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THE EFFECTS THAT LIQUID AND SOLID CATTLE MANURE HAVE ON THE WATER QUALITY OF DRAINAGE DITCHES IN PUTNAM COUNTY, OHIO

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HONORS PROJECT

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Introduction

The Problems:

Lake Erie

In the 1960s and the early 1970s, when the lake was experiencing high nutrient loads and poor water quality, including eutrophication, the cause was largely point source pollution. However, since the early 1980s, nonpoint sources of nutrient loading have been more frequent, as shown in Figure 1. Lake Erie has experienced harmful algal blooms with increased frequency since the mid-1990s. These blooms affect water quality, and thus potentially affect human health and recreational usage of the lake. The most common species found in Lake Erie between 2002-2007 was *Microcystis aeruginosa*, a type of cyanobacteria that forms blooms during the summer months. *Lyngbya,* another type of cyanobacteria, has been known to form large benthic mats along shorelines, like it did in the fall of 2006 ("Lake Erie Symposium Morning Session"). These blooms form when algae that are present normally in the lake grow vigorously. Cyanobacterial harmful algal blooms can use up all the oxygen in the water, produce toxins, and make people and animals sick (United States. Department of Health and Human Services). Many factors can be contributed to harmful algal blooms (HABs), such as excess nutrients, warmer water temperatures, low-flow or low-wind conditions, and sunlight ("Harmful Algal Blooms in Ohio Waters"). The increases in phytoplankton biomass and algal blooms in the western basin of Lake Erie are often attributed to increased phosphorus loads. The Lake Erie Phosphorus Task Force was formed to identify the sources of the increased levels of soluble phosphorus and to make recommendations to lower levels loaded into the lake.

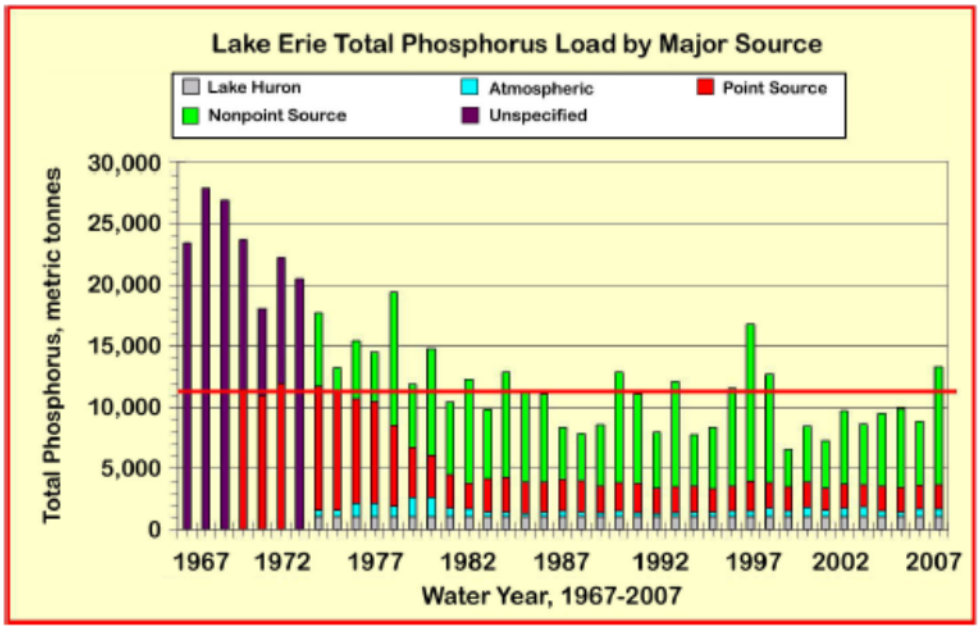

Figure 1: Annual Loading of Total Phosphorus to Lake Erie by Various Sources (Korleski 2010)

In 2011, Lake Erie experienced the largest harmful algal bloom in its recorded history (Mickalak et al. 2013), as seen in Figure 2. Meteorological conditions were right that year to spur algal growth, specifically *Microcystis* and *Anabaena*, both potentially toxic cyanobacteria. Also contributing to this bloom were the long-term trends in agricultural practices, which were consistent with the increased phosphorus loading to the western basin of the lake (Michalak et al. 2013). In recent years, the Maumee River, a tributary to the western basin of Lake Erie, has been linked to bloom development by delivering phosphorus and nitrogen, nutrients that algae take up, to the lake ("Lake Erie Symposium Morning Session"). The Maumee River carries water to the western basin of Lake Erie from northwest Ohio, where row crop agriculture is the predominant land use, as shown in Figure 3. Agriculture has been linked to water quality problems, such as excess nutrient and sediment loads, as shown in Figure 4 below. These nonpoint source pollutants are transferred by runoff to surface water and by leaching to groundwater. Surface runoff occurs when the infiltration capacity of the soil is exceeded by precipitation rate or snow melt. Subsurface drainage can also carry nutrients to waterways. This source of nonpoint source pollution has contributed to eutrophication, algal blooms, degradation of water quality, and various human health and environmental impacts.

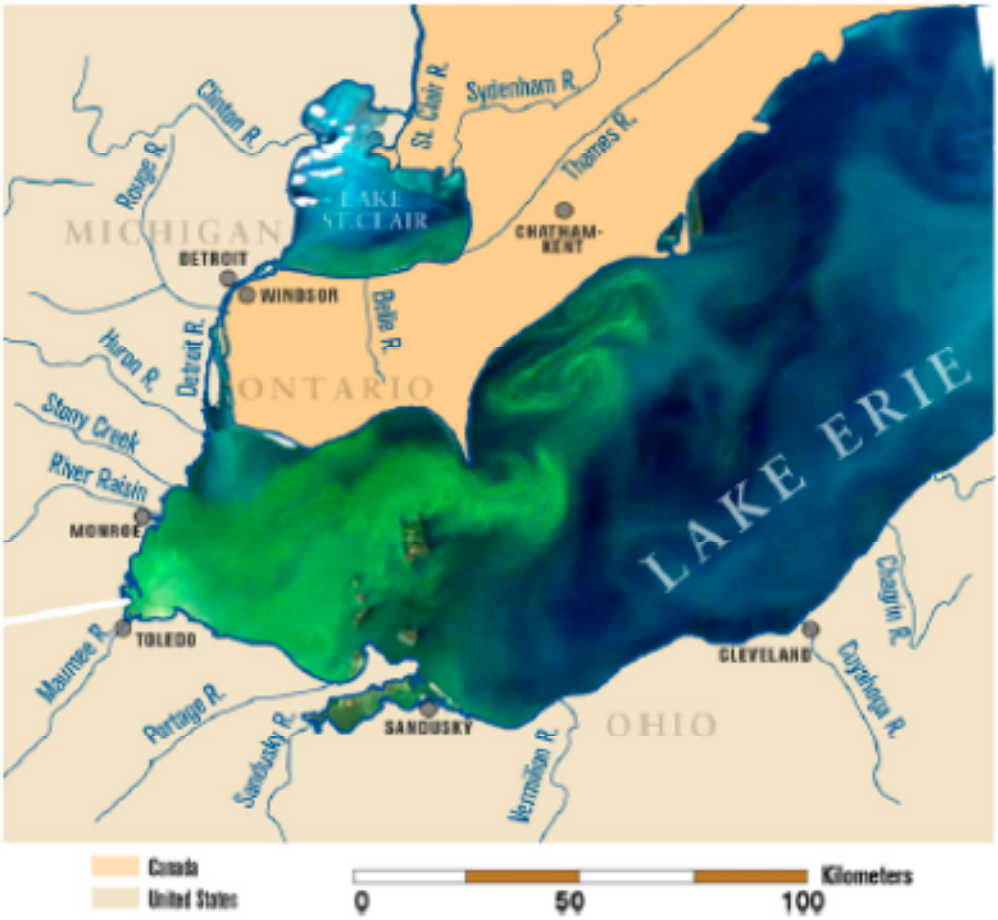

Figure 2: MODIS Satellite Image of Lake Erie on in September 2011 Showing the 2011 Algal Bloom (Michalak et al. 2013)

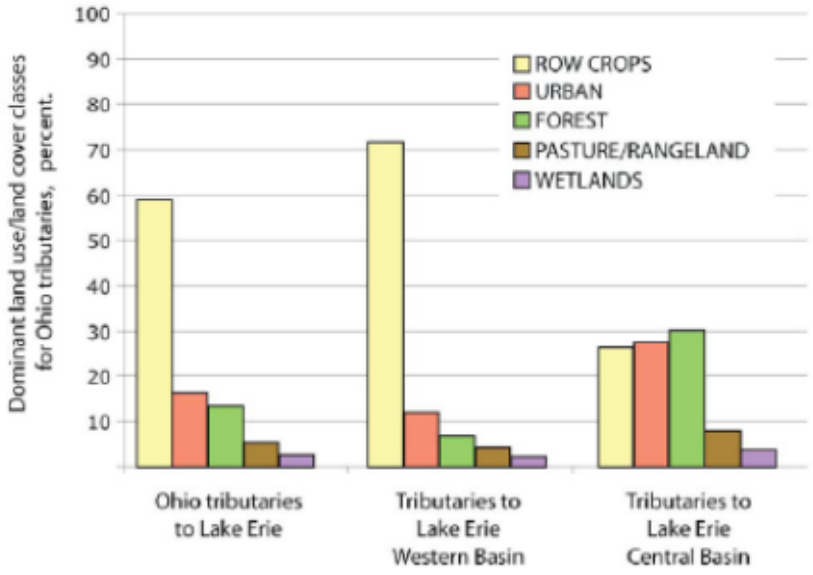

Figure 3: Dominant Land Use for Lake Erie Basin Tributaries (Korleski 2010)

Waterbody type	Leading cause of impairment	Source(s) of impairment
Rivers and streams *39% impaired*	Bacteria (pathogens) *35% of impaired streams*	Animal feeding operations Crop production
Lakes, reservoirs, and ponds *45% impaired*	Nutrients (N and P) *50% of impaired waters*	Riparian pasture grazing Crop production
Coastal resources		
Tidal estuaries *51% impaired*	Metals (primarily Hg)	Sewage treatment plants
Great Lakes shoreline *78% impaired*	Toxic organic chemicals	Contaminated sediments
Ocean shoreline *14% impaired*	Pathogens	Urban runoff Storm sewers
Coral reefs	Nutrients[a]	Animal manures Human septage
Wetlands	Wetland loss (degradation is also an issue)	Agriculture (sedimentation)
Groundwater	Chemical	Underground storage tanks Animal feedlots (8th leading cause out of 20)

Figure 4: Influence of CAFO Manure-Related Pollution on Water Quality of Various Waterbodies (Bowman 2010)

The western basin of Lake Erie receives water from the Maumee and other Rivers, such as the Portage and Sandusky, which carry water from streams, ditches, and smaller rivers located throughout predominantly agricultural areas in northwest Ohio. Ditches are important because they are essential for crop production for direct drainage or as conduits for tile drainage (a series of underground tiles that are installed on poorly drained soils to improve water infiltration) and irrigation effluent (Needelman et al. 2007). They are also important because they often harbor rare

species or species not found in other farmland habitats (Herzon and Helenius 2008). These small tributaries carry fertilizer, pesticide, and manure runoff into the rivers, which then carry the pollution to Lake Erie, having cumulative effects on the degradation of the lake and contributing to the algal blooms that it has been experiencing in the past two decades. This runoff also threatens the health of the ditch, river, and lake ecosystems in which many species live and depend on. Application of animal manures to soils with subsurface drainage has been linked to contamination of the effluent with nutrients, bacteria, and veterinary antibiotics (Hoorman et al. 2007).

Agriculture and Manure

Agriculture in the Lake Erie Watershed takes place on intensively tile-drained soils with high clay content, as seen in Figure 5 below. The tile drainage contributes to high delivery of nutrients to streams and ditches in the area, while the high clay content contributes to rapid surface runoff during rainfall events, carrying dissolved pollutants and fine-grained sediments (Korleski 2010). In fact, agricultural sources are responsible for 66% of suspended solids, 74% of total phosphorus, and 95% of pesticide loadings in surface water (Hatfield 1998). Some of the nutrients in waterways come from animal manure, both liquid and solid sources, which are applied to fields as a source of nutrients.

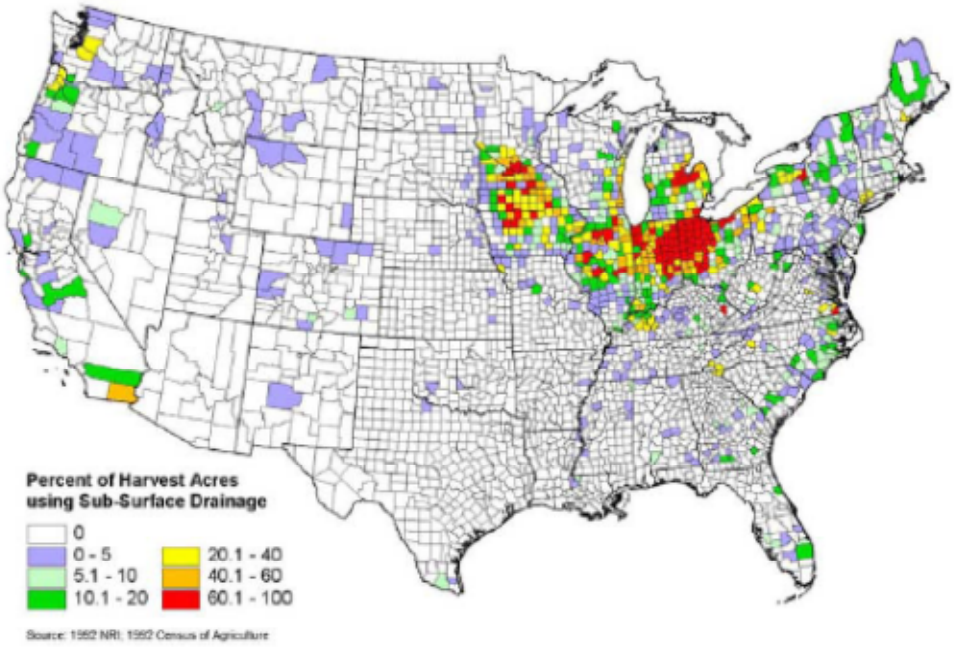

Figure 5: Percent of Harvest Acres in the United State Using Subsurface Drainage in 1992 (Korleski 2010)

Manure can be handled as a solid, semi-solid, slurry, or liquid. Manure of less than 4-5% solids can be handled as a liquid, manure of 5-10% solids can be handled as a slurry, manure of 10-15% solids can be handled as a semi-solid, and manure above 20% solids can be handled as a solid (Ritter and Shirmohammadi 2001). Solid manure is often mixed with urine, bedding, soil, water, and other holding materials, whereas liquid manure is mixed with urine and water. Manure is applied by a variety of methods, including land surface spreading, spraying, and subsurface injection.

In the past several decades, the number of total farms has decreased, but the number of animals per farm has increased, as seen in Figure 6 below. Beginning in the late 1990s, a trend towards construction of large, confined dairy facilities began to occur, with many of them being located in the Lake Erie basin, with the bulk of manure being liquid (Korleski 2010). This is concerning because farms now have more animal manure to apply to the land. They also often have no way of storing the extra manure, so they have to apply the manure to their fields more often or in greater volume. This leads to increased runoff to ditches and streams, which ultimately means increased nutrient loads to Lake Erie. Land application of manure is estimated to contribute about 27% of annual fertilizer input in the Lake Erie basin, with 45-70% of the P in manure being inorganic, and the rest being organic (Korleski 2010).

