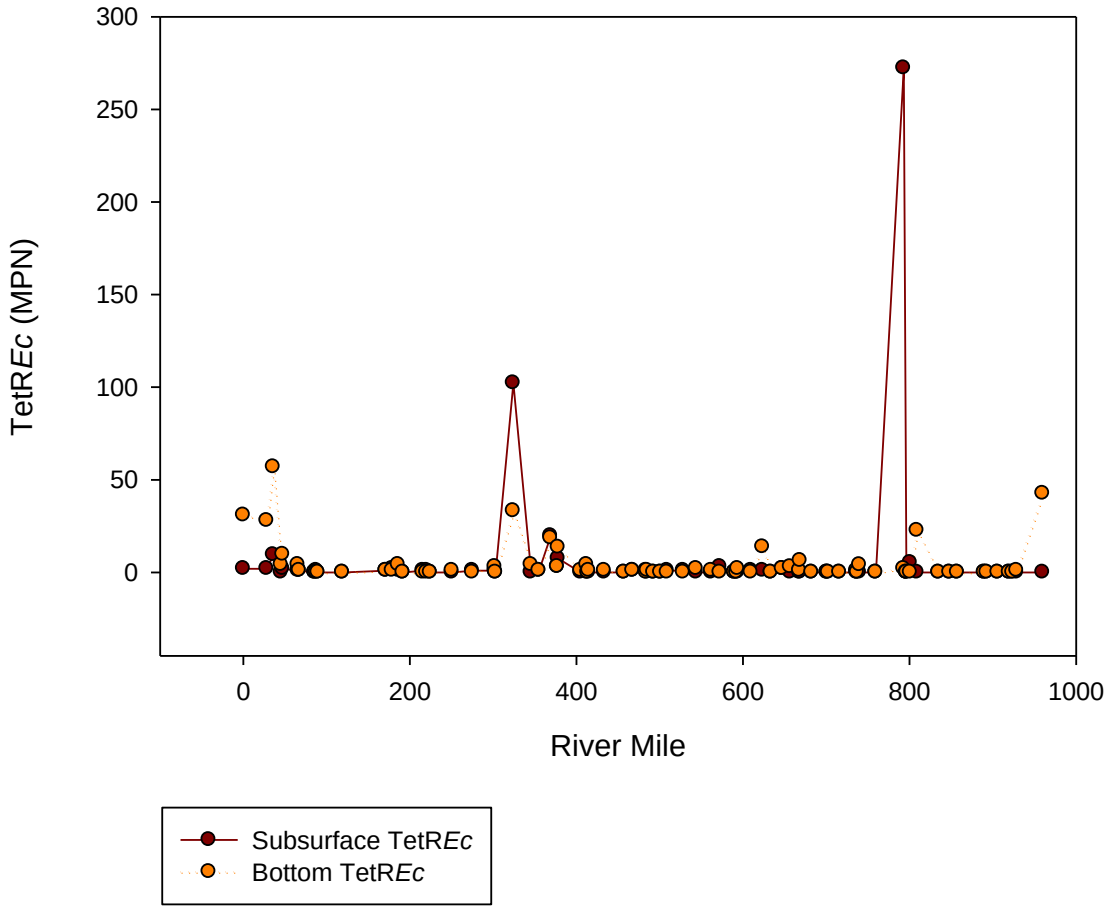



**FIGURE 12:** Ohio River Study 2007, Subsurface vs. Bottom Tetracycline Resistant *E. coli* (TetR*Ec*) Bacteria Counts by River Mile

Spikes in subsurface TetR*Ec* counts are shown at river miles 324.2 (MPN 102.2) and 793.0 (MPN 272.3). Spikes in bottom TetR*Ec* relative to their subsurface samples are shown at river miles 0.2 (MPN 30.9), 28.4 (MPN 27.9), 36.1 (MPN 56.9), 324.2 (MPN 33.2), 623.7 (MPN 13.7), 809.1 (MPN 22.6) and 960 (MPN 42.6)

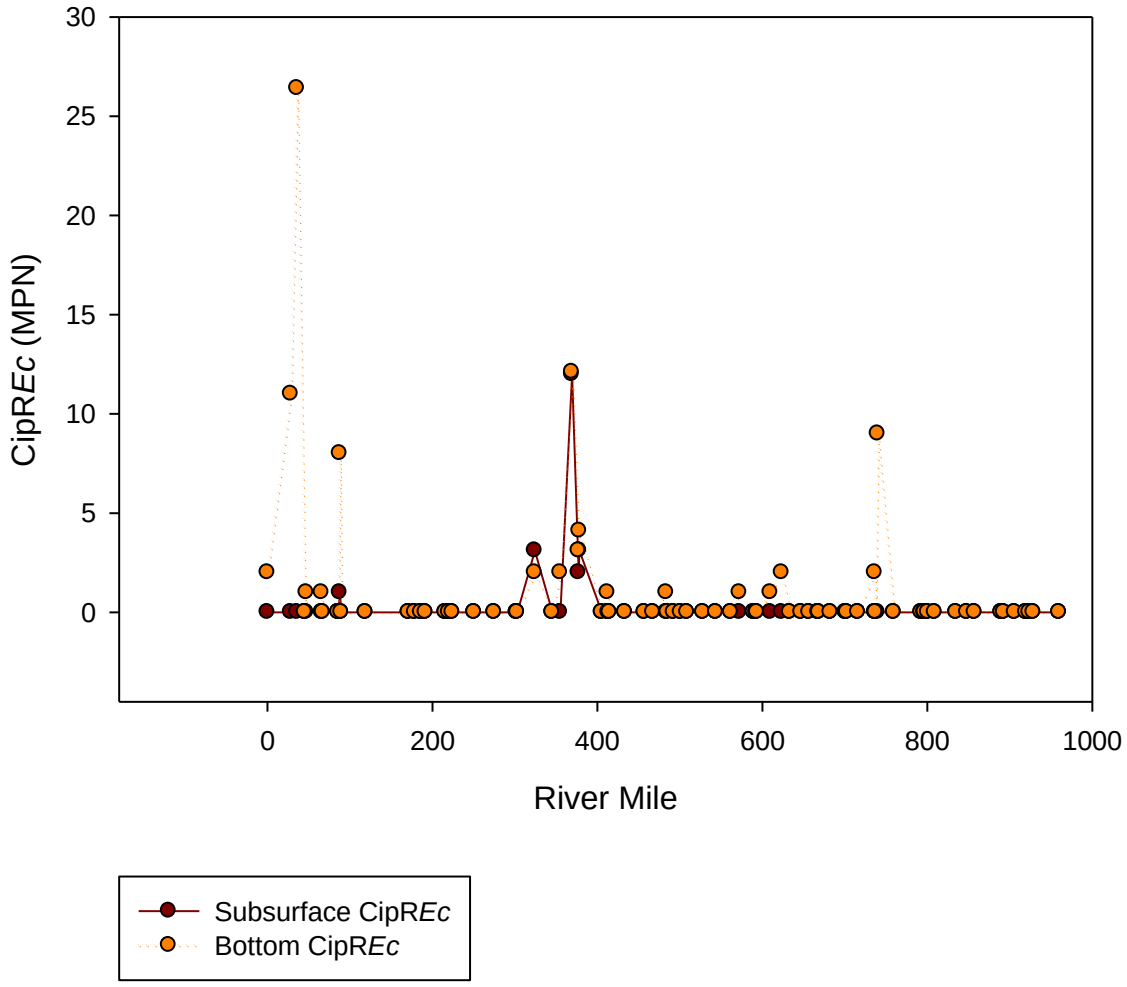



**FIGURE 13:** Ohio River Study 2007, Subsurface vs. Bottom Ciprofloxacin Resistant *E. coli* (CipR*Ec*) Bacteria Counts by River Mile.

Spikes in bottom CipR*Ec* bacteria counts relative to their subsurface samples are shown at river miles; 28.4 (MPN 11), 36.1 (MPN 26.4), 87.7 (MPN 8), and 740.0 (MPN 9). River mile 369.2 shows a spike in both subsurface and bottom samples with MPN 12 and 12.1, respectively.

## Statistical Analyses

### Mann-Whitney Rank Sum

All subsurface versus bottom samples for each bacteria type were then compared for median and sum rank differences and plotted. Point plot Figures 14 through 18 show these results for TetR coliforms, CipR coliforms, total *E. coli*, TetR*Ec*, and CipR*Ec.*, respectively. Note that a comparison between total coliforms is exempt due to a majority of the samples having MPN > 2419.6, with no exact value obtained.

### ANOVA on Ranks

When an ANOVA on ranks (Kruskal-Wallis One Way Analysis of Variance on Ranks) was run to determine the correlation between river quadrant and coliforms/*E. coli*, only total subsurface *E. coli* median values among the left bank samples showed a significant difference from the rest of the quadrants ($p=0.013$). A Pairwise Multiple Comparison Procedure (Dunn's Method) reveals that samples taken from the left bank show significant differences in subsurface total *E. coli* counts compared to the right and center channel samples. All other quadrant versus bacteria correlations were not significant among all samples taken in the Ohio River.

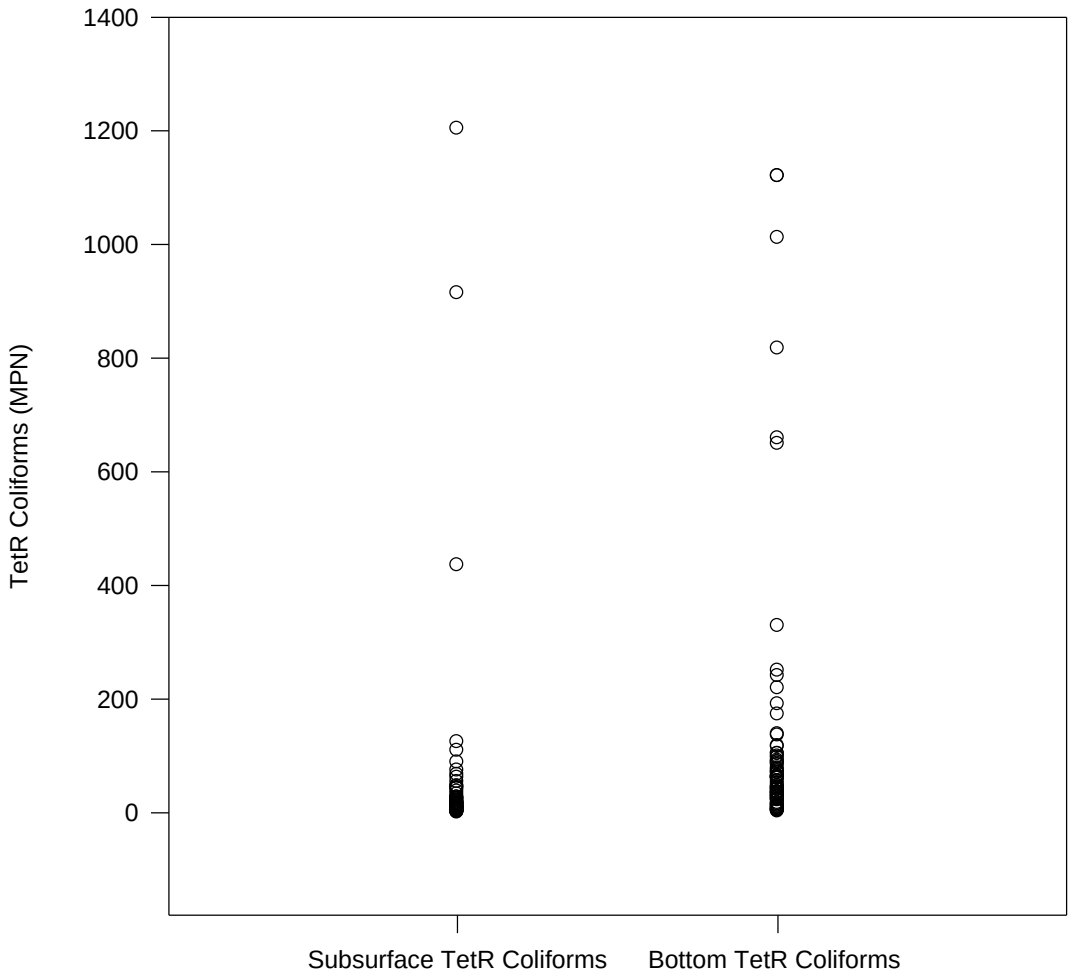



**FIGURE 14:** Ohio River Study 2007, Mann-Whitney Sun Rank Test Point Plot Analysis for Comparing Subsurface (top) vs. Bottom Tetracycline Resistant Coliforms (TetR)

Sampling sites with maximum values (MPN >2419.6) were not used in this analysis. Median values equal 9.75 and 52.35 for subsurface and bottom TetR coliforms, respectively. Mann-Whitney U Statistic = 4583.5; The difference in the rank sum between the two groups is greater than would be expected by chance; there is a statistically significant difference ($P < 0.001$)

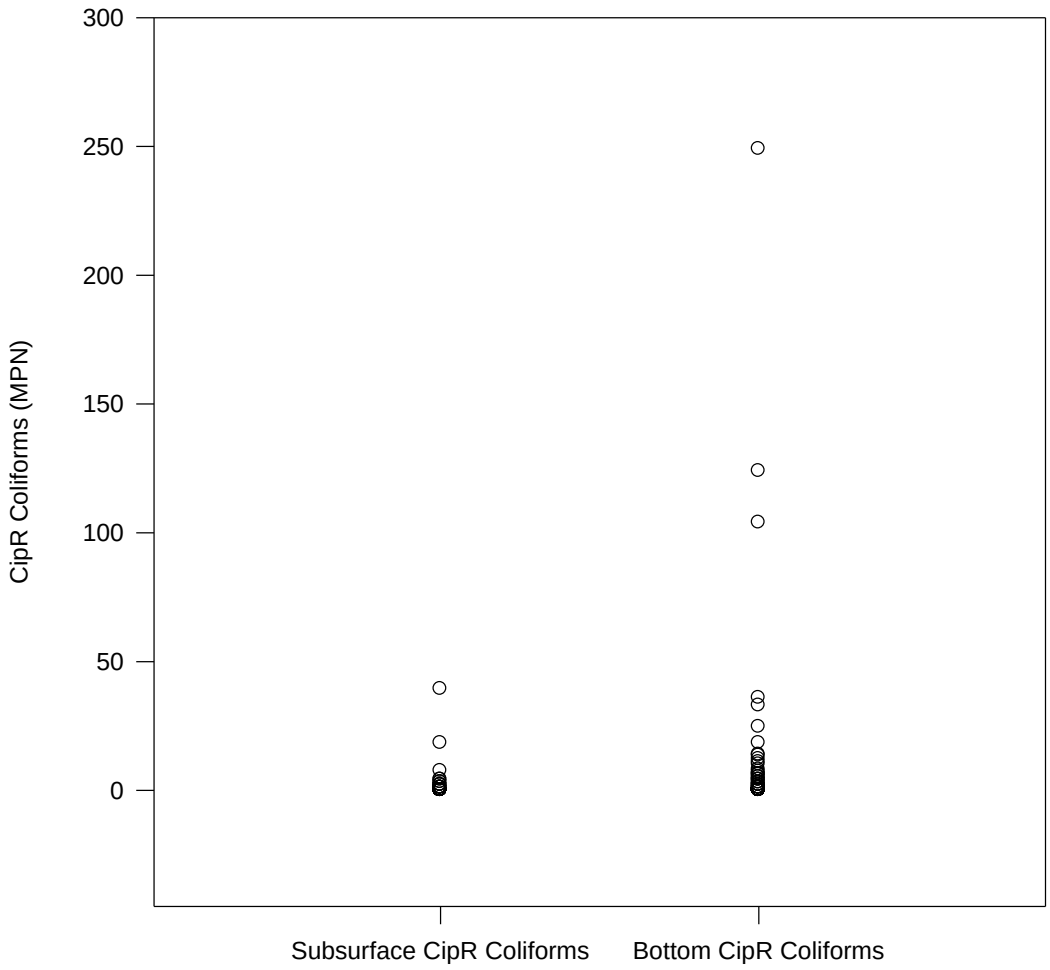



**FIGURE 15:** Ohio River Study 2007, Mann-Whitney Sum Rank Test Point Plot Analysis for Comparing Subsurface (top) vs. Bottom Ciprofloxacin Resistant Coliforms (CipR)

Mann-Whitney U Statistic = 3693.5; Both median values for subsurface and bottom coliforms are equal to zero; however, the sum of their ranks is larger for bottom CipR coliforms. The difference in values between the two groups is greater than would be expected by chance; there is a statistically significant difference ($P = 0.002$)

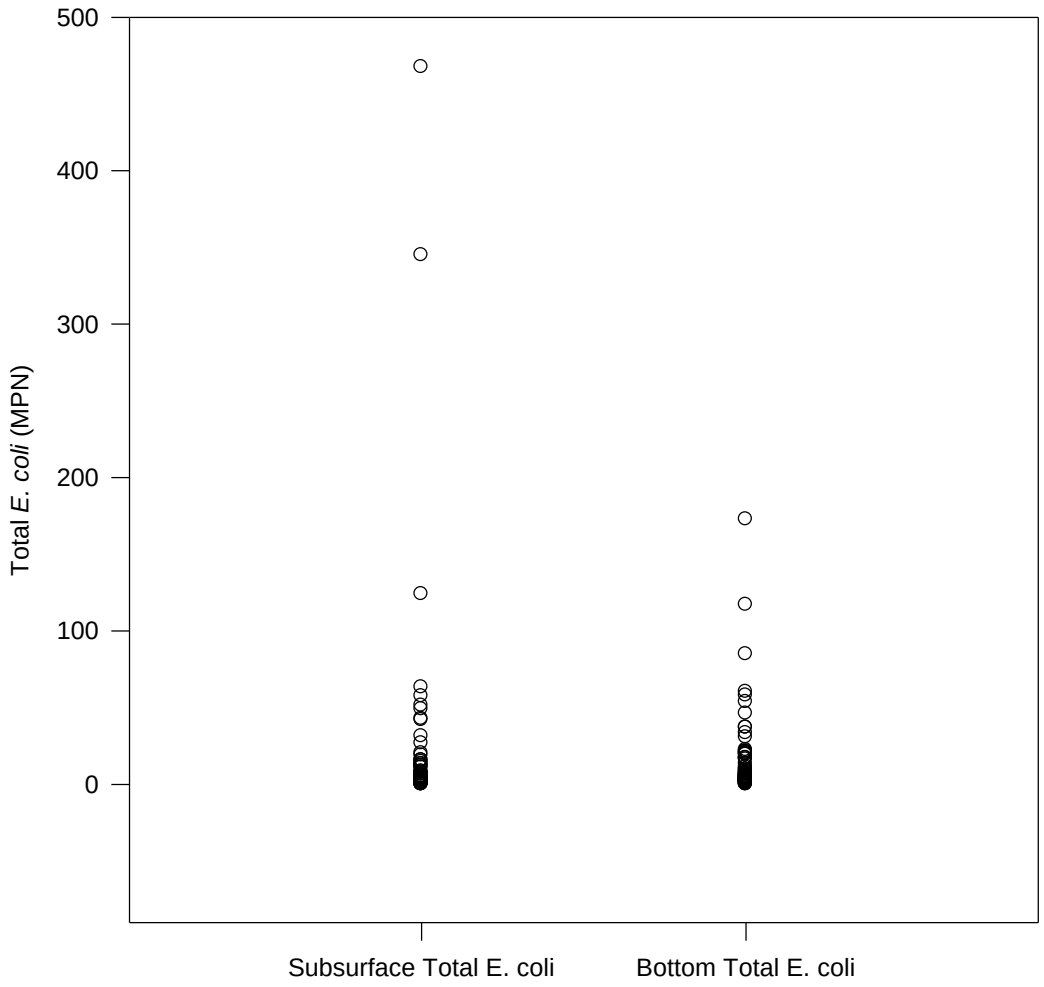



**FIGURE 16:** Ohio River Study 2007, Mann-Whitney Sum Rank Test Point Plot Analysis for Comparing Subsurface (top) vs. Bottom Total *E. coli*

Mann-Whitney U Statistic = 3458.0; Median values equal 4.1 and 5.1 for subsurface and bottom total *E. coli*, respectively. The difference in the rank sum between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference ($P = 0.074$)

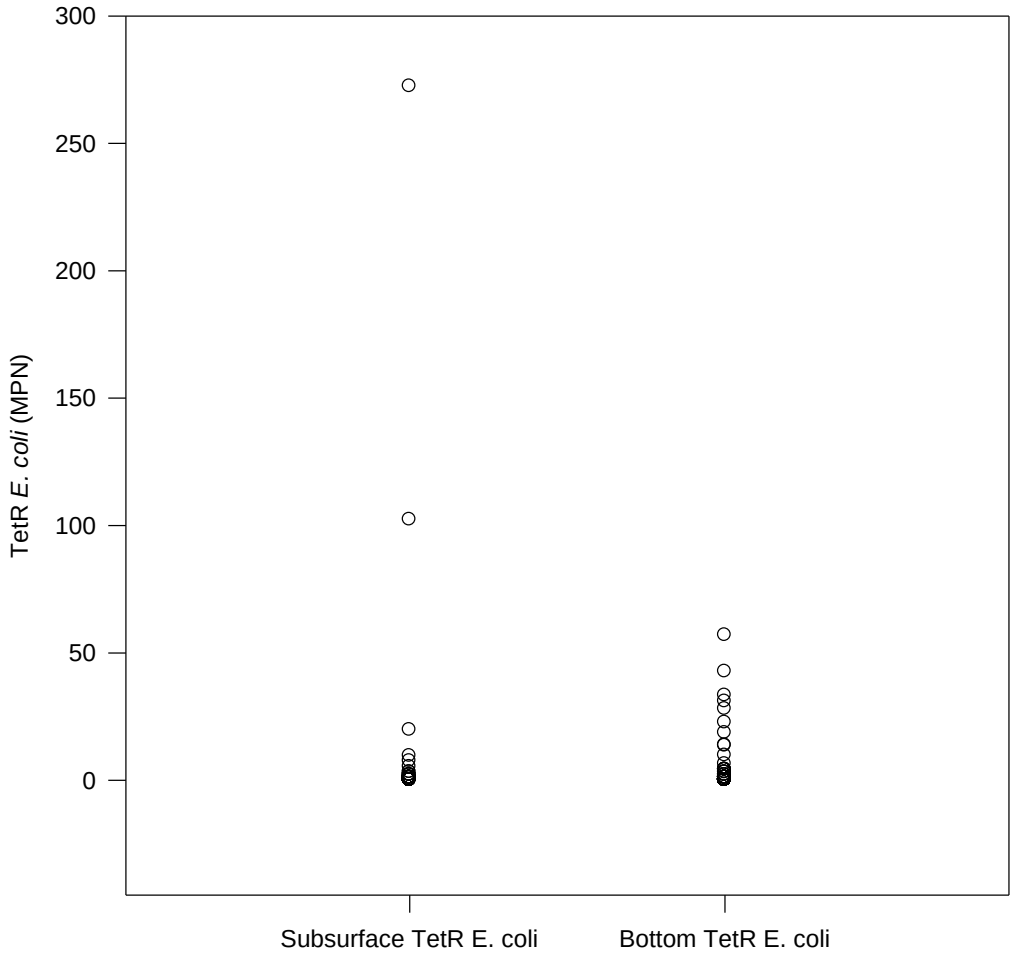



**FIGURE 17**: Ohio River Study 2007, Mann-Whitney Sum Rank Test Point Plot Analysis for Comparing Subsurface (top) vs. Bottom Tetracycline Resistant *E. coli*

Mann-Whitney U Statistic = 3412.5; Both median values for subsurface and bottom TetR *E. coli* equal zero. The difference in the rank sum between the two groups is not great enough to exclude the possibility that the difference is due to random sampling variability; there is not a statistically significant difference *(P* = 0.074)

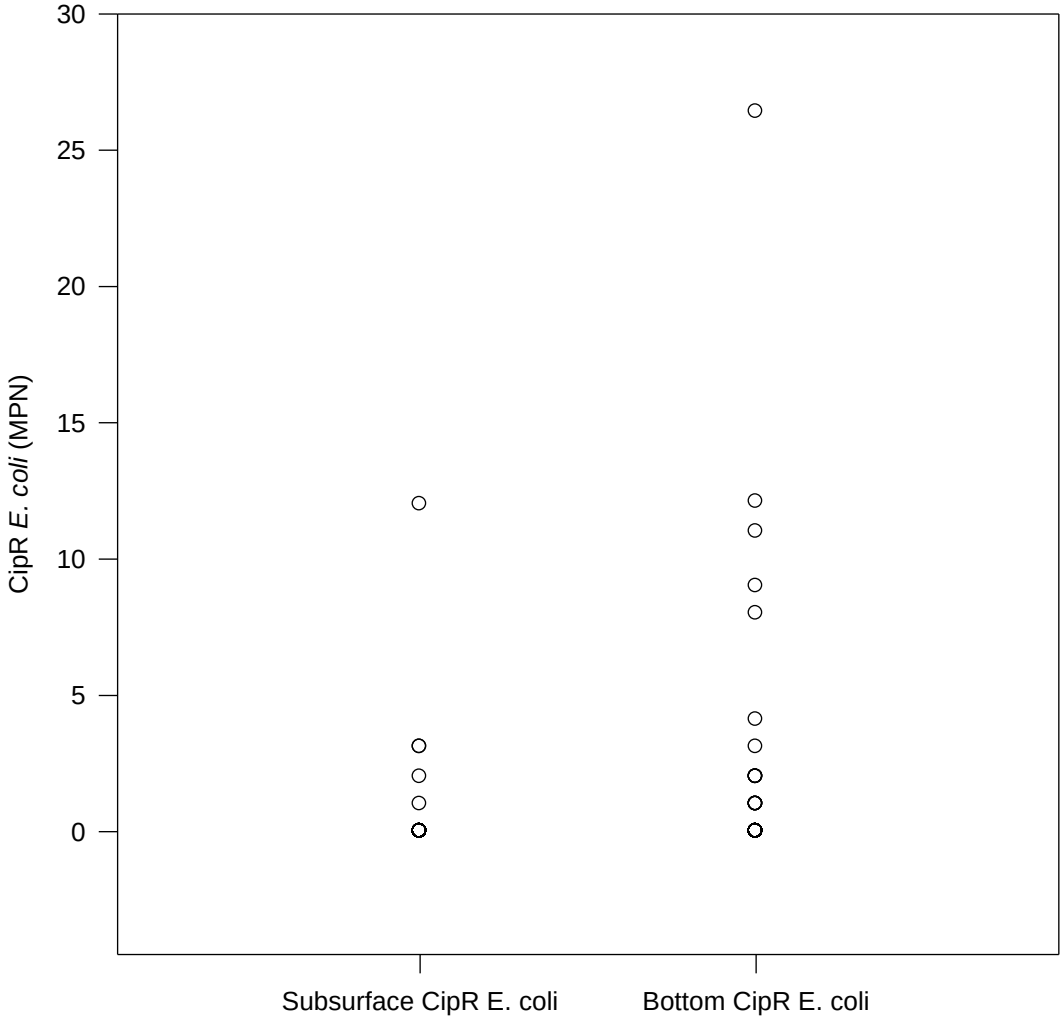



**FIGURE 18:** Ohio River Study 2007, Mann-Whitney Sum Rank Test Point Plot Analysis for Comparing Subsurface (top) vs. Bottom Ciprofloxacin Resistant *E. coli*

Mann-Whitney U Statistic = 3459.5; Both median values for CipR *E. coli* equal zero; however, the sum of their ranks is greater for bottom CipR *E. coli*. The difference between the two groups is greater than would be expected by chance; there is a statistically significant difference ($P$ = 0.004)

## SPEARMAN'S CORRELATION

A spearman's rho analysis was performed on the entire data set. However, the goal of this test was to determine how the bacteria populations near the surface and at the bottom of the river correlate to each other. The Mann-Whitney Rank Sum Test determined that certain populations were independent of each other meaning they represent different bacterial communities; however, this test reveals that although the samples may represent different populations, these populations may tend to increase or decrease together based on their correlation coefficients. All of the subsurface versus bottom correlations for each bacteria type were significant ($P$ <0.05) and showed positive correlation coefficients (ρ). These positive ρ values and significant $P$-values indicate that as subsurface bacteria values tend to increase, bottom sample bacteria values will likely increase as well. The Spearman's Rho Analysis in Figure 19 represents only the $P$-values for the subsurface versus bottom correlations. The values for the entire data set are summarized in Table 3 in Appendix A

| Bottom \ Top | Top Total coliforms | Top TetR coliforms | Top CipR coliforms | Top Total *E. coli* | Top TetR *E. coli* | Top CipR *E.coli* |
|---|---|---|---|---|---|---|
| Bot Total coliforms | 0.0001 | | | | | |
| Bot TetR coliforms | | 0.0212 | | | | |
| Bot CipR coliforms | | | 0.0001 | | | |
| Bot Total *E. coli* | | | | 0.0001 | | |
| Bot TetR *E. coli* | | | | | 0.0016 | |
| Bot CipR *E. coli* | | | | | | 0.0001 |

**FIGURE 19:** Ohio River Study 2007, Subsurface (top) vs. Bottom Spearman's Rho Analysis Output of *P* - values

## *PART 2: OHIO RIVER/GUYANDOTTE RIVER, 2008*

### THE OHIO RIVER UPSTREAM OF THE GUYANDOTTE RIVER

To begin analyzing this data, a Pearson's Product Moment Correlation (PPMC) was first performed on the data set of each individual location separately. The samples taken in the Ohio River upstream of the Guyandotte River were analyzed independently to determine any relationships between the variables tested at that location. The PPMC involved the following variables: river quadrant (designated by numbers 1-5), depth in feet, temperature in degrees Celsius, percent dissolved oxygen, pH, turbidity (NTU), total *E. coli*, total cultivable bacteria (TCB), ciprofloxacin resistant cultivable bacteria (CipR), tetracycline resistant cultivable bacteria (TetR), virginiamycin resistant cultivable bacteria (VirR), and the amount of sediment per sample (mg/ml). The PPMC output of p-values for this specific location within the Ohio River upstream of the Guyandotte River is shown in Figure 20. A statistical summary of Pearson's *r*- values as well as *P*- values are shown in Table's 9-11 located in Appendix B.

Linear regression analysis was performed on all statistically significant correlations within the data set. These results are shown in Figures 21-27.

| Quad | Quad | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Depth** | 0.5953 | **Depth** | | | | | | | | | |
| **Temp** | **0.0109*** | 0.8087 | **Temp** | | | | | | | | |
| **% DO** | 0.2620 | 0.4923 | 0.4538 | **% DO** | | | | | | | |
| **pH** | 0.9017 | 0.8131 | 0.9621 | 0.6254 | **pH** | | | | | | |
| **NTU** | 0.4928 | **0.0009*** | 0.4925 | 0.9421 | 0.9685 | **NTU** | | | | | |
| ***E. coli*** | 0.3970 | 0.7822 | 0.0799 | 0.2179 | 0.1484 | 0.5465 | **E. coli** | | | | |
| **TCB** | 0.1072 | 0.7699 | 0.4445 | **0.0124*** | 0.4979 | 0.7533 | 0.3235 | **TCB** | | | |
| **CipR** | 0.6447 | 0.6899 | 0.0927 | 0.7961 | 0.8262 | 0.0554 | 0.0835 | 0.9365 | **CipR** | | |
| **TetR** | 0.9604 | 0.8806 | 0.1409 | **0.0034*** | 0.6788 | 0.1452 | **0.0279*** | **0.0149*** | **0.0408*** | **TetR** | |
| **VirR** | 0.5004 | 0.0639 | 0.1772 | 0.1001 | 0.6949 | 0.2293 | 0.5350 | 0.4799 | 0.6829 | 0.2376 | **VirR** |
| **Sediment** | 0.5142 | 0.0587 | 0.7370 | 0.5551 | 0.5781 | 0.1200 | 0.2697 | 0.7144 | 0.5372 | 0.9504 | 0.5274 |

**FIGURE 20:** 2008 Pearson's Product Moment Correlation (PPMC) Output of p-values for the Ohio River Sites Upstream of the Guyandotte River

Statistically significant *P* -values ($P < 0.05$) are **bold** and indicated with asterisks*

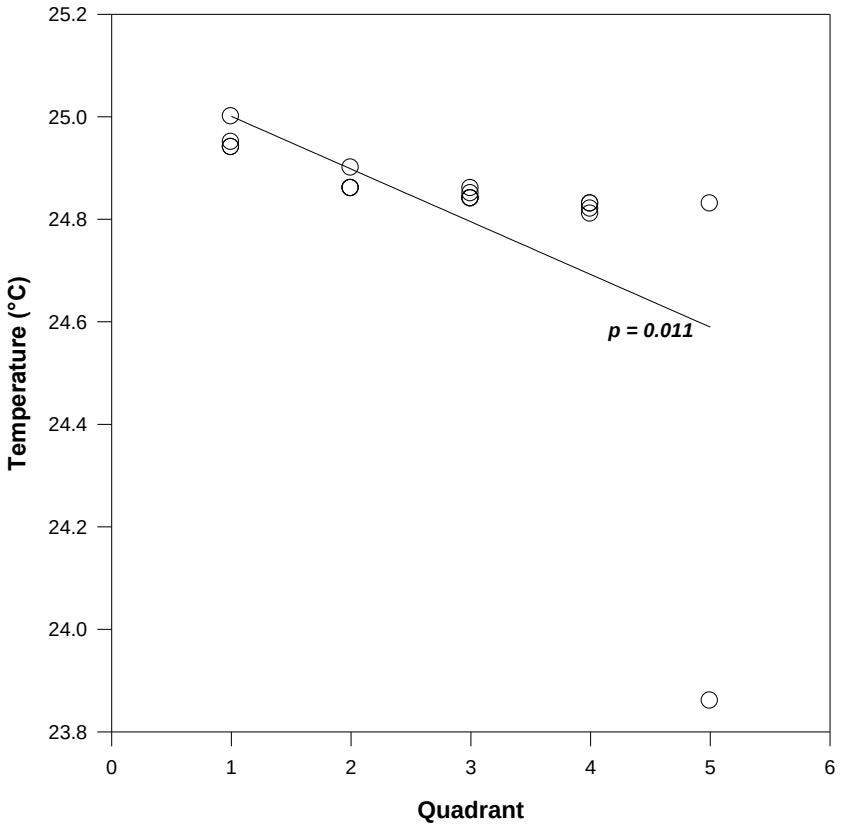



**FIGURE 21:** 2008 Linear Regression Analysis of the Ohio River Sites Upstream of the Guyandotte River, River Quadrant vs. Temperature

River Quadrants designated by (1) Left Bank, (2) Left Channel, (3) Center Channel, (4) Right Channel, and (5) Right Bank