Type of livestock	Size of operation	1982	1997	Percent increase
Milking cows	300–999	1,281,300	1,835,832	43
	>1,000	578,223	2,135,205	265
Beef	150–299	647,880	721,624	11
	300–999	615,890	836,548	36
	>1,000	325,150	508,268	56
Swine	150–299	948,702	1,196,911	26
	300–999	654,301	2,113,110	223
	>1,000	213,048	2,851,534	1,238
Poultry	150–299	651,816	1,264,537	94
	300–999	881,644	1,650,785	87
	>1,000	835,889	1,832,509	119

Figure 6: Changes in Livestock Production Operations Between 1982 and 1997 (Bowman 2010)

Manure not only carries excess nutrients to waterways and contributes to their pollution, but is also a source of diseases. Livestock manure contains large quantities of microorganisms from the intestine of animals, and are therefore potential sources of approximately 150 diseases. Even though the incidence of human diseases attributed to manure is infrequent (Ritter and Shirmohammadi 2001), it is still a topic of interest and public concern.

Nutrients

Nutrients like nitrogen and phosphorus are essential for crop production, but too much of the nutrients results in runoff that can increase eutrophication in receiving waters. In fact, eutrophication of freshwater ecosystems resulting from anthropogenic nutrient loading to waters has become a global problem. Lake Erie and water bodies around the Midwest have had problems with consistently high levels of nutrients over the past several years. The effects of this are water bodies being choked with vegetation or algae, changes in aquatic flora and fauna composition, increased fluctuations of dissolved oxygen levels, and an increase in total organic load ("Water Quality Parameters & Indicators"). This places stress on the aquatic fauna, reduced aesthetic quality of the water body, and results in odors.

Ditches can transport large amounts of nitrogen (N). Ditches often have high concentrations of N and tend to be N-saturated, compared to other water courses (Needelman et al. 2007). N is added to water bodies and tributaries through mineralization of dissolved and suspended soil organic matter, and through fertilizer and animal manure applications (Hoorman et al. 2007).

Ditches can yield loads of phosphorus (P) that are of environmental concern. Autumn fertilizer application, fertilizer being applied on the surface rather than injected into the soil, and conservation tillage are three management practices that can create conditions for enhanced dissolved reactive phosphorus (DRP) runoff. These management practices have increased in the region in the past ten years, and are trends that have been linked to the 2011 bloom in Lake Erie. These trends are consistent with the observed 218% increase in DRP loadings between 1995 and 2011 from the Maumee River, while runoff increased by just 42% (Michalak et al. 2013). Excess P contributes to hypoxic conditions in Lake Erie and its tributaries. It can also limit their uses for drinking, recreation, and industry. Manure P loss is highly affected by the amount of rain that occurs and the runoff-to-rain ratio during an event (Vadas et al. 2011). Figure 7, below, shows how the variability in weather can affect the year-to-year nonpoint source P loading.

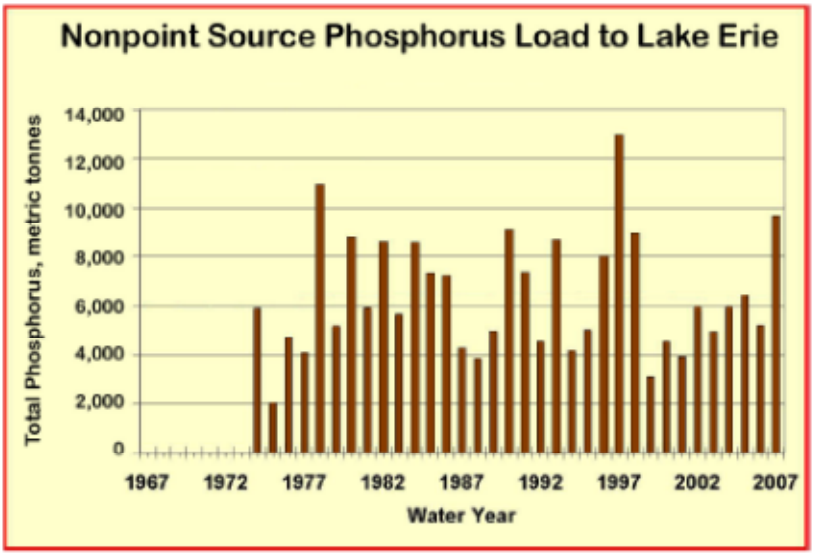

Figure 7: Weather-induced Variability of Nonpoint Source Phosphorus Loading to Lake Erie (Korleski 2010)

Nutrient transport is highly dependent on runoff volume, which depends on factors such as rainfall and soil moisture content. The majority of nutrient loading occurs during storm events (Reutter et al. 2011). Nutrients, including N, P, and potassium (K), that accumulate on the farm or are lost off-farm account for over 80% of all incoming nutrients to lakes and rivers. Because of this, agricultural, primarily the dairy industry in the northeastern United States, is considered the source of most nutrient water pollution. Thus, it is responsible for the deterioration of over 1,500 water bodies (Bowman 2009).

Coliforms and Escherichia coli (E. coli)

Cattle in the United States produce 40 million tons of net manure per year (Bowman 2009). This manure can carry a number of pathogens, with the types of pathogens carried being dependent on the type of animal manure, as seen in Figure 8 below. Livestock manure is considered one of the primary causes of bacterial contamination of surface and ground waters. The application of manure to land and the transport of pathogens with tile drainage to surface waters has been identified as a major pathogen transport pathway (Jamieson et al. 2002). Pathogens are transported with the movement of infiltrating water, surface runoff, and sediment and waste particles.

Pathogen and related disease	Hogs	Poultry		Cattle	
		Turkeys	Chickens	Beef	Dairy
Bacteria					
Escherichia coli				•	•
Colibacillosis					
Salmonella sp.	•	•	•	•	•
Salmonellosis					
Campylobacter sp.		•	•	•	•
Campylobacteriosis					
Listeria monocytogenes					
Listeriosis				•	•
Yersinia sp.					
Yersiniosis	•				
Protozoa				•	•
Cryptosporidium parvum				•	•
Cryptosporidiosis					
Giardia lamblia				•	•
Giardiasis					
Viruses	•	•	•	•	•
Endotoxin	•	•	•	•	•

Figure 8: Bacterial Pathogens, Fungi, Viruses, and Endotoxins That Occur in CAFOs (Bowman 2010)

Fecal coliforms are naturally occurring bacteria found in the intestines of all warm blooded animals and birds. Some are pathogenic, but others indicate that pathogenic bacteria and viruses may be present ("Water Quality Parameters & Indicators"). Bacterial manure pathogens threaten water quality and human health. They are the leading stressor in impaired rivers and streams and the fourth leading stressor in impaired estruaries. Over 150 pathogen, such as those in Figures 9a and 9b below, found in livestock manure are associated with risks to humans, including E. coli 0157:H7, which has caused many waterborne outbreaks related to contamination of drinking water. E. coli and other bacterial species can cause outbreaks in humans due to the ingestion of manure-contaminated food or water (Bowman 2009).

Pathogen	Human disease	Comments
Escherichia coli including *E. coli* O157:H7 and other Shiga toxin-producing serotypes such as O111:NM and O104:H21	1. Diarrhea; 2. diarrhea with bleeding (dysentery); 3. renal failure; 4. hemolytic-uremic syndrome (HUS); 5. arthritis and other rheumatological syndromes	*E. coli* O157:H7 is the most common cause of renal failure in children in the United States.
Campylobacter jejuni	1. Diarrhea; 2. dysentery; 3. systemic infections; 4. Guillain-Barré syndrome (neuromuscular paralysis); 5. arthritis and other rheumatological syndromes	In many countries, *Campylobacter* infections outnumber infections from *Salmonella* species. Commonly found in poultry. Human isolates may be resistant to antibiotics that the prior animal host was exposed to. Most common cause of Guillain-Barré syndrome worldwide.
Salmonella species, including *S. typhimurium* DT104	1. Diarrhea; 2. dysentery; 3. systemic infections that spread from the intestinal tract to the other parts of the body; 4. food poisoning; 5. arthritis and other rheumatological syndromes	Very common human pathogen. More than 3000 different isovars are known, and the number of animal species involved is very large. Like *Campylobacter*, antibiotic resistance in human isolates appears related to antibiotic use in animals.
Yersinia enterocolitica	1. Intestinal infection mimicking appendicitis; 2. diarrhea; 3. arthritis and other rheumatological syndromes	Major reservoir is swine.
Listeria monocytogenes	1. Diarrhea; 2. systemic infections; 3. meningitis	Although the total number of reported cases of human listeriosis in the United States is not high, they are often severe with high fatality rates.
Leptospira species	1. Systemic illness that varies from simple fever to multiple organ failure with hemorrhage (Weil's disease)	This disease is poorly diagnosed in the United States because clinicians do not frequently think of it. Rodent urine is a common vehicle.

Figure 9a: Pathogens and the Human Disease Syndromes That They Cause (Bowman 2010)

Pathogen	Human disease	Comments
Brucella abortus	1. Brucellosis, a systemic illness with a pattern of relapses	Uncommon in North America but common in countries such as Saudi Arabia.
Coxiella burnetti	1. Q fever (nonspecific fever, pneumonia, hepatitis); 2. chronic forms include endocarditis, hepatitis, osteomyelitis	This difficult-to-culture organism is increasingly recognized as a pathogen. A spore-like form can survive for years at room temperature.
Mycobacteria species	Unknown	Unknown
Cryptosporidium species	1. Diarrhea that can be asymptomatic, acute but short-lived, chronic, or quite severe and cholera-like; 2. pneumonia; 3. biliary system obstruction and pain	Essentially unrecognized in the human population before the HIV pandemic; in combination with microsporidia, it causes most AIDS-related diarrhea. Clinically evident pneumonia, biliary disease, or cholera-like diarrhea is seen only in the immunocompromised host. The transmissive form is the oocyst, a cyst-like structure.
Microsporidian species	1. Diarrhea that varies from the subclinical to the profound; 2. systemic infection with fever and weight loss	Recent evidence suggests widespread asymptomatic infection in the human population, with serious disease in the immunocompromised population. The transmissive form is the spore.
Giardia lamblia	1. Diarrhea	Ubiquitous; the transmissive form is the cyst.
Toxoplasma gondii	1. Systemic parasitic infection that can range from asymptomatic disease to a mononucleosis-like, prolonged syndrome, to ocular disease, to fatal progressive disease in people with AIDS; 2. infection of the fetus leading to blindness, seizures, or death	Worldwide pathogen with the curious characteristic that the parasites only form oocysts (the transmissive stage) when infecting cats. Thus, manure-related transmission can occur only when cat feces are present in the manure.

Figure 9b: Pathogens and the Human Disease Syndromes That They Cause (Bowman 2010)

For years, farmers were doing what was considered “green”, recycling manure by applying it to their fields. However, recent research indicates that pathogens from manure can survive on fields and in runoff. The problem with manure pathogens has developed because of farms becoming larger. Water quality expectations have been raised and this has led to concerns about animal waste and drinking water.

Antibacterial Resistance

In North America, antibiotics have been used in beef cattle production since the 1950s (Alexander et al. 2008). Cattle in North America are routinely fed subtherapeutic levels of antimicrobials to prevent disease and improve growth efficiency. This practice has been shown to promote antimicrobial resistance (AMR) in intestinal microflora, including E.coli. It is also found that AMR bacteria are able to persist in the bovine gut in absence of antimicrobial

resistance pressure. (Mirzaagha et al. 2011). Improper use of antimicrobials has implicated development of bacterial resistance to antibiotics. Although this use of various antibiotics may reduce production costs, antibiotic-resistant bacteria may be able to be transferred from livestock to humans through the ingestion of manure-contaminated water or food. This may have tragic effects on human health.

Another issue is that over 75% of the amount of antibiotics ingested is excreted either unchanged or as bioactive metabolites. This can increase the abundance of antibiotic-resistant microorganisms in the biota. Antibiotics may also reduce the anaerobic digestion of manure through their effects on anaerobic bacteria (Bowman 2009). This may lead to even more nutrient loading to water ways.

Currently, little information is available regarding the concentration of antibiotics in animal wastes, or on their fate and transport in the environment. Use of antibiotics is increasing, which could be contributing to the emergence of more antibiotic-resistant pathogens, along with strains that are growing more resistant (Bowman 2009). This can increase the severity of diseases and limit treatment options for sickened individuals.

My Honors Project

This project was interdisciplinary and combined microbiology, chemistry, economics, agriculture, soil science, watershed management, and biology. I explored the effects that the application of solid and liquid cattle manure had on the water quality of subsurface and surface drainage to ditches on the lacustrine clay soils on farms in Putnam County, Ohio. Putnam Count is a rural, highly agricultural county with primarily small to medium-sized farms in Northwest Ohio that is part of the Maumee River Watershed.

I took water samples that led to ditches, and from ditches, as they are some of the only practical locations where quantitative measurements of pollutant transport can occur. I used the equipment available to me in the laboratories of my project advisors, Dr. Robert Midden and Dr. George Bullerjahn. Dr. Midden had lab equipment to test for E.coli, coliforms, ammonia, nitrate, and DRP, and Dr. Bullerjahn had lab equipment to test for lactose-negative and lactose-positive (most likely E.coli) coliforms and antibiotic resistance to ampicillin. Therefore, I tested for these components.

I decided that this is the project that I wanted to do because my specialization is Watershed Management, I have an interest in improving agricultural practices, and I have a strong interest in water quality, especially that of Lake Erie. The problems associated with water pollution and nutrient-loading from manure application to fields are tough economic and scientific problems to solve, and a lot of research and efforts are going into fixing the problems of pollution and antibiotic resistance. I felt like I could contribute to this research by exploring the issues, doing my own field and academic research, and providing possible solutions. Midden and Espen state that “data analysis would be greatly improved if we could obtain manure application records” (2010). This is part of what my research contributes to the issue, including timing and rates of application, as I obtained manure application records from the farmers and have used what they have told me to draw conclusions in this report.

Materials and Methods

Information-Gathering

I began my honors project by researching information related to manure management, non-point source pollution, agriculture and runoff, water quality, and the Lake Erie algal blooms. I also researched soil types in Putnam County, Ohio using www.putnamcountygis.com. I then looked up small dairy farms in Putnam County that resided within the same soil type. This soil type has a high clay content. Before I spoke with farmers about their farms, I assured them that I was doing an independent undergraduate research project, and the point of the project was to allow me to gain experience developing research projects and performing tasks in field and laboratory environments, and I would not give out their names and locations in any reports or presentations I did in order to protect their identities. Once they were assured that I was collecting information and water samples only for educational purposes, the farmers were more comfortable talking to me.

I spoke with several farmers, asking them questions about their farms, the size of their farms, the number of dairy cattle they owned, and the manure they hauled. After I gathered this information, I sorted through the farms and compared the answers that I received. I chose three similar farms that I had easy access to and that had owners who were willing to let me take water samples from their land. I spoke with each of the farmers about the details of their farms, including planting information for the year before sampling (2012) and the growing year of sampling (2013), and yields, as can be seen in Table 1. I converted total crop yields to yields in pounds for each crop field using the bushel size information obtained from University of North Carolina's website (Russ 2001). I also asked where drainage tiles let out, where surface drainage occurred, and about their manure management. I also received records of past soil tests and manure samples, and types and rates of application of fertilizers and any chemicals that they applied to their fields. I chose sites around the county that I would be collecting water samples from.

The first time I collected water samples, I collected from three farms around the county. However, due to time constraints and the abundance of sites I had, my advisors and I decided to focus on a few sites that I could obtain good quality data and information from instead of obtaining only fair data from a large number of sites.

I kept in close contact with the farmers so that I could find out when there was subsurface and/or surface drainage while I was in Bowling Green. I obtained precipitation records, as seen in Table 2 and Table 3, manure application information, and dates in which manure was applied to fields from the farmers as well, as seen in Table 4. I also kept daily precipitation records that I received from www.wunderground.com, using records I collected from a weather station within the county that was close to each of the farms that I was collecting data from.

Field and Laboratory Materials and Methods

The first time I collected water samples, I used a Global Positioning System (GPS) to record the spot that I took samples from. I made sure to use the GPS each time I collected samples in order to find my sampling points and take samples at the exact same points every time. I flagged the points as well. I took samples using leak-proof plastic bags or clean plastic containers. During some samplings I was able to obtain new, clean containers, but other times I reused containers, which I cleaned using distilled water. In order to get samples from subsurface drainage, I put the sample containers under the drainage tile when the water was running, filling up the container. When I collected surface drainage running from a field to a drainage ditch, I would put the container into the water and try to collect as little sediment as possible. Sometimes surface drainage systems were stationary, or there was not enough water so there were just pools of water in the drainage system or on the fields. I collected samples under these conditions, and I

was sure to note drainage condition (running, stationary, pools on field surface) in my field notes. Each time I collected water samples, I always collected samples that directly drain a single application field within 24 hours following a rainstorm or snowmelt on a manure-applied field, or as soon as I could get out to the field site. I kept the samples cool in a refrigerator during any and all storage that took place. During travel from sample sites to the laboratories at Bowling Green, I kept the samples in a cooler with ice packs to keep them cool. All sampling information can be seen in Table 5.

For chemical analysis of water samples, I used a Seal Analytical AQ2 Automated Discrete Analyzer to obtain readings of phosphates (oP) (specifically DRP, which consists of both inorganic and organic forms of P that are dissolved in water and is largely bio-available), nitrates (NOx), and ammonia (NH_3). First, I took a small volume of the water samples I had collected and passed it through a syringe with a nylon 0.45 micrometer microfilter. I then used Pasteur pipets to transfer the filtered water to plastic tubes to be analyzed in the Seal Analyzer. I ran the tests and saved the results to analyze later with Dr. Midden, one of my project advisors.

Fecal coliforms are the most commonly used indicator organisms for estimating the persistence of enteric bacteria in the environment (Jamieson et al. 2002). Therefore, I used fecal coliforms to determine the load of bacteria in the water samples. Two different methods, Colilert tests and Eosin Methylene Blue (EMB) and MacConkey Agar plates, were used to analyze the water samples for biological analysis. It is important to point out that plating on EMB and MacConkey Agar is an independent measure of the microbes from Colilert tests, and the techniques measure slightly different things. The Colilert tests give results for E. coli and coliforms, while the EMB and MacConkey Agar plates give results for total gram negative CFU and the subset of CFU that are lactose positive.

For the method in Dr. Midden's lab, I performed Colilert tests. I put the water samples I collected into IDEXX 120 milliliter vessels with sodium thiosulfate. I then made 1:10 dilutions of each water sample. For this, I made a 1000 milliliter buffer solution by taking 1.25 milliliters of 0.2M sodium phosphate (pH 7.2) and 5 milliliters of 0.4M MgCl2 and adding them to a 1 liter flask, to which I then added distilled water to the 1 liter mark. I took 10 milliliters of the water sample from the undiluted vessel and added it to a separate vessel, to which I then added buffer to the 100 milliliter mark. I did this for each of the water samples I collected so that there were approximately 100 milliliters of undiluted and 100 milliliters of diluted sample. I used diluted and undiluted tap water as a control. I then added Colilert reagent, which consists of bacterial nutrients and color metric reagents, for 100 milliliter samples by IDEXX Laboratories, Inc. to each vessel in order to provide nutrients to promote growth of bacteria, and shook the vessels until the reagent was dissolved. I put each vessel's contents into an IDEXX Quanti-Tray, sealed them, and put them in the incubator at 35 degrees Celsius for approximately 24 hours. I then analyzed the trays, using the IDEXX Quanti-Tray ® 2000 MPN Table, counting coliforms as positive yellow in the large and small wells of the trays. I also counted E. coli as positive fluorescence using a 365 nanometer long wave ultraviolet light.

For the method in Dr. Bullerjahn's lab, I first had to make EMB plates using 37.4 grams of Levine Eosin Methylene Blue Agar, distilled water, and an autoclave. The first time I collected and analyzed water samples, I also made MacConkey Agar plates. However, comparing the EMB and MacConkey Agar plates, I decided to use EMB plates for the remaining time of my honors project. This was decided because both types gave the same results, and the color reaction on EMB plates is more intense and easier to observe. I diluted the water samples by putting 10 milliliters of sterile water in plastic tubes. I then added 100 microliters of each water sample into their corresponding

tubes to make a 1:100 dilution. During some analyse, I would make 1:1000 dilutions as well by adding 10 microliters of each water sample into their corresponding tubes. Doing 1:100 and 1:1000 dilutions allowed me to compare the results between the different dilutions, and allowed me to have 1:1000 dilution plates with fewer colonies that were easier to count in case the 1:100 dilution plates had too many colonies to count. I capped and flipped the tubes to mix them. I added 100 microliters of water from each tube to their corresponding plates, and streaked the sample using a sterile inoculating loop in order to cover the entire plate surface. I put them in the incubator at 37 degrees Celsius for 24 hours. I checked on the plates, but most of the time I left the plates in the incubator for a full 48 hours to allow the colonies to grow larger. I counted the total colony-forming units (cfu) and lactose positive cfu. The lactose-positive cfu were dark purple with a green sheen on EMB plates, and were most likely E.coli.

In addition to chemical and biological analyses, I analyzed some of my water samples for antibiotic resistance. I obtained records of the antibiotics the farmers give to their dairy cattle. One of these antibiotics was ampicillin, an antibiotic given to dairy cows to treat mastitis. This is a common antibiotic used on dairy farms, so I decided to test coliform and E. Coli resistance to ampicillin. The first step in this analysis was to make agar plates. I made EMB agar, allowed it to cool slightly, and added 50 micrograms/milliliter of ampicillin. This is what Mirzaagha et al. did, but with MacConkey agar (2011). I then poured plates and allowed them to cool. After colonies had formed on the regular plates, I transferred the colonies to the ampicillin EMB plates using a sterile inoculating loop and put them in an incubator at 37 degrees Celsius for 48 hours. Those colonies that grew were ampicillin-resistant. From this data, I calculated the total colonies, lactose-positive colonies, and coliform colonies (not lactose-positive) that were antibiotic resistant.

First Sampling

I first collected water samples on the evening of January 20 and the morning of January 21, 2013, after harvesting yet before the farmers began applying manure to the fields. I collected samples from all of the sites that I flagged from all three farms. The data I obtained represents the baseline data (the data without any manure application since the last rain or melting event) that I could use later on to compare manure runoff samples to. The water was not running, so I collected samples directly from the ditches and canals, and some from the surface drainage that I could. I sampled before and after the site at which the runoff would enter the water way, in order to see if there was any effect that the field currently had on the water quality, if any, at that particular time.

Second Sampling

I collected samples for the second time on March 10, 2013. This was after the farmers had applied manure to the fields and right after the first rainfall since the applications.

Third Sampling

I collected samples for the third time on March 26, 2013.

I began analyzing the colonies for antibiotic resistance, specifically for ampicillin resistance, on April 18, 2013, in which I made EMB plates using 40 grams of agar solution. On April 19, I streaked plates with 250 total colonies from the colonies that grew on the EMB plates between January 22 and March 27, the first through the third

time I water sampled. On April 22, I made more agar plates using 40 grams of agar solution. Ampicillin and cycloheximide, an anti-fungal drug, were then added after autoclaving, and I poured plates. On April 23, I plated the colonies that had grown on the EMB plates onto the ampicillin plates. On April 24, I counted the colonies that grew and calculated the percentage of colonies that were antibiotic resistant (the ones that grew). Since mold growth did not seem to be a problem during incubation, cycloheximide was omitted from further ampicillin plates.

Fourth Sampling

The summer of 2013 I was out of state completing an internship, so I did not do any water sampling. The fall of 2013 was dry, so I was not able to obtain water samples again until December. On December 21, a farmer collected samples for me from my sites because I was unable to get out to the sites before the water level in the ditch reached over the drainage tiles.

I tested for antibiotic resistance a second time. I made ampicillin plates using 50 milligrams/milliliter of ampicillin on December 30, 2013. After the colonies grew on the EMB plates, I streaked the colonies onto ampicillin plates. Two days were given in order to see if more colonies grew or grew larger.

Fifth Sampling

I collected water samples on February 21, 2014. It had rained and large amounts of snow had melted, but were beginning to refreeze, so the water at most spots were a little slushy.

For the ampicillin resistance test, I counted the colonies that grew on the ampicillin plates and waited an additional day to see if the colonies grew bigger, and counted them again.

Results

Nutrient Results

Nutrient levels were analyzed according to the following guidelines: Phosphate levels below 0.1 ppm (parts per million) are low, between 0.1-0.5 are moderate, between 0.5-1 are moderately high, and above 1.0 are high. Ammonia levels above 0.1 ppm are high. Nitrate levels below 5 ppm are low, between 5-10 are low moderate, between 10-15 are moderately high, and above 15 are high (*Advanced Wastewater Treatment to Achieve Low Concentration of Phosphorus).* The results I obtained can be seen in Table 6, with the sites I analyzed consistently and the results highlighted in yellow. Figures 10 through 16, below, show site-by-site results from each water sampling. Figure 17 displays the nutrient runoff data from each of the sampling sites on 12/21/2013, the date in which most of the sites experienced their highest ammonia, nitrate, and/or phosphate flows. Table 7 shows the concentration levels of ammonia, nitrates, and phosphates from the water samples.

The largest number of sites with high ammonia and phosphate concentrations occurred on 12/21/2013, with the water samples showing low, low-moderate, and moderately high concentrations of nitrates on this date. However, the highest concentrations of both ammonia and phosphates were obtained from water samples from site 2B on 2/21/2014, while the highest concentration of nitrates came from site 6C on 3/26/2013.

Site 1B shows consistently high ammonia concentrations from all three dates I obtained water samples from it. Nitrates were never above the low-moderate range, while phosphate varied between moderate and high. The date with the highest concentration levels for 1B was 2/21/2014

Site 2B shows normal levels of ammonia, all below 0.1 ppm, except for on 2/21/2014, when the concentration is extremely high at 17.3 ppm, the highest seen throughout the course of the project. Nitrates are low in every water sample, while phosphates vary from moderately high to high, with the highest concentration of phosphates seen throughout this project seen on 2/21/2014. This is also the date with the highest concentration levels for 2B.

Site 1C shows low ranges of ammonia on 1/20/2013-1/21/2013, but high concentrations on 12/21/2013 and 2/21/2014. Nitrate levels were consistently low, while phosphates varied from moderate to high, with the highest concentration was from 12/21/2013. This was also the date in which the highest concentration levels for 1C could be seen.

Site 5C shows high levels of ammonia on 12/21/2013 and 2/21/2014. Nitrates were low on all sampling dates, while phosphates were always high. Ammonia and phosphates were both high on 12/21/2013 and 2/21/2014.

Site 6C showed low ammonia concentrations on 3/10/2013, but high concentrations on 12/21/2013. Nitrate levels were low on 3/10/2013, but were moderately high on 12/21/2013, and were the second highest out of all the water samples on this date. Phosphate levels varied from moderately high to high. The date with the highest concentrations levels was 12/21/2013.

Site 1D showed low levels of ammonia on the first sampling date of 1/20/2013-1-21-2013, but high levels on the 3/2013 sampling dates. Nitrates were consistently low, while phosphate levels were consistently moderate. The date with the highest concentration levels was 3/10/2013.

Site 2D showed high levels of ammonia on all sampling dates, while nitrates varied from low to low-moderate to moderately high. The highest nitrate concentration from the project came from site 2D on 3/26/2013. Phosphate concentrations varied from moderate to moderately high. The date with the highest ammonia and phosphate concentrations came from 3/10/2013.