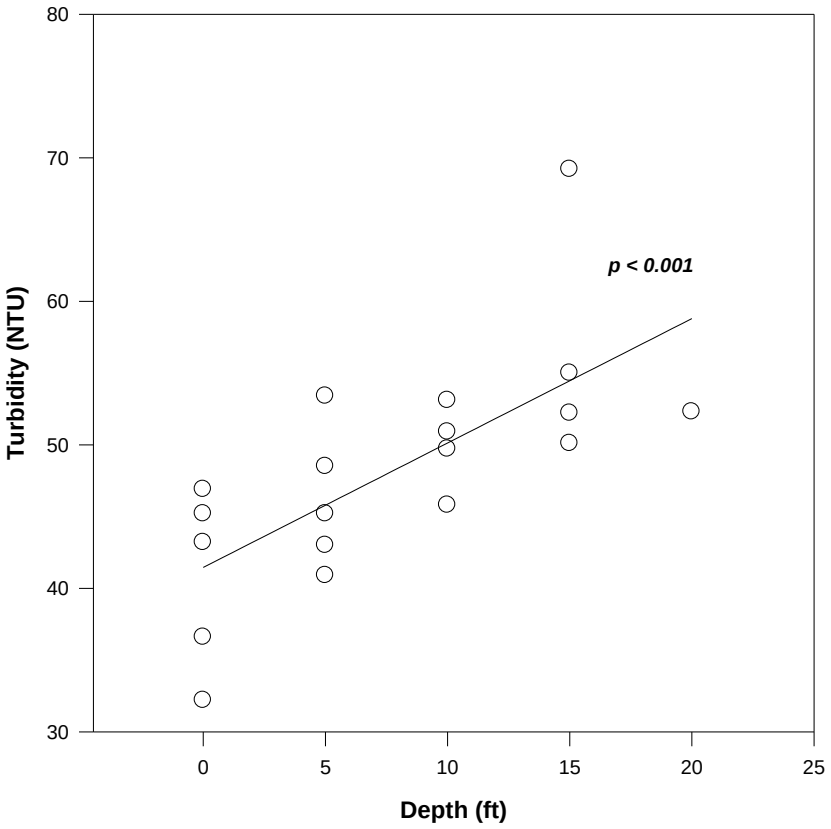



**FIGURE 22:** 2008 Linear Regression Analysis of the Ohio River Sites Upstream of the Guyandotte River, Depth (ft) vs. Turbidity (NTU)

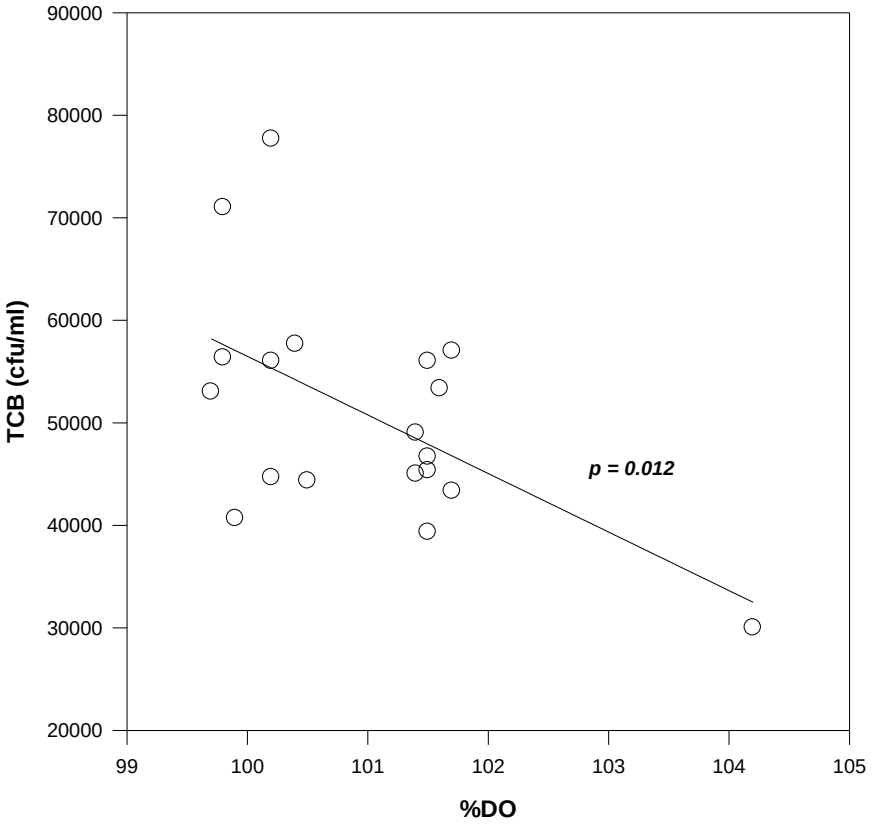



**FIGURE 23:** 2008 Linear Regression Analysis of the Ohio River Sites Upstream of the Guyandotte River, Percent Dissolved Oxygen (%DO) vs. Total Cultivable Bacteria (TCB)

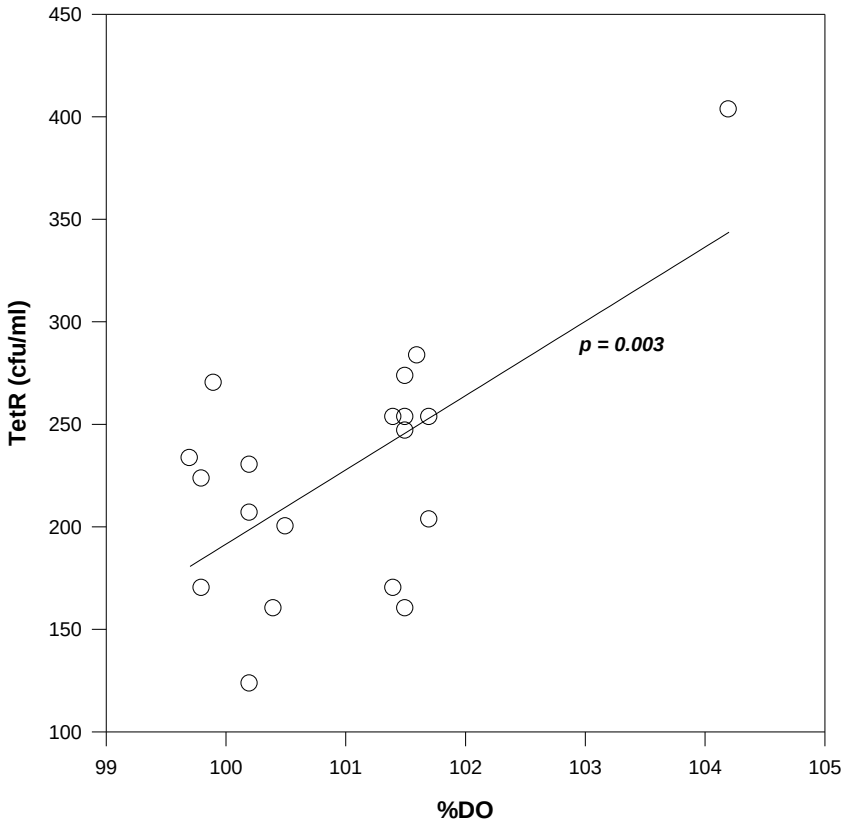



**FIGURE 24:** 2008 Linear regression Analysis of the Ohio River Sites Upstream of the Guyandotte River, Percent Dissolved Oxygen (%DO) vs. Tetracycline Resistant Cultivable Bacteria (TetR)

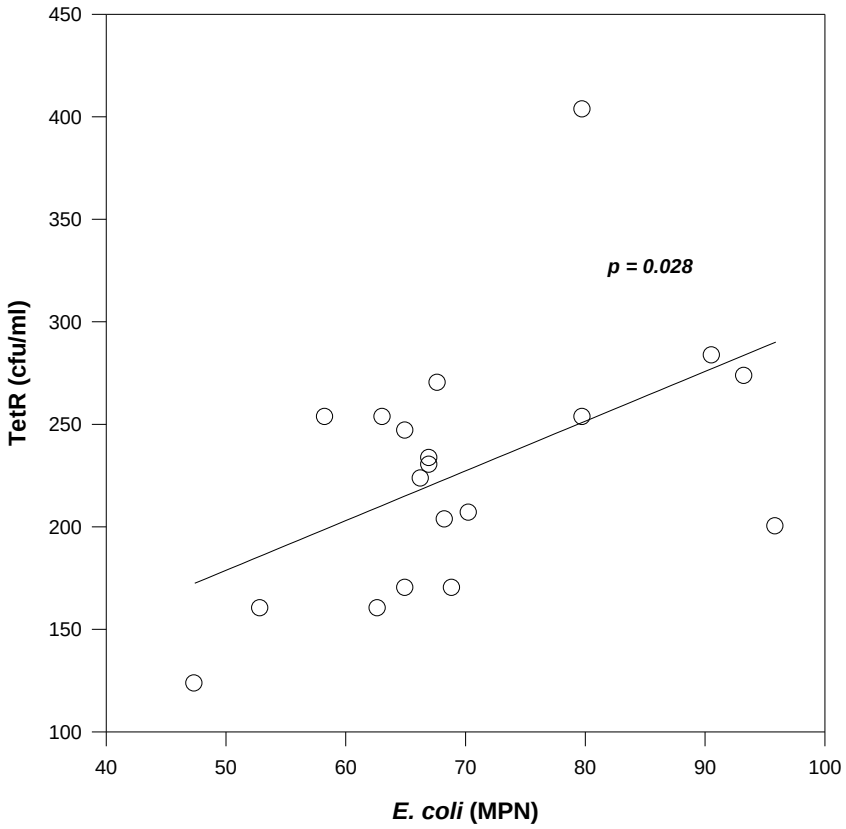



**FIGURE 25:** 2008 Linear Regression Analysis of the Ohio River Sites Upstream of the Guyandotte River, Total *E. coli* vs. Tetracycline Resistant Cultivable Bacteria (TetR)

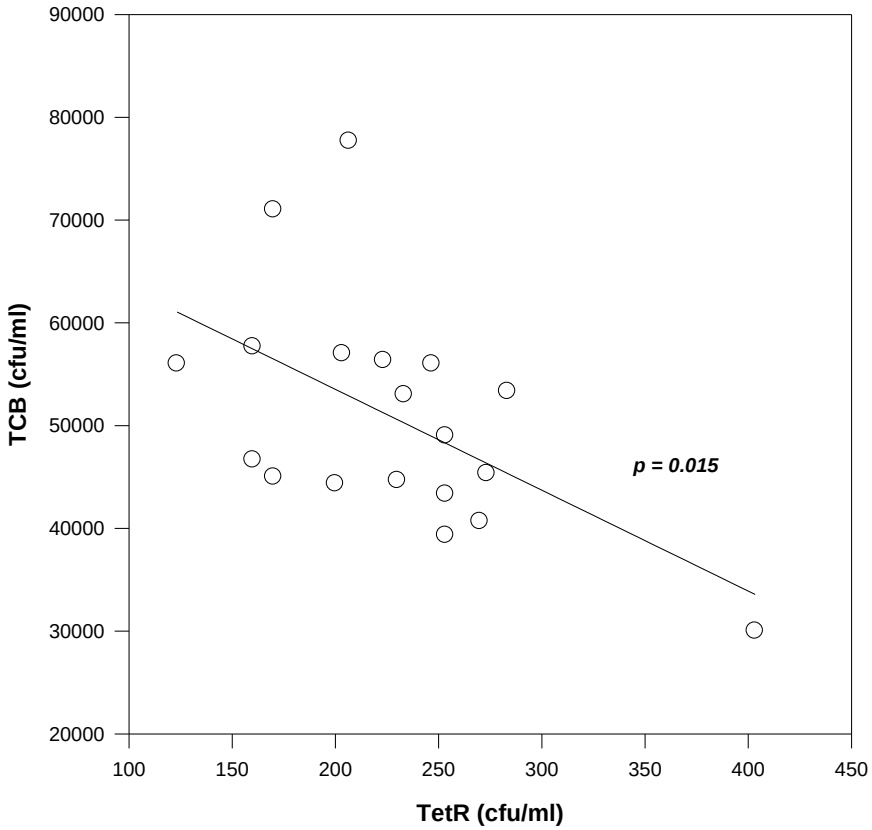



**FIGURE 26:** 2008 Linear Regression Analysis of the Ohio River Sites Upstream of the Guyandotte River, Tetracycline Resistant Cultivable Bacteria (TetR) vs. Total Cultivable Bacteria (TCB)

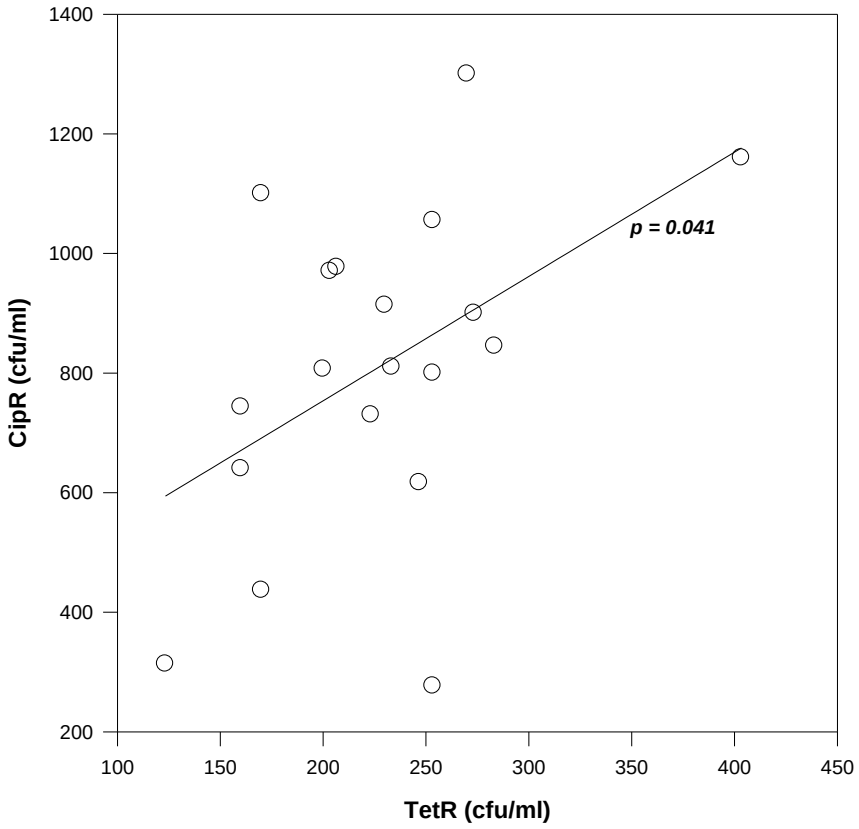



**FIGURE 27:** 2008 Linear Regression Analysis of the Ohio River Sites Upstream of the Guyandotte River, Tetracycline Resistant Cultivable Bacteria (TetR) vs. Ciprofloxacin Resistant Cultivable Bacteria (CipR)

## THE GUYANDOTTE RIVER

The samples taken within the Guyandotte River were also analyzed independently at first to determine whether there were any significant relationships between the variables of this population. A separate PPMC was performed with the same variables as described for Ohio River above the Guyandotte. The output of *P*-values for this location is shown in Figure 28 and the statistical summary is shown in Table 10 located in Appendix B. Linear regressions in Figures 29 - 34 indicate the significant correlations for the Guyandotte River sample set.

| Quad | Quad | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Depth | No value | Depth | | | | | | | | | |
| Temp | **0.0099*** | 0.4970 | Temp | | | | | | | | |
| % DO | 0.1910 | 0.1081 | **0.0326*** | % DO | | | | | | | |
| pH | 0.9245 | **0.0188*** | 0.5333 | 0.2451 | pH | | | | | | |
| NTU | 0.8625 | 0.1377 | 0.5827 | 0.2040 | 0.2148 | NTU | | | | | |
| *E. coli* | 0.2421 | 0.2387 | 0.0670 | **0.0039*** | 0.4185 | 0.2231 | *E. coli* | | | | |
| TCB | 0.7180 | 0.7236 | 0.8596 | 0.6550 | 0.2830 | 0.8862 | 0.3701 | TCB | | | |
| CipR | 0.4741 | 0.2333 | 0.6528 | 0.6462 | 0.7167 | 0.4036 | 0.7057 | 0.4889 | CipR | | |
| TetR | 0.9043 | 0.1388 | 0.8019 | 0.5687 | 0.1838 | 0.9100 | 0.8938 | 0.3677 | 0.4827 | TetR | |
| VirR | 0.2846 | 0.3305 | 0.3817 | 0.8246 | 0.5995 | 0.8604 | 0.5642 | 0.6632 | 0.0705 | 0.1591 | VirR |
| Sediment | 0.0916 | 0.2988 | 0.2331 | 0.8432 | 0.5541 | 0.6114 | 0.7504 | 0.9555 | **0.0274*** | 0.3479 | **0.0169*** |

**FIGURE 28:** 2008 Pearson's Product Moment Correlation (PPMC) Output of *P* - values for the Guyandotte River Sites

Statistically significant *P*-values ($P < 0.05$) are **bold** and indicated with asterisks *

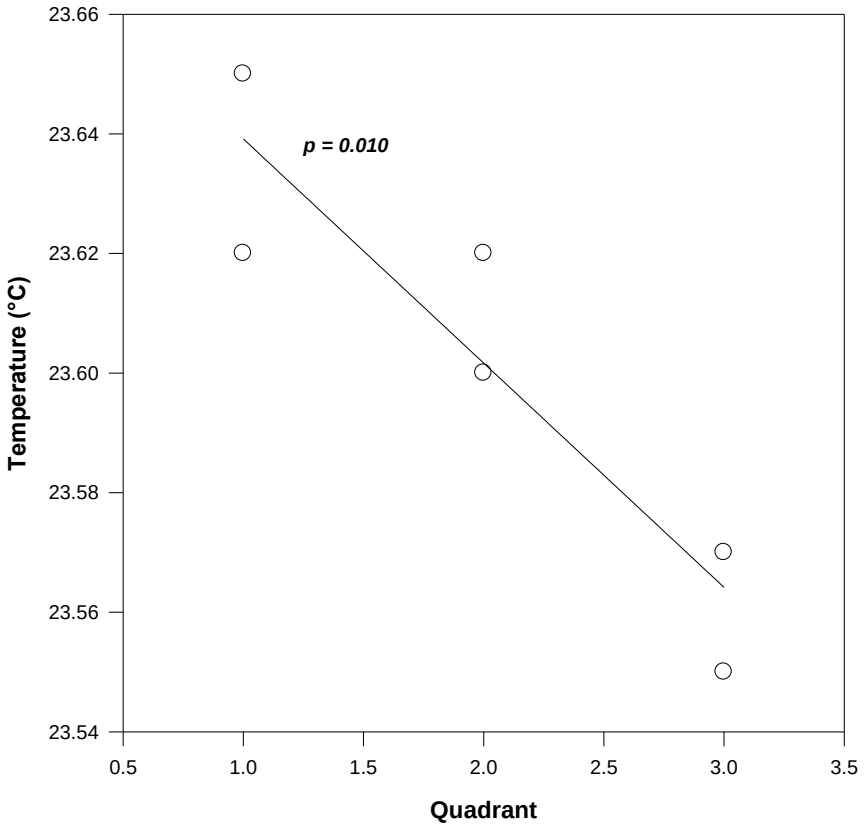



**FIGURE 29:** 2008 Linear Regression Analysis for the Guyandotte River Samples, River Quadrant vs. Temperature

River Quadrants designated by (1) Left Bank, (2) Left Channel, (3) Center Channel, (4) Right Channel, and (5) Right Bank

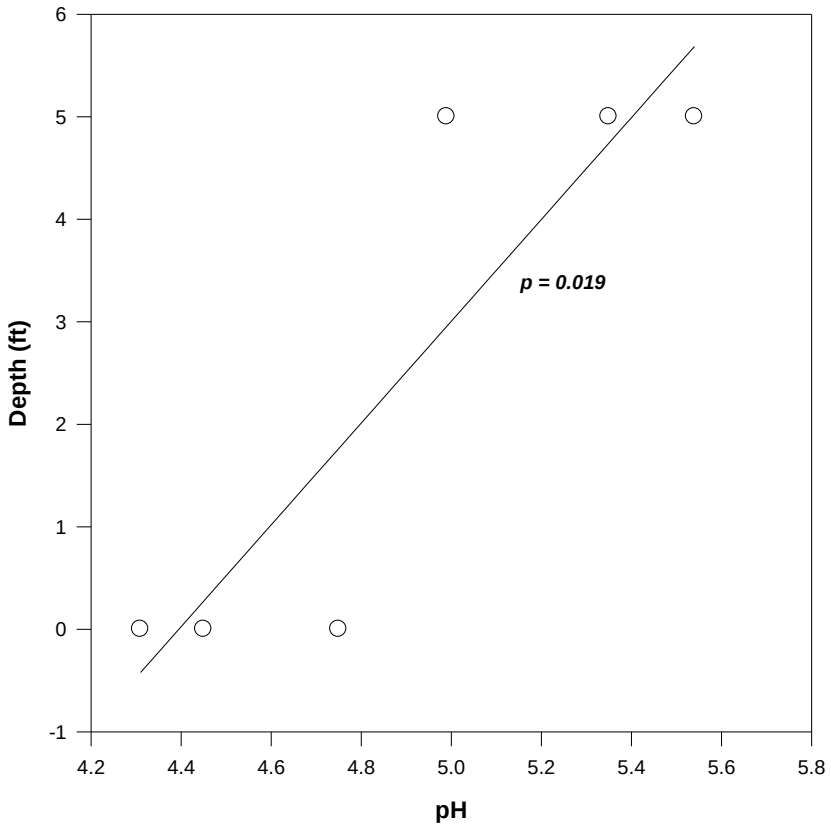



**FIGURE 30:** 2008 Linear Regression Analysis of the Guyandotte River Sites, pH vs. Depth (ft)

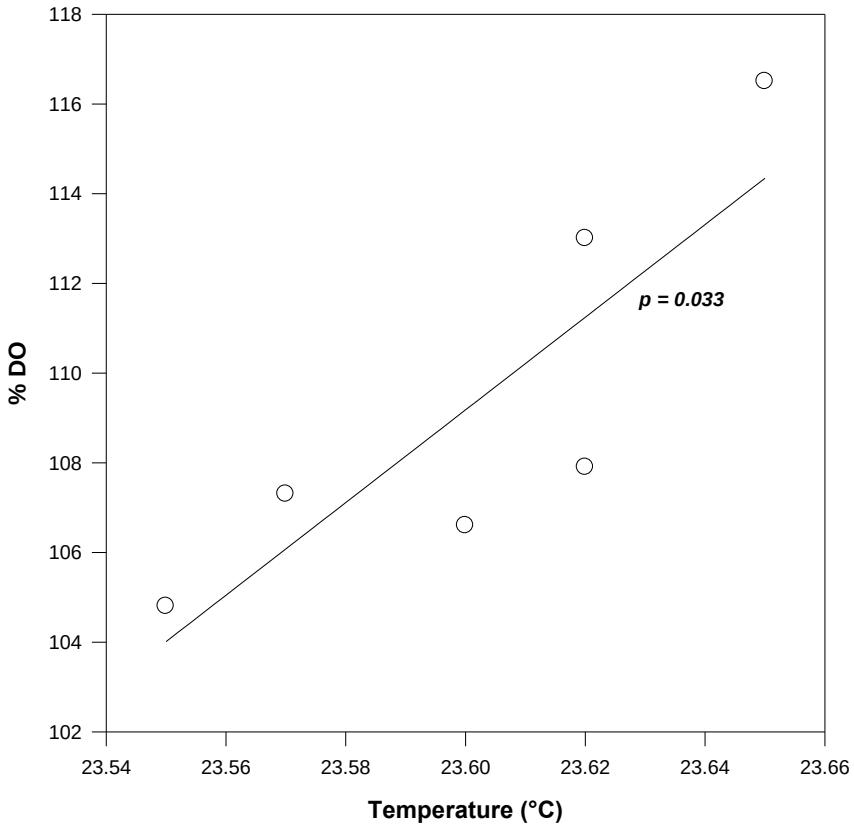



**FIGURE 31:** 2008 Linear Regression Analysis of the Guyandotte River Sites, Temperature vs. Percent Dissolved Oxygen (%DO)

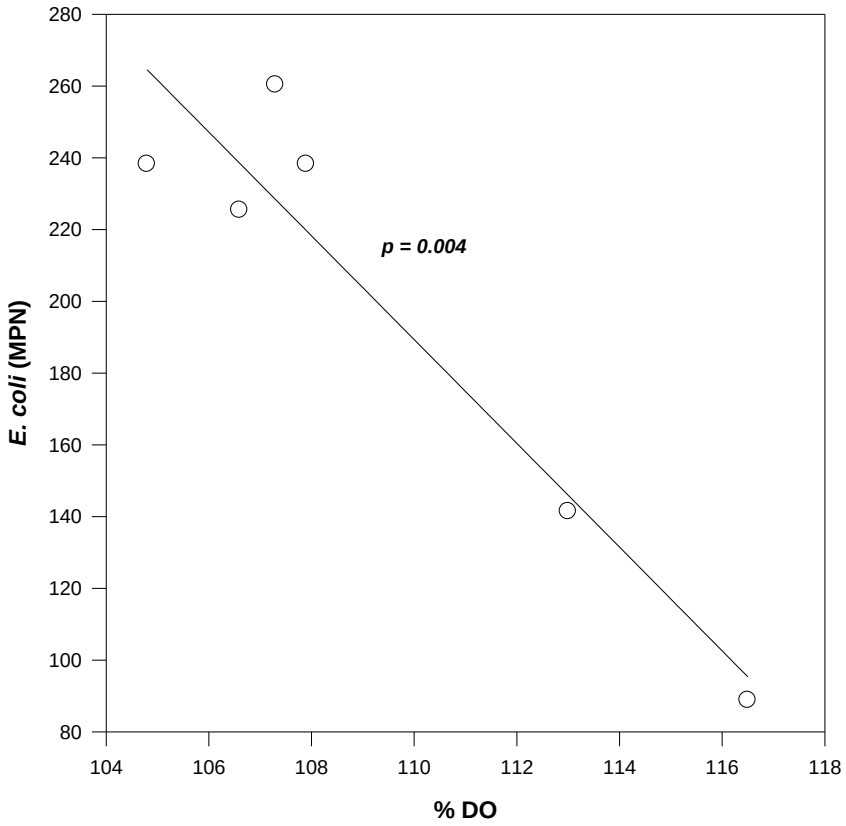



**FIGURE 32:** 2008 Linear Regression Analysis of the Guyandotte River Sites, Percent Dissolved Oxygen (%DO) vs. Total *E. coli*

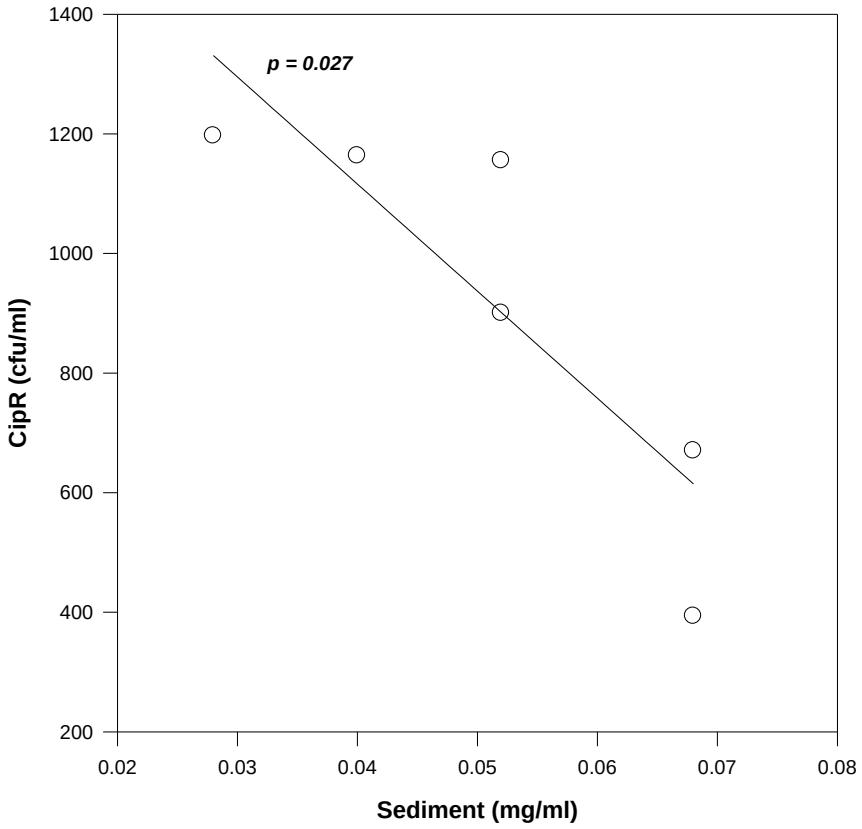



**FIGURE 33:** 2008 Linear Regression Analysis of the Guyandotte River Sites, Amount of Sediment (mg/ml) vs. Ciprofloxacin Resistant Cultivable Bacteria (CipR)

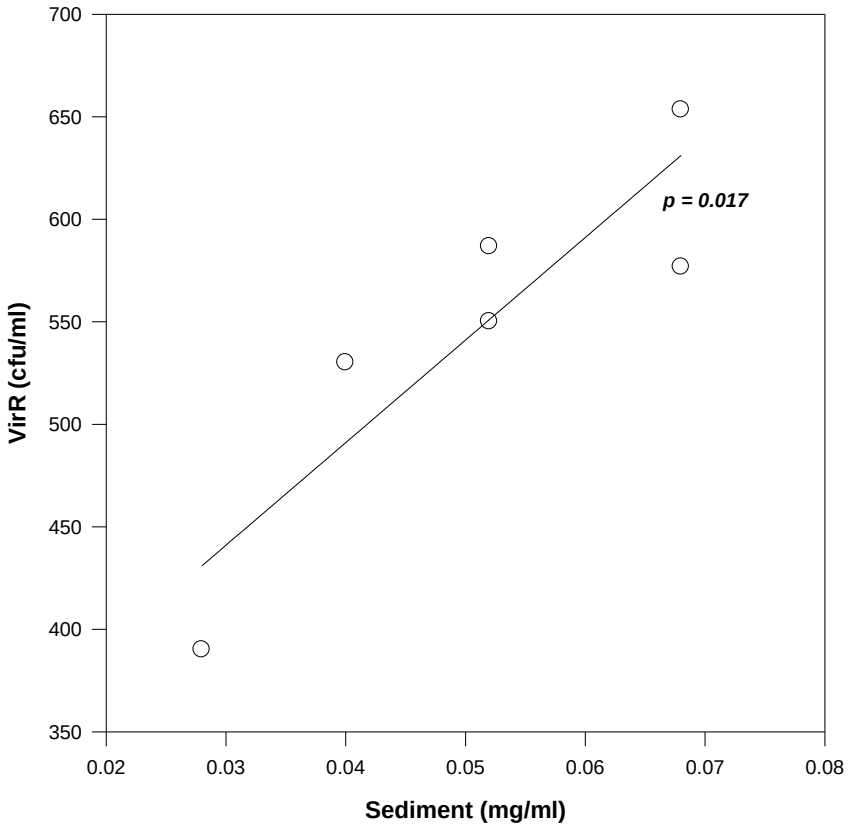



**FIGURE 34:** 2008 Linear Regression Analysis of the Guyandotte River Sites, Amount of Sediment (mg/ml) vs. Virginiamycin Resistant Cultivable Bacteria (VirR)

## THE OHIO RIVER DOWNSTREAM OF THE GUYANDOTTE RIVER

The PPMC results for the samples taken in the Ohio River downstream of the Guyandotte River are shown in Figure 35. The output of *P*- values and statistical summary for this location is shown in Table 11 in Appendix B. Figures 36 - 41 show the correlations that are significantly related within this data set.

| Quad | Quad | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Depth | 0.8717 | Depth | | | | | | | | | |
| Temp | **0.0222*** | 0.8926 | Temp | | | | | | | | |
| % DO | 0.5646 | 0.5604 | 0.5255 | % DO | | | | | | | |
| pH | **0.0019*** | 0.8722 | 0.1797 | 0.5986 | pH | | | | | | |
| NTU | 0.4326 | **0.0052*** | 0.3044 | 0.1554 | 0.9194 | NTU | | | | | |
| *E. coli* | **0.0029*** | 0.4708 | **0.0000*** | 0.3496 | 0.0508 | 0.1247 | *E. coli* | | | | |
| TCB | 0.2950 | 0.2982 | 0.6280 | 0.5203 | 0.1442 | 0.6635 | 0.8100 | TCB | | | |
| CipR | 0.8266 | 0.1289 | 0.2190 | 0.9074 | 0.7139 | 0.6923 | 0.4243 | 0.1979 | CipR | | |
| TetR | 0.7947 | 0.2973 | 0.2531 | 0.9183 | 0.8179 | 0.2410 | 0.2770 | 0.4583 | 0.1592 | TetR | |
| VirR | 0.0711 | 0.1114 | 0.1165 | 0.8144 | 0.2741 | 0.0756 | 0.1046 | 0.6476 | 0.0739 | **0.0018*** | VirR |
| Sediment | 0.3825 | 0.2437 | 0.6771 | 0.4173 | 0.2530 | 0.1322 | 0.9224 | 0.7675 | 0.4437 | 0.7417 | 0.4659 |

**FIGURE 35:** 2008 Pearson's Product Moment Correlation (PPMC) output of *P*- values for the Ohio River Sites Downstream of the Guyandotte River

Statistically significant *P*-values ($P < 0.05$) are **bold** and indicated with asterisks*

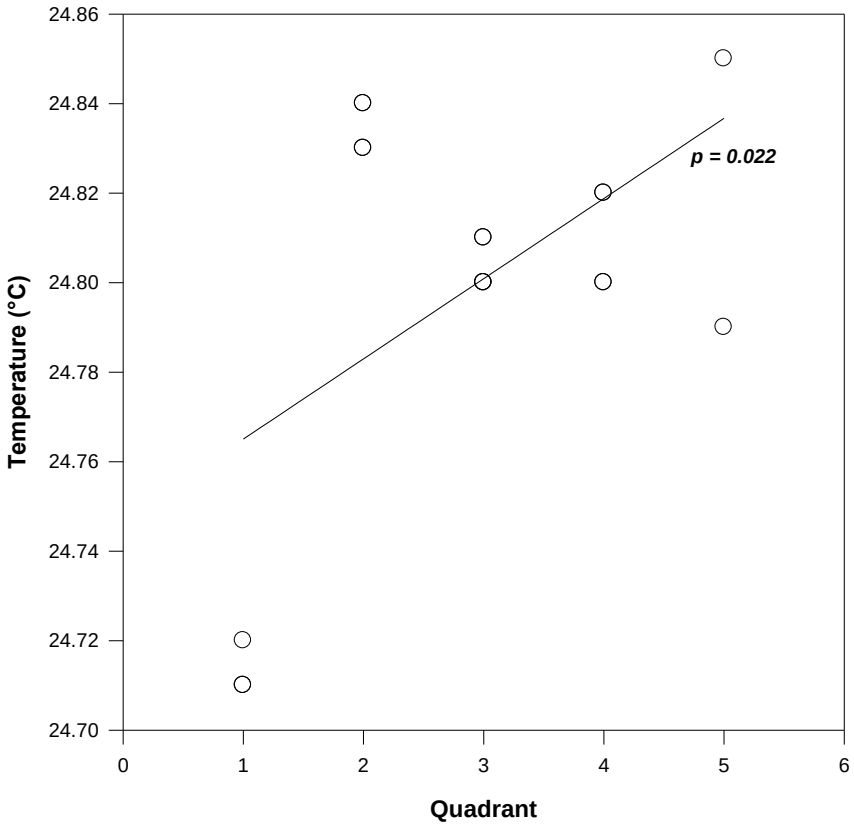



**FIGURE 36:** 2008 Linear Regression Analysis of the Ohio River Sites Downstream of the Guyandotte River, River Quadrant vs. Temperature

River Quadrants designated by (1) Left Bank, (2) Left Channel, (3) Center Channel, (4) Right Channel, and (5) Right Bank

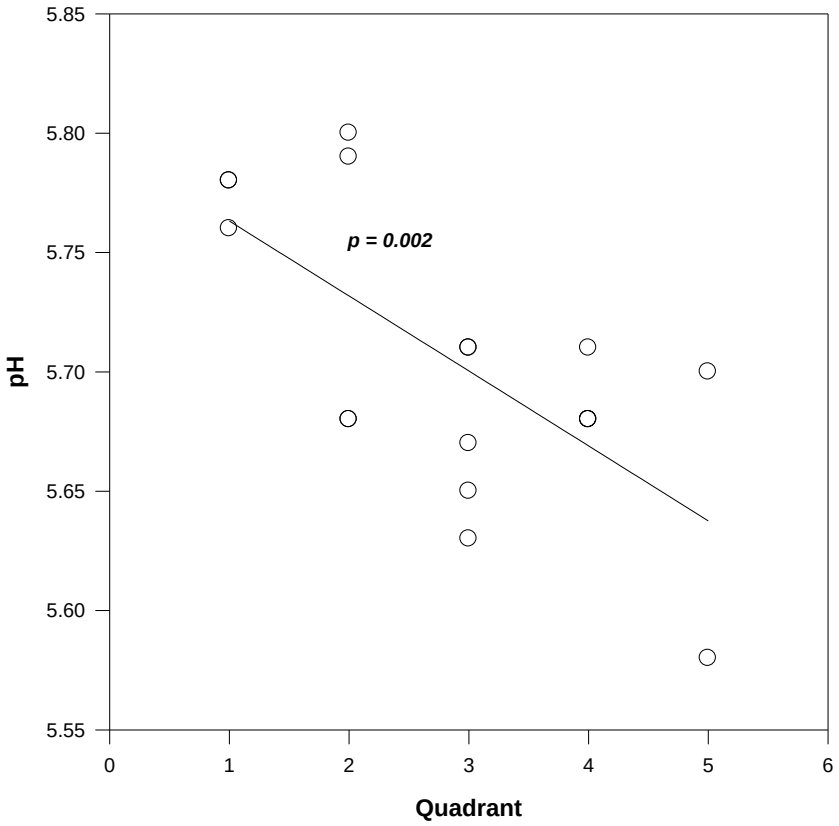



**FIGURE 37:** 2008 Linear Regression Analysis of the Ohio River Sites Downstream of the Guyandotte River, River Quadrant vs. pH

River Quadrants designated by (1) Left Bank, (2) Left Channel, (3) Center Channel, (4) Right Channel, and (5) Right Bank

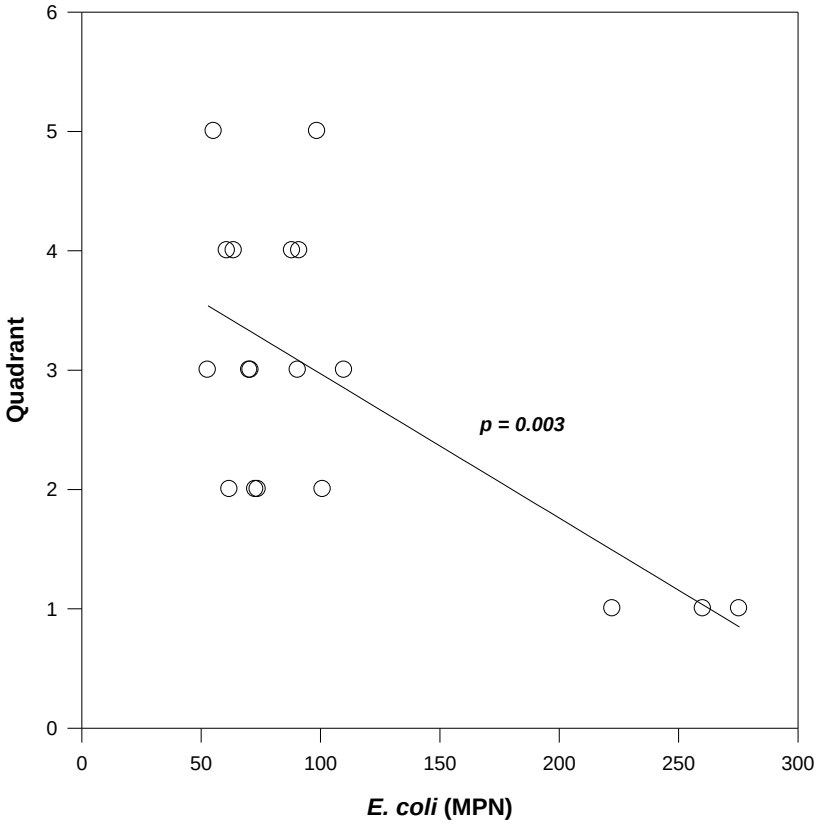



**FIGURE 38:** 2008 Linear Regression Analysis of the Ohio River Sites Downstream of the Guyandotte River, Total *E. coli* vs. River Quadrant

River Quadrants designated by (1) Left Bank, (2) Left Channel, (3) Center Channel, (4) Right Channel, and (5) Right Bank

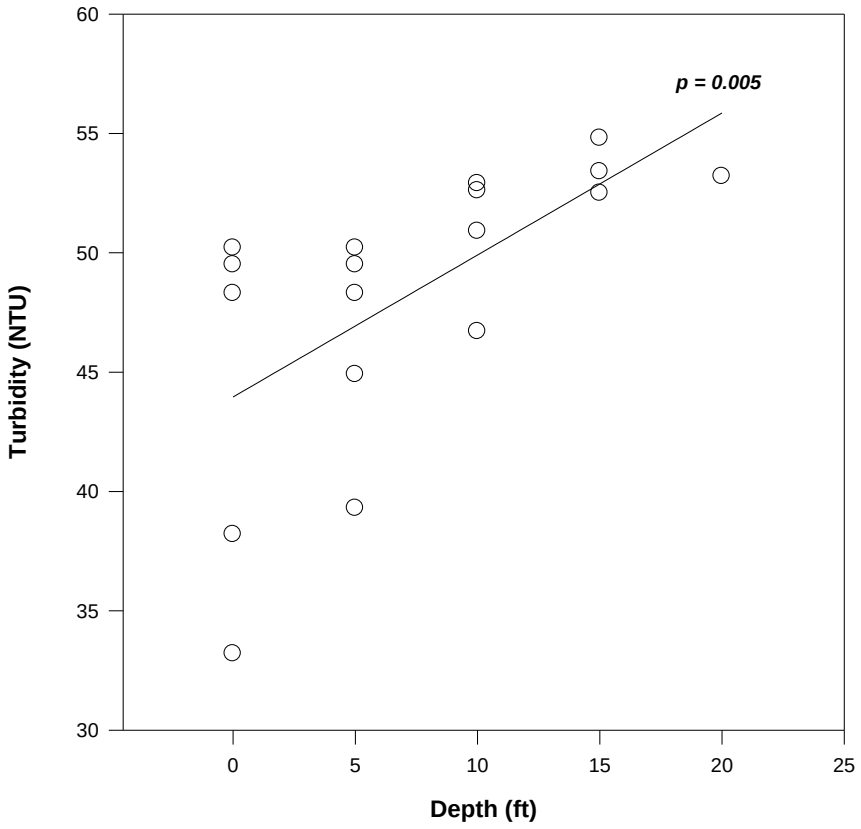



**FIGURE 39:** 2008 Linear Regression Analysis of the Ohio River Sites Downstream of the Guyandotte River, Depth (ft) vs. Turbidity (NTU)

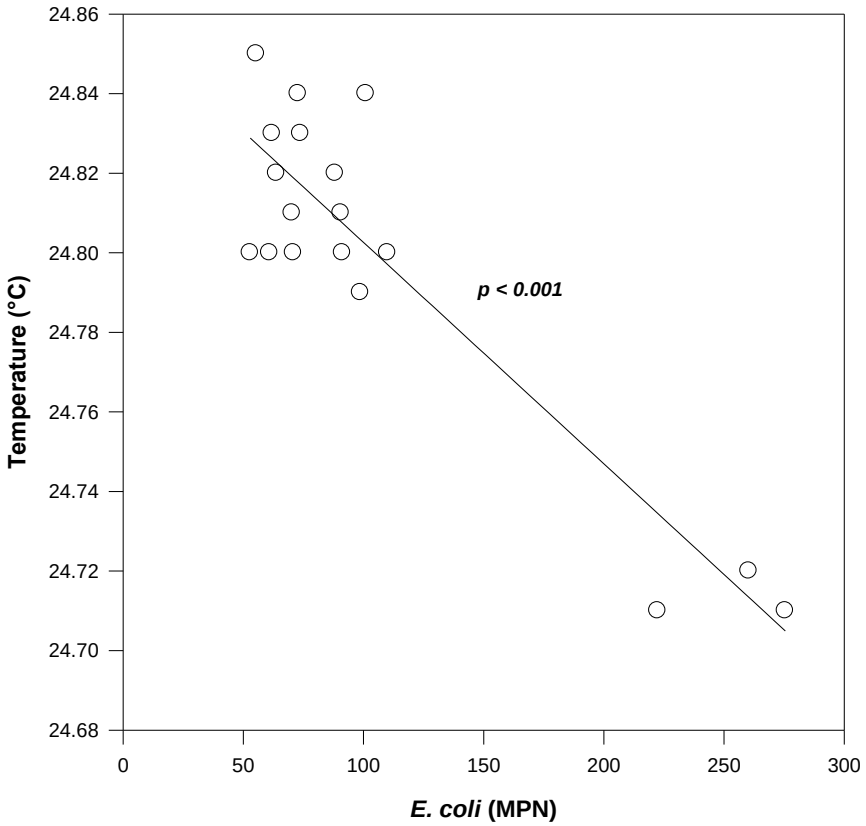



**FIGURE 40:** 2008 Linear Regression Analysis of the Ohio River Sites Downstream of the Guyandotte River, Total *E. coli* vs. Temperature

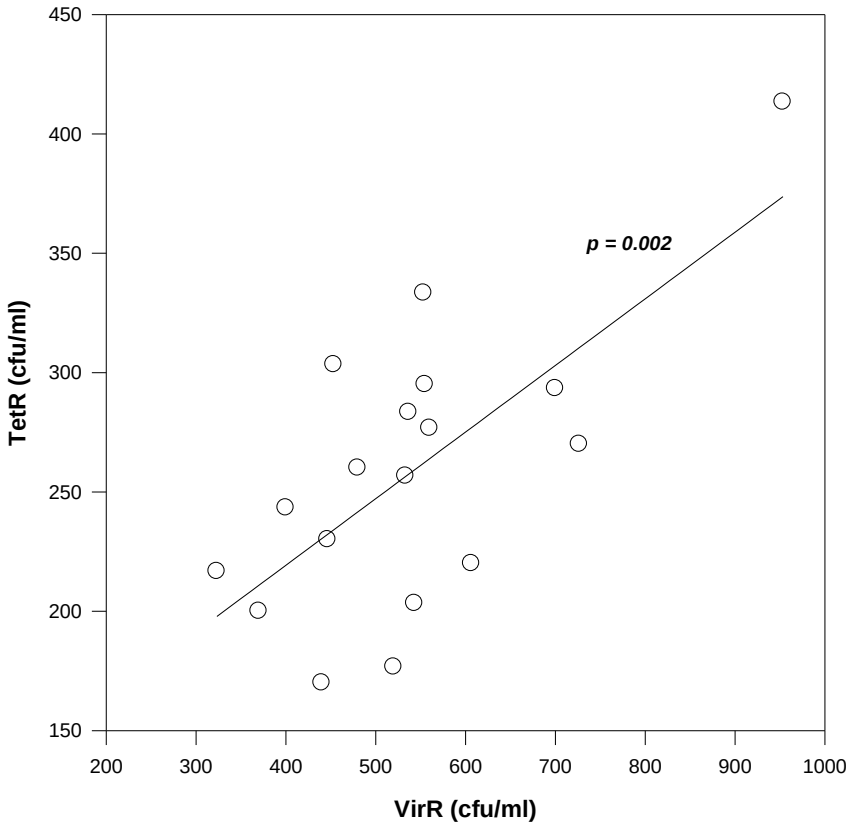



**FIGURE 41:** 2008 Linear Regression Analysis of the Ohio River Sites Downstream of the Guyandotte River, Virginiamycin Resistant Cultivable Bacteria (VirR) vs. Tetracycline Resistant Cultivable Bacteria (TetR)

## Depth & River Quadrant On Bacteria

Once each river location was analyzed independently, the PPMC results revealed that depth had no significant effect on the amount of bacteria present in the samples at any location. Box plots are shown relating all bacterial samples with depth in Figures 42 – 46. Due to this finding, further analyses on bacteria counts were based on arithmetic mean of counts taken at each site. The PPMC results also revealed TCB, TetR, CipR, and VirR bacteria counts were not significantly related to river quadrant. However, the results did show a significant relationship ($P<0.05$) between *E. coli* and river quadrant for the samples taken in the Ohio River below the mouth of the Guyandotte River. To further analyze *E. coli* in the Ohio River, an ANOVA t-test was performed on *E. coli* (averaged by depth) grouped by location (upstream or downstream) and river quadrant (LB, LC, C, RC, RB). The t-test results for each relationship are shown in Table 12 of Appendix B. A simple bar graph was used to compare *E. coli* counts upstream of the Guyandotte River to *E. coli* downstream of the Guyandotte River by river quadrant [Fig. 47].

## Sediment and Bacteria

To analyze whether the amount of sediment per sample was significant in determining the amount of bacteria per sample, all bacterial samples (TCB, total *E. coli,* TetR, CipR and VirR) were plotted against the amount of sediment measured in each sample. A linear regression analysis was performed to determine significance [Fig. 48]. Note that all points were analyzed independently, regardless of the location or depth at which the sample was taken.

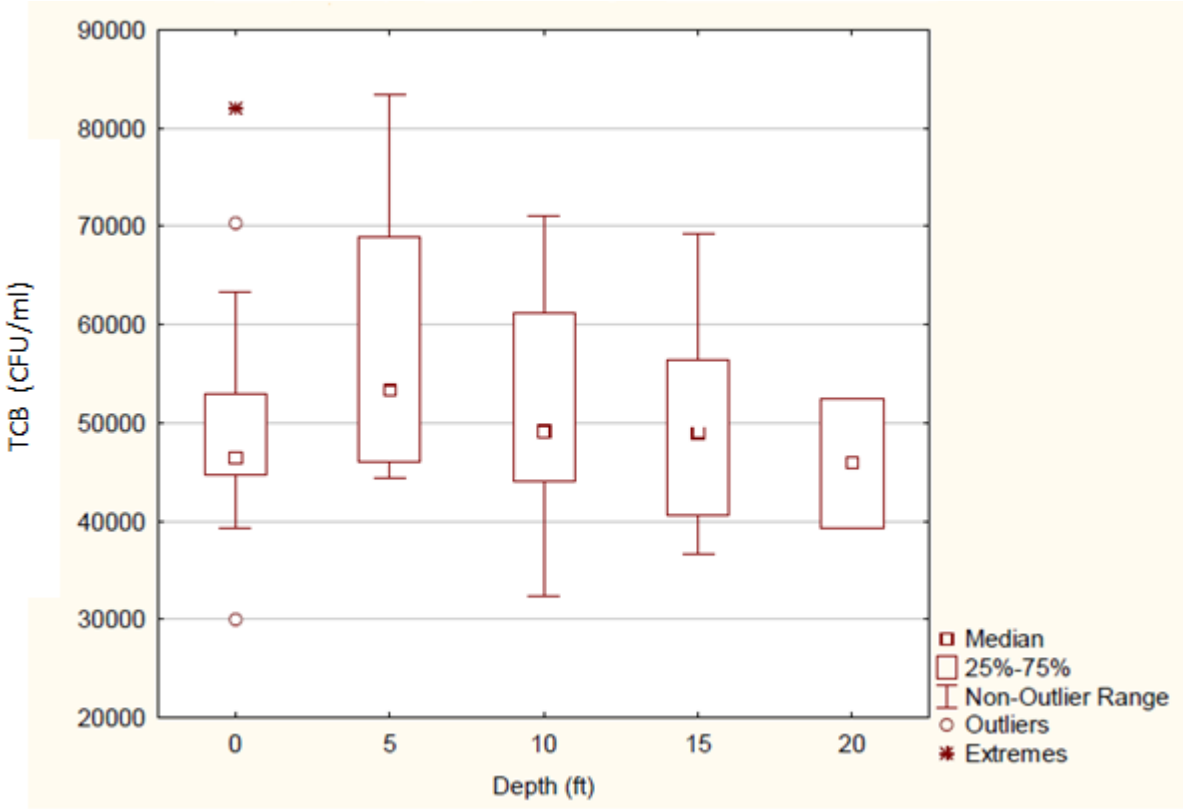



**FIGURE 42:** 2008 Box Plot Analysis of Total Cultivable Bacteria (TCB) grouped by Depth (ft)

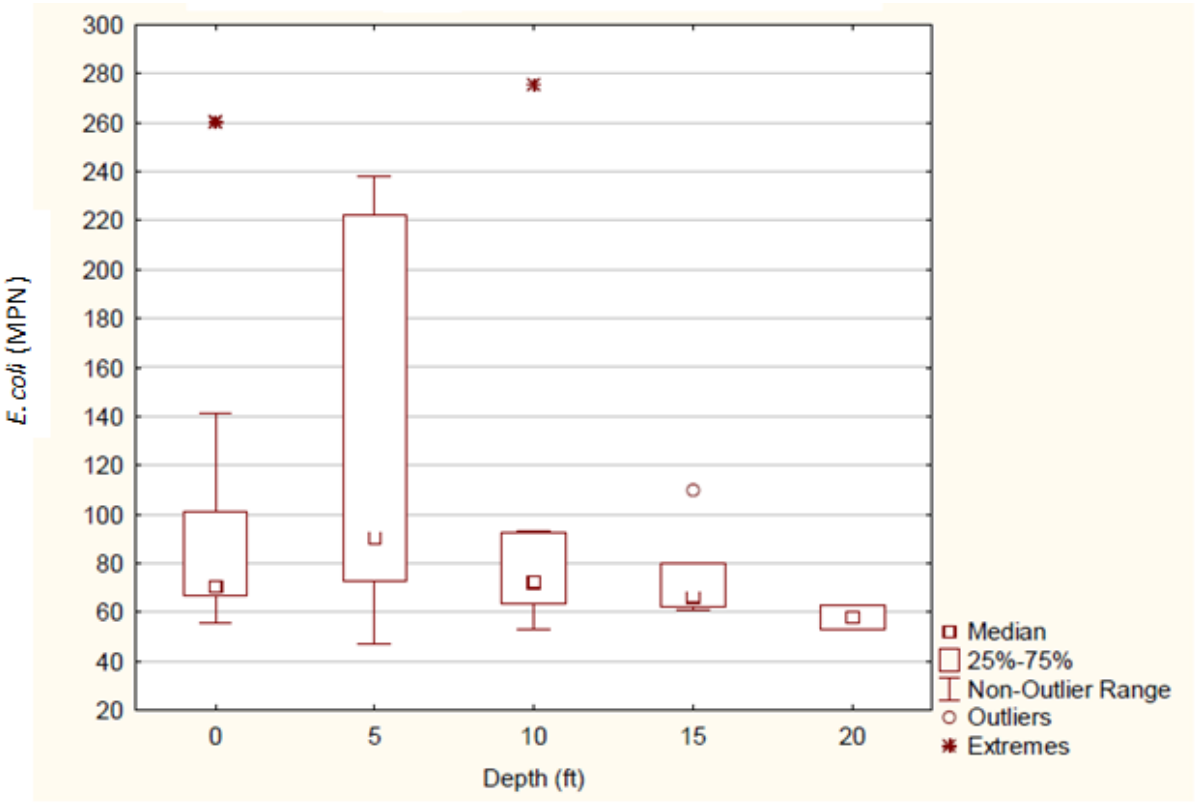



**FIGURE 43:** 2008 Box Plot Analysis of Total *E. coli* grouped by Depth (ft)

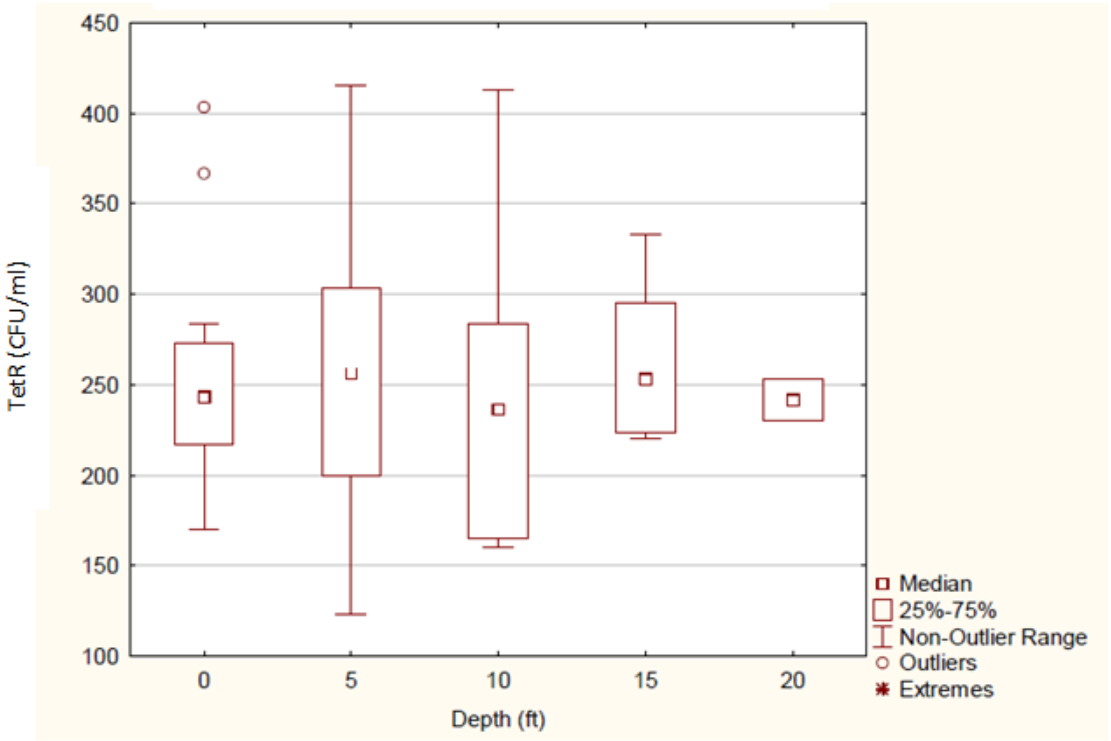



**FIGURE 44:** 2008 Box Plot Analysis of Tetracycline Resistant Cultivable Bacteria (TetR) grouped by Depth (ft)

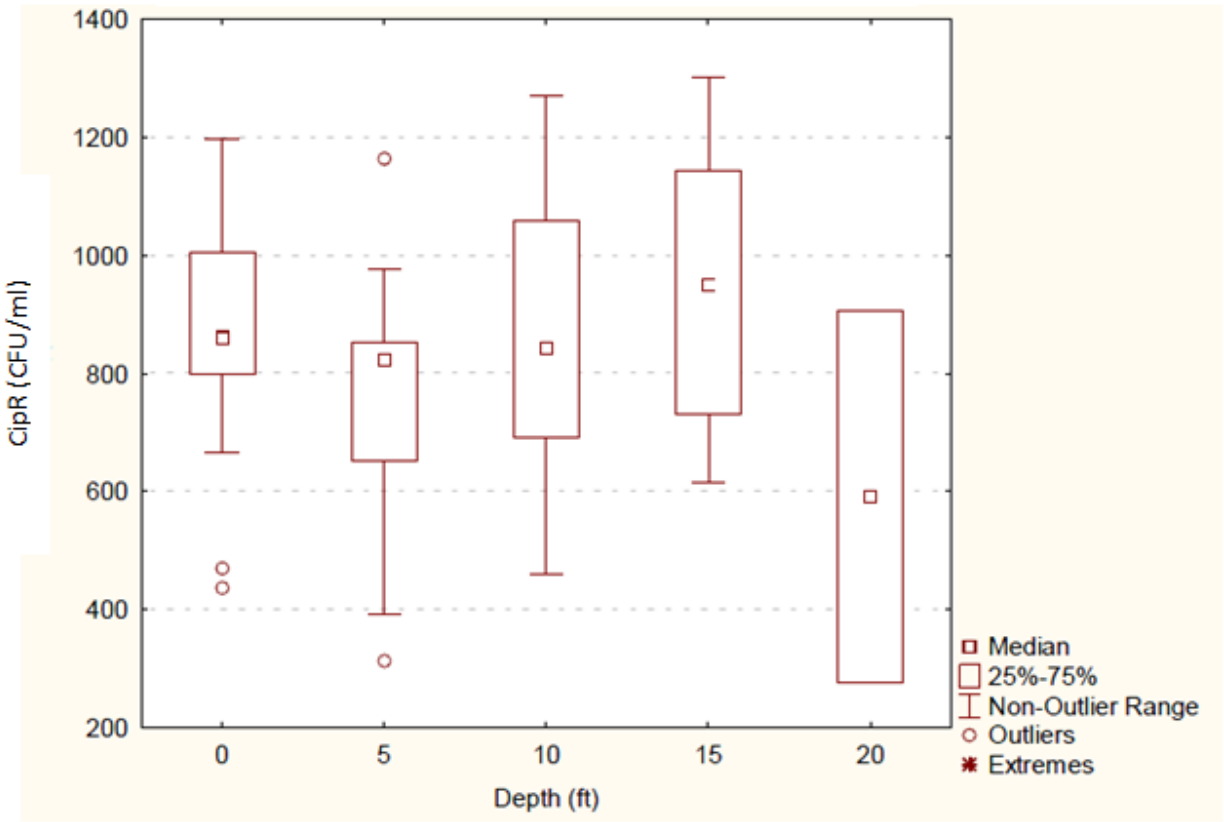



**FIGURE 45:** 2008 Box Plot Analysis of Ciprofloxacin Resistant Cultivable Bacteria (CipR) grouped by Depth (ft)

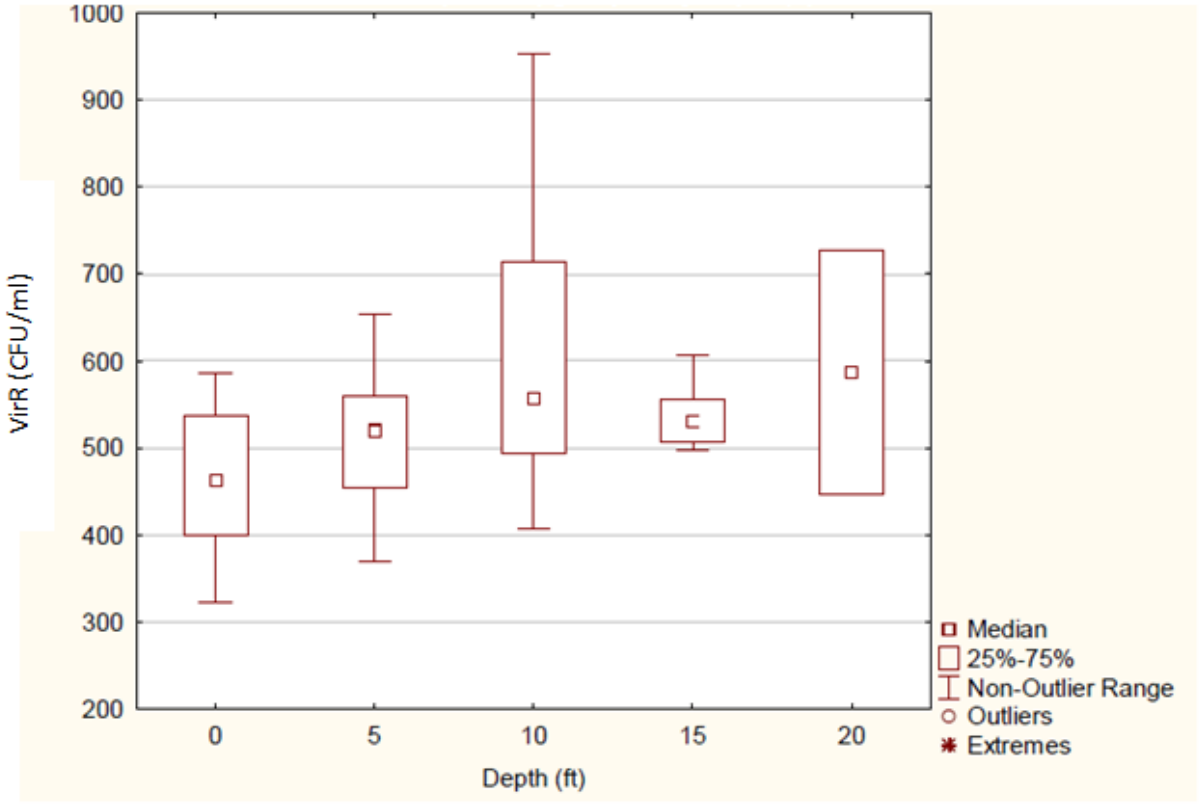



**FIGURE 46:** 2008 Box Plot Analysis of Virginiamycin Resistant Cultivable Bacteria (VirR) grouped by Depth (ft)

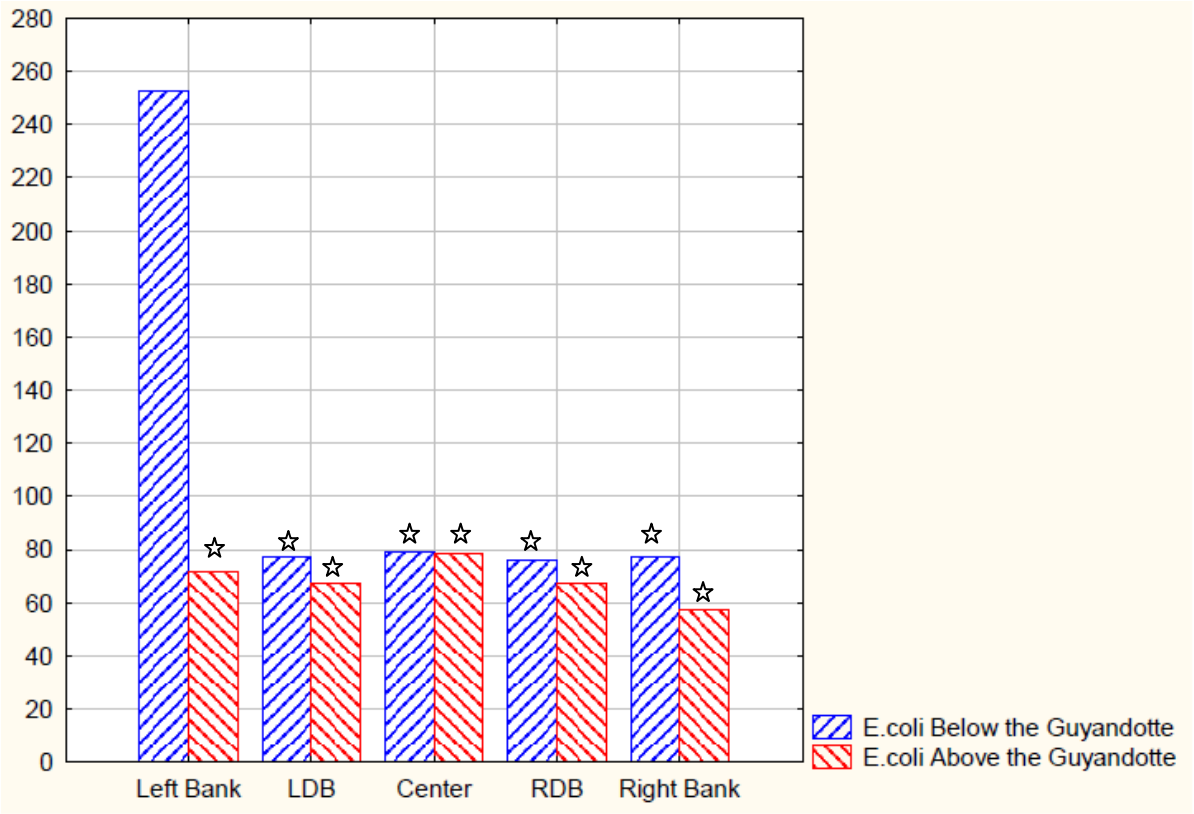



**FIGURE 47:** 2008 E. coli Mean Bar Graph, Ohio River upstream of the Guyandotte River vs Ohio River downstream of the Guyandotte River by river quadrant

☆ indicates a significant difference ($p < 0.01$) from the left bank below the Guyandotte River

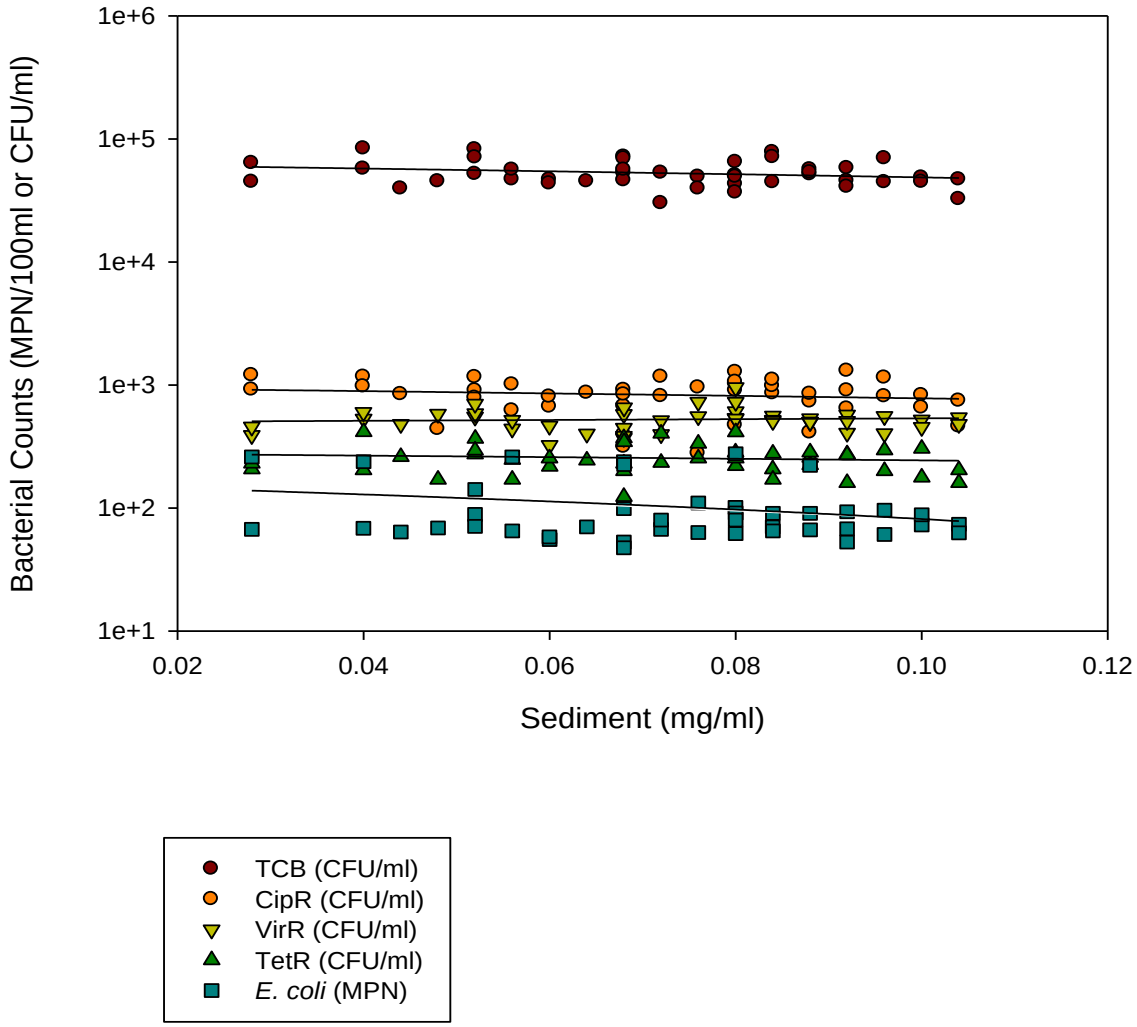



**FIGURE 48:** 2008 Linear Regression Analysis, Bacteria Counts vs Amount of Sediment per Sample

*P*-values and $r^2$ values for each correlation: Total Cultivable Bacteria (TCB), *P*=0.1381, $r^2$=0.0529; Ciprofloxacin Resistant (CipR), *P*=0.1210, $r^2$=0.0213; Virginiamycin Resistant (VirR), *P*=0.6579, $r^2$=0.0048; Tetracycline Resistant (TetR), *P*=0.4846, $r^2$=0.0120; *E. coli*, *P*=0.1210, $r^2$=0.0576.