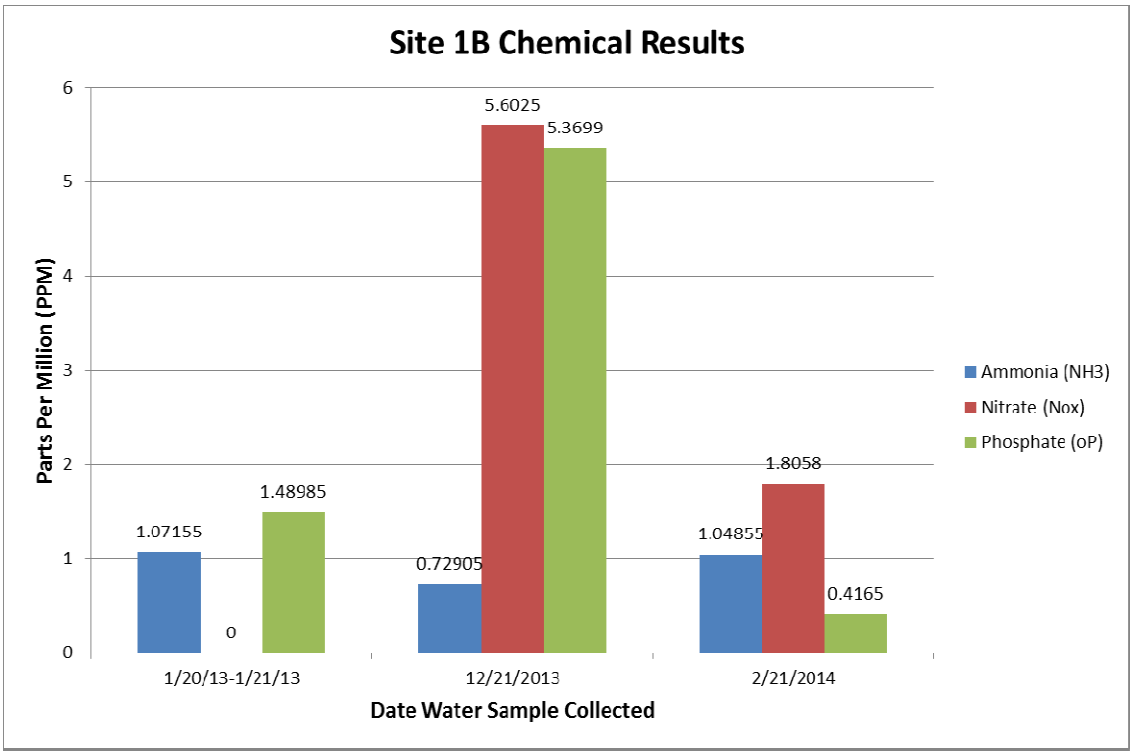

Figure 10: Site 1B Nutrient Runoff Results

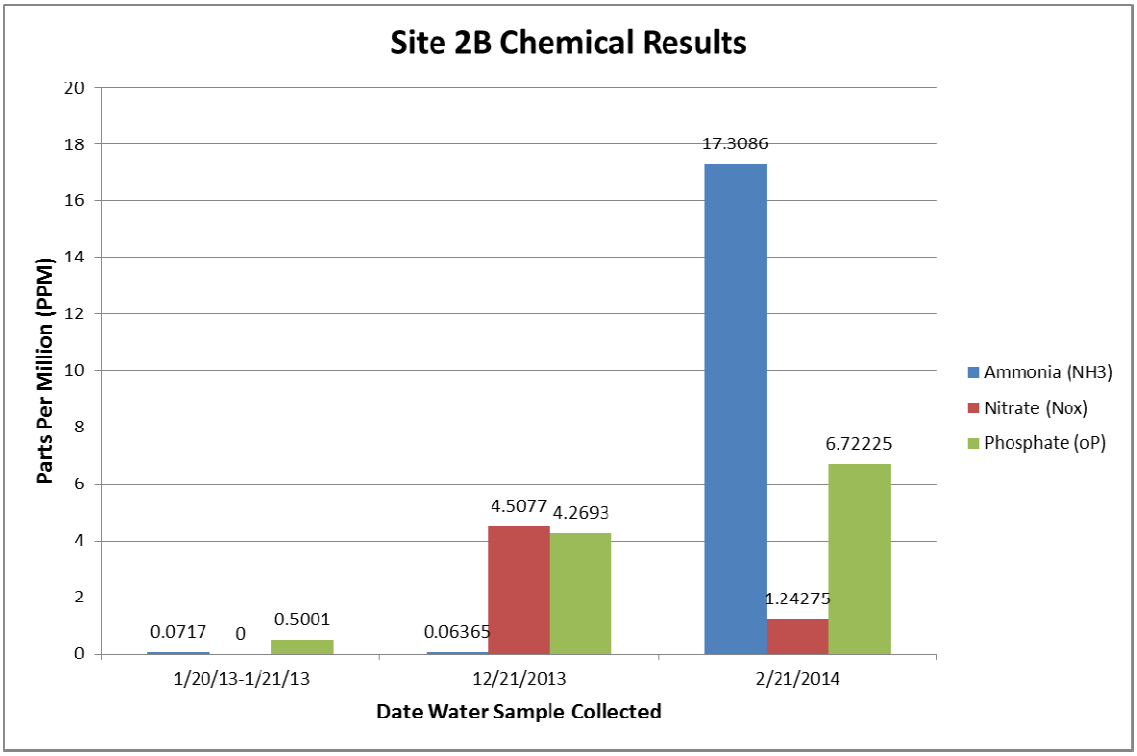

Figure 11: Site 2B Nutrient Runoff Results

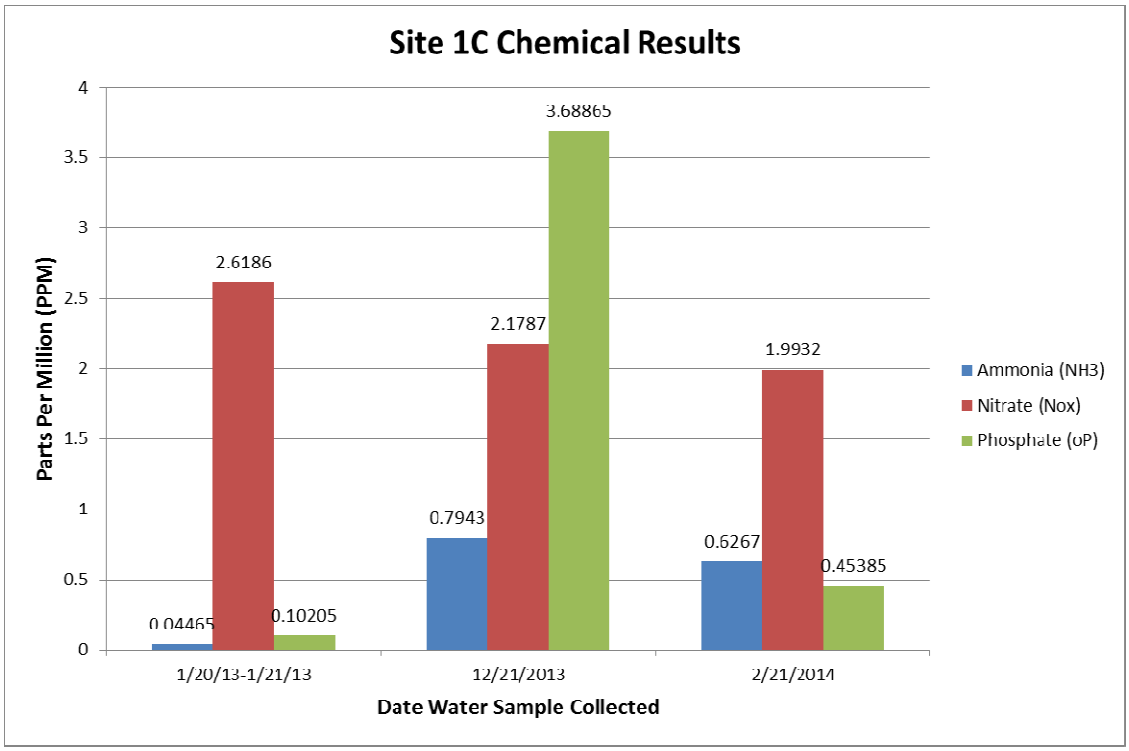

Figure 12: Site 1C Nutrient Runoff Results

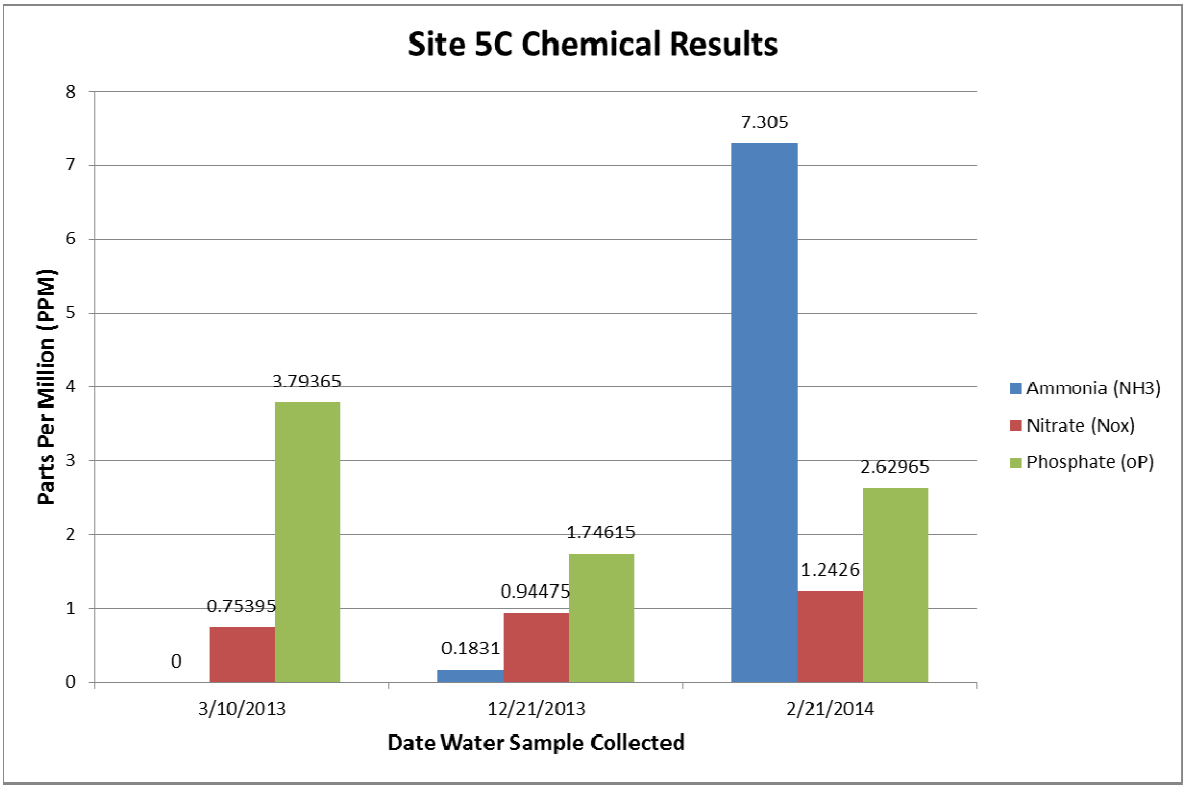

Figure 13: Site 5C Nutrient Runoff Results

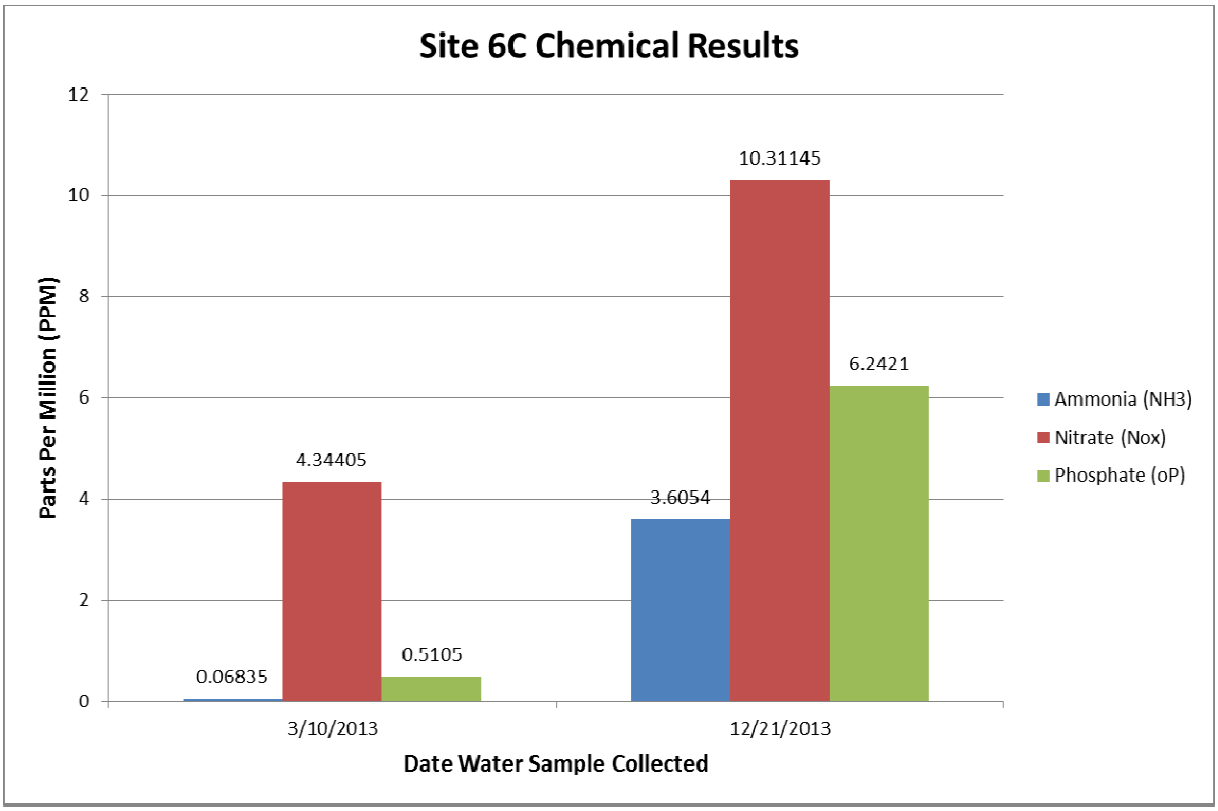

Figure 14: Site 6C Nutrient Runoff Results

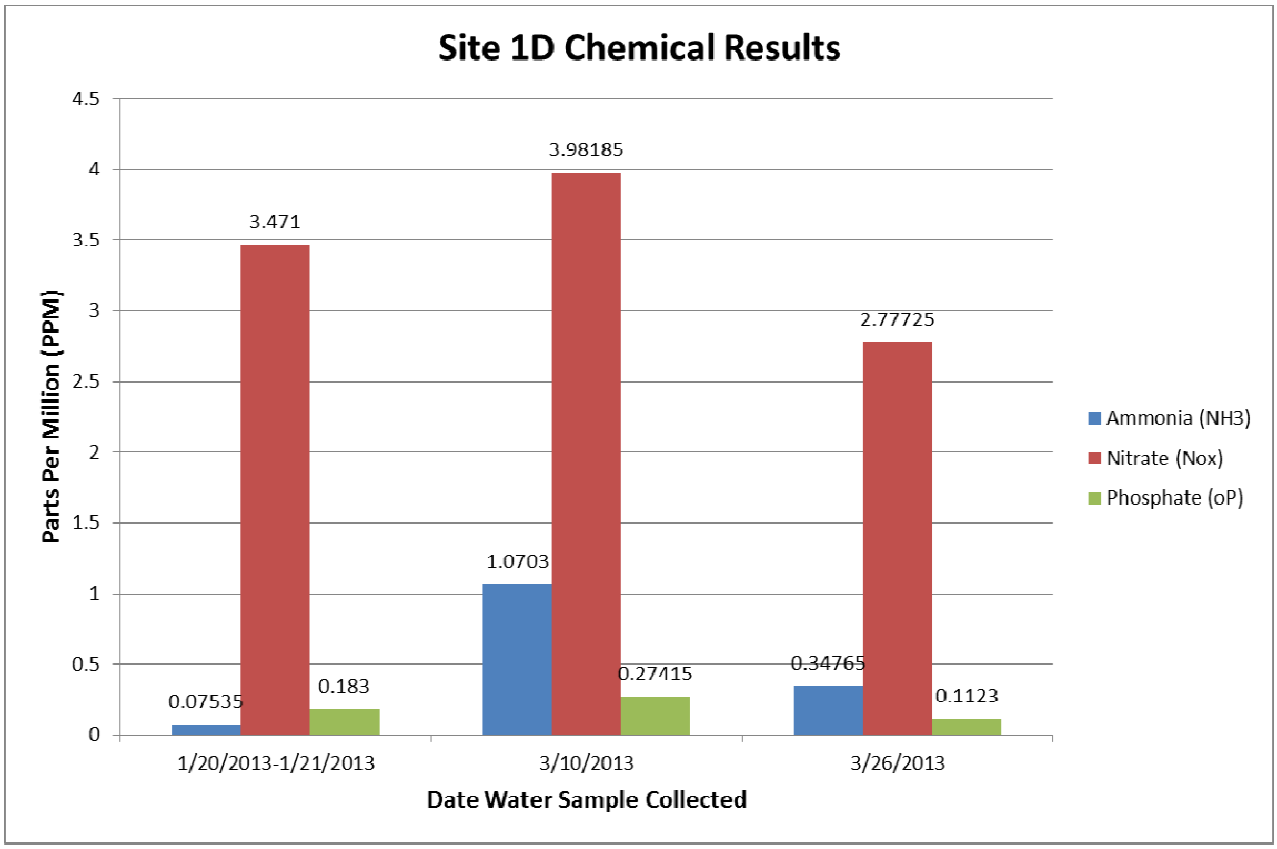

Figure 15: Site 1D Nutrient Runoff Results

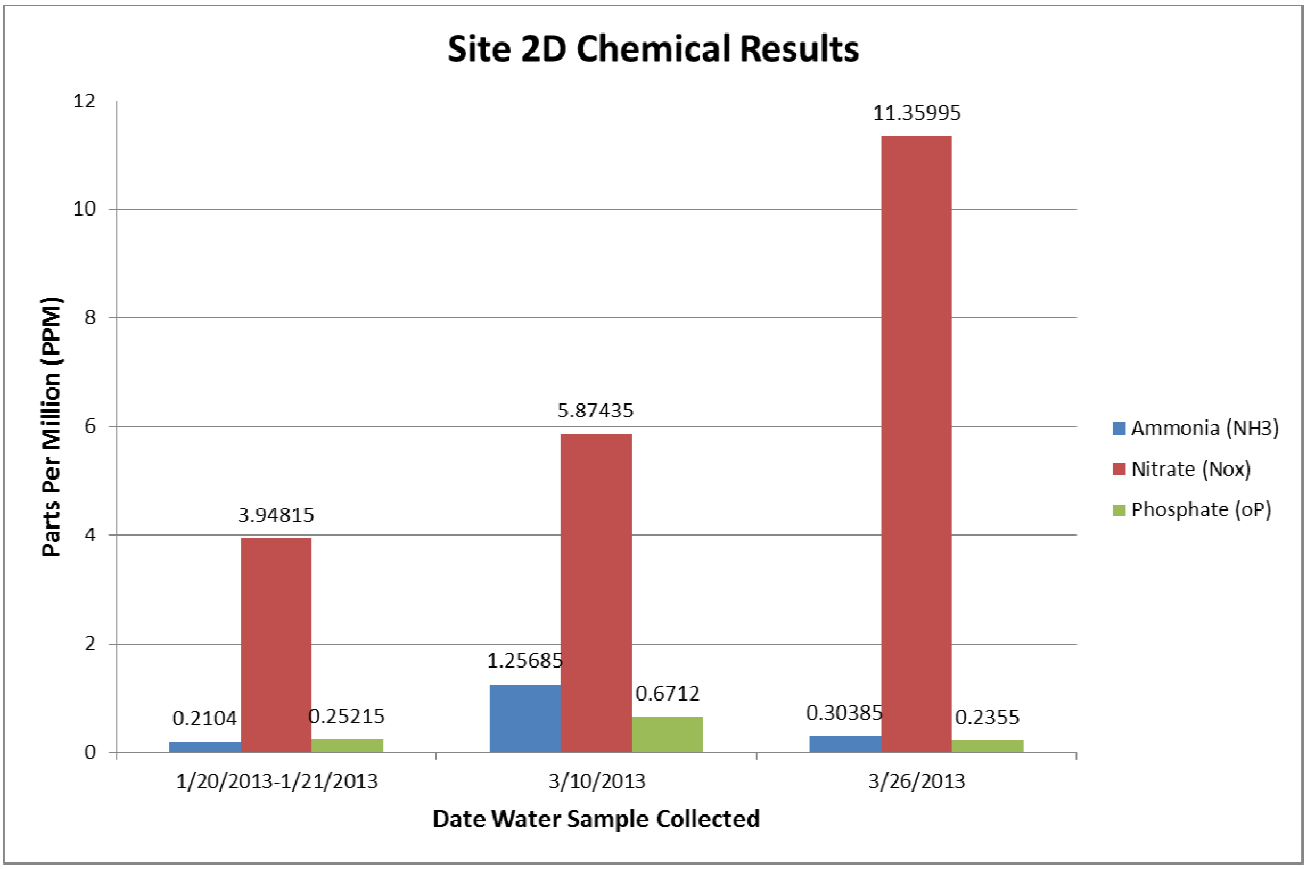

Figure 16: Site 2D Nutrient Runoff Results

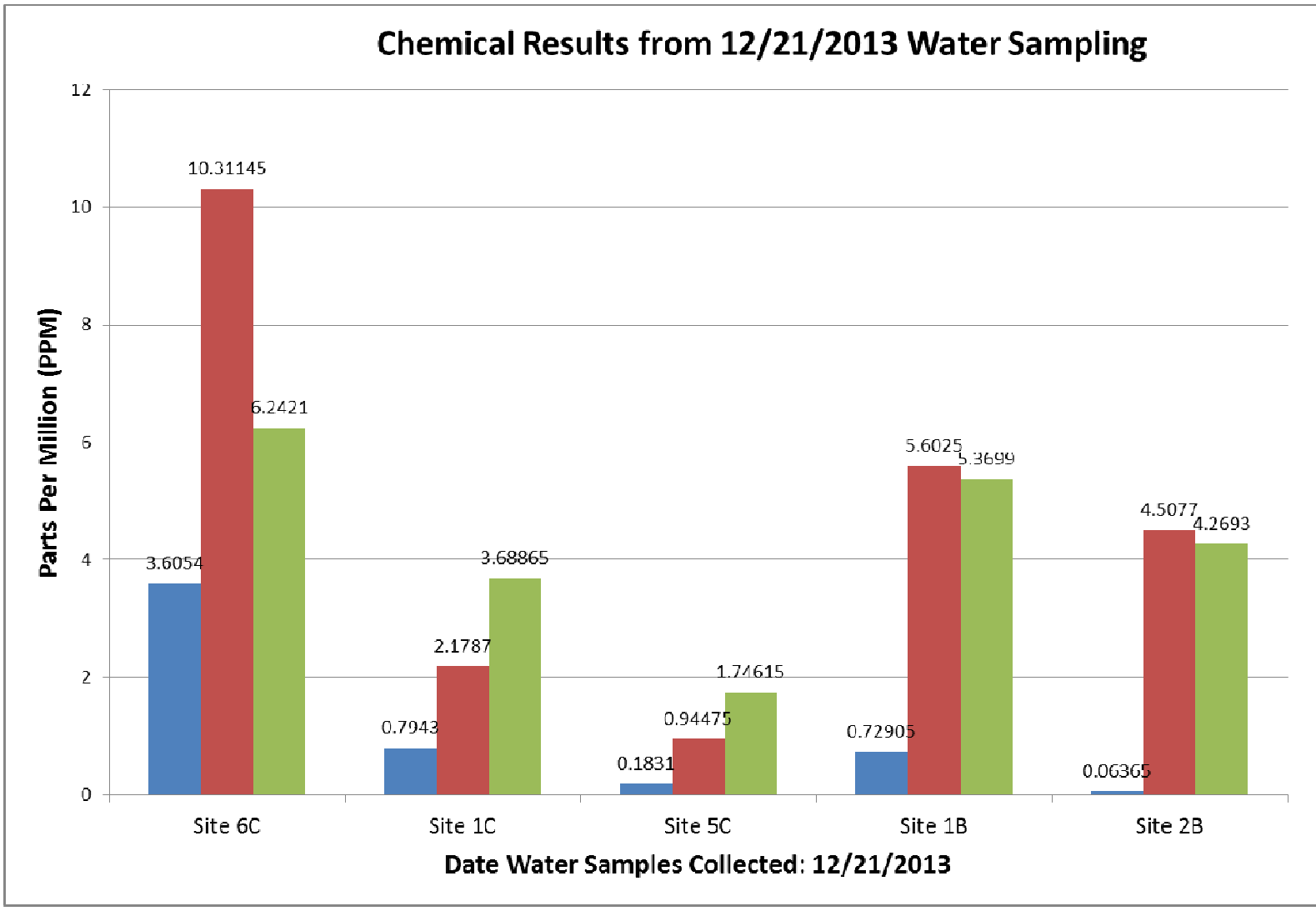

Figure 17: Nutrient Results From All Sites Where Water Samples Were Collected on 12/21/2013 (The Date In Which the Most Sites Had There Maximum Nutrient [Ammonia, Nitrate, or Phosphate])

Biological Results: Colilert Water Trays

As stated earlier, two independent biological measures of coliforms were performed throughout this project. The Colilert tests gave results for total coliforms and E. coli present in the water samples, while EMB plates gave total gram negative CFU and lactose positive CFU. The lactose positive CFU is a proxy for E. coli, but is not a guarantee that E. coli is present. Although the results of both tests can be compared to one another to some degree, they measure slightly different things.

Colilert tests work differently than plating water samples. In the Colilert tests, two chemicals are present. One is broken down by coliforms and causes a yellow color to form, while the other is broken down by E. coli and forms a fluorescent color that can be seen under UV light. There are 97 total wells in the water trays. By counting the wells that turn yellow and the wells that are fluorescent, I was able to estimate the number of coliform and E. coli present in

the water samples. The IDEXX Quanti-Tray ® 2000 MPN Table was used to indicate the MPN (most probable number) of colonies, which is comparable to CFU (colony forming units). This test counts up to 2419.6 colonies. A 1:10 dilution is performed in addition to the undiluted water sample test because it can allow for up to 10 times the number of colonies to be detected compared to the undiluted test. The diluted tests allowed me to estimate higher numbers of colonies present. However, the dilution test is not as reliable as the undiluted test at lower concentrations, due to the reduced resolution. Therefore, if smaller numbers of colonies are observed, it is best to rely on the undiluted sample for more accurate results. However, if the maximum number of colonies that the tests and table can detect is observed, the 1:10 dilution test is better to rely on. For analysis, if the undiluted sample is less than 2419.6 MPN/100ml (the maximum value), then the mean of that value and the 1:10 dilution value should be taken. If the undiluted sample had all water wells be positive (had the maximum value), then only the 1:10 dilution value is taken. This pertains to total coliforms and E. coli. The results from the water samples I collected can be seen in Table 6, with the sites I analyzed consistently and the results highlighted in yellow. I analyzed the values from this table in order to determine the final total coliform and total E. coli from each sample according to the guidelines just specified. This information can be seen in Table 8.

E. coli levels can be analyzed according to the following guidelines: For bathing beach waters: 235 colony forming units (CFU)/100 ml for designated beach areas, 298 CFU/100 ml for moderate full body contact recreation, 409 CFU/100 ml for lightly used full body contact recreation, and 575 CFU/100 ml for infrequently used full body contact recreation. For primary contact waters, referring to activities where an individual is completely immerse in water: 126 CFU/100 ml for Class A, 161 cfu/100 ml for Class B, and 206 cfu/100 ml for Class C. For secondary contact waters: 1030 cfu/100 ml (*Ohio 2014 Integrated Report: Section F: Evaluating Beneficial Use: Recreation*). According to the Erie County Department of Health, E. coli levels of 235 CFU/100 ml is the standard level of contamination that means people should limit direct contact with the water ("Beach Sampling"). It is the single-sample bathing-water standard in Ohio (Francy et al. 2003). The possibility of illness increases with higher levels of E.coli. Therefore, if E.coli levels are between 235 – 1,000 CFU/100ml, Lake Erie beaches are posted with a swimming advisory, meaning swimming is permitted but the public should take precautions. If E. coli levels are higher than 1,000 CFU/100ml, beaches will be posted with a swimming restriction and swimming is not allowed ("Beach Sampling"). To make comparisons between the Colilert water tray results and the EMB plate results, I used the Erie County Department of Health's information about E.Coli contamination of beaches to analyze my data. Results were analyzed as to whether they had E.Coli levels below 235 CFU/100 ml, between 235 and 1,000 CFU/100 ml, and above 1,000 CFU/100 ml. Table 8 displays these results.