# CHAPTER V: DISCUSSION

## *PART 1: OHIO RIVER SURVEY, 2007*

### ANALYZING TOTAL COLIFORM & ANTIBIOTIC RESISTANT COLIFORM COUNTS

Coliform bacteria are normally present in all environments, including aquatic environments. The Ohio River provides a suitable habitat for coliform survival. The majority of samples taken from the river had coliform counts above the maximum number MPN >2419.6 that could be enumerated by this method. In the results, all coliform counts >2419.6 MPN were plotted as an exact 2500 MPN to show the majority of samples were maxed out for TC bacteria. Because this data set failed the normality test, nonparametric statistics were used to analyze the data. Spearman's rho analysis shows a positive correlation relationship for subsurface total coliform bacteria and bottom total coliform bacteria, meaning their values tend to increase together. When comparing subsurface versus bottom total coliform counts the null hypothesis is that these two samples were drawn from populations with the same median. The Mann Whitney Rank Sum Test results showed the difference between the two groups to be statistically significant ($P<0.0001$), rejecting the null hypothesis, concluding the samples were drawn from different populations. This test revealed that the bottom samples had a greater median value for total coliform bacteria. The results also seem to show a slight trend with total bottom coliform counts being the greatest furthest downstream beginning at river mile ~500 to the mouth of the Ohio River before it enters the Mississippi River [Fig. 8].

Antibiotic resistant (TetR and CipR) coliforms were also enumerated in subsurface and bottom samples. Spearman's rho analysis showed a positive correlation between subsurface and bottom TetR coliforms, and subsurface and bottom CipR coliforms. This positive correlation indicates populations of bacteria at the surface and at the bottom tend to increase together. To determine whether these populations are independent of each other, the median values of each correlation were compared statistically using the Mann Whitney Rank Sum Test. These results showed that the bottom TetR coliform median value (x) was significantly greater than the subsurface TetR coliform median value (y; $P< 0.001$). The results for CipR coliforms also showed a statistically significant difference in median values with a *P*-value of 0.002 when comparing CipR subsurface versus CipR bottom coliforms. These tests indicate that all coliform samples taken in the Ohio River represented independent bacterial communities and the median values suggest that samples taken from the bottom of the river contained more antibiotic resistant coliforms than the subsurface samples.

## Assessing Spikes in Antibiotic Resistant Coliform Bacteria Along the Ohio River

To determine potential antibiotic resistant problem areas of the Ohio River, point plots of each bacterial group plotted by river mile were analyzed and river mile sites were observed more closely for land use (ie. industrial, agricultural) and possible inputs (ie. tributaries, CSOs) that may have influenced these "spikes" in antibiotic resistant bacteria. The following resources were used to investigate these sites; GIS, Google Earth, and USACOE Ohio River Navigational Charts.

Spikes in subsurface and bottom TetR coliforms were found at river miles 0.2, 36.1, 87.7, 324.2, 414.8, 528.4, 623.7, 740.0 and 793.0. Spikes in CipR coliforms were consistent with almost all of the same sites as TetR coliforms, but with lower counts. Descriptions of these sites are given in Figure 49a and b. Many of these sites were located right along the banks or just downstream of urbanized cities and towns, large metropolitan areas, near industrial parks (ie. power plants, wastewater treatment plants) and/or near rural areas where land is used mostly for farming and agriculture. Urbanization, industrialization, and agriculture are three major potential sources that could be linked to antibiotic resistant bacteria in the Ohio River due to higher concentrations of waste materials (ie. sewage, fertilizers, medical waste, heavy metals) being released straight into the river from these areas. These waste materials may contain concentrations of tetracycline and/or ciprofloxacin due to their uses in human medicine and animal husbandry. Also, heavy metal contaminants from industrial waste have been shown to be linked to increases in antibiotic resistant bacteria counts (Calomiris, Armstrong, & Seidler, 1984) (Dhalkephalkar & Chopade, 1994).

## ANALYZING TOTAL *E. COLI* AND ANTIBIOTIC RESISTANT *E. COLI*

A specific type of coliform bacteria, *Escherichia coli* (*E. coli*), is used to assess water quality as an indicator of fecal contamination. The presence of *E. coli* in a water source indicates an increased risk to public health for those coming in contact with the water source. Random samples along the length of the Ohio River were taken and enumerated for the MPN of *E. coli* in each sample. The current Ohio River standard for contact recreation safety states that a single 100 ml sample of water cannot exceed 240 MPN (ORSANCO River Facts/Conditions) Only a few of the samples came close to or exceeded this standard (RM 89.7 & 324.2). The majority of

samples contained less than 100 MPN of total *E. coli (TE).* To compare the median values and determine whether the subsurface populations and bottom populations are distributed equally for total and antibiotic resistant *E. coli*, the Mann-Whitney Rank Sum Test was used. For total *E. coli (TE)* and tetracycline resistant *E. coli* (TetR*Ec*), the differences in the median values between the subsurface samples and bottom samples were not great enough to exclude the possibility that the differences were due to random sampling variability; in other words, no significant differences were found ($P$=0.074 for *TE* and TetR*Ec* ). This result fails to reject the null hypothesis that these two samples were drawn from a single population. However, it was determined that the median values between subsurface ciprofloxacin resistant *E. coli* (CipR*Ec*) and bottom CipR*Ec* were greater than expected by chance; therefore, the difference was significant ($P$=0.004). This indicates these two populations for ciprofloxacin resistant *E. coli* were independent, and bottom counts were higher than surface counts.

## ASSESSING SPIKES IN TOTAL *E. COLI* AND ANTIBIOTIC RESISTANT *E. COLI* ALONG THE OHIO RIVER

To assess potential areas in and along the Ohio River that may be at risk for fecal contamination, all *E. coli* counts were graphed by river mile and "spikes" in *E. coli* at specific river miles were noted. Two subsurface samples along the length of the river exceeded the criteria for recreational contact for total *E. coli* at river miles 89.7 and 324.2. These sites are both near urbanized areas, and sewer outlets were noted along the left bank of the river in which these two samples were grabbed. No bottom total *E. coli* counts exceeded the contact recreation standard. Two "spikes" in subsurface TetR *E. coli* were noted at river miles 324.2 and 793.0.

Although there is no criteria for antibiotic resistant bacteria present in a single sample, river mile 793.0 exceeded 240 MPN, the criteria for total *E. coli* in one sample. The discrepancy between subsurface total *E. coli* and TetR*E.c* at river mile 793.0 is questionable and no reasonable conclusion can be made at this time on why TetR*E.c* showed over a 200 fold increase in MPN to total *E. coli* at this particular site. Based on all sites; however, total *E. coli* and TetR*E.c* subsurface and bottom populations were not different, and statistically insignificant. Like CipR coliforms, the majority of CipR *E. coli* counts for subsurface and bottom samples also recorded zero MPN. Descriptions of the sites showing "spikes" in total *E. coli* and antibiotic resistant *E. coli* are shown with the coliform data in Figure 49a and b. The Ohio River is likely less exposed to the antibiotic ciprofloxacin or CipR bacteria and more exposed to tetracycline or TetR bacteria due to tetracycline not only being used in human medicine but in veterinary medicine and agriculture as well. There is more opportunity along the Ohio River for tetracycline resistance to occur based solely on the land use of the Ohio River Basin.

(a)

| River Mile Site | Bacteria "spikes" | Site Description |
|---|---|---|
| 0.2 | Bottom TetR coliforms<br>Bottom CipR coliforms | At the confluence of the Allegheny and Monongahela Rivers located in the downtown metropolis city of Pittsburgh, PA. This site is likely to be highly impacted by the city and it's industrialization |
| 36.1 | Subsurface TetR coliforms<br>Bottom TetR coliforms<br>Bottom CipR coliforms | This site is located just downstream of Shippingport, PA. This area is highly industrialized harboring a major power station along the left bank of the river. |
| 87.7 | Bottom TetR coliforms<br>Bottom CipR coliforms | This site is located along the bank of Martins's Ferry, OH. |
| 89.7 | Subsurface Total *E. coli* | This site is located downstream of a sewer outlet located at ~ RM88 emptying into the Ohio River on the left bank |
| 186.2 | Subsurface Total *E. coli*<br>Bottom Total *E. coli* | This site is located just downstream of Parkersburg, WV. The Little Kanawha River empties into the Ohio River just upstream of this site. |
| 302.2 | Subsurface *E. coli* | This site is located upstream of Huntington,WV right off the banks of Proctorville, OH. |
| 324.2 | Subsurface TetR coliforms<br>Subsurface CipR coliforms<br>Subsurface Total *E. coli*<br>Subsurface TetR *E.coli* | This site is located between Ashland, KY on the left bank and Ironton, OH on the right bank. Between RM 321-323, there are 5 CSOs that empty into the Ohio River in that area on the KY side. This site is likely to be impacted by industrialization and urbanization |
| 369.2 | Subsurface CipR coliforms | This site is located right off the banks of Garrison,KY. Kinniconick Creek empties into the Ohio River just upstream of this site |

| | | |
|---|---|---|
| 414.8 | Bottom TetR coliforms | This site is located between the banks of Moranburg, KY and Ripley, OH. The site is just downstream of a powerstation located on the left bank |
| 528.4 | Subsurface TetR coliforms | This site is located along the bank of Warsaw, KY. Two industrial discharge points are located on the river in this area. Bryant's Creek also enters the river just upstream of this site |
| 623.7 | Bottom TetR coliforms | This site is located between the banks of Sugar Grove, IN and Orell, KY. The land use surround this site includes mostly fields and agricultural land along the right bank, while the left bank is civilized and industrialized. |
| 740.0 | Bottom TetR coliforms | This site is mostly surrounded by fields and agricultural land. It is located just downstream of the two small towns of Lewisport, KY, and Grandview, IN. |
| 793.0 | Subsurface TetR coliforms | This site is located along the banks of Evansville, IN, a large metropolitan city that sits right along the Ohio River. This site is likely to be highly impacted by the city and its industrialization |

(b)

| River Mile / Bacteria | 0.2 | 36.1 | 87.7 | 89.7 | 186.2 | 302.2 | 324.2 | 369.2 | 414.8 | 528.4 | 623.7 | 740 | 793 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Subsurface TetR coliforms | | X | X | | | | X | | | X | | | X |
| Subsurface CipR coliforms | | | | | | | X | X | | | | | |
| Subsurface Total *E. coli* | | | | X | X | X | X | | | | | | |
| Subsurface TetR *E. coli* | | | | | | | X | | | | | | |
| Subsurface CipR *E. coli* | | | | | | | | | | | | | |
| Bottom TetR coliforms | X | X | | | | | | | X | | X | X | |
| Bottom CipR coliforms | X | X | X | | | | | | | | | | |
| Bottom Total *E. coli* | | | | | X | | | | | | | | |
| Bottom TetR *E. coli* | | | | | | | | | | | | | |
| Bottom CipR *E. coli* | | | | | | | | | | | | | |
| | | | | | | | | | | | | | |

**FIGURE 49:** (a) Descriptions of Sites with Noted "Spikes" in Bacterial Counts. (b) Chart depicting bacterial spikes by river mile.

# CHAPTER VI : CONCLUSIONS

## *PART I: OHIO RIVER SURVEY 2007*

Subsurface and bottom samples for some bacteria counts showed the samples represent different populations, while other comparisons between top and bottom samples were not significantly different. Antibiotic resistant coliform bacteria were found in greater numbers from samples taken from the bottom of the river compared to subsurface samples taken. This was consistent throughout the entire river. With that it is reasonable to say that other types of bacteria may also be present in greater numbers at the bottom of the river that were not tested. Upon analyzing the 2007 results, a few testable hypotheses were formed; (i) bacterial populations are different at different locations in the stream, including different depths and (ii) samples taken from the bottom of the river likely contain more sediment; thus providing substrate for the accumulation of bacterial populations and the potential for spread of resistance genes among these populations. With lower numbers of antibiotic resistant coliforms and *E. coli* present from the previous study and the lack of significance between *TE* and Tet*R**E.c* subsurface and bottom sample populations, the new hypotheses were tested by analyzing the amount of total cultivable bacteria, and antibiotic resistant cultivable bacteria at different depths, as well as total *E. coli*. The amount of sediment per sample was also compared to the amount of bacteria in each sample. This way the analysis wasn't limited to only coliform bacteria, but included all bacteria that can be cultivated in the laboratory. This hypothesis was tested during the 2008 sample season, summarized and analyzed in Part 2 of this Chapter.

During rain events, runoff is likely to expose the river to even greater amounts of coliform bacteria, *E. coli* and ARB; however, due to the lack of rainfall during this 2007 sampling period and normal flow conditions, these data may represent baseline bacterial data for the areas tested along the Ohio River. These results can also be used to further investigate fluctuations in bacterial counts along the river as a result of other physical changes such as season, precipitation, flow etc. Some areas of concern were also noted, specifically with sites containing fecal contamination; therefore, monitoring of these sites should be continued in order to identify sources of contamination.

## *PART 2: OHIO /GUYANDOTTE RIVER STUDY, 2008*

To further investigate how bacteria, including antibiotic resistant bacteria are distributed throughout a large river system a second study was performed on a much smaller area of the Ohio River, including samples within the Ohio River upstream and downstream of the mouth of the Guyandotte River and samples within the Guyandotte River. This section addresses the questions of whether a single surface sample provides a representative measurement of bacteria for an entire large river at that location and whether sediments play a critical role in the distribution of antibiotic resistant bacteria throughout the water column of a large river system based on the results found.

### DEPTH AND RIVER QUADRANT VS. BACTERIA

The samples taken in the Ohio River and in the Guyandotte River were analyzed separately to first determine whether depth and location across the river (also referred to as quadrant) correlated with the amount of bacteria in each sample, including total cultivable bacteria (TCB), total *E. coli* (TE), tetracycline resistant bacteria (TetR), ciprofloxacin resistant bacteria (CipR), and virginiamycin resistant bacteria (VirR). The evidence shows neither depth nor river quadrant was correlated with any bacteria samples taken in the Ohio River upstream of the Guyandotte River and samples taken within the Guyandotte River. However, samples taken from the Ohio River downstream of the mouth of the Guyandotte revealed that *E. coli* was not correlated with depth but was significantly correlated with river quadrant. *E. coli* counts taken from the left bank samples of the Ohio River were approximately three orders of magnitude greater than samples taken from any other quadrant downstream, as well as samples taken

upstream of the Guyandotte River. This provides evidence that flow from the Guyandotte River is carrying greater amounts of *E. coli* thus causing a heterogeneous distribution of fecal bacteria in the Ohio River just downstream of the Guyandotte. It is important to emphasize that there were no significant differences in bacteria taken upstream of the tributary or in samples taken in the tributary, concluding that a single grab sample can be a reasonable representation of a river transect, except where major local changes can be expected to create local heterogeneity (ie. the entrance of a major tributary or other large input).

## WATER CHEMISTRY

Correlation statistics also included water chemistry data. Linear regressions performed on the significant correlations revealed temperature changes occurred dependent on the where the sample was taken in relation to river quadrant for all river locations. When comparing each location and regression, the results suggest that the Guyandotte River water was cooler than the mainstem Ohio River and the left bank samples taken below the Guyandotte River revealed cooler water temperatures as well. Linear regression analysis also revealed that turbidity significantly increased with depth, but only in the Ohio River samples. Turbidity is the measurement of the light scattering properties of water due to suspended particles. More samples were taken in the Ohio River across each transect and at more depths creating better results for a linear regression analysis. Turbidity is suspected to increase with depth in a large river system due to suspended solids settling towards the bottom. It is also likely that resuspension may occur at greater depths due to bottom feeders stirring up the substrate. The lack of a significant correlation between turbidity and depth in the Guyandotte River is most likely due to the limited amount of samples taken in the shallower river, and the fact that the

river was well stirred up. pH readings revealed fairly low, acidic conditions for all samples. It is questionable whether the YSI was accurately calibrated for pH prior to taking these readings; and therefore, the pH correlations are being considered inconclusive at this time. Percent dissolved oxygen levels for the Ohio River were considered good with most samples containing 99-100%. The Guyandotte River measurements indicated slightly higher percents, with an average of 110% DO, likely due to algal bloom activity.

## SEDIMENT

Upon analyzing the amount of bacteria in each sample and the amount of sediment in each sample, only samples taken from the Guyandotte River showed any correlation between bacteria counts and sediment. CipR bacteria decreased with increasing amounts of sediment, while VirR bacteria increased as the amount of sediment increased. These findings may be a result of some other unknown factor. At this time no conclusion has been made regarding these differences. In order to compare all of the bacteria samples taken to the amount of sediment measured in each sample, location and depth at which the sample was taken was disregarded and a linear regression revealed no significant relationship between the amount of sediment per sample and the amount of total bacteria, ARB, or *E. coli* per sample. The linear regression results fail to support the hypothesis that more sediment in a sample provides substrate for greater bacterial populations. Figure 48 shows statistically powerful yet surprising results, indicating that sediments are not associated bacterial populations. With contradicting evidence, we conclude that sediment cannot be used as a proxy for bacterial counts.

## *SUMMARY*

Several factors play a role in determining whether a single surface sample can provide a representative bacterial measurement for an entire river at that location. The two key factors considered in this study were location of the sample and depth of the sample; however, there are many other factors such as, size of the watershed, flow at that particular time, past and present precipitation events, surrounding land use, and possibly most importantly, whether there are direct inputs into the system that may affect that particular sample. In the Ohio River 2007 study subsurface samples and bottom samples showed "spikes" in highly urbanized areas as well as areas where streams or local inputs were likely to occur. In the 2008 study, depth had no significance; however, location was found to be a factor due to the direct input of water and bacteria from the Guyandotte River, a major tributary of the Ohio River. For a large river system, surface samples will only provide representative bacterial measurements for sample locations where the water is well mixed and homogenized. In the case of a tributary or another large input, this single surface sample would not provide an accurate enough measurement for that stretch of river. In conclusion, for large rivers like the Ohio River, single grab samples are sufficient for most sites; however, intense sampling is needed in order to detect the degree of impact of specific source like a tributary in order to define local heterogeneity. In cases where sources of contamination are not yet known, intense sampling techniques would aid in finding these potentially harmful sources or in cases where contaminants are known, but the source is yet to be found, more intense sampling techniques may lead to a resolution to the problem.

Since the presence of antibiotics and/or ARB is not currently used in the assessment of stream quality and the distribution of ARB and fecal indicator bacteria in the Ohio River Basin have been shown to be distinct (Smith & Somerville, 2003) it is questionable whether or not current water quality standards are reliable in assessing public health risks. Maryland is currently using the Antibiotic Resistance Analysis (ARA) Bacteria Source Tracking methodology to determine the sources of bacterial pollution. "This method uses enterococci patterns of antibiotic resistance for identifying bacterial sources" and is based on the assumption that human fecal bacteria are resistant to different concentrations and different types of antibiotics than domestic animal fecal bacteria, while wildlife fecal bacteria should be less resistant than both humans and domestic animals due to less exposure to antibiotics through therapeutic practices (Maryland Dept of the Environment). Many other researchers and agencies have adopted this method to identify sources of fecal pollution in surface and groundwater (Whitlock, Jones, & Harwood, 2002) (Wiggens, et al., 1999); however, these source tracking efforts are distinct from this study. No method involving the quantification of ARB has been used to assess public health risks or set standards for the quality and safety of surface waters. We believe that monitoring of ARB is important to understanding the impacts humans and development have on the resource. Assessing the safety and quality of the most important resource on this earth, fresh water, is the first step to maintaining a clean and healthy ecosystem for all life to flourish.

## *FUTURE DIRECTIONS*

Several testable hypotheses have stemmed from the results and conclusions of this study. Previous studies on the Ohio River show spikes in fecal indicator and antibiotic resistant bacteria particularly after heavy rain events, likely due to overland flow and runoff. The results of this study are surprising yet statistically powerful, indicating that sediments are not associated with fecal and/or ARB. Because this study was performed under dry weather conditions, it is questionable whether high bacteria counts are associated with sediment loading occurring in a stream after a rain event. Resident sediments within a stream may not be associated with bacterial contamination, which were tested in this study. However, one hypothesis is that sediments carried into a river or stream by overland flow may provide a different answer to the same question, are sediments critical to the distribution of fecal indicator and ARB? Sediments washed into a river or stream after it rains may carry bacterial populations along with them; therefore, a continuation study on sediments during wet weather conditions would provide a great source of comparable data to this study.

This thesis showed that subsurface samples are sufficient for large river sampling in areas where the water is well mixed. Recall that samples taken in the Ohio River above the Guyandotte River showed no significant differences in bacteria counts across the horizontal transect. This indicates that in instances where the river is homogenized, a single surface sample provides a good representation of bacteria populations in that area. However, in a heterogeneous area of the river (ie. where a tributary enters), our results show that a subsurface sample taken

from the middle of the river would not provide a good representation of the bacteria present where the tributary enters. Knowing where to sample to provide the best representation of bacteria counts in a specific location of a large river is key to making sure bacteria criteria are met. A more intensive study on tributary impacts to large river systems could provide greater details to how flow and mixing occurs between the two systems, and where the two become homogenized within the mainstem.

# LITERATURE CITED


1. Aarestrup, F. M., Seyfarth, A. M., Hanne-Dorthe, E., Pedersen, K., Hendriksen, R. S., & Bager, F. (2001). Effect of Abolishment of the Use of Antimicrobial Agents for Growth Promotio on Occurence of Antimicrobial Resistance in Fecal Enterococci from Food Animals in Denmark. *Antimicrobial Agents and Chemotherapy* , 2054-2059.

2. Agerse, Y. (2007). The tetracycline resistance gene tet(E) is frequently occuring and prsent on large horizontally transferable plasmids in Aeromonas spp. from fish farms. *Journal of Aquaculture* .

3. Angulo, F. (2000). Antimicrobial agents in aquaculture: potential impact on public health. *APUA Newsletter , 18* (1).

4. Armstrong, J. L., Shigeno, D. S., Calomiris, J. J., & Seidler, R. J. (1981). Antibiotic Resistant Bacteria in Drinking Water. *Applied and Environmental Microbiology* , 277-283.

5. Australia's Cooperative Research Centre for Water Quality and Treatment. (2006, May). Consumer's Guide to Drinking Water.

6. Benke, A. C., & Cushing, C. E. (2005). *Rivers of North America: The Natural History.* Academic Press.

7. Calomiris, J. J., Armstrong, J. L., & Seidler, R. J. (1984). Association of metal tolerance with multiple antibiotic resistance of bacteria isolated from drinking water. *Applied and Environmental Biology* , 1238-1242.

8. Chaturvedi, S., Chandra, R., & Rai, V. (2008). Multiple antibiotic resistance patterns of rhizospheric bacteria isolated from Phragmites australis growing in constructed wetland for distillery effluent treatment. *Journal of Environmental Biology* , 117-124.

9. Chee-Sanford, J. C., Aminov, R. I., Krapac, I. J., Garrigues-Jeanjean, N., & Mackie, R. I. (2001). Occurence and Diversity of Tetracycline Resistance Genes in Lagoons and Groundwater Underlying Two Swine Production Facilities. *Applied Environmental Microbiology , 67* (4), 1494-1502.

10. Cometta, A., Calandra, T., Bille, J., & Glauser, M. P. (1994). Escherichia coli resistant to fluoroquinolones in patients with cancer and neutropenia. *North England Journal of Medicine , 330* (1240).

11. DePaola, D., Flynn, P. A., McPhearson, R. M., & Levy, S. B. (1988). Phenotypic and genotypic characterization of tetraycycline-and oxytetraycycline-resistant Aeromonas hydrophila from cultured channel catfish (Ictalurus punctatus) and their environments. *Applied Environmental Microbiology , 54* (7), 1861-1863.

12. Dhalkephalkar, P. K., & Chopade, B. A. (1994). High levels of multiple metal reistance and its correlation to antibiotic resistance in environmental isolates of Acinetobacter. *Biometals , 7*, 67-74.

13. Dotson, T. (2008, May 21). Factors that influence the distribution of antibiotic resistant bacteria in the Mud River, WV. *Marshall University Electronic Theses* . Huntington, WV.

14. Harvey, R., Funk, J., Wittum, T. E., & Hoet, A. E. (2009). A metagenomic approach for determiningg prevalence of tetraycycline resistance genes in the fecal flora of conventionally raised feedlot steers and feedlot steers raised without antimicrobials. *American Journal of Veterinary Research , 70* (2), 198-202.

15. James, W. (1994). *Current Practices in Modeling the Management of Stormwater Impacts.* CRC Press.

16. Jin Jun, L., Jeong, J. B., Huh, M. D., Chung, J. K., Choi, D., Lee, C. H., et al. (2004). Detection of tetracycline-resistance determinants by multiplex polymerase chain reaction in Edwardsiella tarda isolated from fish farms in Korea. *Journal of Aquaculture* .

17. Kern, W. V., Andriof, E., Oethinger, M., Kern, P., Hacker, J., & Marre, R. (1994). Emergence of fluoroquinolone-resistant Escherichia coli at a cancer center. *Antimicrobial Agents of Chemotherapy , 38*, 681-687.

18. Kern, W. V., Markus, A., & Andriof, E. (1994). Bacterimia due to fluoroquinolone-resistant Escherichia coli in two immunocompromised patients. *European Journal of Clinical Microbial Infectious Diseases , 13*, 161-165.

19. Kummerer, K. (2001). *Pharmaceuticals in the environment: sources, fate, effects and risks.* Heidelberg, Germany: Springer.

20. Kummerer, K. (2004). Resistance in the environment. *Journal of Antimicrobial Chemotherapy , 54*, 311-320.

21. Levy, S. (2002). *The Antibiotic Paradox: How the Misuse of Antibiotics Destroys Their Curative Powers* (2nd ed.). Perseus Publishing.

22. Livermore, D. M. (2002). *Trends in fluoroquinolone (ciprofloxacin) resistance in enterobacteriaceae from bacteremias, England and Wales, 1990-1999.* CDC.

23. Maryland Dept of the Environment. (n.d.). *Bacteriological Water Quality Monitoring.* Retrieved March 2009, from http://www.mde.state.md.us/

24. McManus, P. S., & Stockwell, V. O. (2001). Antibiotic use for plant disease management in the United States. *Plant Health Progress* .

25. MedicineNet, Inc. (1996-2009). *Definition of Antibiotic*. Retrieved 2009 January, from MedicineNet: http://www.medterms.com/script/main/art.asp?articlekey=8121

26. Mehnam, S., Beighton, D., Philpott-howard, J., & Woodford, N. (2000). Mechanisms of resistance to quinupristin-dalfopristin among isolates of Enterococcus faecium from animals, raw meat, and hospital patients in Western Europe. *Antimicrobial Agents and Chemotherapy* , 433-436.

27. Murray, B. E. (1997). Antibiotic resistance. *Advances in Internal Medicine , 42*, 339-367.

28. Nobel Lectures. (1964). *Physiology or Medicine, 1942-1962.* Amsterdam: Elsevier Publishing Company.

29. Ohio Historical Society. (2005, July 1). *Ohio River.* Retrieved March 2009, from Ohio History Central: http://www.ohiohistorycentral.org/entry.php?rec=784

30. ORSANCO. (1997, November). Identification of Longitudinal/Bacteria Impacts from CSOs: Investigating CSOs and their impacts on the Ohio River. *1* .

31. ORSANCO. (n.d.). *Ohio River Facts*. Retrieved March 2007, from Ohio River Valley Ohio Sanitation Commission: http://www.orsanco.org

32. ORSANCO River Facts/Conditions. (n.d.). *Is it safe to swim in the Ohio River?* Retrieved July 2009, from http://www.orsanco.org/index.php/is-it-safe-to-swim-in-the-ohio-river

33. ORSANCO. (2006, April 18). Wet Weather Proposal May Lower Ohio River Water Quality Standards. *2008 Biennial Assessment* . Cincinnati, OH.

34. Silvia, J. (2006). Frequency of transferable multiple antibiotic resistance amongst coliform bacteria isolated from a treated sewage effluent in Antofagasta, Chile. *Electronic Journal of Biotechnology , 9* (5), 533-540.

35. Smith, L. M., & Somerville, C. C. (2003). Antibiotic resistant and fecal coliform bacteria in the Ohio River belong to distinct microbial populations. *ASB Bulletin, 50:173.*

36. Somerville, C. C. (2008). *Antibiotic resistant bacteria in huntington tap water.*

37. Somerville, C. C., Saunders, C. B., & Van Meter, S. A. (May 2002). Frequency and distribution of antibiotic resistant bacteria in the Ohio River. *102nd Annual Meetings of the American Society for Microbiology*, (pp. 12-23). Salt Lake City, Utah.

38. Somerville, C. C., Smith, L. M., Loughman, K. R., & Johnson, A. N. (2004). Antibiotic resistant and fecal coliform bacteria in the Ohio River: comparisons to land

use patterns. *104th General Meeting of the American Society of Microbiology*, (pp. 23-27). New Orleans, LA.

39. Speer, B. S., Shoemaker, N. B., & Salyers, A. A. (1992). Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. *Clinical Microbiology Reviews , 5* (4), 387-399.

40. Upper Guyandotte Water Association. (2006). *Problems Facing Our Watershed*. Retrieved July 2008, from www.wvugwa.org

41. US FDA. (2000, April 19). Risk Assessment of the Public Health Impact of Streptogramin Resistance in Enterococcus faecium Attributable to the Use of Streptogramins in Animals. *65*(76).

42. US FDA. (2004, November 24). Virginiamycin Risk Assessment Available for Review and Comment.

43. West Virginia Rivers Coalition. (2005, September). Helping Solve Local Wastewater Problems: A Guide for West Virginia Watershed Organizations. Wardensville, WV.

44. Whitlock, J. E., Jones, D. T., & Harwood, V. J. (2002). Identification of the Sources of Fecal Coliforms in an Urban Watershed using Antibiotic Resistance Analysis. *Water Research , 36* (17), 4273-4282.

45. Wiggens, B. A., Andrews, R. W., Conway, R. A., Corr, C. L., Dobratz, E. J., Dougherty, D. P., et al. (1999). Use of Antibiotic Resistance Analysis to Identify Nonpoint Source Pollution. *Applied and Enviromental Microbiology* , 3483-3486.

46. WV EPA & ORSANCO. (1994). Basinwide survey of Ohio and Tennessee River Valley. *Section II ,* 50-70.

47. WV EPA. (2004, March 30). Metals, fecal coliform and pH TMDLs for the Guyandotte River Watershed. WV.

48. Yonath, A. (2005). Antibiotics targeting ribosomes: Resistance, selectivity, synergism and cellular regulation. *Annual Review of Biochemistry* (74), 649-679.

49. Zwenger, S., Welsch, T., & Gillock, E. (2008). Bacteria resistant to ciprofloxacin, chloramphienicol, and tetracycline isolated from western kentucky feedlots. *Transactions of the Kansas Academy of Science , 111* (1), 125-135.