Coliform bacteria occur in nature and are often, but not always, related to the presence of human activity. Since coliforms occur naturally, I did not use coliform bacteria concentrations as a criterion to analyze by assigning low, moderate, and high values to like I did for E. coli. High coliform numbers can, however, indicate the presence of E. coli, so the data is still important to obtain. For example, site 2B showed the maximum and highest level of E. coli on 2/21/2014 out of all other sites and sampling dates throughout the project, and also showed the maximum and highest level of coliforms. Comparatively, from the 3/26/2013 sampling, site 1D showed the maximum number of coliforms present, but the lowest number of E. coli colonies present. Therefore, the present of high coliform levels does not necessarily indicate the presence of high levels of E. coli.

The 12/21/2013 and 2/21/2014 sampling dates show samples from two sites having high concentrations of E. coli, and one site having low concentrations. Two sites in December showed moderate E. coli levels in the water, and one site in February of 2014 shows moderate E. coli levels in the water sample. 12/21/2013 and 2/21/2014 were the dates in which the water samples from the sites were the most concentrated with E. coli.

Site 1B showed high levels of E. coli contamination in samples taken from 12/21/2013 and 2/21/2013, but low levels from the 1/20/2013-1/21/2013 sampling.

As previously stated, on 2/21/2014, site 2B showed the highest E. coli levels seen from all of the Colilert tests throughout the project, with high levels of coliform bacteria also present. Site 2B displayed low levels of E. coli on the other two dates it was sampled.

Site 1C showed various E. coli contamination results. Sampling from 1/20/2013-1/21/2013 showed low levels, 12/21/2013 showed high levels, and 2/21/2014 showed moderate levels.

Site 5C showed low E. coli levels from the 3/10/2013 and 2/21/2013 water samplings, but moderate levels from the 12/21/2013 sampling.

E. coli levels for site 6C were low the first time I collected a water sample for the site on 3/10/2013, but were moderate from the 12/21/2013 sampling.

Levels of E. coli for site 1D varied from moderate from the 1/20/2013-1/21/2013 water sampling, but were low for the two March samples. Site 1D had the lowest E. coli levels of all the sites throughout the project on 3/26/2013.

Site 2D had low E. coli levels in the January and 3/10/2013 water samples, but moderate levels in the water sample from 3/26/2013.

Due to the location of the sites, I can rule out the possibility that any of the samples were contaminated by drainage from household septic tanks in the area.

Biological Results: Plates

Lactose positive colony levels, which is most probably E. coli colony levels, resulting from plating were analyzed the same way that levels in the Colilert water trays were. The results from the water samples I collected can be seen in Table 9, with the sites I analyzed consistently and the results highlighted in yellow. Various levels of lactose positive coliform contamination can be seen in Table 10, which breaks up the results. Figures 18 through 24 below show site-by-site lactose positive results. Due to the EMB plates being used consistently throughout the project, I will discuss the results from just the EMB plates and not the MacConkey plates.

2/21/2014 was the date showing the highest levels of lactose positive colonies and therefore was the date of the highest contamination. The water sample from 2B on 2/21/2013 was observed to contain the highest concentration of lactose positive colonies throughout the project. Site 1D may have been the least contaminated, as levels of lactose positive colonies on all three sampling dates were low.

Site 1B showed high levels of lactose positive colonies in the 1/20/2013-1/21/2013 water sample, and low levels in the 2/21/2014 water samples. The 1:100 dilution plates showed low levels on 12/21/2013, while the 1:1000 dilution plates showed moderate levels.

Site 2B showed low levels of lactose positive colonies in the water samples from 1/20/2013-1/21-2013 and the 1:100 dilutions from 12/21/2013, but moderate levels in the 1:1000 dilutions from 12/21/2013. Site 2B showed the high lactose positive colony levels from the 2/21/2014 sampling. This is also the highest level seen amongst all the sites throughout the project.

Site 1C showed low levels of lactose positive colonies in the 1/20/2013-1/21/2013 water samples and in the 1:100 dilutions of the 12/21/2013 water samples. Moderate levels were shown in the 1:1000 dilutions of the 12/21/2013 water samples and the 2/21/2014 water samples.

Site 5C showed various levels of lactose positive colonies throughout the project. Low levels were seen in the 3/10/2013 sample and the 1:100 dilutions of the 12/21/2013 samples. Moderate levels were seen in the 1:1000 dilution samples from12/21/2013 and in one of the plates from 2/21/2014. High levels were observed on the other plate from 2/21/2014

Site 6C showed low levels of lactose positive colonies present in the 3/10/2013 water sample and the 1:100 dilutions from 12/21/2013. Moderate levels were observed in the 1:1000 dilution plates from 12/21/2013.

Site 1D showed low concentration levels of lactose positive colonies in all samples from all three dates in which water was sampled there. Site 1D, therefore, seems to be the least contaminated site in terms of concentration of lactose positive bacteria

Site 2D showed low concentrations of lactose positive colonies in samples from 1/20/2013-1/21/2013 and 3/10/2013 water samplings. The sampling from 3/26/2013 were observed to contain moderate levels of lactose positive colonies.

Key:

Below 235 CFU/100 ml
Between 235 and 1,000 CFU/100 ml
Above 1,000 CFU/100 ml

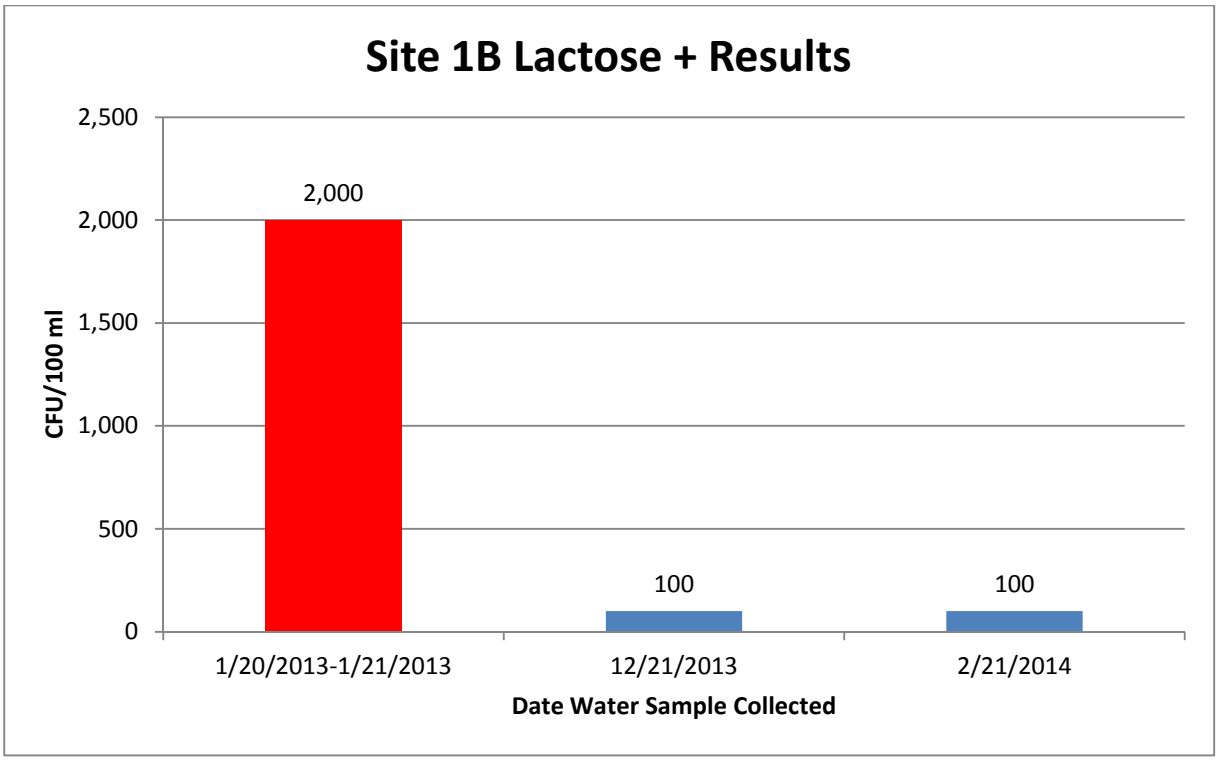

Figure 18: Site 1B Lactose Positive Results

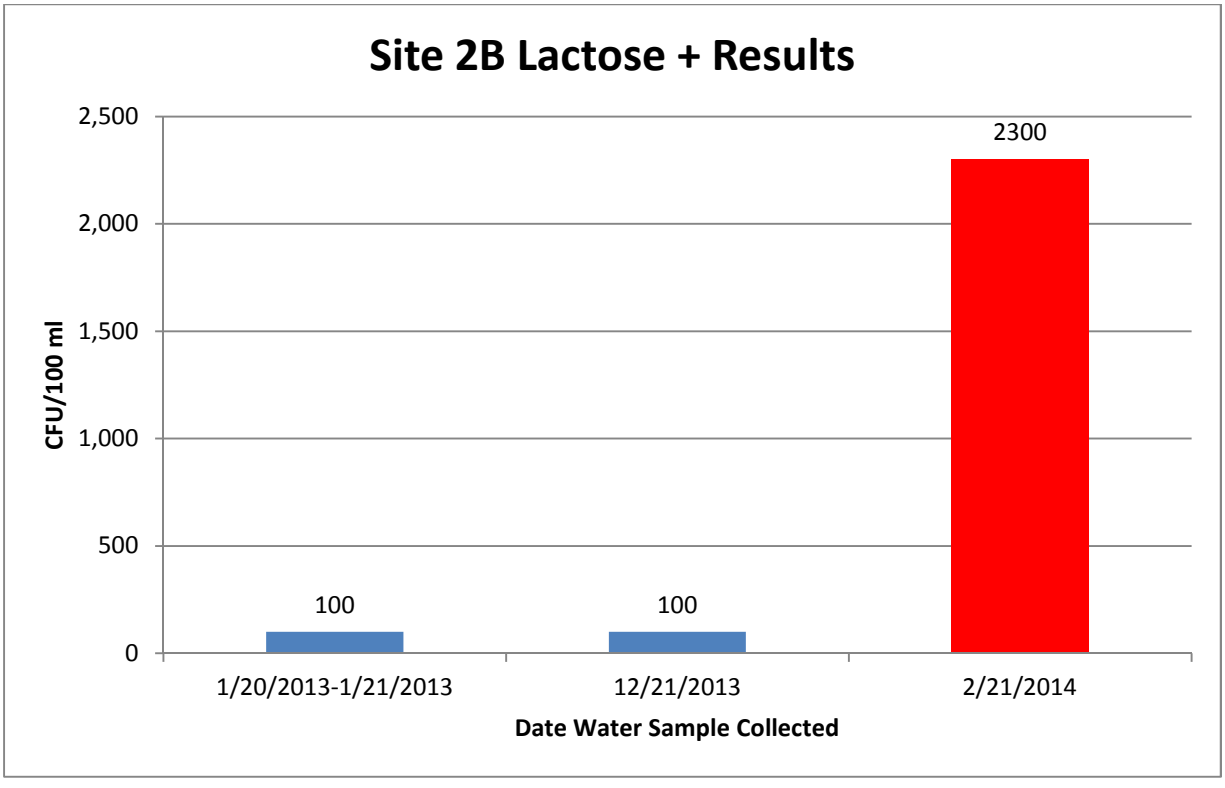

Figure 19: Site 2B Lactose Positive Results

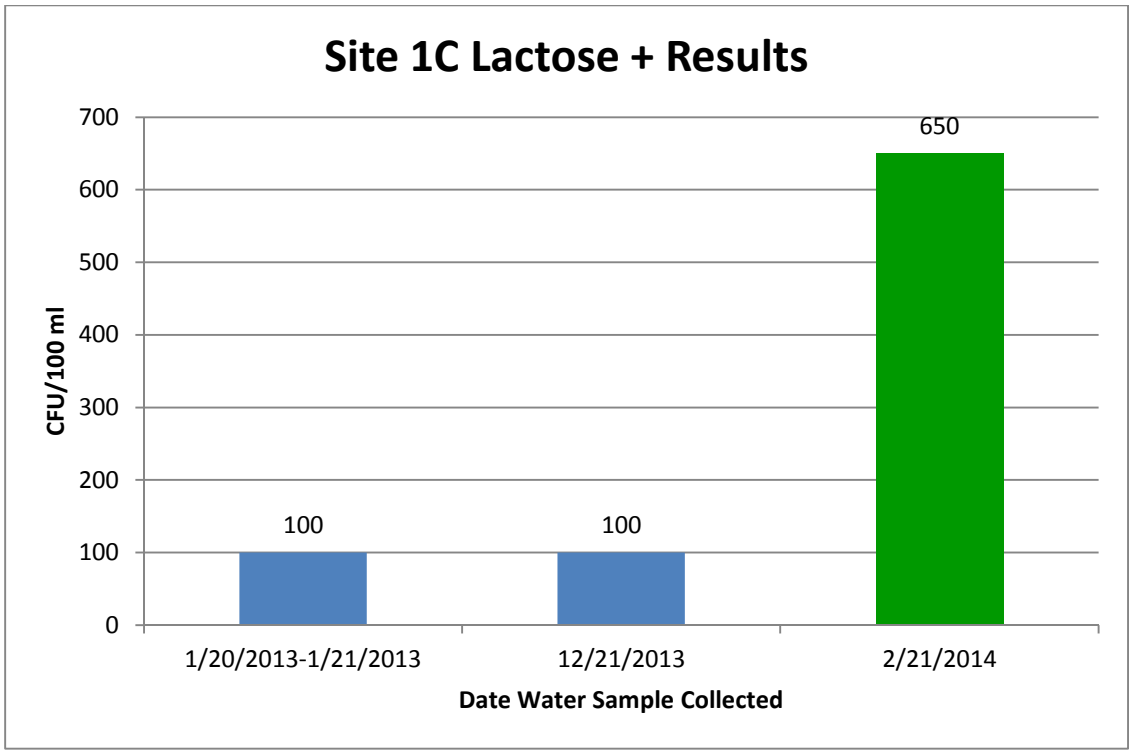

Figure 20: Site 1C Lactose Positive Results

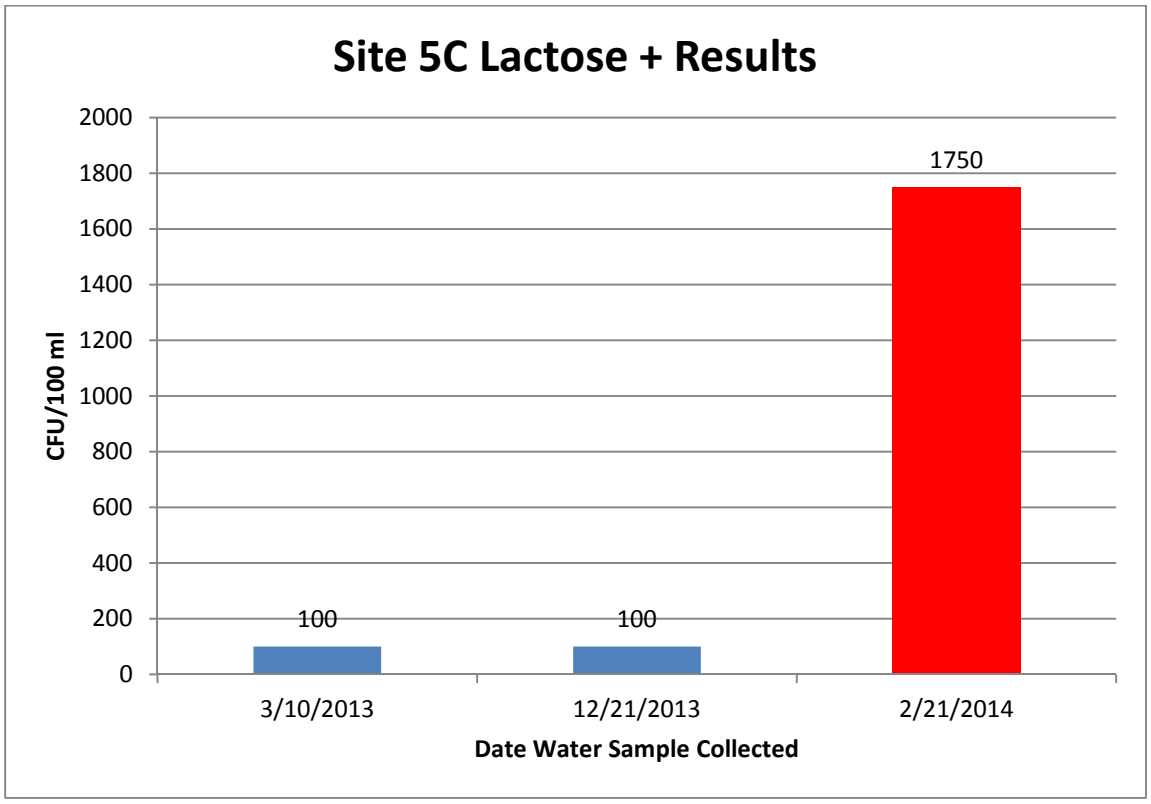

Figure 21: Site 5C Lactose Positive Results

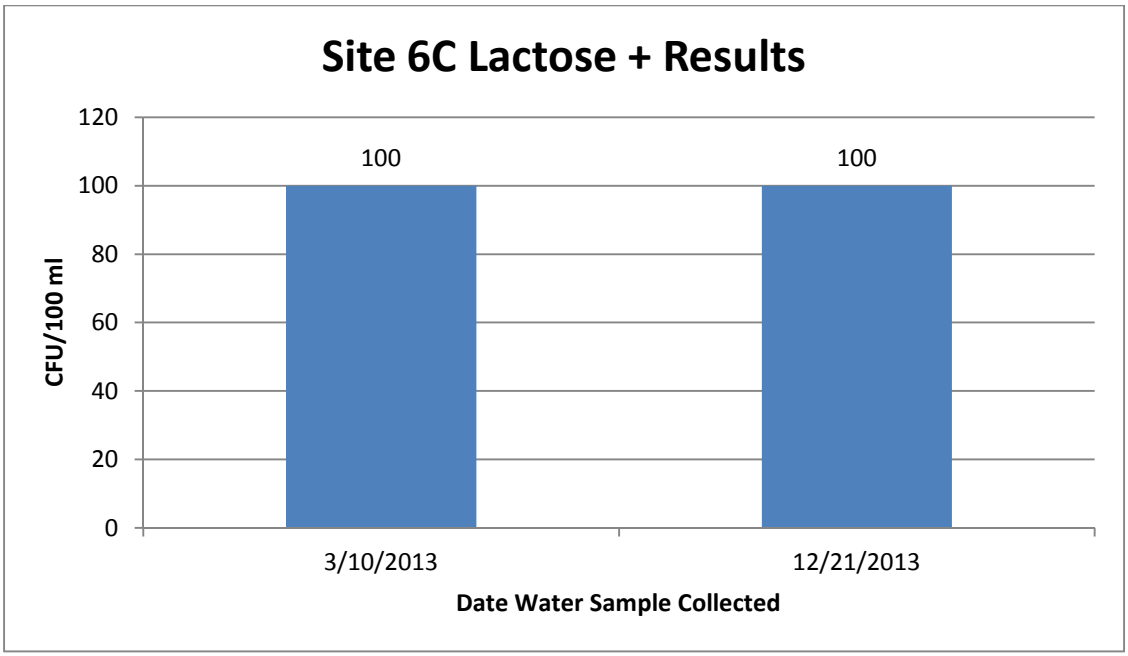

Figure 22: Site 6C Lactose Positive Results

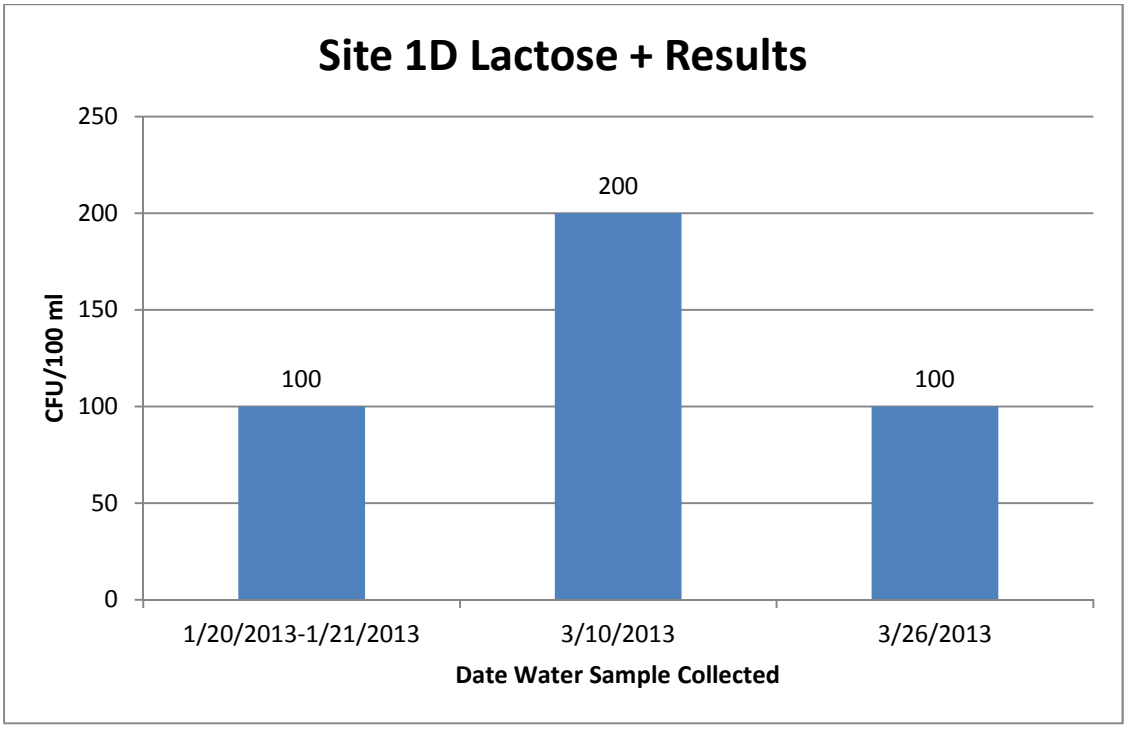

Figure 23: Site 1D Lactose Positive Results

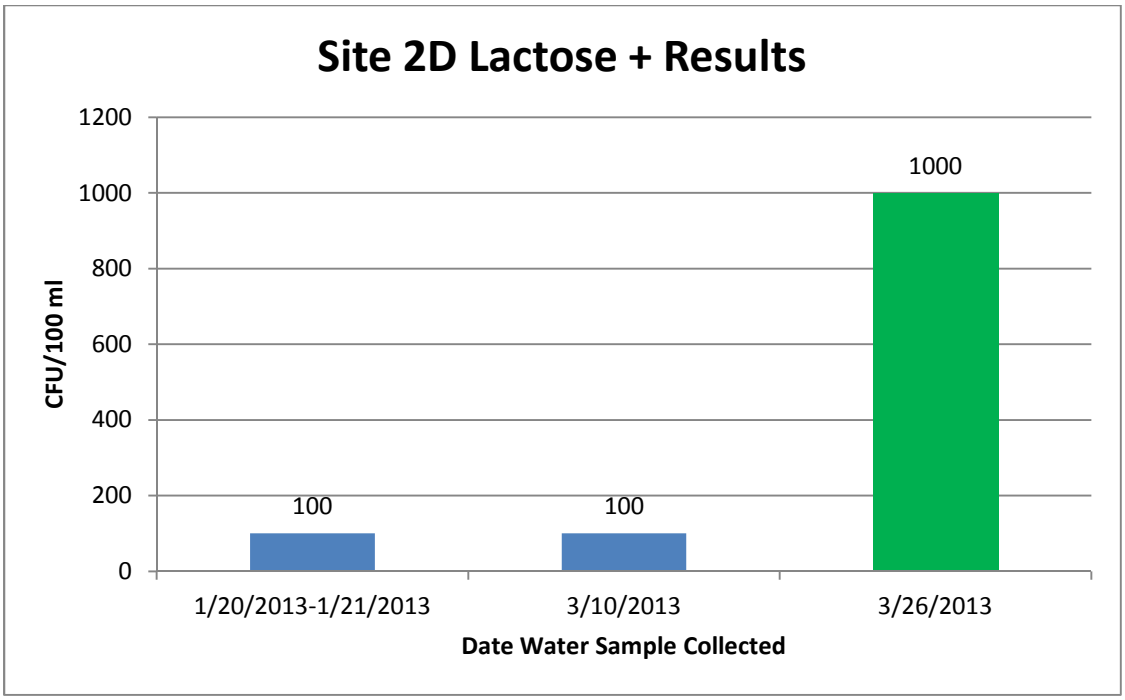

Figure 24: Site 2D Lactose Positive Results

Antibiotic Resistance Results

I tested for high level resistance to the antibiotic ampicillin, as I plated 50 micrograms/ml. I performed ampicillin resistance analyses three times: Once on April 23, 2013 for colonies that grew on EMB plates from the January and March 2013 water samplings, once on January 3, 2014 for colonies that grew on EMB plates from the December 2013 water sampling, and once on February 27, 2014 for colonies that grew on EMB plates from the February 2014 water sampling. A summary of these results showing how resistant the community is can be seen in Table 11. In total, the percentage of colonies that grew on AMP plates that were resistant was 60.28%, the percentage of colonies that grew on AMP plates that were lactose positive was 29.36%, and the percentage of lactose positive colonies that grew on AMP plates that were resistant was 56.58%.

Discussion

Results: 1/20/2013-1/21/2013

Sampling from January 2013 was meant to represent baseline data before manure was applied to the fields later on in the year. Water was obtained from the ditches themselves, or from the surface drainage for site 1B and 1D. Most of the nutrient values were low or moderate for the water samples taken from the sampling sites. Site 1B, however, contained high values of ammonia and phosphate. In this case, this could make sense because this suggests that site 1B was carrying more concentrated water than those other sites, which carried higher volumes of snow and ice melt. Site 1B also occurs next to a gravel road, and runoff from this road was likely carried in the surface drainage by site 1B. However, it is not clear what the effect of the road runoff would be. It is not definite that road runoff

would cause high nutrient concentrations. These reasons may also explain the high concentration of lactose + colonies observed on EMB plates, and the highest concentration of coliforms out of all the sites on this date.

Results: 3/10/2013

Water sampled from site 2D on this date did not allow for an accurate measurement of nutrients and biological data on this date because the water draining from the tile was mixing with water from the ditch. Samples from site 1D, however were accurate. Site 1D is a site of surface drainage, draining fields 15 and 14, in which solid manure had been applied in February and early March. It therefore makes sense that ammonia levels are higher due to the manure runoff. However, E. coli concentrations were the lowest at this site, which was unexpected.

Sites 5C and 6C, both surface drained sites, had water samples collected from them in order to obtain more baseline data, with surface water coming directly from fields instead of from the ditches themselves. Both had low ammonia and nitrate levels, which makes sense because no manure had been applied to the fields. Phosphate levels were moderately high to high, which suggests that the soil may already have a buildup of phosphates. If this is true, then phosphate application in terms of manure and other fertilizers should be decreased in order to decrease nutrient pollution to the ditch.

It is possible that the soil at this site may have a buildup of phosphates. I obtained soil test results from a small farm in Putnam County, and three out of the five fields tested had soils with very high phosphate concentrations. One field had soils with high concentrations, and one field had soils with low concentrations of phosphates. This is from a farm with fewer cattle than the farm that sites 5C and 6C were on. The soil types on the two farms were the same and contain high clay content. These results suggest that the sites 5C and 6C may be on fields that had high phosphate concentrations in its soils to begin with, which may have contributed to the high concentrations in the runoff.

Results: 3/26/2013

Site 1D, a surface drained site draining fields 14 and 15, in which solid manure was spread, and site 2D, a subsurface drained site carrying runoff from the solid manure-applied field 32, were sampled from in late March of 2013. Both sites contained large levels of ammonia in their water samples and moderate levels of phosphates. The sample from 1D contained low levels of nitrates, but the sample from 2D contained moderately high levels of nitrates. The nitrogen levels may vary because fields 14 and 15 were applied with solid strawpack manure (which is what the fields throughout this project were applied with when they were applied with solid manure), whereas field 32 was applied with just solid manure without strawpack. This may have led to the nitrogen level found at site 2D to be higher. Since 2D was a subsurface drained site, the high level of nitrates may also suggest that the soil in field 32 and surrounding fields had a buildup of nitrates, which would have caused the excess nitrates to leach through the soil and into the runoff water. Site 2D had a moderate concentration of E. coli colonies, compared to the low concentration of 1D. The difference is not significant, but may be due to the higher manure ratio in the solid manure applied to field 32, compared to the lower manure content in the strawpack manure applied to fields 14 and 15.

Results: 12/21/2013

Due to the large amount of rain leading to runoff on 12/21/2013 after months of solid and liquid manure applications and rainfall volumes that were not significant enough to allow for water to flow from the drainage tiles or in surface drainages, it makes sense that the largest number of sites with high ammonia and phosphate concentrations occurred on 12/21/2013. The accumulation of manure on the land coupled with the significant volume of rainfall lead to concentrated runoff shortly after the drainage tiles and surface drainages began running.

Site 6C, which is surface drained and drained a field that was applied with liquid manure, was the only site to possess moderately high nitrate concentrations on this date, and the highest nitrate concentration of all of the sites. This may be because the runoff was carried directly to the ditch, and didn't pass through the ground and through tiles before it entered the ditch, causing the nitrate levels to be higher. This may also explain why site 6C experienced the highest ammonia and phosphate concentrations compared to other sites on this date. Comparable levels of lactose +/E. coli colonies were observed in the water trays and on the EMB plates, which were low-moderate levels.

Site 1C drains field 6, which received several loads of solid manure before this precipitation event, by surface drainage. Site 5C drains fields 1, 5, and 6, but only field 6 had manure applied to it. Site 5C also is surface drained. Since site 5C drains three fields, in which only one was applied with manure, it makes sense that nutrient runoff levels were slightly lower than at site 1C, which drains only the one field. It also makes sense that site 1C contained higher E. coli levels in the water trays than did site 5C, due to the higher concentrated manure runoff that probably occurred.