# APPENDIX A

## Latitude and Longitude Coordinates for 2007 Ohio River Sites

| River Mile | Quadrant | Latitude | Longitude |
|---|---|---|---|
| 0.2 | 2 | 40.44327 | -80.0171 |
| 28.4 | 1 | 40.67188 | -80.3415 |
| 36.1 | 1 | 40.62674 | -80.4587 |
| 45.6 | 2 | 40.62267 | -80.6105 |
| 47.4 | 3 | 40.60927 | -80.6395 |
| 65.7 | 5 | 40.38452 | -80.6242 |
| 67.3 | 4 | 40.36766 | -80.6073 |
| 85.8 | 3 | 40.12839 | -80.7088 |
| 87.7 | 5 | 40.10177 | -80.7086 |
| 89.7 | 5 | 40.07929 | -80.7281 |
| 119.2 | 1 | 39.74654 | -80.8595 |
| 171.4 | 3 | 39.40866 | -81.4416 |
| 178.8 | 4 | 39.34714 | -81.5482 |
| 186.2 | 1 | 39.27421 | -81.5967 |
| 191.9 | 4 | 39.26089 | -81.6928 |
| 215.9 | 2 | 39.00682 | -81.7638 |
| 220.2 | 2 | 38.94987 | -81.7724 |
| 224.5 | 4 | 38.93224 | -81.7983 |
| 250.7 | 3 | 39.02248 | -82.0378 |
| 275.1 | 5 | 38.73975 | -82.1901 |
| 302.2 | 5 | 38.43897 | -82.3373 |
| 302.9 | 4 | 38.43714 | -82.3513 |
| 324.2 | 5 | 38.49808 | -82.663 |
| 345.5 | 1 | 38.70805 | -82.8769 |
| 355.1 | 3 | 38.72714 | -82.9896 |
| 369.2 | 1 | 38.61575 | -83.1758 |
| 377.3 | 3 | 38.59698 | -83.3041 |
| 378.2 | 4 | 38.60489 | -83.3173 |
| 405.3 | 4 | 38.63685 | -83.706 |
| 412.5 | 5 | 38.69911 | -83.7932 |
| 413.5 | 4 | 38.70353 | -83.8104 |
| 414.8 | 2 | 38.71369 | -83.8291 |
| 433.7 | 3 | 38.78393 | -84.1268 |
| 457.3 | 3 | 39.03633 | -84.3518 |
| 467.8 | 1 | 39.11801 | -84.4729 |
| 483.7 | 5 | 39.12443 | -84.7134 |
| 485.1 | 4 | 39.13883 | -84.7289 |
| 492.8 | 1 | 39.09223 | -84.838 |
| 501.1 | 5 | 39.00468 | -84.8512 |
| 509.1 | 3 | 38.91089 | -84.8749 |
| 528.4 | 2 | 38.78214 | -84.9103 |
| 544 | 5 | 38.69167 | -85.1584 |
| 562.1 | 2 | 38.71332 | -85.4486 |

| | | | |
|---|---|---|---|
| 572.5 | 3 | 38.56933 | -85.4135 |
| 589.7 | 3 | 38.41605 | -85.6177 |
| 592.5 | 4 | 38.3768 | -85.6307 |
| 593.8 | 2 | 38.36049 | -85.6394 |
| 609.9 | 1 | 38.26409 | -85.8317 |
| 623.7 | 4 | 38.08564 | -85.908 |
| 633.7 | 2 | 37.99652 | -86.0173 |
| 647.3 | 3 | 38.01279 | -86.1899 |
| 657 | 5 | 38.1171 | -86.2738 |
| 667.9 | 2 | 38.16518 | -86.3654 |
| 668.7 | 1 | 38.15961 | -86.353 |
| 682.7 | 1 | 38.10122 | -86.4589 |
| 701 | 3 | 37.91759 | -86.5294 |
| 702.7 | 3 | 37.9172 | -86.556 |
| 716.1 | 2 | 37.89065 | -86.6467 |
| 736.5 | 5 | 37.96091 | -86.885 |
| 737.4 | 1 | 37.95058 | -86.9002 |
| 740 | 4 | 37.93643 | -86.939 |
| 759.7 | 4 | 37.80793 | -87.1393 |
| 793 | 4 | 37.97108 | -87.5896 |
| 796.1 | 3 | 37.93618 | -87.6139 |
| 796.9 | 4 | 37.92688 | -87.6209 |
| 800.9 | 5 | 37.88298 | -87.5889 |
| 809.1 | 4 | 37.82825 | -87.6747 |
| 835.2 | 4 | 37.85833 | -87.9237 |
| 848.4 | 4 | 37.80116 | -88.027 |
| 857.5 | 2 | 37.69634 | -88.1225 |
| 889.8 | 1 | 37.43138 | -88.3157 |
| 893 | 3 | 37.40182 | -88.3602 |
| 906.1 | 1 | 37.31794 | -88.5004 |
| 919.9 | 5 | 37.14861 | -88.425 |
| 923.9 | 4 | 37.10184 | -88.4368 |
| 928.8 | 2 | 37.05643 | -88.4952 |
| 960 | 5 | 37.22623 | -88.9936 |

*River Quadrants: (1) Left Bank, (2) Left Channel, (3) Center Channel, (4) Right Channel, (5) Right Bank*

| TABLE 1 | | Total Coliforms (MPN) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Surface | | | Bottom | | |
| River Mile | Quadrant | Total/Control | TetR | CipR | Total/Control | TetR | CipR |
| 0.2 | 2 | 1986.3 | 20.3 | 3.1 | 2419.6 | 658.6 | 103.9 |
| 28.4 | 1 | >2419.6 | 13.4 | 4.1 | >2419.6 | 240 | 18.3 |
| 36.1 | 1 | >2419.6 | 913.9 | 2 | >2419.6 | 1011.2 | 123.9 |
| 45.6 | 2 | 648.8 | 8.5 | 0 | 1986.3 | 34.1 | 0 |
| 47.4 | 3 | 517.2 | 7.4 | 0 | >2419.6 | 328.2 | 32.9 |
| 65.7 | 5 | 920.8 | 7.5 | 2 | >2419.6 | 103.4 | 13.4 |
| 67.3 | 4 | >2419.6 | 13.5 | 2 | >2419.6 | 31.3 | 1 |
| 85.8 | 3 | 980.4 | 6.3 | 0 | >2419.6 | 91.3 | 3 |
| 87.7 | 5 | 913.9 | 42.8 | 1 | >2419.6 | 1119.9 | 248.9 |
| 89.7 | 5 | 1732.9 | 5.2 | 0 | 1119.9 | 64.2 | 1 |
| 119.2 | 1 | 770.1 | 24.1 | 0 | 1732.9 | 190.4 | 0 |
| 171.4 | 3 | 920.8 | 26.6 | 1 | 866.4 | 59.4 | 0 |
| 178.8 | 4 | 1203.3 | 88.2 | 0 | >2419.6 | 62.4 | 0 |
| 186.2 | 1 | 1299.7 | 23.8 | 0 | 2419.6 | 40.4 | 0 |
| 191.9 | 4 | 248.9 | 16.8 | 0 | 1011.2 | 218.7 | 6 |
| 215.9 | 2 | >2419.6 | 15.8 | 0 | 1732.9 | 12.1 | 0 |
| 220.2 | 2 | 275.5 | 1 | 0 | 579.4 | 4.1 | 0 |
| 224.5 | 4 | >2419.6 | 73.8 | 0 | >2419.6 | 88.8 | 0 |
| 250.7 | 3 | 410.6 | 6.3 | 0 | 1203.3 | 5.2 | 0 |
| 275.1 | 5 | 344.8 | 2 | 1 | >2419.6 | 42.6 | 6.3 |
| 302.2 | 5 | 613.1 | 2 | 0 | 1732.9 | 60.2 | 3 |
| 302.9 | 4 | 231 | 5.2 | 0 | 960.6 | 28.5 | 0 |
| 324.2 | 5 | >2419.6 | 1203.3 | 39.3 | >2419.6 | 98.7 | 24.6 |
| 345.5 | 1 | >2419.6 | 19.9 | 1 | >2419.6 | 50.4 | 2 |
| 355.1 | 3 | 9.8 | 16 | 4.1 | >2419.6 | 30.1 | 10.9 |
| 369.2 | 1 | >2419.6 | 53.8 | 18.3 | >2419.6 | 88.6 | 12.1 |
| 377.3 | 3 | >2419.6 | 31.8 | 3.1 | >2419.6 | 34.6 | 5.2 |
| 378.2 | 4 | 2419.6 | 17.3 | 7.5 | >2419.6 | 70.3 | 6.3 |
| 405.3 | 4 | 1986.3 | 11 | 0 | 1046.2 | 2 | 0 |
| 412.5 | 5 | 1553.1 | 13.2 | 0 | >2419.6 | 172.5 | 4.1 |
| 413.5 | 4 | 1203.3 | 7.5 | 0 | 1732.9 | 16.8 | 0 |
| 414.8 | 2 | 365.4 | 6.3 | 0 | >2419.6 | 816.4 | 0 |
| 433.7 | 3 | 1553.1 | 7.4 | 0 | >2419.6 | 35 | 0 |
| 457.3 | 3 | 2419.6 | 41.7 | 0 | >2419.6 | 23.3 | 0 |
| 467.8 | 1 | >2419.6 | 4.1 | 0 | >2419.6 | 22.5 | 0 |
| 483.7 | 5 | 1553.1 | 5.2 | 2 | >2419.6 | 14.6 | 1 |
| 485.1 | 4 | 1986.6 | 5.2 | 0 | >2419.6 | 54.3 | 4.1 |
| 492.8 | 1 | >2419.6 | 16.1 | 0 | >2419.6 | 32.7 | 0 |
| 501.1 | 5 | >2419.6 | 9.7 | 0 | >2419.6 | 3.1 | 0 |
| 509.1 | 3 | 1986.3 | 3.1 | 0 | >2419.6 | 35.9 | 0 |
| 528.4 | 2 | >2419.6 | >2419.6 | 0 | >2419.6 | 135.5 | 0 |

| Table 1 | (Cont.) | Total Coliforms (MPN) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Surface | | | Bottom | | |
| River Mile | Quadrant | Total/Control | TetR | CipR | Total/Control | TetR | CipR |
| 572.5 | 3 | >2419.6 | 9.8 | 0 | >2419.6 | 86 | 2 |
| 589.7 | 3 | 2419.6 | 3.1 | 0 | >2419.6 | 9.7 | 0 |
| 592.5 | 4 | 870.4 | 8.6 | 0 | >2419.6 | 8.6 | 0 |
| 593.8 | 2 | >2419.6 | 5.2 | 0 | >2419.6 | 46.5 | 0 |
| 609.9 | 1 | >2419.6 | 45.7 | 0 | >2419.6 | 115.3 | 2 |
| 623.7 | 4 | >2419.6 | 36.9 | 1 | >2419.6 | 1119.9 | 13.8 |
| 633.7 | 2 | 2419.6 | 14.6 | 0 | >2419.6 | 68.7 | 0 |
| 647.3 | 3 | 866.4 | 11 | 0 | 1732.9 | 3.1 | 0 |
| 657 | 5 | 2419.6 | 7.5 | 0 | >2419.6 | 21.3 | 5 |
| 667.9 | 2 | 2419.6 | 123.9 | 0 | 2419.6 | 44.1 | 0 |
| 668.7 | 1 | 1119.9 | 1 | 0 | >2419.6 | 72.4 | 8 |
| 682.7 | 1 | >2419.6 | 2 | 0 | >2419.6 | 135.4 | 0 |
| 701 | 3 | 816.4 | 6.3 | 0 | >2419.6 | 39.9 | 0 |
| 702.7 | 3 | >2419.6 | 46.2 | 0 | >2419.6 | 96 | 0 |
| 716.1 | 2 | 648.8 | 1 | 0 | >2419.6 | 77.6 | 7.2 |
| 736.5 | 5 | 1203.3 | 3.1 | 0 | >2419.6 | 61.4 | 10 |
| 737.4 | 1 | 1413.6 | 6.3 | 0 | >2419.6 | 61.6 | 1 |
| 740 | 4 | 1299.7 | 2 | 0 | >2419.6 | 648.8 | 35.8 |
| 759.7 | 4 | 2419.6 | 5.2 | 0 | >2419.6 | 7.5 | 4 |
| 793 | 4 | >2419.6 | 435.2 | 0 | >2419.6 | 27.2 | 1 |
| 796.1 | 3 | >2419.6 | 26.5 | 0 | >2419.6 | 22.8 | 0 |
| 796.9 | 4 | >2419.6 | 66.3 | 0 | >2419.6 | 43.9 | 0 |
| 800.9 | 5 | >2419.6 | 61.3 | 0 | >2419.6 | 116.9 | 0 |
| 809.1 | 4 | >2419.6 | 13.4 | 0 | >2419.6 | 249.5 | 0 |
| 835.2 | 4 | >2419.6 | 4.1 | 0 | >2419.6 | 39.9 | 0 |
| 848.4 | 4 | >2419.6 | 108.6 | 0 | >2419.6 | 137.6 | 0 |
| 857.5 | 2 | >2419.6 | 7.5 | 0 | >2419.6 | 26.2 | 0 |
| 889.8 | 1 | 1299.7 | 7.4 | 0 | 1986.3 | 6.3 | 0 |
| 893 | 3 | 248.1 | 0 | 0 | >2419.6 | 74.7 | 0 |
| 906.1 | 1 | 816.4 | 6.3 | 0 | >2419.6 | 4.1 | 0 |
| 919.9 | 5 | 1553.1 | 7.4 | 0 | >2419.6 | 27.5 | 0 |
| 923.9 | 4 | >2419.6 | 9.7 | 0 | >2419.6 | 14.5 | 0 |
| 928.8 | 2 | >2419.6 | 14.8 | 1 | >2419.6 | 103.1 | 0 |
| 960 | 5 | >2419.6 | 23.1 | 0 | >2419.6 | 83.6 | 0 |

***Table 1:*** *River Run 2007 bacteria data by river mile and quadrant. All quadrants are designated by (1) Left Bank, (2) Left Channel, (3) Center/Navigational Channel, (4) Right Channel, (5) Right Bank. Total coliform counts include total coliforms (control), Tetracycline resistant coliforms (TetR), and Ciprofloxacin resistant coliforms (CipR) for surface and bottom samples at each site. All bacteria count units are in MPN (Most Probable Number).*

| Table 2 | | *E-coli* (MPN) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Surface | | | Bottom | | |
| River Mile | Quadrant | Total/Control | TetR | CipR | Total/Control | TetR | CipR |
| 0.2 | 2 | 48.8 | 2 | 0 | 172.6 | 30.9 | 2 |
| 28.4 | 1 | 41.7 | 2 | 0 | 57.8 | 27.9 | 11 |
| 36.1 | 1 | 11 | 9.5 | 0 | 36.8 | 56.9 | 26.4 |
| 45.6 | 2 | 1 | 0 | 0 | 1 | 4.1 | 0 |
| 47.4 | 3 | 4.1 | 2 | 0 | 84.7 | 9.7 | 1 |
| 65.7 | 5 | 14.5 | 1 | 0 | 9.6 | 4.1 | 1 |
| 67.3 | 4 | 51.2 | 1 | 0 | 33.2 | 1 | 0 |
| 85.8 | 3 | 0 | 0 | 0 | 3.1 | 0 | 0 |
| 87.7 | 5 | 7.5 | 1 | 1 | 53.5 | 0 | 8 |
| 89.7 | 5 | 467.4 | 0 | 0 | 1 | 0 | 0 |
| 119.2 | 1 | 6.3 | 0 | 0 | 3.1 | 0 | 0 |
| 171.4 | 3 | 1 | 1 | 0 | 6.3 | 1 | 0 |
| 178.8 | 4 | 18.7 | 2 | 0 | 36.9 | 1 | 0 |
| 186.2 | 1 | 57.3 | 1 | 0 | 116.9 | 4.1 | 0 |
| 191.9 | 4 | 4.1 | 0 | 0 | 8.4 | 0 | 0 |
| 215.9 | 2 | 13.1 | 1 | 0 | 2 | 0 | 0 |
| 220.2 | 2 | 1 | 1 | 0 | 2 | 0 | 0 |
| 224.5 | 4 | 15.6 | 0 | 0 | 22.3 | 0 | 0 |
| 250.7 | 3 | 5.2 | 0 | 0 | 4.1 | 1 | 0 |
| 275.1 | 5 | 3.1 | 1 | 0 | 10.9 | 0 | 0 |
| 302.2 | 5 | 123.9 | 1 | 0 | 16.9 | 3.1 | 0 |
| 302.9 | 4 | 2 | 0 | 0 | 4.1 | 0 | 0 |
| 324.2 | 5 | 344.8 | 102.2 | 3.1 | 60.2 | 33.2 | 2 |
| 345.5 | 1 | 13.2 | 0 | 0 | 21.3 | 4.1 | 0 |
| 355.1 | 3 | 0 | 1 | 0 | 46 | 1 | 2 |
| 369.2 | 1 | 63.1 | 19.7 | 12 | 30.5 | 18.5 | 12.1 |
| 377.3 | 3 | 26.6 | 3.1 | 2 | 17.1 | 3.1 | 3.1 |
| 378.2 | 4 | 42.6 | 7.5 | 3.1 | 19.7 | 13.5 | 4.1 |
| 405.3 | 4 | 4.1 | 0 | 0 | 4.1 | 1 | 0 |
| 412.5 | 5 | 10.7 | 0 | 0 | 20.4 | 4.1 | 1 |
| 413.5 | 4 | 11.9 | 1 | 0 | 5.1 | 0 | 0 |
| 414.8 | 2 | 1 | 0 | 0 | 13.2 | 1 | 0 |
| 433.7 | 3 | 1 | 0 | 0 | 4.1 | 1 | 0 |
| 457.3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 467.8 | 1 | 2 | 1 | 0 | 3 | 1 | 0 |
| 483.7 | 5 | 4.1 | 1 | 0 | 21.1 | 0 | 1 |
| 485.1 | 4 | 15.5 | 0 | 0 | 19.7 | 1 | 0 |
| 492.8 | 1 | 2 | 0 | 0 | 5.1 | 0 | 0 |
| 501.1 | 5 | 6.3 | 0 | 0 | 16 | 0 | 0 |
| 509.1 | 3 | 4.1 | 1 | 0 | 7.2 | 0 | 0 |
| 528.4 | 2 | 1 | 1 | 0 | 2 | 0 | 0 |

| Table 2 (Cont.) | | *E-coli* (MPN) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Surface | | | Bottom | | |
| River Mile | Quadrant | Total/Control | TetR | CipR | Total/Control | TetR | CipR |
| 572.5 | 3 | 20.1 | 3.1 | 0 | 3.1 | 0 | 1 |
| 589.7 | 3 | 0 | 0 | 0 | 5.2 | 0 | 0 |
| 592.5 | 4 | 31.3 | 0 | 0 | 4.1 | 0 | 0 |
| 593.8 | 2 | 1 | 0 | 0 | 2 | 2 | 0 |
| 609.9 | 1 | 1 | 1 | 0 | 3 | 0 | 1 |
| 623.7 | 4 | 2 | 1 | 0 | 17 | 13.7 | 2 |
| 633.7 | 2 | 8.4 | 0 | 0 | 6.2 | 0 | 0 |
| 647.3 | 3 | 2 | 2 | 0 | 3 | 2 | 0 |
| 657 | 5 | 7.5 | 0 | 0 | 7.4 | 3 | 0 |
| 667.9 | 2 | 1 | 0 | 0 | 7.1 | 1 | 0 |
| 668.7 | 1 | 1 | 0 | 0 | 0 | 6.3 | 0 |
| 682.7 | 1 | 3.1 | 0 | 0 | 4.1 | 0 | 0 |
| 701 | 3 | 7.4 | 0 | 0 | 2 | 0 | 0 |
| 702.7 | 3 | 1 | 0 | 0 | 4.1 | 0 | 0 |
| 716.1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| 736.5 | 5 | 2 | 0 | 0 | 5.2 | 1 | 2 |
| 737.4 | 1 | 3 | 0 | 0 | 2 | 0 | 0 |
| 740 | 4 | 0 | 0 | 0 | 19.1 | 4 | 9 |
| 759.7 | 4 | 3.1 | 0 | 0 | 2 | 0 | 0 |
| 793 | 4 | 1 | 272.3 | 0 | 1 | 2 | 0 |
| 796.1 | 3 | 6.3 | 0 | 0 | 8.6 | 0 | 0 |
| 796.9 | 4 | 4.1 | 0 | 0 | 9.7 | 0 | 0 |
| 800.9 | 5 | 4.1 | 5.2 | 0 | 5.2 | 0 | 0 |
| 809.1 | 4 | 1 | 0 | 0 | 1 | 22.6 | 0 |
| 835.2 | 4 | 1 | 0 | 0 | 6.3 | 0 | 0 |
| 848.4 | 4 | 2 | 0 | 0 | 2 | 0 | 0 |
| 857.5 | 2 | 1 | 0 | 0 | 6.3 | 0 | 0 |
| 889.8 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| 893 | 3 | 0 | 0 | 0 | 0 | 0 | 0 |
| 906.1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 919.9 | 5 | 3.1 | 0 | 0 | 0 | 0 | 0 |
| 923.9 | 4 | 3 | 0 | 0 | 3 | 0 | 0 |
| 928.8 | 2 | 0 | 0 | 0 | 3.1 | 1 | 0 |
| 960 | 5 | 5.1 | 0 | 0 | 5.1 | 42.6 | 0 |

***Table 2:*** *River Run 2007 bacteria data by river mile and quadrant. All quadrants are designated by (1) Left Bank, (2) Left Channel, (3) Center/Navigational Channel, (4) Right Channel, (5) Right Bank. Total E.coli counts include total E.coli (control), Tetracycline resistant E.coli (TetR), and Ciprofloxacin resistant E.coli (CipR) for surface and bottom samples at each site. All bacteria count units are in MPN (Most Probable Number).*

**Table 3**: 2007 River Run Data: Top Coliforms and *E. coli* vs Bottom Coliforms and *E. coli*.
Nonparametric: Spearman's ρ

| **Variable** | **by Variable** | **Spearman ρ** | **Prob > lpl** |
|---|---|---|---|
| Top TetR Coliforms | Top Total Coliforms | 0.4525 | **<.0001** |
| Top CipR Coliforms | Top Total Coliforms | 0.1062 | 0.3579 |
| Top CipR Coliforms | Top TetR Coliforms | 0.2992 | **0.0082** |
| Bot Total Coliforms | Top Total Coliforms | 0.4361 | **<.0001** |
| Bot Total Coliforms | Top TetR Coliforms | 0.0477 | 0.6801 |
| Bot Total Coliforms | Top CipR Coliforms | 0.1383 | 0.2302 |
| Bot TetR Coliforms | Top Total Coliforms | 0.0844 | 0.4657 |
| Bot TetR Coliforms | Top TetR Coliforms | 0.2623 | **0.0212** |
| Bot TetR Coliforms | Top CipR Coliforms | 0.2612 | **0.0217** |
| Bot TetR Coliforms | Bot Total Coliforms | 0.2374 | **0.0376** |
| Bot CipR Coliforms | Top Total Coliforms | -0.0963 | 0.4046 |
| Bot CipR Coliforms | Top TetR Coliforms | -0.0125 | 0.9138 |
| Bot CipR Coliforms | Top CipR Coliforms | 0.5766 | **<.0001** |
| Bot CipR Coliforms | Bot Total Coliforms | 0.2110 | 0.0653 |
| Bot CipR Coliforms | Bot TetR Coliforms | 0.4634 | **<.0001** |
| Top Total *E. coli* | Top Total Coliforms | 0.1669 | 0.1468 |
| Top Total *E. coli* | Top TetR Coliforms | 0.2432 | **0.0331** |
| Top Total *E. coli* | Top CipR Coliforms | 0.3241 | **0.004** |
| Top Total *E. coli* | Bot Total Coliforms | -0.0965 | 0.4038 |
| Top Total *E. coli* | Bot TetR Coliforms | 0.0982 | 0.3956 |
| Top Total *E. coli* | Bot CipR Coliforms | 0.2896 | **0.0106** |
| Top TetR *E. coli* | Top Total Coliforms | 0.0870 | 0.4518 |
| Top TetR *E. coli* | Top TetR Coliforms | 0.3456 | **0.0021** |
| Top TetR *E. coli* | Top CipR Coliforms | 0.5717 | **<.0001** |
| Top TetR *E. coli* | Bot Total Coliforms | -0.0291 | 0.8015 |
| Top TetR *E. coli* | Bot TetR Coliforms | 0.1779 | 0.1217 |
| Top TetR *E. coli* | Bot CipR Coliforms | 0.4127 | **0.0002** |
| Top TetR *E. coli* | Top Total *E. coli* | 0.3837 | **0.0006** |
| Top CipR *E. coli* | Top Total Coliforms | 0.1268 | 0.272 |
| Top CipR *E. coli* | Top TetR Coliforms | 0.2970 | **0.0087** |
| Top CipR *E. coli* | Top CipR Coliforms | 0.5513 | **<.0001** |

| | | | |
|---|---|---|---|
| Top CipR *E. coli* | Bot Total Coliforms | 0.1388 | 0.2287 |
| Top CipR *E. coli* | Bot TetR Coliforms | 0.1635 | 0.1555 |
| Top CipR *E. coli* | Bot CipR Coliforms | 0.3790 | **0.0007** |
| Top CipR *E. coli* | Top Total *E. coli* | 0.3499 | **0.0018** |
| Top CipR *E. coli* | Top TetR *E. coli* | 0.4252 | **0.0001** |
| Bot Total *E. coli* | Top Total Coliforms | 0.0482 | 0.6769 |
| Bot Total *E. coli* | Top TetR Coliforms | 0.3039 | **0.0072** |
| Bot Total *E. coli* | Top CipR Coliforms | 0.5686 | **0.0001** |
| Bot Total *E. coli* | Bot Total Coliforms | 0.1156 | 0.3168 |
| Bot Total *E. coli* | Bot TetR Coliforms | 0.3012 | **0.0078** |
| Bot Total *E. coli* | Bot CipR Coliforms | 0.4631 | **<.0001** |
| Bot Total *E. coli* | Top Total *E. coli* | 0.5112 | **<.0001** |
| Bot Total *E. coli* | Top TetR *E. coli* | 0.4286 | **0.0001** |
| Bot Total *E. coli* | Top CipR *E. coli* | 0.3288 | **0.0035** |
| Bot TetR *E. coli* | Top Total Coliforms | 0.0982 | 0.3957 |
| Bot TetR *E. coli* | Top TetR Coliforms | 0.1800 | 0.1173 |
| Bot TetR *E. coli* | Top CipR Coliforms | 0.4512 | **<.0001** |
| Bot TetR *E. coli* | Bot Total Coliforms | 0.0312 | 0.7877 |
| Bot TetR *E. coli* | Bot TetR Coliforms | 0.3097 | **0.0061** |
| Bot TetR *E. coli* | Bot CipR Coliforms | 0.4715 | **<.0001** |
| Bot TetR *E. coli* | Top Total *E. coli* | 0.2697 | **0.0177** |
| Bot TetR *E. coli* | Top TetR *E. coli* | 0.3529 | **0.0016** |
| Bot TetR *E. coli* | Top CipR *E. coli* | 0.2613 | **0.0217** |
| Bot TetR *E. coli* | Bot Total *E. coli* | 0.3974 | **0.0003** |
| Bot CipR *E. coli* | Top Total Coliforms | 0.0615 | 0.595 |
| Bot CipR *E. coli* | Top TetR Coliforms | 0.2177 | 0.0572 |
| Bot CipR *E. coli* | Top CipR Coliforms | 0.6637 | **<.0001** |
| Bot CipR *E. coli* | Bot Total Coliforms | 0.2294 | **0.0448** |
| Bot CipR *E. coli* | Bot TetR Coliforms | 0.4492 | **<.0001** |
| Bot CipR *E. coli* | Bot CipR Coliforms | 0.7544 | **<.0001** |
| Bot CipR *E. coli* | Top Total *E. coli* | 0.2734 | **0.0161** |
| Bot CipR *E. coli* | Top TetR *E. coli* | 0.5546 | **<.0001** |
| Bot CipR *E. coli* | Top CipR *E. coli* | 0.5279 | **<.0001** |
| Bot CipR *E. coli* | Bot Total *E. coli* | 0.5460 | **<.0001** |
| Bot CipR *E. coli* | Bot TetR *E. coli* | 0.4556 | **<.0001** |

## Normality Test (Kolmogorov-Smirnov)

| | | | |
|---|---|---|---|
| Quadrant: | K-S Dist. = 0.182 | P < 0.001 | Failed |
| Top_Total_Coliforms: | K-S Dist. = 0.292 | P < 0.001 | Failed |
| Top_TetR_Coliforms: | K-S Dist. = 0.421 | P < 0.001 | Failed |
| Top_CipR_Coliforms: | K-S Dist. = 0.404 | P < 0.001 | Failed |
| Bot_Total_Coliforms: | K-S Dist. = 0.448 | P < 0.001 | Failed |
| Bot_TetR_Coliforms : | K-S Dist. = 0.331 | P < 0.001 | Failed |
| Bot_CipR_Coliforms : | K-S Dist. = 0.390 | P < 0.001 | Failed |
| Top_Total_E.coli: | K-S Dist. = 0.378 | P < 0.001 | Failed |
| Top_TetR_E.coli: | K-S Dist. = 0.455 | P < 0.001 | Failed |
| Top_CipR_E.coli : | K-S Dist. = 0.510 | P < 0.001 | Failed |
| Bot_Total_E.coli : | K-S Dist. = 0.288 | P < 0.001 | Failed |
| Bot_TetR_E.coli: | K-S Dist. = 0.365 | P < 0.001 | Failed |
| Bot_CipR_E.coli : | K-S Dist. = 0.389 | P < 0.001 | Failed |

*A test that fails indicates that the data varies significantly from the pattern expected if the data was drawn from a population with a normal distribution.*
*A test that passes indicates that the data matches the pattern expected if the data was drawn from a population with a normal distribution.*

# APPENDIX B

## Latitude and Longitude Coordinates for the 2008 Guyandotte and Ohio River Sites

| River and Location | Quadrant Location | Latitude | Longitude |
|---|---|---|---|
| Guyandotte | Right Bank | 38.42815 | -82.39139 |
| Guyandotte | Center Channel | 38.42807 | -82.3916 |
| Guyandotte | Left Bank | 38.42811 | -82.3918 |
| Ohio R. Upstream of Guyandotte | Right Bank | 38.43532 | -82.38362 |
| Ohio R. Upstream of Guyandotte | Right Channel | 38.43478 | -82.38393 |
| Ohio R. Upstream of Guyandotte | Center Channel | 38.43353 | -82.38343 |
| Ohio R. Upstream of Guyandotte | Left Channel | 38.43223 | -82.38397 |
| Ohio R. Upstream of Guyandotte | Left Bank | 38.43138 | -82.38411 |
| Ohio R. Downstream of Guyandotte | Right Bank | 38.44028 | -82.4062 |
| Ohio R. Downstream of Guyandotte | Right Channel | 38.43971 | -82.40681 |
| Ohio R. Downstream of Guyandotte | Center Channel | 38.43829 | -82.40605 |
| Ohio R. Downstream of Guyandotte | Left Channel | 38.43704 | -82.40725 |
| Ohio R. Downstream of Guyandotte | Left Bank | 38.43623 | -82.4066 |

**Table 1**

| DATE AND TIME OF EACH SAMPLE | | | | | | |
|---|---|---|---|---|---|---|
| **Date** | **Time** | **Sample #** | **River** | **Location** | **Quad** | **Depth (ft)** |
| 7/3/2008 | 9:37 AM | 1 | Guyandotte | Guyandotte | LB | 0 |
| 7/3/2008 | 9:38 AM | 2 | Guyandotte | Guyandotte | LB | 5 |
| 7/3/2008 | 9:46 AM | 3 | Guyandotte | Guyandotte | C | 0 |
| 7/3/2008 | 9:50 AM | 4 | Guyandotte | Guyandotte | C | 5 |
| 7/3/2008 | 9:55 AM | 5 | Guyandotte | Guyandotte | RB | 0 |
| 7/3/2008 | 9:57 AM | 6 | Guyandotte | Guyandotte | RB | 5 |
| | | | | | | |
| 7/3/2008 | 10:55 AM | 7 | Ohio | Downstream of Guy | LB | 0 |
| 7/3/2008 | 11:00 AM | 8 | Ohio | Downstream of Guy | LB | 5 |
| 7/3/2008 | 11:00 AM | 9 | Ohio | Downstream of Guy | LB | 10 |
| 7/3/2008 | 11:14 AM | 10 | Ohio | Downstream of Guy | LC | 0 |
| 7/3/2008 | 11:19 AM | 11 | Ohio | Downstream of Guy | LC | 5 |
| 7/3/2008 | 11:19 AM | 12 | Ohio | Downstream of Guy | LC | 10 |
| 7/3/2008 | 11:20 AM | 13 | Ohio | Downstream of Guy | LC | 15 |
| 7/3/2008 | 11:30 AM | 14 | Ohio | Downstream of Guy | C | 0 |
| 7/3/2008 | 11:35 AM | 15 | Ohio | Downstream of Guy | C | 5 |
| 7/3/2008 | 11:36 AM | 16 | Ohio | Downstream of Guy | C | 10 |
| 7/3/2008 | 11:37 AM | 17 | Ohio | Downstream of Guy | C | 15 |
| 7/3/2008 | 11:40 AM | 18 | Ohio | Downstream of Guy | C | 20 |
| 7/3/2008 | 11:50 AM | 19 | Ohio | Downstream of Guy | RC | 0 |
| 7/3/2008 | 11:54 AM | 20 | Ohio | Downstream of Guy | RC | 5 |
| 7/3/2008 | 11:55 AM | 21 | Ohio | Downstream of Guy | RC | 10 |
| 7/3/2008 | 11:56 AM | 22 | Ohio | Downstream of Guy | RC | 15 |
| 7/3/2008 | 12:06 PM | 23 | Ohio | Downstream of Guy | RB | 0 |
| 7/3/2008 | 12:07 PM | 24 | Ohio | Downstream of Guy | RB | 5 |
| | | | | | | |
| 7/3/2008 | 12:19 PM | 25 | Ohio | Upstream of Guy | RB | 0 |
| 7/3/2008 | 12:20 PM | 26 | Ohio | Upstream of Guy | RB | 5 |
| 7/3/2008 | 12:48 PM | 27 | Ohio | Upstream of Guy | RC | 0 |
| 7/3/2008 | 12:50 PM | 28 | Ohio | Upstream of Guy | RC | 5 |
| 7/3/2008 | 12:51 PM | 29 | Ohio | Upstream of Guy | RC | 10 |
| 7/3/2008 | 12:52 PM | 30 | Ohio | Upstream of Guy | RC | 15 |
| 7/3/2008 | 1:00 PM | 31 | Ohio | Upstream of Guy | C | 0 |
| 7/3/2008 | 1:02 PM | 32 | Ohio | Upstream of Guy | C | 5 |
| 7/3/2008 | 1:05 PM | 33 | Ohio | Upstream of Guy | C | 10 |
| 7/3/2008 | 1:06 PM | 34 | Ohio | Upstream of Guy | C | 15 |
| 7/3/2008 | 1:08 PM | 35 | Ohio | Upstream of Guy | C | 20 |
| 7/3/2008 | 1:17 PM | 36 | Ohio | Upstream of Guy | LC | 0 |
| 7/3/2008 | 1:19 PM | 37 | Ohio | Upstream of Guy | LC | 5 |
| 7/3/2008 | 1:20 PM | 38 | Ohio | Upstream of Guy | LC | 10 |
| 7/3/2008 | 1:21 PM | 39 | Ohio | Upstream of Guy | LC | 15 |
| 7/3/2008 | 1:32 PM | 40 | Ohio | Upstream of Guy | LB | 0 |
| 7/3/2008 | 1:33 PM | 41 | Ohio | Upstream of Guy | LB | 5 |
| 7/3/2008 | 1:34 PM | 42 | Ohio | Upstream of Guy | LB | 10 |
| 7/3/2008 | 1:37 PM | 43 | Ohio | Upstream of Guy | LB | 15 |

**Table 2**

| WATER CHEMISTRY DATA SUMMARY | | | | | | | |
|---|---|---|---|---|---|---|---|
| **River** | **Location** | **Quad** | **Depth (ft)** | **Temp_C** | **DO_%** | **pH** | **NTU** |
| Guyandotte | Guyandotte | LB | 0 | 23.65 | 116.5 | 4.75 | 26.8 |
| Guyandotte | Guyandotte | LB | 5 | 23.62 | 107.9 | 5.35 | 29.4 |
| Guyandotte | Guyandotte | C | 0 | 23.62 | 113 | 4.31 | 21.3 |
| Guyandotte | Guyandotte | C | 5 | 23.6 | 106.6 | 4.99 | 32 |
| Guyandotte | Guyandotte | RB | 0 | 23.57 | 107.3 | 4.45 | 28.3 |
| Guyandotte | Guyandotte | RB | 5 | 23.55 | 104.8 | 5.54 | 28.3 |
| | | | | | | | |
| Ohio R. | Downstream of Guy | LB | 0 | 24.72 | 99.8 | 5.78 | 38.2 |
| Ohio R. | Downstream of Guy | LB | 5 | 24.71 | 99.6 | 5.78 | 44.9 |
| Ohio R. | Downstream of Guy | LB | 10 | 24.71 | 99.5 | 5.76 | 46.7 |
| Ohio R. | Downstream of Guy | LC | 0 | 24.84 | 100 | 5.68 | 50.2 |
| Ohio R. | Downstream of Guy | LC | 5 | 24.84 | 100 | 5.68 | 50.2 |
| Ohio R. | Downstream of Guy | LC | 10 | 24.83 | 100 | 5.8 | 52.9 |
| Ohio R. | Downstream of Guy | LC | 15 | 24.83 | 100 | 5.79 | 54.8 |
| Ohio R. | Downstream of Guy | C | 0 | 24.81 | 101.4 | 5.71 | 49.5 |
| Ohio R. | Downstream of Guy | C | 5 | 24.81 | 101.4 | 5.71 | 49.5 |
| Ohio R. | Downstream of Guy | C | 10 | 24.8 | 101.1 | 5.63 | 52.6 |
| Ohio R. | Downstream of Guy | C | 15 | 24.8 | 101 | 5.67 | 52.5 |
| Ohio R. | Downstream of Guy | C | 20 | 24.8 | 100.9 | 5.65 | 53.2 |
| Ohio R. | Downstream of Guy | RC | 0 | 24.82 | 99.4 | 5.68 | 48.3 |
| Ohio R. | Downstream of Guy | RC | 5 | 24.82 | 99.4 | 5.68 | 48.3 |
| Ohio R. | Downstream of Guy | RC | 10 | 24.8 | 99.3 | 5.71 | 50.9 |
| Ohio R. | Downstream of Guy | RC | 15 | 24.8 | 99.2 | 5.68 | 53.4 |
| Ohio R. | Downstream of Guy | RB | 0 | 24.85 | 99.6 | 5.7 | 33.2 |
| Ohio R. | Downstream of Guy | RB | 5 | 24.79 | 99.4 | 5.58 | 39.3 |
| | | | | | | | |
| Ohio R. | Upstream of Guy | RB | 0 | 25 | 101.4 | 5.82 | 32.2 |
| Ohio R. | Upstream of Guy | RB | 5 | 24.95 | 100.5 | 5.67 | 43 |
| Ohio R. | Upstream of Guy | RC | 0 | 24.94 | 100.4 | 5.64 | 45.8 |
| Ohio R. | Upstream of Guy | RC | 5 | 24.94 | 99.9 | 5.63 | 69.2 |
| Ohio R. | Upstream of Guy | RC | 10 | 24.9 | 101.7 | 6.2 | 43.2 |
| Ohio R. | Upstream of Guy | RC | 15 | 24.86 | 101.7 | 5.68 | 48.5 |
| Ohio R. | Upstream of Guy | C | 0 | 24.86 | 101.5 | 5.75 | 49.7 |
| Ohio R. | Upstream of Guy | C | 5 | 24.86 | 101.4 | 5.73 | 50.1 |
| Ohio R. | Upstream of Guy | C | 10 | 24.86 | 104.2 | 5.52 | 46.9 |
| Ohio R. | Upstream of Guy | C | 15 | 24.84 | 101.6 | 5.4 | 53.4 |
| Ohio R. | Upstream of Guy | C | 20 | 24.84 | 101.5 | 8.48 | 53.1 |
| Ohio R. | Upstream of Guy | LC | 0 | 24.84 | 101.5 | 5.45 | 55 |
| Ohio R. | Upstream of Guy | LC | 5 | 24.85 | 101.5 | 5.42 | 52.3 |
| Ohio R. | Upstream of Guy | LC | 10 | 24.83 | 100.2 | 5.59 | 45.2 |
| Ohio R. | Upstream of Guy | LC | 15 | 24.83 | 100.2 | 5.59 | 45.2 |
| Ohio R. | Upstream of Guy | LB | 0 | 24.82 | 99.8 | 5.49 | 50.9 |
| Ohio R. | Upstream of Guy | LB | 5 | 24.81 | 99.8 | 5.34 | 52.2 |
| Ohio R. | Upstream of Guy | LB | 10 | 24.83 | 99.7 | 5.79 | 36.6 |
| Ohio R. | Upstream of Guy | LB | 15 | 23.86 | 100.2 | 5.82 | 40.9 |

**Table 3**

| TOTAL COLIFORMS & TOTAL E. COLI (MPN) | | | | | |
|---|---|---|---|---|---|
| **River** | **Location** | **Quad** | **Depth (ft)** | **Total Coliforms (MPN)** | ***E. coli* (MPN)** |
| Guyandotte | Guyandotte | LB | 0 | >2419.6 | 88.8 |
| Guyandotte | Guyandotte | LB | 5 | >2419.6 | 238.2 |
| Guyandotte | Guyandotte | C | 0 | >2419.6 | 141.4 |
| Guyandotte | Guyandotte | C | 5 | >2419.6 | 225.4 |
| Guyandotte | Guyandotte | RB | 0 | >2419.6 | 260.3 |
| Guyandotte | Guyandotte | RB | 5 | >2419.6 | 238.2 |
| | | | | | |
| Ohio R. | Downstream of Guy | LB | 0 | >2419.6 | 260.3 |
| Ohio R. | Downstream of Guy | LB | 5 | >2419.6 | 222.4 |
| Ohio R. | Downstream of Guy | LB | 10 | >2419.6 | 275.5 |
| Ohio R. | Downstream of Guy | LC | 0 | >2419.6 | 101.0 |
| Ohio R. | Downstream of Guy | LC | 5 | >2419.6 | 72.8 |
| Ohio R. | Downstream of Guy | LC | 10 | >2419.6 | 73.8 |
| Ohio R. | Downstream of Guy | LC | 15 | >2419.6 | 62.0 |
| Ohio R. | Downstream of Guy | C | 0 | >2419.6 | 70.3 |
| Ohio R. | Downstream of Guy | C | 5 | >2419.6 | 90.6 |
| Ohio R. | Downstream of Guy | C | 10 | 2419.6 | 70.8 |
| Ohio R. | Downstream of Guy | C | 15 | >2419.6 | 110.0 |
| Ohio R. | Downstream of Guy | C | 20 | >2419.6 | 52.9 |
| Ohio R. | Downstream of Guy | RC | 0 | >2419.6 | 63.8 |
| Ohio R. | Downstream of Guy | RC | 5 | >2419.6 | 88.2 |
| Ohio R. | Downstream of Guy | RC | 10 | >2419.6 | 91.2 |
| Ohio R. | Downstream of Guy | RC | 15 | >2419.6 | 60.9 |
| Ohio R. | Downstream of Guy | RB | 0 | >2419.6 | 55.4 |
| Ohio R. | Downstream of Guy | RB | 5 | >2419.6 | 98.7 |
| | | | | | |
| Ohio R. | Upstream of Guy | RB | 0 | >2419.6 | 67.0 |
| Ohio R. | Upstream of Guy | RB | 5 | >2419.6 | 47.4 |
| Ohio R. | Upstream of Guy | RC | 0 | >2419.6 | 67.0 |
| Ohio R. | Upstream of Guy | RC | 5 | >2419.6 | 70.3 |
| Ohio R. | Upstream of Guy | RC | 10 | >2419.6 | 65.0 |
| Ohio R. | Upstream of Guy | RC | 15 | >2419.6 | 66.3 |
| Ohio R. | Upstream of Guy | C | 0 | >2419.6 | 79.8 |
| Ohio R. | Upstream of Guy | C | 5 | >2419.6 | 90.6 |
| Ohio R. | Upstream of Guy | C | 10 | >2419.6 | 93.3 |
| Ohio R. | Upstream of Guy | C | 15 | >2419.6 | 65.0 |
| Ohio R. | Upstream of Guy | C | 20 | >2419.6 | 63.1 |
| Ohio R. | Upstream of Guy | LC | 0 | >2419.6 | 58.3 |
| Ohio R. | Upstream of Guy | LC | 5 | >2419.6 | 68.3 |
| Ohio R. | Upstream of Guy | LC | 10 | >2419.6 | 62.7 |
| Ohio R. | Upstream of Guy | LC | 15 | >2419.6 | 79.8 |
| Ohio R. | Upstream of Guy | LB | 0 | 1986.3 | 68.9 |
| Ohio R. | Upstream of Guy | LB | 5 | >2419.6 | 95.9 |
| Ohio R. | Upstream of Guy | LB | 10 | >2419.6 | 52.9 |
| Ohio R. | Upstream of Guy | LB | 15 | >2419.6 | 67.7 |

**Table 4**

| TOTAL CULTIVABLE BACTERIA TOTALS (cfus/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **River** | **Location** | **Quad** | **Depth (ft)** | **AVG TCB** | **x Dilution** | **Total Cult. Bact.** |
| Guyandotte | Guyandotte | LB | 0 | 82.00 | 1000 | 82000.0 |
| Guyandotte | Guyandotte | LB | 5 | 71.33 | 1000 | 71333.3 |
| Guyandotte | Guyandotte | C | 0 | 70.33 | 1000 | 70333.3 |
| Guyandotte | Guyandotte | C | 5 | 69.00 | 1000 | 69000.0 |
| Guyandotte | Guyandotte | RB | 0 | 63.33 | 1000 | 63333.3 |
| Guyandotte | Guyandotte | RB | 5 | 83.33 | 1000 | 83333.3 |
| | | | | | | |
| Ohio R. | Downstream of Guy | LB | 0 | 46.67 | 1000 | 46666.7 |
| Ohio R. | Downstream of Guy | LB | 5 | 51.33 | 1000 | 51333.3 |
| Ohio R. | Downstream of Guy | LB | 10 | 42.67 | 1000 | 42666.7 |
| Ohio R. | Downstream of Guy | LC | 0 | 50.33 | 1000 | 50333.3 |
| Ohio R. | Downstream of Guy | LC | 5 | 48.33 | 1000 | 48333.3 |
| Ohio R. | Downstream of Guy | LC | 10 | 32.33 | 1000 | 32333.3 |
| Ohio R. | Downstream of Guy | LC | 15 | 36.67 | 1000 | 36666.7 |
| Ohio R. | Downstream of Guy | C | 0 | 45.00 | 1000 | 45000.0 |
| Ohio R. | Downstream of Guy | C | 5 | 44.33 | 1000 | 44333.3 |
| Ohio R. | Downstream of Guy | C | 10 | 51.67 | 1000 | 51666.7 |
| Ohio R. | Downstream of Guy | C | 15 | 49.00 | 1000 | 49000.0 |
| Ohio R. | Downstream of Guy | C | 20 | 52.50 | 1000 | 52500.0 |
| Ohio R. | Downstream of Guy | RC | 0 | 39.33 | 1000 | 39333.3 |
| Ohio R. | Downstream of Guy | RC | 5 | 44.67 | 1000 | 44666.7 |
| Ohio R. | Downstream of Guy | RC | 10 | 64.67 | 1000 | 64666.7 |
| Ohio R. | Downstream of Guy | RC | 15 | 69.33 | 1000 | 69333.3 |
| Ohio R. | Downstream of Guy | RB | 0 | 46.50 | 1000 | 46500.0 |
| Ohio R. | Downstream of Guy | RB | 5 | 46.00 | 1000 | 46000.0 |
| | | | | | | |
| Ohio R. | Upstream of Guy | RB | 0 | 53.00 | 1000 | 53000.0 |
| Ohio R. | Upstream of Guy | RB | 5 | 56.00 | 1000 | 56000.0 |
| Ohio R. | Upstream of Guy | RC | 0 | 44.67 | 1000 | 44666.7 |
| Ohio R. | Upstream of Guy | RC | 5 | 77.67 | 1000 | 77666.7 |
| Ohio R. | Upstream of Guy | RC | 10 | 71.00 | 1000 | 71000.0 |
| Ohio R. | Upstream of Guy | RC | 15 | 56.33 | 1000 | 56333.3 |
| Ohio R. | Upstream of Guy | C | 0 | 30.00 | 1000 | 30000.0 |
| Ohio R. | Upstream of Guy | C | 5 | 53.33 | 1000 | 53333.3 |
| Ohio R. | Upstream of Guy | C | 10 | 45.33 | 1000 | 45333.3 |
| Ohio R. | Upstream of Guy | C | 15 | 56.00 | 1000 | 56000.0 |
| Ohio R. | Upstream of Guy | C | 20 | 39.33 | 1000 | 39333.3 |
| Ohio R. | Upstream of Guy | LC | 0 | 43.33 | 1000 | 43333.3 |
| Ohio R. | Upstream of Guy | LC | 5 | 57.00 | 1000 | 57000.0 |
| Ohio R. | Upstream of Guy | LC | 10 | 46.67 | 1000 | 46666.7 |
| Ohio R. | Upstream of Guy | LC | 15 | 49.00 | 1000 | 49000.0 |
| Ohio R. | Upstream of Guy | LB | 0 | 45.00 | 1000 | 45000.0 |
| Ohio R. | Upstream of Guy | LB | 5 | 44.33 | 1000 | 44333.3 |
| Ohio R. | Upstream of Guy | LB | 10 | 57.67 | 1000 | 57666.7 |
| Ohio R. | Upstream of Guy | LB | 15 | 40.67 | 1000 | 40666.7 |

**Table 5**

| TETRACYCLINE RESISTANT TOTALS (cfus/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **River** | **Location** | **Quad** | **Depth (ft)** | **AVG TetR** | **x Dilution** | **Total TetR** |
| Guyandotte | Guyandotte | LB | 0 | 27 | 10 | 273.3 |
| Guyandotte | Guyandotte | LB | 5 | 37 | 10 | 370.0 |
| Guyandotte | Guyandotte | C | 0 | 37 | 10 | 366.7 |
| Guyandotte | Guyandotte | C | 5 | 34 | 10 | 343.3 |
| Guyandotte | Guyandotte | RB | 0 | 21 | 10 | 206.7 |
| Guyandotte | Guyandotte | RB | 5 | 42 | 10 | 415.0 |
| | | | | | | |
| Ohio R. | Downstream of Guy | LB | 0 | 17 | 10 | 170.0 |
| Ohio R. | Downstream of Guy | LB | 5 | 26 | 10 | 256.7 |
| Ohio R. | Downstream of Guy | LB | 10 | 41 | 10 | 413.3 |
| Ohio R. | Downstream of Guy | LC | 0 | 28 | 10 | 283.3 |
| Ohio R. | Downstream of Guy | LC | 5 | 18 | 10 | 176.7 |
| Ohio R. | Downstream of Guy | LC | 10 | 20 | 10 | 203.3 |
| Ohio R. | Downstream of Guy | LC | 15 | 22 | 10 | 220.0 |
| Ohio R. | Downstream of Guy | C | 0 | 24 | 10 | 243.3 |
| Ohio R. | Downstream of Guy | C | 5 | 28 | 10 | 276.7 |
| Ohio R. | Downstream of Guy | C | 10 | 29 | 10 | 293.3 |
| Ohio R. | Downstream of Guy | C | 15 | 33 | 10 | 333.3 |
| Ohio R. | Downstream of Guy | C | 20 | 23 | 10 | 230.0 |
| Ohio R. | Downstream of Guy | RC | 0 | 26 | 10 | 260.0 |
| Ohio R. | Downstream of Guy | RC | 5 | 30 | 10 | 303.3 |
| Ohio R. | Downstream of Guy | RC | 10 | 27 | 10 | 270.0 |
| Ohio R. | Downstream of Guy | RC | 15 | 30 | 10 | 295.0 |
| Ohio R. | Downstream of Guy | RB | 0 | 22 | 10 | 216.7 |
| Ohio R. | Downstream of Guy | RB | 5 | 20 | 10 | 200.0 |
| | | | | | | |
| Ohio R. | Upstream of Guy | RB | 0 | 23 | 10 | 233.3 |
| Ohio R. | Upstream of Guy | RB | 5 | 12 | 10 | 123.3 |
| Ohio R. | Upstream of Guy | RC | 0 | 23 | 10 | 230.0 |
| Ohio R. | Upstream of Guy | RC | 5 | 21 | 10 | 206.7 |
| Ohio R. | Upstream of Guy | RC | 10 | 17 | 10 | 170.0 |
| Ohio R. | Upstream of Guy | RC | 15 | 22 | 10 | 223.3 |
| Ohio R. | Upstream of Guy | C | 0 | 40 | 10 | 403.3 |
| Ohio R. | Upstream of Guy | C | 5 | 28 | 10 | 283.3 |
| Ohio R. | Upstream of Guy | C | 10 | 27 | 10 | 273.3 |
| Ohio R. | Upstream of Guy | C | 15 | 25 | 10 | 246.7 |
| Ohio R. | Upstream of Guy | C | 20 | 25 | 10 | 253.3 |
| Ohio R. | Upstream of Guy | LC | 0 | 25 | 10 | 253.3 |
| Ohio R. | Upstream of Guy | LC | 5 | 20 | 10 | 203.3 |
| Ohio R. | Upstream of Guy | LC | 10 | 16 | 10 | 160.0 |
| Ohio R. | Upstream of Guy | LC | 15 | 25 | 10 | 253.3 |
| Ohio R. | Upstream of Guy | LB | 0 | 17 | 10 | 170.0 |
| Ohio R. | Upstream of Guy | LB | 5 | 20 | 10 | 200.0 |
| Ohio R. | Upstream of Guy | LB | 10 | 16 | 10 | 160.0 |
| Ohio R. | Upstream of Guy | LB | 15 | 27 | 10 | 270.0 |

**Table 6**

| CIPROFLOXACIN RESISTANT TOTALS (cfus/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **River** | **Location** | **Quad** | **Depth (ft)** | **AVG CiproR** | **x Dilution** | **Total CiproR** |
| Guyandotte | Guyandotte | LB | 0 | 116 | 10 | 1155.0 |
| Guyandotte | Guyandotte | LB | 5 | 67 | 10 | 670.0 |
| Guyandotte | Guyandotte | C | 0 | 90 | 10 | 900.0 |
| Guyandotte | Guyandotte | C | 5 | 39 | 10 | 393.3 |
| Guyandotte | Guyandotte | RB | 0 | 120 | 10 | 1196.7 |
| Guyandotte | Guyandotte | RB | 5 | 116 | 10 | 1163.3 |
| | | | | | | |
| Ohio R. | Downstream of Guy | LB | 0 | 101 | 10 | 1005.0 |
| Ohio R. | Downstream of Guy | LB | 5 | 41 | 10 | 410.0 |
| Ohio R. | Downstream of Guy | LB | 10 | 127 | 10 | 1270.0 |
| Ohio R. | Downstream of Guy | LC | 0 | 47 | 10 | 470.0 |
| Ohio R. | Downstream of Guy | LC | 5 | 82 | 10 | 823.3 |
| Ohio R. | Downstream of Guy | LC | 10 | 46 | 10 | 460.0 |
| Ohio R. | Downstream of Guy | LC | 15 | 90 | 10 | 900.0 |
| Ohio R. | Downstream of Guy | C | 0 | 86 | 10 | 860.0 |
| Ohio R. | Downstream of Guy | C | 5 | 85 | 10 | 853.3 |
| Ohio R. | Downstream of Guy | C | 10 | 78 | 10 | 783.3 |
| Ohio R. | Downstream of Guy | C | 15 | 95 | 10 | 950.0 |
| Ohio R. | Downstream of Guy | C | 20 | 91 | 10 | 906.7 |
| Ohio R. | Downstream of Guy | RC | 0 | 84 | 10 | 840.0 |
| Ohio R. | Downstream of Guy | RC | 5 | 65 | 10 | 653.3 |
| Ohio R. | Downstream of Guy | RC | 10 | 102 | 10 | 1016.7 |
| Ohio R. | Downstream of Guy | RC | 15 | 114 | 10 | 1143.3 |
| Ohio R. | Downstream of Guy | RB | 0 | 67 | 10 | 665.0 |
| Ohio R. | Downstream of Guy | RB | 5 | 83 | 10 | 833.3 |
| | | | | | | |
| Ohio R. | Upstream of Guy | RB | 0 | 81 | 10 | 810.0 |
| Ohio R. | Upstream of Guy | RB | 5 | 31 | 10 | 313.3 |
| Ohio R. | Upstream of Guy | RC | 0 | 91 | 10 | 913.3 |
| Ohio R. | Upstream of Guy | RC | 5 | 98 | 10 | 976.7 |
| Ohio R. | Upstream of Guy | RC | 10 | 110 | 10 | 1100.0 |
| Ohio R. | Upstream of Guy | RC | 15 | 73 | 10 | 730.0 |
| Ohio R. | Upstream of Guy | C | 0 | 116 | 10 | 1160.0 |
| Ohio R. | Upstream of Guy | C | 5 | 85 | 10 | 845.0 |
| Ohio R. | Upstream of Guy | C | 10 | 90 | 10 | 900.0 |
| Ohio R. | Upstream of Guy | C | 15 | 62 | 10 | 616.7 |
| Ohio R. | Upstream of Guy | C | 20 | 28 | 10 | 276.7 |
| Ohio R. | Upstream of Guy | LC | 0 | 80 | 10 | 800.0 |
| Ohio R. | Upstream of Guy | LC | 5 | 97 | 10 | 970.0 |
| Ohio R. | Upstream of Guy | LC | 10 | 74 | 10 | 743.3 |
| Ohio R. | Upstream of Guy | LC | 15 | 106 | 10 | 1055.0 |
| Ohio R. | Upstream of Guy | LB | 0 | 44 | 10 | 436.7 |
| Ohio R. | Upstream of Guy | LB | 5 | 81 | 10 | 806.7 |
| Ohio R. | Upstream of Guy | LB | 10 | 64 | 10 | 640.0 |
| Ohio R. | Upstream of Guy | LB | 15 | 130 | 10 | 1300.0 |

**Table 7**

| VIRGINIAMYCIN RESISTANT TOTALS (cfus/ml) | | | | | | |
|---|---|---|---|---|---|---|
| **River** | **Location** | **Quad** | **Depth (ft)** | **AVG VirR** | **x Dilution** | **Total VirR** |
| Guyandotte | Guyandotte | LB | 0 | 55 | 10 | 550.0 |
| Guyandotte | Guyandotte | LB | 5 | 58 | 10 | 576.7 |
| Guyandotte | Guyandotte | C | 0 | 59 | 10 | 586.7 |
| Guyandotte | Guyandotte | C | 5 | 65 | 10 | 653.3 |
| Guyandotte | Guyandotte | RB | 0 | 39 | 10 | 390.0 |
| Guyandotte | Guyandotte | RB | 5 | 53 | 10 | 530.0 |
| | | | | | | |
| Ohio R. | Downstream of Guy | LB | 0 | 44 | 10 | 440.0 |
| Ohio R. | Downstream of Guy | LB | 5 | 53 | 10 | 533.3 |
| Ohio R. | Downstream of Guy | LB | 10 | 95 | 10 | 953.3 |
| Ohio R. | Downstream of Guy | LC | 0 | 54 | 10 | 536.7 |
| Ohio R. | Downstream of Guy | LC | 5 | 52 | 10 | 520.0 |
| Ohio R. | Downstream of Guy | LC | 10 | 54 | 10 | 543.3 |
| Ohio R. | Downstream of Guy | LC | 15 | 61 | 10 | 606.7 |
| Ohio R. | Downstream of Guy | C | 0 | 40 | 10 | 400.0 |
| Ohio R. | Downstream of Guy | C | 5 | 56 | 10 | 560.0 |
| Ohio R. | Downstream of Guy | C | 10 | 70 | 10 | 700.0 |
| Ohio R. | Downstream of Guy | C | 15 | 55 | 10 | 553.3 |
| Ohio R. | Downstream of Guy | C | 20 | 45 | 10 | 446.7 |
| Ohio R. | Downstream of Guy | RC | 0 | 48 | 10 | 480.0 |
| Ohio R. | Downstream of Guy | RC | 5 | 45 | 10 | 453.3 |
| Ohio R. | Downstream of Guy | RC | 10 | 73 | 10 | 726.7 |
| Ohio R. | Downstream of Guy | RC | 15 | 56 | 10 | 555.0 |
| Ohio R. | Downstream of Guy | RB | 0 | 32 | 10 | 323.3 |
| Ohio R. | Downstream of Guy | RB | 5 | 37 | 10 | 370.0 |
| | | | | | | |
| Ohio R. | Upstream of Guy | RB | 0 | 40 | 10 | 396.7 |
| Ohio R. | Upstream of Guy | RB | 5 | 38 | 10 | 383.3 |
| Ohio R. | Upstream of Guy | RC | 0 | 46 | 10 | 456.7 |
| Ohio R. | Upstream of Guy | RC | 5 | 52 | 10 | 516.7 |
| Ohio R. | Upstream of Guy | RC | 10 | 51 | 10 | 506.7 |
| Ohio R. | Upstream of Guy | RC | 15 | 50 | 10 | 496.7 |
| Ohio R. | Upstream of Guy | C | 0 | 51 | 10 | 513.3 |
| Ohio R. | Upstream of Guy | C | 5 | 50 | 10 | 500.0 |
| Ohio R. | Upstream of Guy | C | 10 | 57 | 10 | 570.0 |
| Ohio R. | Upstream of Guy | C | 15 | 52 | 10 | 516.7 |
| Ohio R. | Upstream of Guy | C | 20 | 73 | 10 | 726.7 |
| Ohio R. | Upstream of Guy | LC | 0 | 46 | 10 | 463.3 |
| Ohio R. | Upstream of Guy | LC | 5 | 60 | 10 | 600.0 |
| Ohio R. | Upstream of Guy | LC | 10 | 48 | 10 | 480.0 |
| Ohio R. | Upstream of Guy | LC | 15 | 53 | 10 | 530.0 |
| Ohio R. | Upstream of Guy | LB | 0 | 58 | 10 | 580.0 |
| Ohio R. | Upstream of Guy | LB | 5 | 40 | 10 | 403.3 |
| Ohio R. | Upstream of Guy | LB | 10 | 41 | 10 | 406.7 |
| Ohio R. | Upstream of Guy | LB | 15 | 51 | 10 | 506.7 |

**Table 8**

| AMOUNT OF SEDIMENT PER SAMPLE (mg/ml) | | | | | |
|---|---|---|---|---|---|
| **River** | **Location** | **Quad** | **Depth (ft)** | **Meas. (g/25 ml)** | **Final (mg/ml)** |
| Guyandotte | Guyandotte | LB | 0 | 0.0013 | 0.052 |
| Guyandotte | Guyandotte | LB | 5 | 0.0017 | 0.068 |
| Guyandotte | Guyandotte | C | 0 | 0.0013 | 0.052 |
| Guyandotte | Guyandotte | C | 5 | 0.0017 | 0.068 |
| Guyandotte | Guyandotte | RB | 0 | 0.0007 | 0.028 |
| Guyandotte | Guyandotte | RB | 5 | 0.001 | 0.04 |
| | | | | | |
| Ohio R. | Downstream of Guy | LB | 0 | 0.0014 | 0.056 |
| Ohio R. | Downstream of Guy | LB | 5 | 0.0022 | 0.088 |
| Ohio R. | Downstream of Guy | LB | 10 | 0.002 | 0.08 |
| Ohio R. | Downstream of Guy | LC | 0 | 0.002 | 0.08 |
| Ohio R. | Downstream of Guy | LC | 5 | 0.0025 | 0.1 |
| Ohio R. | Downstream of Guy | LC | 10 | 0.0026 | 0.104 |
| Ohio R. | Downstream of Guy | LC | 15 | 0.002 | 0.08 |
| Ohio R. | Downstream of Guy | C | 0 | 0.0016 | 0.064 |
| Ohio R. | Downstream of Guy | C | 5 | 0.0021 | 0.084 |
| Ohio R. | Downstream of Guy | C | 10 | 0.0013 | 0.052 |
| Ohio R. | Downstream of Guy | C | 15 | 0.0019 | 0.076 |
| Ohio R. | Downstream of Guy | C | 20 | 0.0017 | 0.068 |
| Ohio R. | Downstream of Guy | RC | 0 | 0.0011 | 0.044 |
| Ohio R. | Downstream of Guy | RC | 5 | 0.0025 | 0.1 |
| Ohio R. | Downstream of Guy | RC | 10 | 0.002 | 0.08 |
| Ohio R. | Downstream of Guy | RC | 15 | 0.0024 | 0.096 |
| Ohio R. | Downstream of Guy | RB | 0 | 0.0015 | 0.06 |
| Ohio R. | Downstream of Guy | RB | 5 | 0.0017 | 0.068 |
| | | | | | |
| Ohio R. | Upstream of Guy | RB | 0 | 0.0012 | 0.048 |
| Ohio R. | Upstream of Guy | RB | 5 | 0.0024 | 0.096 |
| Ohio R. | Upstream of Guy | RC | 0 | 0.0023 | 0.092 |
| Ohio R. | Upstream of Guy | RC | 5 | 0.0023 | 0.092 |
| Ohio R. | Upstream of Guy | RC | 10 | 0.0015 | 0.06 |
| Ohio R. | Upstream of Guy | RC | 15 | 0.001 | 0.04 |
| Ohio R. | Upstream of Guy | C | 0 | 0.0026 | 0.104 |
| Ohio R. | Upstream of Guy | C | 5 | 0.002 | 0.08 |
| Ohio R. | Upstream of Guy | C | 10 | 0.0018 | 0.072 |
| Ohio R. | Upstream of Guy | C | 15 | 0.0022 | 0.088 |
| Ohio R. | Upstream of Guy | C | 20 | 0.0023 | 0.092 |
| Ohio R. | Upstream of Guy | LC | 0 | 0.0014 | 0.056 |
| Ohio R. | Upstream of Guy | LC | 5 | 0.0019 | 0.076 |
| Ohio R. | Upstream of Guy | LC | 10 | 0.0007 | 0.028 |
| Ohio R. | Upstream of Guy | LC | 15 | 0.0021 | 0.084 |
| Ohio R. | Upstream of Guy | LB | 0 | 0.0021 | 0.084 |
| Ohio R. | Upstream of Guy | LB | 5 | 0.0022 | 0.088 |
| Ohio R. | Upstream of Guy | LB | 10 | 0.0018 | 0.072 |
| Ohio R. | Upstream of Guy | LB | 15 | 0.0017 | 0.068 |

# Ohio River Upstream of the Guyandotte River:

# Pearson's Correlation Statistical Summary Table

**Table 9**

| Variables | Pearson's r value | P value of significance |
|---|---|---|
| Quad:Quad | r = 1.0000 | p = --- |
| Depth:Quad | r = -0.1302 | p = 0.5953 |
| Temp (C):Quad | r = -0.5695 | p = 0.0109* |
| DO (%):Quad | r = -0.2709 | p = 0.2620 |
| pH:Quad | r = -0.0304 | p = 0.9017 |
| NTU:Quad | r = -0.1676 | p = 0.4928 |
| E.coli:Quad | r = -0.2062 | p = 0.3970 |
| Control:Quad | r = 0.3813 | p = 0.1072 |
| CipR:Quad | r = -0.1131 | p = 0.6447 |
| TetR:Quad | r = -0.0122 | p = 0.9604 |
| VirR:Quad | r = -0.1647 | p = 0.5004 |
| Sediment:Quad | r = -0.1595 | p = 0.5142 |
| Depth:Depth | r = 1.0000 | p = --- |
| Temp (C):Depth | r = 0.0595 | p = 0.8087 |
| DO (%):Depth | r = -0.1678 | p = 0.4923 |
| pH:Depth | r = -0.0581 | p = 0.8131 |
| NTU:Depth | r = 0.6989 | p = 0.0009* |
| E.coli:Depth | r = -0.0680 | p = 0.7822 |
| Control:Depth | r = 0.0719 | p = 0.7699 |
| CipR:Depth | r = -0.0980 | p = 0.6899 |
| TetR:Depth | r = -0.0370 | p = 0.8806 |
| VirR:Depth | r = 0.4332 | p = 0.0639 |
| Sediment:Depth | r = 0.4411 | p = 0.0587 |
| Temp (C):Temp (C) | r = 1.0000 | p = --- |
| DO (%):Temp (C) | r = 0.1828 | p = 0.4538 |
| pH:Temp (C) | r = -0.0117 | p = 0.9621 |
| NTU:Temp (C) | r = 0.1677 | p = 0.4925 |
| E.coli:Temp (C) | r = 0.4117 | p = 0.0799 |
| Control:Temp (C) | r = -0.1865 | p = 0.4445 |
| CipR:Temp (C) | r = 0.3967 | p = 0.0927 |
| TetR:Temp (C) | r = 0.3508 | p = 0.1409 |
| VirR:Temp (C) | r = 0.3231 | p = 0.1772 |
| Sediment:Temp (C) | r = 0.0803 | p = 0.7437 |
| DO (%):DO (%) | r = 1.0000 | p = --- |
| pH:DO (%) | r = 0.1197 | p = 0.6254 |
| NTU:DO (%) | r = -0.0179 | p = 0.9421 |
| E.coli:DO (%) | r = 0.2964 | p = 0.2179 |
| Control:DO (%) | r = -0.5611 | p = 0.0124* |
| CipR:DO (%) | r = 0.0635 | p = 0.7961 |
| TetR:DO (%) | r = 0.6364 | p = 0.0034* |
| VirR:DO (%) | r = 0.3886 | p = 0.1001 |
| Sediment:DO (%) | r = -0.1445 | p = 0.5551 |
| pH:pH | r = 1.0000 | p = --- |

| | | |
|---|---|---|
| NTU:pH | r = 0.0097 | p = 0.9685 |
| E.coli:pH | r = 0.3447 | p = 0.1484 |
| Control:pH | r = -0.1657 | p = 0.4979 |
| CipR:pH | r = 0.0540 | p = 0.8262 |
| TetR:pH | r = 0.1017 | p = 0.6788 |
| VirR:pH | r = 0.0963 | p = 0.6949 |
| Sediment:pH | r = 0.1362 | p = 0.5781 |
| NTU:NTU | r = 1.0000 | p = --- |
| E.coli:NTU | r = 0.1476 | p = 0.5465 |
| Control:NTU | r = -0.0772 | p = 0.7533 |
| CipR:NTU | r = 0.4463 | p = 0.0554 |
| TetR:NTU | r = 0.3473 | p = 0.1452 |
| VirR:NTU | r = 0.2895 | p = 0.2293 |
| Sediment:NTU | r = 0.3690 | p = 0.1200 |
| E.coli:E.coli | r = 1.0000 | p = --- |
| Control:E.coli | r = -0.2394 | p = 0.3235 |
| CipR:E.coli | r = 0.4073 | p = 0.0835 |
| TetR:E.coli | r = 0.5036 | p = 0.0279* |
| VirR:E.coli | r = 0.1518 | p = 0.5350 |
| Sediment:E.coli | r = 0.2667 | p = 0.2697 |
| Control:Control | r = 1.0000 | p = --- |
| CipR:Control | r = 0.0196 | p = 0.9365 |
| TetR:Control | r = -0.5489 | p = 0.0149* |
| VirR:Control | r = -0.1726 | p = 0.4799 |
| Sediment:Control | r = 0.0899 | p = 0.7144 |
| CipR:CipR | r = 1.0000 | p = --- |
| TetR:CipR | r = 0.4730 | p = 0.0408* |
| VirR:CipR | r = -0.1003 | p = 0.6829 |
| Sediment:CipR | r = 0.1510 | p = 0.5372 |
| TetR:TetR | r = 1.0000 | p = --- |
| VirR:TetR | r = 0.2846 | p = 0.2376 |
| Sediment:TetR | r = -0.0153 | p = 0.9504 |
| VirR:VirR | r = 1.0000 | p = --- |
| Sediment:VirR | r = -0.1546 | p = 0.5274 |
| Sediment:Sediment | r = 1.0000 | p = --- |
| | | *indicates significance of p <0.05 |

# Guyandotte River:

# Pearson's Correlation Statistical Summary Table

**Table 10**

| Variables | Pearson's r value | p value of significance |
|---|---|---|
| Quad:Quad | r = 1.0000 | p = --- |
| Depth:Quad | r = 0.0000 | p = --- |
| Temp (C):Quad | r = -0.9174 | p = 0.0099* |
| DO %:Quad | r = -0.6181 | p = 0.1910 |
| pH:Quad | r = -0.0503 | p = 0.9245 |
| NTU:Quad | r = 0.0250 | p = 0.9625 |
| E.coli:Quad | r = 0.5656 | p = 0.2421 |
| Control:Quad | r = -0.1903 | p = 0.7180 |
| CipR:Quad | r = 0.3671 | p = 0.4741 |
| TetR:Quad | r = -0.0639 | p = 0.9043 |
| VirR:Quad | r = -0.5252 | p = 0.2846 |
| Sediment:Quad | r = -0.7415 | p = 0.0916 |
| Depth:Depth | r = 1.0000 | p = --- |
| Temp (C):Depth | r = -0.3496 | p = 0.4970 |
| DO %:Depth | r = -0.7180 | p = 0.1081 |
| pH:Depth | r = 0.8857 | p = 0.0188* |
| NTU:Depth | r = 0.6794 | p = 0.1377 |
| E.coli:Depth | r = 0.5689 | p = 0.2387 |
| Control:Depth | r = 0.1865 | p = 0.7236 |
| CipR:Depth | r = -0.5743 | p = 0.2333 |
| TetR:Depth | r = 0.6780 | p = 0.1388 |
| VirR:Depth | r = 0.4842 | p = 0.3305 |
| Sediment:Depth | r = 0.5123 | p = 0.2988 |
| Temp (C):Temp (C) | r = 1.0000 | p = --- |
| DO %:Temp (C) | r = 0.8488 | p = 0.0326* |
| pH:Temp (C) | r = -0.3223 | p = 0.5333 |
| NTU:Temp (C) | r = -0.2860 | p = 0.5827 |
| E.coli:Temp (C) | r = -0.7805 | p = 0.0670 |
| Control:Temp (C) | r = 0.0939 | p = 0.8596 |
| CipR:Temp (C) | r = -0.2358 | p = 0.6528 |
| TetR:Temp (C) | r = -0.1328 | p = 0.8019 |
| VirR:Temp (C) | r = 0.4408 | p = 0.3817 |
| Sediment:Temp (C) | r = 0.5745 | p = 0.2331 |
| DO %:DO % | r = 1.0000 | p = --- |
| pH:DO % | r = -0.5626 | p = 0.2451 |
| NTU:DO % | r = -0.6042 | p = 0.2040 |
| E.coli:DO % | r = -0.9489 | p = 0.0039* |
| Control:DO % | r = 0.2343 | p = 0.6550 |
| CipR:DO % | r = 0.2405 | p = 0.6462 |
| TetR:DO % | r = -0.2962 | p = 0.5687 |
| VirR:DO % | r = 0.1175 | p = 0.8246 |
| Sediment:DO % | r = 0.1049 | p = 0.8432 |
| pH:pH | r = 1.0000 | p = --- |
| NTU:pH | r = 0.5930 | p = 0.2148 |

| | | |
|---|---|---|
| E.coli:pH | r = 0.4107 | p = 0.4185 |
| Control:pH | r = 0.5267 | p = 0.2830 |
| CipR:pH | r = -0.1912 | p = 0.7167 |
| TetR:pH | r = 0.6259 | p = 0.1838 |
| VirR:pH | r = 0.2738 | p = 0.5995 |
| Sediment:pH | r = 0.3069 | p = 0.5541 |
| NTU:NTU | r = 1.0000 | p = --- |
| E.coli:NTU | r = 0.5845 | p = 0.2231 |
| Control:NTU | r = -0.0760 | p = 0.8862 |
| CipR:NTU | r = -0.4228 | p = 0.4036 |
| TetR:NTU | r = -0.0601 | p = 0.9100 |
| VirR:NTU | r = 0.0934 | p = 0.8604 |
| Sediment:NTU | r = 0.2653 | p = 0.6114 |
| E.coli:E.coli | r = 1.0000 | p = --- |
| Control:E.coli | r = -0.4504 | p = 0.3701 |
| CipR:E.coli | r = -0.1988 | p = 0.7057 |
| TetR:E.coli | r = 0.0709 | p = 0.8938 |
| VirR:E.coli | r = -0.2995 | p = 0.5642 |
| Sediment:E.coli | r = -0.1680 | p = 0.7504 |
| Control:Control | r = 1.0000 | p = --- |
| CipR:Control | r = 0.3558 | p = 0.4889 |
| TetR:Control | r = 0.4524 | p = 0.3677 |
| VirR:Control | r = 0.2285 | p = 0.6632 |
| Sediment:Control | r = 0.0297 | p = 0.9555 |
| CipR:CipR | r = 1.0000 | p = --- |
| TetR:CipR | r = -0.3604 | p = 0.4827 |
| VirR:CipR | r = -0.7746 | p = 0.0705 |
| Sediment:CipR | r = -0.8617 | p = 0.0274* |
| TetR:TetR | r = 1.0000 | p = --- |
| VirR:TetR | r = 0.6537 | p = 0.1591 |
| Sediment:TetR | r = 0.4692 | p = 0.3479 |
| VirR:VirR | r = 1.0000 | p = --- |
| Sediment:VirR | r = 0.8917 | p = 0.0169* |
| Sediment:Sediment | r = 1.0000 | p = --- |
| | | * indicates significance of $p < 0.05$ |