Site 1B is a site of surface drainage that occurs right before field 8, in which solid manure was applied to before this rain event, and in which no fields upstream of it receive manure application for the year of 2013. Ammonia and phosphate concentrations are still high, and nitrate levels are moderate, which is odd because I suspected that they would be low due to the lack of manure runoff. Site 2B is a site of surface drainage that occurs downstream of site 1B, and occurs directly after field 8, in which manure solid manure was applied. Strangely, ammonia, nitrate, and phosphate concentrations are lower at this point where field runoff occurs than at site 1B, which occurs before the field. Also strange is that E. coli concentrations in the water trays were higher for site 1B than for site 2B, in which the opposite would be expected. These results suggest an error in the collection methods, as these results are not what should be expected. Another possible reason for these results is that runoff from field 8 was mixing with runoff from the fields before it, in which case the water sample obtained from site 1B would not be an accurate respresentation of runoff from fields that were not manure applied.

Results: 2/21/2014

High concentrations of ammonia, phosphates, and E. coli/lactose + colonies were observed in water samples obtained from 2/21/2014. The highest concentrations of both ammonia and phosphates were obtained from water samples from site 2B. These results make sense because manure had been applied on frozen ground before this date, and water samples were obtained when snow and ice melt was occurring. Manure application on frozen ground has been shown to contribute significantly to manure runoff.

All sites showed high concentrations of ammonia. Nitrate levels were low at all sites, and phosphate levels varied from moderate (sites 1C and 1B) to high (sites 5C and 2B). All of these sites were surface drained. Sites 1C and 5C drained field 6, in which solid and frozen liquid manure was applied, whereas sites 1B and 2B drained field 8, to which solid strawpack and frozen liquid manure was applied. Site 1B occurs at a point before field 8 begins and

site 2B occurs at a point after field 8, right before the surface drained water enters the ditch. Therefore, it makes sense that the phosphate, ammonia, and E. coli/ lactose + colonies are found at site 2B compared to site 1B. When I collected the water samples, there was a lot of standing water, so that may be a contributing factor as to why the ammonia levels were so high at 2B. The water tray and plate date that I obtained are comparable to one another for site 2B, but not for 1B. E. coli levels in water trays were much higher than lactose + colonies found on plates. One possible explanation for this may be due to error in plating or putting the sample into the water tray. This is why it is a good idea to have two ways of analyzing water quality data.

Sites 5C and 1C showed similar levels of nitrates, but variable ammonia and phosphate levels. They results were not what I was expecting. I expected the water sample from site 1C to contain higher concentrations of nutrients and bacterial colonies because it drains just field 6, whereas site 5C drains fields 1, 5, and 6. I would expect the water sample from 5C to be more diluted with snow and ice melt, but it was actually more concentrated significantly with ammonia, and more concentrated with phosphates and lactose +c colonies. The water tray and plate results for both sites were not comparable. Site 1C was shown to contain higher concentrations of E. coli, but less lactose + colonies than site 5C. Site 5C was shown to contain high concentrations of lactose + colonies on the plates, but low E. coli concentrations in the water trays. A possible explanation may be that the lactose + colonies observed were something other than E. coli. This would explain the low numbers of E. coli but the higher numbers of lactose + colonies. Another possible explanation could be error in plating or in performing the water tray tests.

Other *Results:*

The dates with the highest levels of nutrient and bacterial contamination appear to be 12/21/2013 and 2/21/2014. 12/21/2013 marked the date of a significant volume of rainfall that allowed the drainage tiles and surface drainage systems to flow after at least four months of several manure application events and no flow. This one precipitation event was significant enough to carry large concentrations of nutrients and coliform colonies off the fields and into the waterways.

2/21/2014 marked the date of snow and ice melt after a period of winter manure application to fields. The farmers did not have access to treatment facilities for their manure or means to store the manure over a long winter, so they had no choice but to apply it to their frozen ground. No or very little cover was on the fields over winter. These factors led to the high concentrations of nutrients and E. coli/lactose + colonies in the field runoff. Much higher concentrations of lactose + colonies could be seen in this sampling event compared to the December 2013 sampling event. These findings provide further evidence to the theory that winter application of manure on frozen ground leads to increase runoff and water contamination.

One of the sites that I ended up monitoring was tile drained, while the rest were surface drained. In the beginning stages of this project, I was aiming for a good mix of sites with surface and subsurface drainage. This did not work out as planned in the end, mainly because I had to work with what the farmers did and where they applied manure to. It is therefore hard for me to compare subsurface versus surface drainage based on my experiences. I can, however, say that surface and subsurface drainage was comparable in phosphate and ammonia concentrations. Nitrate concentrations appear to be higher on average compared to the surface drained sites. E. coli/lactose + colonies tended

to always be low for my subsurface drainage site. However, every field and drainage is different, due to a variety of factors including soil composition, manure application rates, manure composition, and time of year.

Coliform (CFU/100 ml) numbers were consistent with what others have attained in similar studies, with concentrations ranging from thousands to millions (Kistemann et al. 2002).

The high phosphate concentrations can be partly attributed to the fact that manure was applied to the surface rather than being injected into the soil, and no tillage was done to incorporate the manure. The December 2013 and February 2014 high phosphate results may be partly caused by autumn and winter manure applications, as well as the large amount of rain and snow melt contributing to runoff events.

The Colilert and EMB assays measure similar things, but yet there are significant differences in the results. The March 2013 sampling events showed similar results on both tests, as did most of the samples collected in January 2013. However, the December 2013 and February 2014 results differed significantly between the two tests. These were also the dates that showed the highest levels of contamination throughout the project. This may be due to errors in plating technique or other errors, or because of the difference in measurements. The Colilert tests measured E. coli, while the EMB plates measured lactose positive colonies. The lactose positive colonies were most likely E. coli, but not necessarily. In order to resolve the differences, in the future I would collect and analyze more than one water sample at each site that I was collecting from. I may try to see if the colonies grown on the EMB plates were in fact E. coli or were something else.