# Ohio River Downstream of the Guyandotte River:

# Pearson's Correlation Statistical Summary Table

**Table 11**

| Variables | Pearon's r value | p value of significance |
|---|---|---|
| Quad:Quad | r = 1.0000 | p = --- |
| Depth:Quad | r = -0.0410 | p = 0.87170 |
| Temp (C):Quad | r = 0.5349 | p = 0.02220* |
| DO (%):Quad | r = -0.1455 | p = 0.56460 |
| pH:Quad | r = -0.6800 | p = 0.00190* |
| NTU:Quad | r = -0.1973 | p = 0.43260 |
| E.coli:Quad | r = -0.6599 | p = 0.00290* |
| Control:Quad | r = 0.2613 | p = 0.29500 |
| CipR:Quad | r = 0.0556 | p = 0.82660 |
| TetR:Quad | r = -0.0660 | p = 0.79470 |
| VirR:Quad | r = -0.4352 | p = 0.07110 |
| Sediment:Quad | r = -0.2190 | p = 0.38250 |
| Depth:Depth | r = 1.0000 | p = --- |
| Temp (C):Depth | r = -0.0343 | p = 0.89260 |
| DO (%):Depth | r = 0.1470 | p = 0.56040 |
| pH:Depth | r = -0.0408 | p = 0.87220 |
| NTU:Depth | r = 0.6289 | p = 0.00520* |
| E.coli:Depth | r = -0.1816 | p = 0.47080 |
| Control:Depth | r = 0.2596 | p = 0.29820 |
| CipR:Depth | r = 0.3716 | p = 0.12890 |
| TetR:Depth | r = 0.2601 | p = 0.29730 |
| VirR:Depth | r = 0.3882 | p = 0.11140 |
| Sediment:Depth | r = 0.2897 | p = 0.24370 |
| Temp (C):Temp (C) | r = 1.0000 | p = --- |
| DO (%):Temp (C) | r = 0.1602 | p = 0.52550 |
| pH:Temp (C) | r = -0.3310 | p = 0.17970 |
| NTU:Temp (C) | r = 0.2564 | p = 0.30440 |
| E.coli:Temp (C) | r = -0.9066 | p = 0.00000* |
| Control:Temp (C) | r = -0.1226 | p = 0.62800 |
| CipR:Temp (C) | r = -0.3046 | p = 0.21900 |
| TetR:Temp (C) | r = -0.2842 | p = 0.25310 |
| VirR:Temp (C) | r = -0.3832 | p = 0.11650 |
| Sediment:Temp (C) | r = 0.1054 | p = 0.67710 |
| DO (%):DO (%) | r = 1.0000 | p = --- |
| pH:DO (%) | r = -0.1331 | p = 0.59860 |
| NTU:DO (%) | r = 0.3493 | p = 0.15540 |
| E.coli:DO (%) | r = -0.2342 | p = 0.34960 |
| Control:DO (%) | r = -0.1622 | p = 0.52030 |
| CipR:DO (%) | r = -0.0295 | p = 0.90740 |
| TetR:DO (%) | r = 0.0260 | p = 0.91830 |
| VirR:DO (%) | r = -0.0595 | p = 0.81440 |
| Sediment:DO (%) | r = -0.2038 | p = 0.41730 |
| pH:pH | r = 1.0000 | p = --- |
| NTU:pH | r = 0.0257 | p = 0.91940 |
| E.coli:pH | r = 0.4668 | p = 0.05080 |

| | | |
|---|---|---|
| Control:pH | r = -0.3584 | p = 0.14420 |
| CipR:pH | r = -0.0929 | p = 0.71390 |
| TetR:pH | r = -0.0584 | p = 0.81790 |
| VirR:pH | r = 0.2724 | p = 0.27410 |
| Sediment:pH | r = 0.2842 | p = 0.25300 |
| NTU:NTU | r = 1.0000 | p = --- |
| E.coli:NTU | r = -0.3754 | p = 0.12470 |
| Control:NTU | r = 0.1101 | p = 0.66350 |
| CipR:NTU | r = 0.1003 | p = 0.69230 |
| TetR:NTU | r = 0.2912 | p = 0.24100 |
| VirR:NTU | r = 0.4291 | p = 0.07560 |
| Sediment:NTU | r = 0.3687 | p = 0.13220 |
| E.coli:E.coli | r = 1.0000 | p = --- |
| Control:E.coli | r = -0.0610 | p = 0.81000 |
| CipR:E.coli | r = 0.2008 | p = 0.42430 |
| TetR:E.coli | r = 0.2708 | p = 0.27700 |
| VirR:E.coli | r = 0.3952 | p = 0.10460 |
| Sediment:E.coli | r = -0.0247 | p = 0.92240 |
| Control:Control | r = 1.0000 | p = --- |
| CipR:Control | r = 0.3184 | p = 0.19790 |
| TetR:Control | r = 0.1867 | p = 0.45830 |
| VirR:Control | r = 0.1157 | p = 0.64760 |
| Sediment:Control | r = 0.0750 | p = 0.76750 |
| CipR:CipR | r = 1.0000 | p = --- |
| TetR:CipR | r = 0.3463 | p = 0.15920 |
| VirR:CipR | r = 0.4314 | p = 0.07390 |
| Sediment:CipR | r = -0.1927 | p = 0.44370 |
| TetR:TetR | r = 1.0000 | p = --- |
| VirR:TetR | r = 0.6830 | p = 0.00180* |
| Sediment:TetR | r = 0.0835 | p = 0.74190 |
| VirR:VirR | r = 1.0000 | p = --- |
| Sediment:VirR | r = 0.1836 | p = 0.46590 |
| Sediment:Sediment | r = 1.0000 | p = --- |
| | | *indicates significance of $p < 0.05$ |

**Table 12:** ANOVA T-test comparing *E. coli* samples taken in the Ohio River upstream and downstream of the Guyandotte River by river quadrant

| | T-test for Independent Samples (Spreadsheet1 Note: Variables were treated as independent s | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group 1 vs. Group 2 | Mean Group 1 | Mean Group 2 | t-value | df | p | Std.Dev. Group 1 | Std.Dev. Group 2 | F-ratio Variances | p Variances |
| Left Bank E.coli Below the Guyandotte vs. Center E.coli Above the Guyandotte | 252.7333 | 78.36000 | 12.24293 | 6 | 0.000018 | 27.34672 | 14.02188 | 3.8036 | 0.237515 |
| Left Bank E.coli Below the Guyandotte vs. RDB E.coli Below the Guyandotte | 252.7333 | 76.02500 | 10.90083 | 5 | 0.000113 | 27.34672 | 15.88214 | 2.9648 | 0.389464 |
| Left Bank E.coli Below the Guyandotte vs. RDB E.coli Above the Guyandotte | 252.7333 | 67.15000 | 13.97773 | 5 | 0.000034 | 27.34672 | 2.25758 | 146.7319 | 0.002036 |
| Left Bank E.coli Below the Guyandotte vs. Right Bank Below the Guyandotte | 252.7333 | 77.05000 | 6.75770 | 3 | 0.006620 | 27.34672 | 30.61772 | 1.2535 | 0.758576 |
| Left Bank E.coli Below the Guyandotte vs. Right Bank Above the Guyandotte | 252.7333 | 57.20000 | 9.03058 | 3 | 0.002867 | 27.34672 | 13.85929 | 3.8934 | 0.674706 |
| Left Bank E.coli Above the Guyandotte vs. Center E.coli Above the Guyandotte | 71.3500 | 78.36000 | -0.66112 | 7 | 0.529686 | 17.91117 | 14.02188 | 1.6317 | 0.632786 |
| Left Bank E.coli Above the Guyandotte vs. RDB E.coli Below the Guyandotte | 71.3500 | 76.02500 | -0.39058 | 6 | 0.709596 | 17.91117 | 15.88214 | 1.2718 | 0.848017 |
| Left Bank E.coli Above the Guyandotte vs. RDB E.coli Above the Guyandotte | 71.3500 | 67.15000 | 0.46530 | 6 | 0.658126 | 17.91117 | 2.25758 | 62.9451 | 0.006609 |
| Left Bank E.coli Above the Guyandotte vs. Right Bank Below the Guyandotte | 71.3500 | 77.05000 | -0.30200 | 4 | 0.777701 | 17.91117 | 30.61772 | 2.9221 | 0.371808 |
| Left Bank E.coli Above the Guyandotte vs. Right Bank Above the Guyandotte | 71.3500 | 57.20000 | 0.96174 | 4 | 0.390643 | 17.91117 | 13.85929 | 1.6702 | 1.000000 |
| LDB E.coli Below the Guyandotte vs. Center E.coli Above the Guyandotte | 77.4000 | 78.36000 | -0.09423 | 7 | 0.927570 | 16.61566 | 14.02188 | 1.4042 | 0.728478 |
| LDB E.coli Below the Guyandotte vs. RDB E.coli Below the Guyandotte | 77.4000 | 76.02500 | 0.11964 | 6 | 0.908672 | 16.61566 | 15.88214 | 1.0945 | 0.942572 |
| LDB E.coli Below the Guyandotte vs. RDB E.coli Above the Guyandotte | 77.4000 | 67.15000 | 1.22254 | 6 | 0.267342 | 16.61566 | 2.25758 | 54.1687 | 0.008241 |
| LDB E.coli Below the Guyandotte vs. Right Bank Below the Guyandotte | 77.4000 | 77.05000 | 0.01924 | 4 | 0.985574 | 16.61566 | 30.61772 | 3.3956 | 0.325200 |
| LDB E.coli Below the Guyandotte vs. Right Bank Above the Guyandotte | 77.4000 | 57.20000 | 1.46044 | 4 | 0.217957 | 16.61566 | 13.85929 | 1.4373 | 1.000000 |
| LDB E.coli Above the Guyandotte vs. Center E.coli Above the Guyandotte | 67.2750 | 78.36000 | -1.35189 | 7 | 0.218461 | 9.29888 | 14.02188 | 2.2738 | 0.525721 |
| LDB E.coli Above the Guyandotte vs. RDB E.coli Below the Guyandotte | 67.2750 | 76.02500 | -0.95087 | 6 | 0.378383 | 9.29888 | 15.88214 | 2.9171 | 0.402706 |
| LDB E.coli Above the Guyandotte vs. RDB E.coli Above the Guyandotte | 67.2750 | 67.15000 | 0.02613 | 6 | 0.980004 | 9.29888 | 2.25758 | 16.9658 | 0.043835 |
| LDB E.coli Above the Guyandotte vs. Right Bank Below the Guyandotte | 67.2750 | 77.05000 | -0.65252 | 4 | 0.549664 | 9.29888 | 30.61772 | 10.8414 | 0.091974 |
| LDB E.coli Above the Guyandotte vs. Right Bank Above the Guyandotte | 67.2750 | 57.20000 | 1.09502 | 4 | 0.335018 | 9.29888 | 13.85929 | 2.2214 | 0.465789 |
| Center E.coli Below the Guyandotte vs. Center E.coli Above the Guyandotte | 78.9200 | 78.36000 | 0.04814 | 8 | 0.962783 | 21.90701 | 14.02188 | 2.4409 | 0.408576 |
| Center E.coli Below the Guyandotte vs. RDB E.coli Below the Guyandotte | 78.9200 | 76.02500 | 0.22071 | 7 | 0.831620 | 21.90701 | 15.88214 | 1.9026 | 0.624191 |
| Center E.coli Below the Guyandotte vs. RDB E.coli Above the Guyandotte | 78.9200 | 67.15000 | 1.05532 | 7 | 0.326336 | 21.90701 | 2.25758 | 94.1629 | 0.003496 |
| Center E.coli Below the Guyandotte vs. Right Bank Below the Guyandotte | 78.9200 | 77.05000 | 0.09350 | 5 | 0.929137 | 21.90701 | 30.61772 | 1.9533 | 0.469519 |
| Center E.coli Below the Guyandotte vs. Right Bank Above the Guyandotte | 78.9200 | 57.20000 | 1.26321 | 5 | 0.262215 | 21.90701 | 13.85929 | 2.4985 | 0.877344 |

**Table 12 (cont)**

| Group 1 vs. Group 2 | T-test for Independent Samples (Spreadsheet<br>Note: Variables were treated as independent s<br>Mean Group 1 | Mean Group 2 | t-value | df | p | Std.Dev. Group 1 | Std.Dev. Group 2 | F-ratio Variances | p Variances |
|---|---|---|---|---|---|---|---|---|---|
| Left Bank E.coli Below the Guyandotte vs. Left Bank E.coli Below the Guyandotte | 252.7333 | 252.7333 | 0.0000 | 4 | 1.000000 | 27.34672 | 27.34672 | 1.000000 | 1.000000 |
| Left Bank E.coli Below the Guyandotte vs. Left Bank E.coli Above the Guyandotte | 252.7333 | 71.3500 | 10.7108 | 5 | 0.000123 | 27.34672 | 17.91117 | 2.331110 | 0.489982 |
| Left Bank E.coli Below the Guyandotte vs. LDB E.coli Below the Guyandotte | 252.7333 | 77.4000 | 10.6483 | 5 | 0.000126 | 27.34672 | 16.61566 | 2.708792 | 0.425530 |
| Left Bank E.coli Below the Guyandotte vs. LDB E.coli Above the Guyandotte | 252.7333 | 67.2750 | 12.9605 | 5 | 0.000049 | 27.34672 | 9.29888 | 8.648671 | 0.113646 |
| Left Bank E.coli Below the Guyandotte vs. Center E.coli Below the Guyandotte | 252.7333 | 78.9200 | 9.9757 | 6 | 0.000059 | 27.34672 | 21.90701 | 1.558276 | 0.631845 |
| Left Bank E.coli Above the Guyandotte vs. Left Bank E.coli Below the Guyandotte | 71.3500 | 252.7333 | -10.7108 | 5 | 0.000123 | 17.91117 | 27.34672 | 2.331110 | 0.489982 |
| Left Bank E.coli Above the Guyandotte vs. Left Bank E.coli Above the Guyandotte | 71.3500 | 71.3500 | 0.0000 | 6 | 1.000000 | 17.91117 | 17.91117 | 1.000000 | 1.000000 |
| Left Bank E.coli Above the Guyandotte vs. LDB E.coli Below the Guyandotte | 71.3500 | 77.4000 | -0.4953 | 6 | 0.638026 | 17.91117 | 16.61566 | 1.162018 | 0.904675 |
| Left Bank E.coli Above the Guyandotte vs. LDB E.coli Above the Guyandotte | 71.3500 | 67.2750 | 0.4038 | 6 | 0.700328 | 17.91117 | 9.29888 | 3.710109 | 0.310117 |
| Left Bank E.coli Above the Guyandotte vs. Center E.coli Below the Guyandotte | 71.3500 | 78.9200 | -0.5561 | 7 | 0.595437 | 17.91117 | 21.90701 | 1.495954 | 0.771534 |
| LDB E.coli Below the Guyandotte vs. Left Bank E.coli Below the Guyandotte | 77.4000 | 252.7333 | -10.6483 | 5 | 0.000126 | 16.61566 | 27.34672 | 2.708792 | 0.425530 |
| LDB E.coli Below the Guyandotte vs. Left Bank E.coli Above the Guyandotte | 77.4000 | 71.3500 | 0.4953 | 6 | 0.638026 | 16.61566 | 17.91117 | 1.162018 | 0.904675 |
| LDB E.coli Below the Guyandotte vs. LDB E.coli Below the Guyandotte | 77.4000 | 77.4000 | 0.0000 | 6 | 1.000000 | 16.61566 | 16.61566 | 1.000000 | 1.000000 |
| LDB E.coli Below the Guyandotte vs. LDB E.coli Above the Guyandotte | 77.4000 | 67.2750 | 1.0635 | 6 | 0.328468 | 16.61566 | 9.29888 | 3.192814 | 0.365847 |
| LDB E.coli Below the Guyandotte vs. Center E.coli Below the Guyandotte | 77.4000 | 78.9200 | -0.1144 | 7 | 0.912161 | 16.61566 | 21.90701 | 1.738326 | 0.677747 |
| LDB E.coli Above the Guyandotte vs. Left Bank E.coli Below the Guyandotte | 67.2750 | 252.7333 | -12.9605 | 5 | 0.000049 | 9.29888 | 27.34672 | 8.648671 | 0.113646 |
| LDB E.coli Above the Guyandotte vs. Left Bank E.coli Above the Guyandotte | 67.2750 | 71.3500 | -0.4038 | 6 | 0.700328 | 9.29888 | 17.91117 | 3.710109 | 0.310117 |
| LDB E.coli Above the Guyandotte vs. LDB E.coli Below the Guyandotte | 67.2750 | 77.4000 | -1.0635 | 6 | 0.328468 | 9.29888 | 16.61566 | 3.192814 | 0.365847 |
| LDB E.coli Above the Guyandotte vs. LDB E.coli Above the Guyandotte | 67.2750 | 67.2750 | 0.0000 | 6 | 1.000000 | 9.29888 | 9.29888 | 1.000000 | 1.000000 |
| LDB E.coli Above the Guyandotte vs. Center E.coli Below the Guyandotte | 67.2750 | 78.9200 | -0.9839 | 7 | 0.357947 | 9.29888 | 21.90701 | 5.550152 | 0.190700 |
| Center E.coli Below the Guyandotte vs. Left Bank E.coli Below the Guyandotte | 78.9200 | 252.7333 | -9.9757 | 6 | 0.000059 | 21.90701 | 27.34672 | 1.558276 | 0.631845 |
| Center E.coli Below the Guyandotte vs. Left Bank E.coli Above the Guyandotte | 78.9200 | 71.3500 | 0.5561 | 7 | 0.595437 | 21.90701 | 17.91117 | 1.495954 | 0.771534 |
| Center E.coli Below the Guyandotte vs. LDB E.coli Below the Guyandotte | 78.9200 | 77.4000 | 0.1144 | 7 | 0.912161 | 21.90701 | 16.61566 | 1.738326 | 0.677747 |
| Center E.coli Below the Guyandotte vs. LDB E.coli Above the Guyandotte | 78.9200 | 67.2750 | 0.9839 | 7 | 0.357947 | 21.90701 | 9.29888 | 5.550152 | 0.190700 |
| Center E.coli Below the Guyandotte vs. Center E.coli Below the Guyandotte | 78.9200 | 78.9200 | 0.0000 | 8 | 1.000000 | 21.90701 | 21.90701 | 1.000000 | 1.000000 |

# APPENDIX C

## IDEXX Quanti-Tray*/2000 MPN Table

| # Large Wells Positive | # Small Wells Positive | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 0 | <1 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 13.0 | 14.1 | 15.1 | 16.1 | 17.1 | 18.1 | 19.1 | 20.2 | 21.2 | 22.2 | 23.3 | 24.3 |
| 1 | 1.0 | 2.0 | 3.0 | 4.0 | 5.0 | 6.0 | 7.1 | 8.1 | 9.1 | 10.1 | 11.1 | 12.1 | 13.2 | 14.2 | 15.2 | 16.2 | 17.3 | 18.3 | 19.3 | 20.4 | 21.4 | 22.4 | 23.5 | 24.5 | 25.6 |
| 2 | 2.0 | 3.0 | 4.1 | 5.1 | 6.1 | 7.1 | 8.1 | 9.2 | 10.2 | 11.2 | 12.2 | 13.3 | 14.3 | 15.4 | 16.4 | 17.4 | 18.5 | 19.5 | 20.6 | 21.6 | 22.7 | 23.7 | 24.8 | 25.8 | 26.9 |
| 3 | 3.1 | 4.1 | 5.1 | 6.1 | 7.2 | 8.2 | 9.2 | 10.3 | 11.3 | 12.4 | 13.4 | 14.5 | 15.5 | 16.5 | 17.6 | 18.6 | 19.7 | 20.8 | 21.8 | 22.9 | 23.9 | 25.0 | 26.1 | 27.1 | 28.2 |
| 4 | 4.1 | 5.2 | 6.2 | 7.2 | 8.3 | 9.3 | 10.4 | 11.4 | 12.5 | 13.5 | 14.6 | 15.6 | 16.7 | 17.8 | 18.8 | 19.9 | 21.0 | 22.0 | 23.1 | 24.2 | 25.3 | 26.3 | 27.4 | 28.5 | 29.6 |
| 5 | 5.2 | 6.3 | 7.3 | 8.4 | 9.4 | 10.5 | 11.5 | 12.6 | 13.7 | 14.7 | 15.8 | 16.9 | 17.9 | 19.0 | 20.1 | 21.2 | 22.2 | 23.3 | 24.4 | 25.5 | 26.6 | 27.7 | 28.8 | 29.9 | 31.0 |
| 6 | 6.3 | 7.4 | 8.4 | 9.5 | 10.6 | 11.6 | 12.7 | 13.8 | 14.9 | 16.0 | 17.0 | 18.1 | 19.2 | 20.3 | 21.4 | 22.5 | 23.6 | 24.7 | 25.8 | 26.9 | 28.0 | 29.1 | 30.2 | 31.3 | 32.4 |
| 7 | 7.5 | 8.5 | 9.6 | 10.7 | 11.8 | 12.8 | 13.9 | 15.0 | 16.1 | 17.2 | 18.3 | 19.4 | 20.5 | 21.6 | 22.7 | 23.8 | 24.9 | 26.0 | 27.1 | 28.3 | 29.4 | 30.5 | 31.6 | 32.8 | 33.9 |
| 8 | 8.6 | 9.7 | 10.8 | 11.9 | 13.0 | 14.1 | 15.2 | 16.3 | 17.4 | 18.5 | 19.6 | 20.7 | 21.8 | 22.9 | 24.1 | 25.2 | 26.3 | 27.4 | 28.6 | 29.7 | 30.8 | 32.0 | 33.1 | 34.3 | 35.4 |
| 9 | 9.8 | 10.9 | 12.0 | 13.1 | 14.2 | 15.3 | 16.4 | 17.6 | 18.7 | 19.8 | 20.9 | 22.0 | 23.2 | 24.3 | 25.4 | 26.6 | 27.7 | 28.9 | 30.0 | 31.2 | 32.3 | 33.5 | 34.6 | 35.8 | 37.0 |
| 10 | 11.0 | 12.1 | 13.2 | 14.4 | 15.5 | 16.6 | 17.7 | 18.9 | 20.0 | 21.1 | 22.3 | 23.4 | 24.6 | 25.7 | 26.9 | 28.0 | 29.2 | 30.3 | 31.5 | 32.7 | 33.8 | 35.0 | 36.2 | 37.4 | 38.6 |
| 11 | 12.2 | 13.4 | 14.5 | 15.6 | 16.8 | 17.9 | 19.1 | 20.2 | 21.4 | 22.5 | 23.7 | 24.8 | 26.0 | 27.2 | 28.3 | 29.5 | 30.7 | 31.9 | 33.0 | 34.2 | 35.4 | 36.6 | 37.8 | 39.0 | 40.2 |
| 12 | 13.5 | 14.6 | 15.8 | 16.9 | 18.1 | 19.3 | 20.4 | 21.6 | 22.8 | 23.9 | 25.1 | 26.3 | 27.5 | 28.6 | 29.8 | 31.0 | 32.2 | 33.4 | 34.6 | 35.8 | 37.0 | 38.2 | 39.5 | 40.7 | 41.9 |
| 13 | 14.8 | 16.0 | 17.1 | 18.3 | 19.5 | 20.6 | 21.8 | 23.0 | 24.2 | 25.4 | 26.6 | 27.8 | 29.0 | 30.2 | 31.4 | 32.6 | 33.8 | 35.0 | 36.2 | 37.5 | 38.7 | 39.9 | 41.2 | 42.4 | 43.6 |
| 14 | 16.1 | 17.3 | 18.5 | 19.7 | 20.9 | 22.1 | 23.3 | 24.5 | 25.7 | 26.9 | 28.1 | 29.3 | 30.5 | 31.7 | 33.0 | 34.2 | 35.4 | 36.7 | 37.9 | 39.1 | 40.4 | 41.6 | 42.9 | 44.2 | 45.4 |
| 15 | 17.5 | 18.7 | 19.9 | 21.1 | 22.3 | 23.5 | 24.7 | 25.9 | 27.2 | 28.4 | 29.6 | 30.9 | 32.1 | 33.3 | 34.6 | 35.8 | 37.1 | 38.4 | 39.6 | 40.9 | 42.2 | 43.4 | 44.7 | 46.0 | 47.3 |
| 16 | 18.9 | 20.1 | 21.3 | 22.6 | 23.8 | 25.0 | 26.2 | 27.5 | 28.7 | 30.0 | 31.2 | 32.5 | 33.7 | 35.0 | 36.3 | 37.5 | 38.8 | 40.1 | 41.4 | 42.7 | 44.0 | 45.3 | 46.6 | 47.9 | 49.2 |
| 17 | 20.3 | 21.6 | 22.8 | 24.1 | 25.3 | 26.6 | 27.8 | 29.1 | 30.3 | 31.6 | 32.9 | 34.1 | 35.4 | 36.7 | 38.0 | 39.3 | 40.6 | 41.9 | 43.2 | 44.5 | 45.9 | 47.2 | 48.5 | 49.8 | 51.2 |
| 18 | 21.8 | 23.1 | 24.3 | 25.6 | 26.9 | 28.1 | 29.4 | 30.7 | 32.0 | 33.3 | 34.6 | 35.9 | 37.2 | 38.5 | 39.8 | 41.1 | 42.4 | 43.8 | 45.1 | 46.5 | 47.8 | 49.2 | 50.5 | 51.9 | 53.2 |
| 19 | 23.3 | 24.6 | 25.9 | 27.2 | 28.5 | 29.8 | 31.1 | 32.4 | 33.7 | 35.0 | 36.3 | 37.6 | 39.0 | 40.3 | 41.6 | 43.0 | 44.3 | 45.7 | 47.1 | 48.4 | 49.8 | 51.2 | 52.6 | 54.0 | 55.4 |
| 20 | 24.9 | 26.2 | 27.5 | 28.8 | 30.1 | 31.5 | 32.8 | 34.1 | 35.4 | 36.8 | 38.1 | 39.5 | 40.8 | 42.2 | 43.6 | 44.9 | 46.3 | 47.7 | 49.1 | 50.5 | 51.9 | 53.3 | 54.7 | 56.1 | 57.6 |
| 21 | 26.5 | 27.9 | 29.2 | 30.5 | 31.8 | 33.2 | 34.5 | 35.9 | 37.3 | 38.6 | 40.0 | 41.4 | 42.8 | 44.1 | 45.5 | 46.9 | 48.4 | 49.8 | 51.2 | 52.6 | 54.1 | 55.5 | 56.9 | 58.4 | 59.9 |
| 22 | 28.2 | 29.5 | 30.9 | 32.3 | 33.6 | 35.0 | 36.4 | 37.7 | 39.1 | 40.5 | 41.9 | 43.3 | 44.8 | 46.2 | 47.6 | 49.0 | 50.5 | 51.9 | 53.4 | 54.8 | 56.3 | 57.8 | 59.3 | 60.8 | 62.3 |
| 23 | 29.9 | 31.3 | 32.7 | 34.1 | 35.5 | 36.8 | 38.3 | 39.7 | 41.1 | 42.5 | 43.9 | 45.4 | 46.8 | 48.3 | 49.7 | 51.2 | 52.7 | 54.2 | 55.6 | 57.1 | 58.6 | 60.2 | 61.7 | 63.2 | 64.7 |
| 24 | 31.7 | 33.1 | 34.5 | 35.9 | 37.3 | 38.8 | 40.2 | 41.7 | 43.1 | 44.6 | 46.0 | 47.5 | 49.0 | 50.5 | 52.0 | 53.5 | 55.0 | 56.5 | 58.0 | 59.5 | 61.1 | 62.6 | 64.2 | 65.8 | 67.3 |
| 25 | 33.6 | 35.0 | 36.4 | 37.9 | 39.3 | 40.8 | 42.2 | 43.7 | 45.2 | 46.7 | 48.2 | 49.7 | 51.2 | 52.7 | 54.3 | 55.8 | 57.3 | 58.9 | 60.5 | 62.0 | 63.6 | 65.2 | 66.8 | 68.4 | 70.0 |
| 26 | 35.5 | 36.9 | 38.4 | 39.9 | 41.4 | 42.8 | 44.3 | 45.9 | 47.4 | 48.9 | 50.4 | 52.0 | 53.5 | 55.1 | 56.7 | 58.2 | 59.8 | 61.4 | 63.0 | 64.7 | 66.3 | 67.9 | 69.6 | 71.2 | 72.9 |
| 27 | 37.4 | 38.9 | 40.4 | 42.0 | 43.5 | 45.0 | 46.5 | 48.1 | 49.6 | 51.2 | 52.8 | 54.4 | 56.0 | 57.6 | 59.2 | 60.8 | 62.4 | 64.1 | 65.7 | 67.4 | 69.1 | 70.8 | 72.5 | 74.2 | 75.9 |
| 28 | 39.5 | 41.0 | 42.6 | 44.1 | 45.7 | 47.3 | 48.8 | 50.4 | 52.0 | 53.6 | 55.2 | 56.9 | 58.5 | 60.2 | 61.8 | 63.5 | 65.2 | 66.9 | 68.6 | 70.3 | 72.0 | 73.7 | 75.5 | 77.3 | 79.0 |
| 29 | 41.7 | 43.2 | 44.8 | 46.4 | 48.0 | 49.6 | 51.2 | 52.8 | 54.5 | 56.1 | 57.8 | 59.5 | 61.2 | 62.9 | 64.6 | 66.3 | 68.0 | 69.8 | 71.5 | 73.3 | 75.1 | 76.9 | 78.7 | 80.5 | 82.4 |
| 30 | 43.9 | 45.5 | 47.1 | 48.7 | 50.4 | 52.0 | 53.7 | 55.4 | 57.1 | 58.8 | 60.5 | 62.2 | 64.0 | 65.7 | 67.5 | 69.3 | 71.0 | 72.9 | 74.7 | 76.5 | 78.3 | 80.2 | 82.1 | 84.0 | 85.9 |
| 31 | 46.2 | 47.9 | 49.5 | 51.2 | 52.9 | 54.6 | 56.3 | 58.1 | 59.8 | 61.6 | 63.3 | 65.1 | 66.9 | 68.7 | 70.5 | 72.4 | 74.2 | 76.1 | 78.0 | 79.9 | 81.8 | 83.7 | 85.7 | 87.6 | 89.6 |
| 32 | 48.7 | 50.4 | 52.1 | 53.8 | 55.6 | 57.3 | 59.1 | 60.9 | 62.7 | 64.5 | 66.3 | 68.2 | 70.0 | 71.9 | 73.8 | 75.7 | 77.6 | 79.5 | 81.5 | 83.5 | 85.4 | 87.5 | 89.5 | 91.5 | 93.6 |
| 33 | 51.2 | 53.0 | 54.8 | 56.5 | 58.3 | 60.2 | 62.0 | 63.8 | 65.7 | 67.6 | 69.5 | 71.4 | 73.3 | 75.2 | 77.2 | 79.2 | 81.2 | 83.2 | 85.2 | 87.3 | 89.3 | 91.4 | 93.6 | 95.7 | 97.8 |
| 34 | 53.9 | 55.7 | 57.6 | 59.4 | 61.3 | 63.1 | 65.0 | 67.0 | 68.9 | 70.8 | 72.8 | 74.8 | 76.8 | 78.8 | 80.8 | 82.9 | 85.0 | 87.1 | 89.2 | 91.4 | 93.5 | 95.7 | 97.9 | 100.2 | 102.4 |
| 35 | 56.8 | 58.6 | 60.5 | 62.4 | 64.4 | 66.3 | 68.3 | 70.3 | 72.3 | 74.3 | 76.3 | 78.4 | 80.5 | 82.6 | 84.7 | 86.9 | 89.1 | 91.3 | 93.5 | 95.7 | 98.0 | 100.3 | 102.6 | 105.0 | 107.3 |
| 36 | 59.8 | 61.7 | 63.7 | 65.7 | 67.7 | 69.7 | 71.7 | 73.8 | 75.9 | 78.0 | 80.1 | 82.3 | 84.5 | 86.7 | 88.9 | 91.2 | 93.5 | 95.8 | 98.1 | 100.5 | 102.9 | 105.3 | 107.7 | 110.2 | 112.7 |
| 37 | 62.9 | 65.0 | 67.0 | 69.1 | 71.2 | 73.3 | 75.4 | 77.6 | 79.8 | 82.0 | 84.2 | 86.5 | 88.8 | 91.1 | 93.4 | 95.8 | 98.2 | 100.6 | 103.1 | 105.6 | 108.1 | 110.7 | 113.3 | 115.9 | 118.6 |
| 38 | 66.3 | 68.4 | 70.6 | 72.7 | 74.9 | 77.1 | 79.4 | 81.6 | 83.9 | 86.2 | 88.6 | 91.0 | 93.4 | 95.8 | 98.3 | 100.8 | 103.4 | 105.9 | 108.6 | 111.2 | 113.9 | 116.6 | 119.4 | 122.2 | 125.0 |
| 39 | 70.0 | 72.2 | 74.4 | 76.7 | 78.9 | 81.3 | 83.6 | 86.0 | 88.4 | 90.9 | 93.4 | 95.9 | 98.4 | 101.0 | 103.6 | 106.3 | 109.0 | 111.8 | 114.6 | 117.4 | 120.3 | 123.2 | 126.1 | 129.2 | 132.2 |
| 40 | 73.8 | 76.2 | 78.5 | 80.9 | 83.3 | 85.7 | 88.2 | 90.8 | 93.3 | 95.9 | 98.5 | 101.2 | 103.9 | 106.7 | 109.5 | 112.4 | 115.3 | 118.2 | 121.2 | 124.3 | 127.4 | 130.5 | 133.7 | 137.0 | 140.3 |
| 41 | 78.0 | 80.5 | 83.0 | 85.5 | 88.0 | 90.6 | 93.3 | 95.9 | 98.7 | 101.4 | 104.3 | 107.1 | 110.0 | 113.0 | 116.0 | 119.1 | 122.2 | 125.4 | 128.7 | 132.0 | 135.4 | 138.8 | 142.3 | 145.9 | 149.5 |
| 42 | 82.6 | 85.2 | 87.8 | 90.5 | 93.2 | 96.0 | 98.8 | 101.7 | 104.6 | 107.6 | 110.6 | 113.7 | 116.9 | 120.1 | 123.4 | 126.7 | 130.1 | 133.6 | 137.2 | 140.8 | 144.5 | 148.3 | 152.2 | 156.1 | 160.2 |
| 43 | 87.6 | 90.4 | 93.2 | 96.0 | 99.0 | 101.9 | 105.0 | 108.1 | 111.2 | 114.5 | 117.8 | 121.1 | 124.6 | 128.1 | 131.7 | 135.4 | 139.1 | 143.0 | 147.0 | 151.0 | 155.2 | 159.4 | 163.8 | 168.2 | 172.8 |
| 44 | 93.1 | 96.1 | 99.1 | 102.2 | 105.4 | 108.6 | 111.9 | 115.3 | 118.7 | 122.3 | 125.9 | 129.6 | 133.4 | 137.4 | 141.4 | 145.5 | 149.7 | 154.1 | 158.5 | 163.1 | 167.9 | 172.7 | 177.7 | 182.9 | 188.2 |
| 45 | 99.3 | 102.5 | 105.8 | 109.2 | 112.6 | 116.2 | 119.8 | 123.6 | 127.4 | 131.4 | 135.4 | 139.6 | 143.9 | 148.3 | 152.9 | 157.6 | 162.4 | 167.4 | 172.6 | 178.0 | 183.5 | 189.2 | 195.1 | 201.2 | 207.5 |
| 46 | 106.3 | 109.8 | 113.4 | 117.2 | 121.0 | 125.0 | 129.1 | 133.3 | 137.6 | 142.1 | 146.7 | 151.5 | 156.5 | 161.6 | 167.0 | 172.5 | 178.2 | 184.2 | 190.4 | 196.8 | 203.5 | 210.5 | 217.8 | 225.4 | 233.3 |
| 47 | 114.3 | 118.3 | 122.4 | 126.6 | 130.9 | 135.4 | 140.1 | 145.0 | 150.0 | 155.3 | 160.7 | 166.4 | 172.3 | 178.5 | 185.0 | 191.8 | 198.9 | 206.4 | 214.2 | 222.4 | 231.0 | 240.0 | 249.5 | 259.5 | 270.0 |
| 48 | 123.9 | 128.4 | 133.1 | 137.9 | 143.0 | 148.3 | 153.9 | 159.7 | 165.8 | 172.2 | 178.9 | 186.0 | 193.5 | 201.4 | 209.8 | 218.7 | 228.2 | 238.2 | 248.9 | 260.3 | 272.3 | 285.1 | 298.7 | 313.0 | 328.2 |
| 49 | 135.5 | 140.8 | 146.4 | 152.3 | 158.5 | 165.0 | 172.0 | 179.3 | 187.2 | 195.6 | 204.6 | 214.3 | 224.7 | 235.9 | 248.1 | 261.3 | 275.5 | 290.9 | 307.6 | 325.5 | 344.8 | 365.4 | 387.3 | 410.6 | 435.2 |

*Quanti-Tray is either a trademark or a registered trademark of IDEXX Laboratories, Inc. in the United States and/or other countries. Covered by U.S. Patent Numbers 4,925,789 ; 5,429,933 ; 5,518,892. Other patents pending.

# IDEXX Quanti-Tray*/2000 MPN Table

| # Large Wells Positive | # Small Wells Positive | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 26 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 |
| 0 | 25.3 | 26.4 | 27.4 | 28.4 | 29.5 | 30.5 | 31.5 | 32.6 | 33.6 | 34.7 | 35.7 | 36.8 | 37.8 | 38.9 | 40.0 | 41.0 | 42.1 | 43.1 | 44.2 | 45.3 | 46.3 | 47.4 | 48.5 | 49.5 |
| 1 | 26.6 | 27.7 | 28.7 | 29.8 | 30.8 | 31.9 | 32.9 | 34.0 | 35.0 | 36.1 | 37.2 | 38.2 | 39.3 | 40.4 | 41.4 | 42.5 | 43.6 | 44.7 | 45.7 | 46.8 | 47.9 | 49.0 | 50.1 | 51.2 |
| 2 | 27.9 | 29.0 | 30.0 | 31.1 | 32.2 | 33.2 | 34.3 | 35.4 | 36.5 | 37.5 | 38.6 | 39.7 | 40.8 | 41.9 | 43.0 | 44.0 | 45.1 | 46.2 | 47.3 | 48.4 | 49.5 | 50.6 | 51.7 | 52.8 |
| 3 | 29.3 | 30.4 | 31.4 | 32.5 | 33.6 | 34.7 | 35.8 | 36.8 | 37.9 | 39.0 | 40.1 | 41.2 | 42.3 | 43.4 | 44.5 | 45.6 | 46.7 | 47.8 | 48.9 | 50.0 | 51.2 | 52.3 | 53.4 | 54.5 |
| 4 | 30.7 | 31.8 | 32.8 | 33.9 | 35.0 | 36.1 | 37.2 | 38.3 | 39.4 | 40.5 | 41.6 | 42.8 | 43.9 | 45.0 | 46.1 | 47.2 | 48.3 | 49.5 | 50.6 | 51.7 | 52.9 | 54.0 | 55.1 | 56.3 |
| 5 | 32.1 | 33.2 | 34.3 | 35.4 | 36.5 | 37.6 | 38.7 | 39.9 | 41.0 | 42.1 | 43.2 | 44.4 | 45.5 | 46.6 | 47.7 | 48.9 | 50.0 | 51.2 | 52.3 | 53.5 | 54.6 | 55.8 | 56.9 | 58.1 |
| 6 | 33.5 | 34.7 | 35.8 | 36.9 | 38.0 | 39.2 | 40.3 | 41.4 | 42.6 | 43.7 | 44.8 | 46.0 | 47.1 | 48.3 | 49.4 | 50.6 | 51.7 | 52.9 | 54.1 | 55.2 | 56.4 | 57.6 | 58.7 | 59.9 |
| 7 | 35.0 | 36.2 | 37.3 | 38.4 | 39.6 | 40.7 | 41.9 | 43.0 | 44.2 | 45.3 | 46.5 | 47.7 | 48.8 | 50.0 | 51.2 | 52.3 | 53.5 | 54.7 | 55.9 | 57.1 | 58.3 | 59.4 | 60.6 | 61.8 |
| 8 | 36.6 | 37.7 | 38.9 | 40.0 | 41.2 | 42.3 | 43.5 | 44.7 | 45.9 | 47.0 | 48.2 | 49.4 | 50.6 | 51.8 | 53.0 | 54.1 | 55.3 | 56.5 | 57.7 | 59.0 | 60.2 | 61.4 | 62.6 | 63.8 |
| 9 | 38.1 | 39.3 | 40.5 | 41.6 | 42.8 | 44.0 | 45.2 | 46.4 | 47.6 | 48.8 | 50.0 | 51.2 | 52.4 | 53.6 | 54.8 | 56.0 | 57.2 | 58.4 | 59.7 | 60.9 | 62.1 | 63.4 | 64.6 | 65.8 |
| 10 | 39.7 | 40.9 | 42.1 | 43.3 | 44.5 | 45.7 | 46.9 | 48.1 | 49.3 | 50.6 | 51.8 | 53.0 | 54.2 | 55.5 | 56.7 | 57.9 | 59.2 | 60.4 | 61.7 | 62.9 | 64.2 | 65.4 | 66.7 | 67.9 |
| 11 | 41.4 | 42.6 | 43.8 | 45.0 | 46.3 | 47.5 | 48.7 | 49.9 | 51.2 | 52.4 | 53.7 | 54.9 | 56.1 | 57.4 | 58.6 | 59.9 | 61.2 | 62.4 | 63.7 | 65.0 | 66.3 | 67.5 | 68.8 | 70.1 |
| 12 | 43.1 | 44.3 | 45.6 | 46.8 | 48.1 | 49.3 | 50.6 | 51.8 | 53.1 | 54.3 | 55.6 | 56.8 | 58.1 | 59.4 | 60.7 | 62.0 | 63.2 | 64.5 | 65.8 | 67.1 | 68.4 | 69.7 | 71.0 | 72.4 |
| 13 | 44.9 | 46.1 | 47.4 | 48.6 | 49.9 | 51.2 | 52.5 | 53.7 | 55.0 | 56.3 | 57.6 | 58.9 | 60.2 | 61.5 | 62.8 | 64.1 | 65.4 | 66.7 | 68.0 | 69.3 | 70.7 | 72.0 | 73.3 | 74.7 |
| 14 | 46.7 | 48.0 | 49.3 | 50.5 | 51.8 | 53.1 | 54.4 | 55.7 | 57.0 | 58.3 | 59.6 | 60.9 | 62.3 | 63.6 | 64.9 | 66.3 | 67.6 | 68.9 | 70.3 | 71.6 | 73.0 | 74.4 | 75.7 | 77.1 |
| 15 | 48.6 | 49.9 | 51.2 | 52.5 | 53.8 | 55.1 | 56.4 | 57.8 | 59.1 | 60.4 | 61.8 | 63.1 | 64.5 | 65.8 | 67.2 | 68.5 | 69.9 | 71.3 | 72.6 | 74.0 | 75.4 | 76.8 | 78.2 | 79.6 |
| 16 | 50.5 | 51.8 | 53.2 | 54.5 | 55.8 | 57.2 | 58.5 | 59.9 | 61.2 | 62.6 | 64.0 | 65.3 | 66.7 | 68.1 | 69.5 | 70.9 | 72.3 | 73.7 | 75.1 | 76.5 | 77.9 | 79.3 | 80.8 | 82.2 |
| 17 | 52.5 | 53.9 | 55.2 | 56.6 | 58.0 | 59.3 | 60.7 | 62.1 | 63.5 | 64.9 | 66.3 | 67.7 | 69.1 | 70.5 | 71.9 | 73.3 | 74.8 | 76.2 | 77.6 | 79.1 | 80.5 | 82.0 | 83.5 | 84.9 |
| 18 | 54.6 | 56.0 | 57.4 | 58.8 | 60.2 | 61.6 | 63.0 | 64.4 | 65.8 | 67.2 | 68.6 | 70.1 | 71.5 | 73.0 | 74.4 | 75.9 | 77.3 | 78.8 | 80.3 | 81.8 | 83.3 | 84.8 | 86.3 | 87.8 |
| 19 | 56.8 | 58.2 | 59.6 | 61.0 | 62.4 | 63.9 | 65.3 | 66.8 | 68.2 | 69.7 | 71.1 | 72.6 | 74.1 | 75.5 | 77.0 | 78.5 | 80.0 | 81.5 | 83.1 | 84.6 | 86.1 | 87.6 | 89.2 | 90.7 |
| 20 | 59.0 | 60.4 | 61.9 | 63.3 | 64.8 | 66.3 | 67.7 | 69.2 | 70.7 | 72.2 | 73.7 | 75.2 | 76.7 | 78.2 | 79.8 | 81.3 | 82.8 | 84.4 | 85.9 | 87.5 | 89.1 | 90.7 | 92.2 | 93.8 |
| 21 | 61.3 | 62.8 | 64.3 | 65.8 | 67.3 | 68.8 | 70.3 | 71.8 | 73.3 | 74.9 | 76.4 | 77.9 | 79.5 | 81.1 | 82.6 | 84.2 | 85.8 | 87.4 | 89.0 | 90.6 | 92.2 | 93.8 | 95.4 | 97.1 |
| 22 | 63.8 | 65.3 | 66.8 | 68.3 | 69.8 | 71.4 | 72.9 | 74.5 | 76.1 | 77.6 | 79.2 | 80.8 | 82.4 | 84.0 | 85.6 | 87.2 | 88.9 | 90.5 | 92.1 | 93.8 | 95.5 | 97.1 | 98.8 | 100.5 |
| 23 | 66.3 | 67.8 | 69.4 | 71.0 | 72.5 | 74.1 | 75.7 | 77.3 | 78.9 | 80.5 | 82.2 | 83.8 | 85.4 | 87.1 | 88.7 | 90.4 | 92.1 | 93.8 | 95.5 | 97.2 | 98.9 | 100.6 | 102.4 | 104.1 |
| 24 | 68.9 | 70.5 | 72.1 | 73.7 | 75.3 | 77.0 | 78.6 | 80.3 | 81.9 | 83.6 | 85.2 | 86.9 | 88.6 | 90.3 | 92.0 | 93.8 | 95.5 | 97.2 | 99.0 | 100.7 | 102.5 | 104.3 | 106.1 | 107.9 |
| 25 | 71.7 | 73.3 | 75.0 | 76.6 | 78.3 | 80.0 | 81.7 | 83.3 | 85.1 | 86.8 | 88.5 | 90.2 | 92.0 | 93.7 | 95.5 | 97.3 | 99.1 | 100.9 | 102.7 | 104.5 | 106.3 | 108.2 | 110.0 | 111.9 |
| 26 | 74.6 | 76.3 | 78.0 | 79.7 | 81.4 | 83.1 | 84.8 | 86.6 | 88.4 | 90.1 | 91.9 | 93.7 | 95.5 | 97.3 | 99.2 | 101.0 | 102.9 | 104.7 | 106.6 | 108.5 | 110.4 | 112.3 | 114.2 | 116.2 |
| 27 | 77.6 | 79.4 | 81.1 | 82.9 | 84.6 | 86.4 | 88.2 | 90.0 | 91.9 | 93.7 | 95.5 | 97.4 | 99.3 | 101.2 | 103.1 | 105.0 | 106.9 | 108.8 | 110.8 | 112.7 | 114.7 | 116.7 | 118.7 | 120.7 |
| 28 | 80.8 | 82.6 | 84.4 | 86.3 | 88.1 | 89.9 | 91.8 | 93.7 | 95.6 | 97.5 | 99.4 | 101.3 | 103.3 | 105.2 | 107.2 | 109.2 | 111.2 | 113.2 | 115.2 | 117.3 | 119.3 | 121.4 | 123.5 | 125.6 |
| 29 | 84.2 | 86.1 | 87.9 | 89.8 | 91.7 | 93.7 | 95.6 | 97.5 | 99.5 | 101.5 | 103.5 | 105.5 | 107.5 | 109.5 | 111.6 | 113.7 | 115.7 | 117.8 | 120.0 | 122.1 | 124.2 | 126.4 | 128.6 | 130.8 |
| 30 | 87.8 | 89.7 | 91.7 | 93.6 | 95.6 | 97.6 | 99.6 | 101.6 | 103.7 | 105.7 | 107.8 | 109.9 | 112.0 | 114.2 | 116.3 | 118.5 | 120.6 | 122.8 | 125.1 | 127.3 | 129.5 | 131.8 | 134.1 | 136.4 |
| 31 | 91.6 | 93.6 | 95.6 | 97.7 | 99.7 | 101.8 | 103.9 | 106.0 | 108.2 | 110.3 | 112.5 | 114.7 | 116.9 | 119.1 | 121.4 | 123.6 | 125.9 | 128.2 | 130.5 | 132.9 | 135.3 | 137.7 | 140.1 | 142.5 |
| 32 | 95.7 | 97.8 | 99.9 | 102.0 | 104.2 | 106.3 | 108.5 | 110.7 | 113.0 | 115.2 | 117.5 | 119.8 | 122.1 | 124.5 | 126.8 | 129.2 | 131.6 | 134.0 | 136.5 | 139.0 | 141.5 | 144.0 | 146.6 | 149.1 |
| 33 | 100.0 | 102.2 | 104.4 | 106.6 | 108.9 | 111.2 | 113.5 | 115.8 | 118.2 | 120.5 | 122.9 | 125.4 | 127.8 | 130.3 | 132.8 | 135.3 | 137.8 | 140.4 | 143.0 | 145.6 | 148.3 | 150.9 | 153.7 | 156.4 |
| 34 | 104.7 | 107.0 | 109.3 | 111.7 | 114.0 | 116.4 | 118.9 | 121.3 | 123.8 | 126.3 | 128.8 | 131.4 | 134.0 | 136.6 | 139.2 | 141.9 | 144.6 | 147.4 | 150.1 | 152.9 | 155.7 | 158.6 | 161.5 | 164.4 |
| 35 | 109.7 | 112.2 | 114.6 | 117.1 | 119.6 | 122.2 | 124.7 | 127.3 | 129.9 | 132.6 | 135.3 | 138.0 | 140.8 | 143.6 | 146.4 | 149.2 | 152.1 | 155.0 | 158.0 | 161.0 | 164.0 | 167.1 | 170.2 | 173.3 |
| 36 | 115.2 | 117.8 | 120.4 | 123.0 | 125.7 | 128.4 | 131.1 | 133.9 | 136.7 | 139.5 | 142.4 | 145.3 | 148.3 | 151.3 | 154.3 | 157.3 | 160.5 | 163.6 | 166.8 | 170.0 | 173.3 | 176.6 | 179.9 | 183.3 |
| 37 | 121.3 | 124.0 | 126.8 | 129.6 | 132.4 | 135.3 | 138.2 | 141.2 | 144.2 | 147.3 | 150.3 | 153.5 | 156.7 | 159.9 | 163.1 | 166.5 | 169.8 | 173.2 | 176.7 | 180.2 | 183.7 | 187.3 | 191.0 | 194.7 |
| 38 | 127.9 | 130.8 | 133.8 | 136.8 | 139.9 | 143.0 | 146.2 | 149.4 | 152.6 | 155.9 | 159.2 | 162.6 | 166.1 | 169.6 | 173.2 | 176.8 | 180.4 | 184.2 | 188.0 | 191.8 | 195.7 | 199.7 | 203.7 | 207.7 |
| 39 | 135.3 | 138.5 | 141.7 | 145.0 | 148.3 | 151.7 | 155.1 | 158.6 | 162.1 | 165.7 | 169.4 | 173.1 | 176.9 | 180.7 | 184.7 | 188.7 | 192.7 | 196.8 | 201.0 | 205.3 | 209.6 | 214.0 | 218.5 | 223.0 |
| 40 | 143.7 | 147.1 | 150.6 | 154.2 | 157.8 | 161.5 | 165.3 | 169.1 | 173.0 | 177.0 | 181.1 | 185.2 | 189.4 | 193.7 | 198.1 | 202.5 | 207.1 | 211.7 | 216.4 | 221.1 | 226.0 | 231.0 | 236.0 | 241.1 |
| 41 | 153.2 | 157.0 | 160.9 | 164.8 | 168.9 | 173.0 | 177.2 | 181.5 | 185.8 | 190.3 | 194.8 | 199.5 | 204.2 | 209.1 | 214.0 | 219.1 | 224.2 | 229.4 | 234.8 | 240.2 | 245.8 | 251.5 | 257.2 | 263.1 |
| 42 | 164.3 | 168.6 | 172.9 | 177.3 | 181.9 | 186.5 | 191.3 | 196.1 | 201.1 | 206.2 | 211.4 | 216.7 | 222.2 | 227.7 | 233.4 | 239.2 | 245.2 | 251.3 | 257.5 | 263.8 | 270.3 | 276.9 | 283.6 | 290.5 |
| 43 | 177.5 | 182.3 | 187.3 | 192.4 | 197.6 | 202.9 | 208.4 | 214.0 | 219.8 | 225.8 | 231.8 | 238.1 | 244.5 | 251.0 | 257.7 | 264.6 | 271.7 | 278.9 | 286.3 | 293.8 | 301.5 | 309.4 | 317.4 | 325.7 |
| 44 | 193.6 | 199.3 | 205.1 | 211.0 | 217.2 | 223.5 | 230.0 | 236.7 | 243.6 | 250.8 | 258.1 | 265.6 | 273.3 | 281.2 | 289.4 | 297.8 | 306.3 | 315.1 | 324.1 | 333.3 | 342.8 | 352.4 | 362.3 | 372.4 |
| 45 | 214.1 | 220.9 | 227.9 | 235.2 | 242.7 | 250.4 | 258.4 | 266.7 | 275.3 | 284.1 | 293.3 | 302.6 | 312.3 | 322.3 | 332.5 | 343.0 | 353.8 | 364.9 | 376.2 | 387.9 | 399.8 | 412.0 | 424.5 | 437.4 |
| 46 | 241.5 | 250.0 | 258.9 | 268.2 | 277.8 | 287.8 | 298.1 | 308.8 | 319.9 | 331.4 | 343.3 | 355.5 | 368.1 | 381.1 | 394.5 | 408.3 | 422.5 | 437.1 | 452.0 | 467.4 | 483.3 | 499.6 | 516.3 | 533.5 |
| 47 | 280.9 | 292.4 | 304.4 | 316.9 | 330.0 | 343.6 | 357.8 | 372.5 | 387.7 | 403.4 | 419.6 | 436.6 | 454.1 | 472.1 | 490.7 | 509.9 | 529.8 | 550.4 | 571.7 | 593.8 | 616.7 | 640.5 | 665.3 | 691.0 |
| 48 | 344.1 | 360.9 | 378.4 | 396.8 | 416.0 | 436.0 | 456.9 | 478.6 | 501.2 | 524.7 | 549.3 | 574.8 | 601.5 | 629.4 | 658.6 | 689.3 | 721.5 | 755.6 | 791.5 | 829.7 | 870.4 | 913.9 | 960.6 | 1011.2 |
| 49 | 461.1 | 488.4 | 517.2 | 547.5 | 579.4 | 613.1 | 648.8 | 686.7 | 727.0 | 770.1 | 816.4 | 866.4 | 920.8 | 980.4 | 1046.2 | 1119.9 | 1203.3 | 1299.7 | 1413.6 | 1553.1 | 1732.9 | 1986.3 | 2419.6 | >2419.6 |

**Antibiotic Stock Solutions**

**1.** The antibiotics, solvents, and concentrations used are shown in Table 1.

**Table 1.** Antibiotics used and recommended concentrations.

| Antibiotic | Catalog No. | Solvent[a] | Stock Conc. | Working Conc. |
|---|---|---|---|---|
| Fungizone | BioWhitaker 17-836R | N/A | 250 μg/ml | 375 ng/ml |
| Ampicillin Sodium Salt | Fisher BP1760-25 | $H_2O$ | 50 mg/ml | 50 μg/ml |
| Ciprofloxacin | Cellgro 61-277-RF | DMSO | 4 mg/ml | 4 μg/ml |
| Erythromycin | Fisher BP920-25 | EtOH:$H_2O$ | 8 mg/ml | 8 μg/ml |
| Streptomycin Sulfate | Fisher BP910-50 | Water | 25 mg/ml | 25 μg/ml |
| Sulfamethizole | Fisher ICN15671125 | DMSO | 128 mg/ml | 128 μg/ml |
| Tetracycline Hydrochloride | Fisher BP912-100 | EtOH:$H_2O$ | 12.5 mg/ml | 12.5 μg/ml |
| Virginiamycin | Fisher 50-213-730 | DMSO | 16 mg/ml | 16 μg/ml |

[a] Fungizone is purchased as a stock solution, it is stored frozen and thawed before use. DMSO = dimethylsulfoxide (Certified ACS). EtOH:$H_2O$ = a mixture of equal parts ethanol (100% USP) and reagent grade water (18 MΩ ).

**2.** Using an analytical balance, weigh out sufficient antibiotic to make a 10 ml stock (see Table 1 and note below) and transfer the antibiotic powder to a sterile 15 ml plastic centrifuge tube (Falcon 2095; Becton Dickinson, Sparks, MD or equivalent).

**Note – for determining amount of antibiotic powder to use**

**a.** Be sure to account for the purity of the antibiotic powder by dividing the weight of pure antibiotic required by the purity. For example, ciprofloxacin may be provided as a powder that contains 803 mg ciprofloxacin per gram. To achieve a stock concentration of 4 mg ciprofloxacin per ml, it is necessary to add 4.98 [or 4.0 mg cipro x (1000 mg powder / 803 mg cipro)] mg powder per ml of stock solution.

**3.** Add 10 ml of the appropriate solvent (see Table 1) to the tube, and vortex to mix.

**4.** In some cases (e.g. when making stock solutions of ciprofloxacin) the tube can be placed in a bath sonicator to facilitate dissolution of the solute. Take care to be certain that all of the antibiotic has gone into solution.

**5.** Draw the antibiotic solution into a sterile 10 ml syringe, and sterilize by forcing the solution through a sterile, 0.2 μm syringe filter (Fisher Scientific cat. no. 09-719C or equivalent) into a second sterile plastic centrifuge tube. ***Do not*** *filter sterilize antibiotics dissolved in DMSO.*

**6.** Store the antibiotic stocks at -20°C until used. Replace antibiotic stocks each month.

**Media Preparation**

**1.** Suspend 9.1 grams Difco R2A agar (Becton Dickinson, Sparks, MD; cat no. 218263) in 500 ml of purified water in a 1,000 ml capacity glass Erlenmeyer flask.

**2.** Add a magnetic stir bar, cover the flask with aluminum foil, place and piece of autoclave tape on the foil, and mark the name of the antibiotic to be added (if appropriate) on the foil.

**3.** Swirl the flask to evenly hydrate the suspended powder, and autoclave at 121°C and 15 psi for 20 minutes on a slow exhaust cycle.

**4.** Move the medium from the autoclave to a 48°C water bath, and hold for at least 30 minutes but not more than 4 hours.

**5.** While the medium is cooling, remove the appropriate antibiotic stock solutions from the freezer and thaw on ice (all solvents except DMSO) or at room temperature (antibiotics in DMSO).

**6.** Place the flask on a magnetic stir plate and stir gently until the medium is well mixed. Be careful not to introduce bubbles. Test the temperature of the medium by touching the side of the flask briefly with your bare hand. It should be warm, but not hot. If the flask is hot to the touch, return it to the water bath until it has cooled enough to be handled comfortably. Do not allow the medium to cool below 48°C.

**7.** Wear disposable latex gloves for the remaining steps of media preparation. When properly tempered, again move the medium to the magnetic stirrer. While stirring gently, ***aseptically*** add 750 μl of fungizone stock.

**8.** Continue stirring for 15 to 30 seconds after the addition of the fungizone to the medium. Tilt the flask to insure that all the fungizone stock solution is transferred to the medium.

**9.** If you are preparing R2A plus fungizone for the enumeration of total cultivable bacteria, aseptically pour 25 ml per plate into pre-sterilized 100 x 15 mm Petri dishes (Falcon 1029, Becton Dickinson, Sparks, MD or equivalent).

**10.** If you are preparing R2A plus fungizone and an additional antibiotic for the enumeration of a particular resistant population, ***aseptically*** add 500 μl of the appropriate antibiotic stock to the flask. Stir gently for an additional 15 seconds and tilt the flask to insure that all the antibiotic stock is transferred to the medium.

**11.** Pour the plates as described in step 9.

**12.** Clearly mark the plates to indicate media content. E.g. “R2Af “ can be used to indicate R2A agar plus fungizone, and “R2Afc” to indicate R2A agar plus fungizone and ciprofloxacin, etc.

**13.** Allow plates to cure at room temperature for at least 48 hours before use. Plates should be inoculated no later than seven days after pouring.

**Sample Collection**

1. Whole water samples must be collected in sterile containers with secure, leak-proof lids. Containers must be clearly labeled with a sample number, and the sample number must be recorded in a notebook in which the location, date and time of sampling are clearly and fully described. If available, include additional information such as: latitude and longitude, air temperature, water temperature, weather conditions, turbidity, level of boating activity, land use patterns, etc.

2. The container should be opened so that the opening is pointing downward, and the inside of the lid does not come into contact with any non-sterile surfaces.

3. Continue holding the opening downward while passing the container through the surface tension layer.

4. When the container is fully submerged, invert it so that it fills with water.

5. Pour off enough water to leave approximately a 10% air headspace.

6. Seal the container and place on ice. Samples should be cultivated within 6 hours of collection.

**Enumeration of Total Cultivable Bacteria**

1. Remove a sample bottle from the ice chest and mix by inversion to re-suspend any sediment that may have settled out during transit.

2. Aseptically transfer 0.1 ml of sample to a sterile 9.9 ml dilution blank in a screw-cap test tube.

3. Tightly cap the tube and mix at full speed on a vortex mixer for at least 5 seconds.

4. Aseptically transfer 0.1 ml of diluted sample to each of three plates of Difco R2A agar plus 375 ng/ml fungizone.

5. Spread the diluted water sample on the surface of the agar plates using a sterile glass spreading rod, a pre-sterilized inoculating loop, or five sterile glass beads (5 mm; see note) until all of the liquid has been absorbed.

   **Note – for use of sterile glass beads**

   **a.** Place six glass beads (Fisher Scientific cat no. 11-312C) into a 1000 ml pipette tip (Biolog cat no. 3001; other tips should be tested for suitability). One set of beads is required for each plate inoculated.

   **b.** Place the tip with beads into the original pipette box, cover all the tips with a sheet of aluminum foil, place the cap on the box, place a piece of autoclave tape on the box, and autoclave at 121°C and 15 psi for 15 minutes.

   **c.** When plating – open the pipette tip box, roll back the aluminum foil to expose a single row of pipette tips, remove one tip at a time, lift the lid of an inoculated plate, and pour the sterile beads onto the agar surface. Normally, one bead remains stuck in the bottom of the tip.

   **d.** Repeat step c for all replicate plates.

**e.** Cover the plates and stack them. Then shake the plates by moving them in a quick back and forth motion while keeping the bottom plate in contact with the bench top - *it is important to avoid allowing the beads to run in a circular motion around the outer edge of the plate*. Shake five times, then rotate the plates by one-quarter turn and shake again five times. Repeat shaking and turning the plates a total of five times.

**f.** Invert the plates and collect the used beads in a beaker containing 70% ethanol.

**6.** Plates must be clearly marked with sample number and date of inoculation.

**7.** Wrap each set of three plates with parafilm and incubate inverted at 25°C for one week (see note)

**Note – for incubation of R2A plates**

**a.** R2A agar plates inoculated with river or lake water will continue to develop new microcolonies for 5 to 6 days after inoculation. Therefore, incubation for at least seven days is recommended. Incubation at temperatures above 25°C is not recommended as it may reduce the number of colony forming units.

**8.** After incubation, count the number of colony forming units (CFU) on each plate and record in a laboratory notebook.

**9.** Determine the mean and standard deviation of CFU counts on replicate plates and record in a laboratory notebook.

**10.** Determine the CFU per ml of total cultivable bacteria in the original sample by multiplying the average CFU value by a dilution factor of 1,000 (accounts for the initial $10^{-2}$ dilution and the plating volume of 0.1 ml). Record this value in the laboratory notebook.

**Enumeration of Antibiotic Resistant Bacteria**

**1.** Remove a sample bottle from the ice chest and mix by inversion to re-suspend any sediment that may have settled out during transit.

**2.** Aseptically transfer 0.1 to 0.2 ml (see note) of undiluted sample to each of three plates of Difco R2A agar plus 375 ng/ml fungizone, plus the appropriate concentration of a single antibiotic (see Table 1).

**Note – for selection of plating volume**

**a.** Preliminary tests to determine the volume of sample to be plated are recommended. A plating volume of 0.1 ml is the default volume, but if the number of antibiotic resistant colony forming units is consistently less than 30 per plate, the volume should be increased to 0.2 ml

**3.** Spread the undiluted water sample on the surface of the agar plates using a sterile glass spreading rod, a pre-sterilized inoculating loop, or five sterile glass beads (5 mm; see note above) until all of the liquid has been absorbed.

**4.** Plates must be clearly marked with sample number and date of inoculation.

**5.** Wrap each set of three plates with parafilm and incubate inverted at 25°C for one week (see note above).

6. After incubation, count the number of colony forming units (CFU) on each plate and record in a laboratory notebook.

7. Determine the mean and standard deviation of CFU counts on replicate plates and record in a laboratory notebook.

8. Determine the CFU per ml of total cultivable bacteria in the original sample by multiplying the average CFU value by a dilution factor of 10 (for a plating volume of 0.1 ml) or 5 (for a plating volume of 0.2 ml). Record this value in the laboratory notebook.

**Determination of Impact Scores**

1. Enter enumeration data for fecal indicators and antibiotic resistant bacteria into an Excel spreadsheet.

2. For each population (i.e. fecal coliforms or ciprofloxacin resistant cells), rank the average count for a site within the population data set of all sites using the PERCENTRANK function. Multiply the PERCENTRANK output by 100 to achieve a percentile score for each data point within the entire population data set (see note).

   **Note – on determining percentile scores**

   **a.** The PERCENTRANK function in Excel cannot simply be copied and pasted from cell to cell. If the function is transferred it will carry the original array size, but the array will be offset and the function will calculate an inappropriate rank. *Therefore, you must set the array to contain the entire population data set for each individual data point.*

3. Choose the boundaries that you wish to apply to the data. For example, an $IS_{90}$ score weights sites with population counts above the $90^{th}$ percentile and below the $10^{th}$ percentile. An $IS_{80}$ score weights sites with population counts above the $80^{th}$ percentile and below the $20^{th}$ percentile. In our hands, $IS_{85}$ to $IS_{90}$ scores provide a useful signal to noise ratio in the index.

4. Assign a population score of 1 to all data points that fall above the upper percentile boundary.

5. Assign a population score of -1 to all data points that fall below the lower percentile boundary.

6. Assign a population score of 0 to all data points that fall between the chosen boundaries.

7. Repeat the determination of population scores for all microbial populations enumerated, i.e. for each antibiotic resistant population measured and for the fecal indicator population.

8. Determine the total impact score (IS) by adding the population scores. For studies that include three antibiotics and one fecal indicator, impact scores can range from -4 to +4. Higher impact scores are indicative of a more impacted water source.

9. Plot IS versus river mile to get a visual representation of water quality variability.

# CURRICULUM VITAE

**Caitlin Nicole Swecker**
**2011 6th Ave Apt 1032**
**Huntington, WV**
**(304) 838-5979**
**Swecker5@marshall.edu**
**Caitlin.swecker@gmail.com**

**Education:**
M.S. Biological Sciences; Marshall University, Summer 2009
Major: Biology
GPA: 3.88
Thesis: *Determining the Distribution of Antibiotic Resistant and Fecal Indicator Bacteria in the Ohio River*

B.S. Biological Sciences; Marshall University, Spring 2007
Major: Biomedical Science
Minor: Chemistry
Magna Cum Laude (Cumulative GPA: 3.71)

**Teaching Experience:**
Marshall University Instructor, Fall 2008 - Spring 2009 *Current*
Department of Biological Sciences
Microbiology

Teaching Assistant, 2007-Summer 2009
Marshall University Biological Sciences Department
Courses:
- Anatomy Lab (Fall 07, Summer 08, Summer 09)
- Physiology Lab (Spring 08)
- Microbiology Lab (Fall 08)
- Genetics Lab (Spring 09)

**Grants/Fellowships:**
Smith Goodno Fellowship, $1500 stipend
Marshall University Biological Sciences Summer Thesis Research Grant, $500
Shelby Pew Research Grant, $300

**Awards/Honors:**
The National Scholars Honor Society, 2008
Gamma Beta Phi, 2003 – 2007
Golden Key, 2004 – 2007
National Society of Collegiate Scholars, 2004 – 2007

**Other Research Experience:**
Distribution of coliform bacteria and *E.coli* throughout the entire Coal River Watershed, St. Albins, WV; Marshall University, Little Coal River Restoration Project, 2008

**Other Skills/Experience:**
Certified Open Water PADI SCUBA diver
Experience using GIS and statistical software: ArcMap, ArcCatalog, SAS (PCA), SigmaStat 3.5, SigmaPlot 10.0, Statistica 8.0