Antibiotic Resistance Results

The results indicate that the bacterial community shows a medium-high level of resistance to the antibiotic ampicillin. Anything that grows on the EMB plates with 50 micrograms/milliliter of ampicillin added represents high level resistance. The AMP resistance results that I obtained are high enough to be of great concern. A big concern is that these bacteria that are resistant to high levels of ampicillin can spread from place to place through water, and can result in other bacterium to acquire resistance (“General Background: About Antibiotic Resistance”). It is likely that resistance bacteria from farms like this are traveling off fields and feedlots and into public waterways.

Economics

In reduced tillage systems, manures are not incorporated or they are incorporated to shallow depths, exacerbating P buildup in the top 2-5 inches of soil (United States. Deparment of Agriculture). This can be seen in Figure 25. This is of economic importance to farmers who must integrate manure P into sustainable nutrient management systems. This is difficult with the excess of manure that farmers often have, due to their large numbers of animals and no place to store the manure and limited resources to process it.

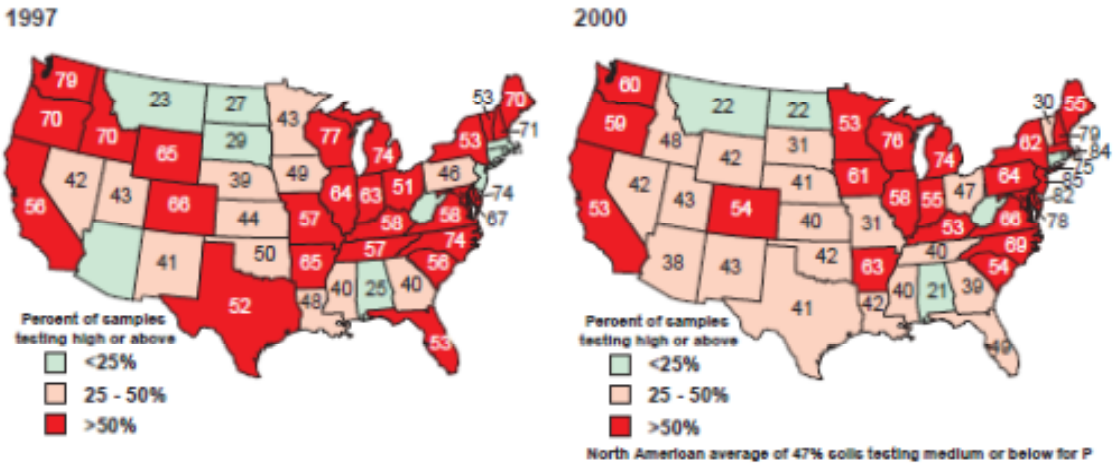

Figure 25: Soil Samples Analyzed in 1997 and 2000 Showing a Regional Buildup of Soil Test P Near P-sensitive Waters (United States. Department of Agriculture 2003)

Long-term waste storage and/or pretreatment, like composting, of livestock manures prior to land application would have the greatest impact on reducing bacterial transport to water bodies (Jamieson et al. 2002). Cattle manure can be composted to produce a high-quality soil amendment, but the cost of processing and the requirement for large-scale operations can be problematic (Bowman 2009). Source and transport and control strategies can also be used to control P loss in agricultural runoff (United States. Department of Agriculture). However, many farmers do not have the money or other resources to implement these types of systems. Smaller producers face higher per-unit costs, and are at a disadvantage relative to larger operations. However, producers who do not adopt pollution control measures put the costs, both in terms of money and health problems, on water users downstream

Increased regulatory activity could arise due to increased public concern and problems with surface water quality and algal blooms. However, livestock farms' annual net income could decline, which may have a dramatic effect on farmers that lack funds to comply to new and increased regulations. Also, the interests of the public and ecological health often take a back seat to economic development and financial growth, including the development of large animal feeding units to supply the demand for an ever-growing human population.

The ultimate goal of manure application systems is to apply manure to the land and minimize environmental change, community relations problems, damage to the land, and cost, while maximizing the use of nutrients in the manure (Ritter and Shirmahammadi 2001). Land application is the cheapest way for farmers to spread manure and return nutrients to the soil, but it is also an efficient way of spreading pathogens over the landscape. A system must be developed to minimize nutrient-loading and the spreading of pathogenic bacteria, and regulations must be set in place. The difficult part is making the systems and regulations cost-effective, especially for farmers with small farms and others who have limited funds. However, there has been success with various smaller-scale systems, and studies are being done to determine how these systems can be effective and affordable. Dr. Robert Midden is currently working on one such system in his laboratory at Bowling Green State University. The village of Ottawa in Putnam County, Ohio approached him in late 2012, asking him if he would be interested in working with them to develop a process to remove the nutrients from liquid manure and sell them as a low-cost fertilizer. His team has developed a process to

bind the nutrients together so they can be filtered out as a solid, making the nutrients less expensive to transport compared to liquid manure. If the costs are kept low in the process, this method of dealing with manure could be economically competitive with simple land application methods and transportation of the manure elsewhere (Sobolewski 2014).

Watershed Management

Watershed management in agricultural areas means dealing with the problems that farmers face. Many farmers with small herds do not have the manure storage systems or adequate manure storage systems, so they spread manure daily or fairly often, even in the winter. This is concerning because spreading manure on frozen or snow-covered ground increases the potential for surface runoff (Ritter and Shirmohammadi 2001). This is one way that P concentration in water is increased and contributes to algal blooms and eutrophication. Many farmers have open lagoon systems to store their excess liquid manure, but even this can be problematic. Natural disasters like floods can wash the large amounts of manures directly into water ways (Bowman 2009). Also problematic are confined animal feeding operations (CAFOs), which often have too many animals and not enough land to apply the large amounts of manure on. They therefore do not have the proper management systems to deal with the excess wastes.

Watershed management plans also have to take geology and soil sciences into consideration of their plans. Runoff may start later in a rain event if soil moisture is high at the time of application but low in the weeks following because the soil could absorb more water at the beginning of a rain event (Vadas et al. 2011). Manure nutrient loss could therefore be higher several days after application than the day after application if more intense rain and runoff events occur later. Soil moisture is therefore an important parameter to consider when collecting and analyzing runoff samples. Also, in some agricultural watersheds, 90% of annual algal-available P export in a watershed comes from only 10% of the land area during just a few relatively large storms (United State. Department of Agriculture). This is important to know in order for watershed managers to come up with plans and regulations to combat this problem and decrease the nutrient loads. Microbial Source Tracking (MST) is a fairly new technology being used by some managers to identify possible sources of fecal contamination, which can also help in watershed management.

Birds, insects, and many other animals depend on ditches as sources of prey and habitat. Plants and animals not found in other habitats often use ditches on a seasonal and year-round basis. Watershed managers must take this into account as well when coming up with management plans. Proper management can decrease pollutant runoff, increase water quality, and improve the biological value of ditches and streams. However, watershed managers must be careful not to create plans and perform actions that conflict with other management strategies. This can be difficult to do, as strategies to reduce bacterial transport may conflict with strategies to mitigate other environmental impacts. For example, tillage can reduce bacterial transport to subsurface drains, but no-till and conservation tillage are promoted to improve soil quality and reduce environmental impacts like erosion. Also, it has been suggested that manure be applied during hot, dry conditions to facilitate bacterial mortality, but these conditions significantly enhance ammonia volatilization (Jamieson et al. 2002). There is no easy solution. Watershed managers may need to weigh the costs and benefits of every part of their proposed plans in order to best deal with the problems that need solutions the most. Manure treatment processes, such as what Dr. Midden is developing (Sobolewski 2014), could also be a potential solution to the pollution problems caused by simply applying manure to the land.

Policies and Regulation

In the United States, manure is managed under restrictions placed on it by the Environmental Protection Agency (EPA), with input from the United States Department of Agriculture (USDA) and the Food and Drug Administration (FDA). Untreated manure is routinely applied within USDA-recommended levels (Bowman 2009). Many regions also have regionally regulations put in place for manure management as well.

Several considerations must be taken into account when policies and regulations are made. For example, topography and management of different sites must be looked into when considering P management. Best management practices should be utilized whenever possible for stream and ditch construction and maintenance, habitat protection, watershed management, and nutrient management systems. Factors specific to the watershed of interest are important to consider and incorporate into policies. For instance, many new, stricter rules have been put in place in recent years within the Grand Lake St. Mary's Watershed in response to algal blooms and degraded waters in Grand Lake St. Mary's. Some of these rules are restricting winter application of manure, restricting manure application on frozen or snow-covered ground, restricting surface application of manure if there is greater than a 50% chance of a certain volume of precipitation within 24 hours of land application, and only allowing manure application on land with at least 90% surface residue cover (*New Rules*).

Every watershed can benefit from well-planned policies and regulations. Currently, there is a need for increased emphasis to be placed in developing watershed-specific regulations for manure application.

Future Predictions

To make predictions about how the future of manure management and agriculture will look, it is helpful to look at the trends. In the United States, the number of no-till acres has tripled from 1990 to 2004 (Vadas et al. 2011), which may be contributing to nutrient and bacterial transport from fields to waterways. Levels of soluble P have continued to increase over the last decade ("Lake Erie Symposium Morning Session"), contributing to increased algal blooms and their severity. In the past three decades, the number of agriculture related Ohio fresh water fish kills has increased by 72% (Hoorman et al. 2007). This information points to traditional agricultural practices and intensive livestock operations as being responsible for the non-point source pollution in northwest Ohio waterways and the Lake Erie western basin. Effects have only increased with the consolidation of production into larger and more concentrated livestock facilities that have limited land for manure application.

Long-term trends in agricultural manure management and other nutrient management practices have the potential for higher nutrient loading into Lake Erie. Observed trends show decreasing wind speeds over the continental United States, which could cause the sort of weak lake circulation and quiescent conditions observed after the 2011 Lake Erie algal bloom onset (Michalak et al. 2013). These trends suggest that long residence times and quiescent conditions may be common in the future, along with reduced wind speeds that could further contribute to the severity of blooms. The 2011 bloom may therefore be a precursor of future blooms in Lake Erie. If the current trends in agriculture and manure production, as well as no action being taken, the problems of eutrophication, polluted water bodies, and antibiotic resistance will continue to grow.

People's concerns about increased nutrients and bacteria on water quality will drive change. If policy and regulation intervention does not take place, socioeconomic factors will continue to drive the current trends in agriculture, increasing the likelihood of algal blooms like the 2011 bloom. There would then be wider fluctuations in pH and a decrease in dissolved oxygen concentrations in Lake Erie and its tributaries, contributing to increased fish kills. If no action takes place, I predict that Lake Erie will experience increasingly severe algal blooms, the tourism and fishing industries will experience decreased incomes due to beach closures and fish kills, humans and animals may experience health problems due to the decreased water quality, and antibiotic resistance of bacteria will be an increasing problem.

Recommendations

Recommendations for Farmers and Manure Management

The fact that manure can be safely recycled on the land in some cases but end up in surface and subsurface water drainage in other cases indicates that manure application and management is a complex system. There is an abundance of various recommendations for farmers and watershed managers for manure management. Many of these recommendations result in a loss of some potential profits to farmers and require additional time. Others require a different attitude and way of thinking when it comes to planning and maintenance. Farmers should do what they can, with support from their local watershed managers, soil and water conservation district, and/or Environmental Protection Agency.

Better management begins with planning. It is recommended that P content of both manure and soil be determined by soil test laboratories before land application of manure (United State. Department of Agriculture). This can tell farmers what fields do and what fields don't need manure applications. Results may be given according to a phosphorus index score, in which case different management methods should be utilized depending on the score obtained. Figure 26, below, gives examples. Sometimes farmers apply manure to their fields on the basis of a crop's N needs. This is not recommended because it often results in the overapplication of P (Bowman 2009). Effective management of livestock and only raising the number of animals that proper manure management allows will also help reduce manure nutrient loads to water ways. Farmers should have a plan and tools in place to capture tile-drained flow if manure effluent does occur. Also, it is best to keep a distance between crops and water ways like ditches, as planting and applying manure right up to the edge increases erosion and runoff. Best Management Practices should be followed as well. The ultimate goal is to improve water quality, meet the drainage needs for crop production, and manage soils in ways that retain crop nutrient resources

Phosphorus index	Management options
< 60 (Low)	*Soil testing:* Test soils for P at least every 3 years to monitor buildup or decline in soil P. *Soil conservation:* Follow good soil conservation practices. Consider effects of changes in tillage practices or land use on potential for increased transport of P from site. *Nutrient management:* Consider effects of any major changes in agricultural practices on P loss *before* implementing them on the farm. Examples include increasing the number of animal units on a farm or changing to crops with a high demand for fertilizer P.
60 to 80 (Medium)	*Soil testing:* Test soils for P at least every 3 years to monitor buildup or decline in soil P. Conduct a more comprehensive soil testing program in areas identified by the P index as most sensitive to P loss by surface runoff, subsurface flow, and erosion. *Soil conservation:* Implement practices to reduce P loss by surface runoff, subsurface flow, and erosion in the most sensitive fields (that is, reduced tillage, field borders, grassed waterways, and improved irrigation and drainage management). *Nutrient management:* Any changes in agricultural practices may affect P loss; carefully consider the sensitivity of fields to P loss before implementing any activity that will increase soil P. Avoid broadcast applications of P fertilizers and apply manures only to fields with low P index values.

(cont.)–

Phosphorus index	Management options
80 to 100 (High)	*Soil testing:* A comprehensive soil testing program should be conducted on the entire farm to determine fields that are most suitable for further additions of P. For fields that are excessive in P, estimates of the time required to deplete soil P to optimum levels should be made for use in long-range planning. *Soil conservation:* Implement practices to reduce P loss by surface runoff, subsurface flow, and erosion in the most sensitive fields (that is, reduced tillage, field borders, grassed waterways, and improved irrigation and drainage management). Consider using crops with high P removal capacities in fields with high P index values. *Nutrient management:* In most situations involving fertilizer P, only a small amount used in starter fertilizers is needed. Manure may be in excess on the farm and should only be applied to fields with lower P index values. A long-term P management plan should be considered.
> 100 (Very high)	*Soil testing:* For fields that are excessive in P, estimate the time required to deplete soil P to optimum levels for use in long-range planning. Consider using new soil testing methods that provide more information on environmental impact of soil P. *Soil conservation:* Implement practices to reduce P loss by surface runoff, subsurface flow, and erosion in the most sensitive fields (that is, reduced tillage, field borders, grassed waterways, and improved irrigation and drainage management). Consider using crops with high P removal capacities in fields with high P index values. *Nutrient management:* Fertilizer and manure P should not be applied for 3 years or more. A comprehensive, long-term P management plan must be developed and implemented for an entire crop rotation.

Figure 26: Phosphorus Index Score and Management Options (United States. Department of Agriculture 2003)

In terms of nutrient management, P loss and erosion can be reduced by conservation tillage, crop residue management, cover crops, and contour farming tillage. Wetlands should be left untouched or should be restored, if possible, to filter and treat any excess nutrients. Parts of floodplains can also be left untouched as they provide similar services as riparian buffers. Shallow injection can be utilized for liquid manure. Some sources say that the best method is no-till and incorporation of manure (Reutter et al. 2011). Constant soil cover is recommended to minimize erosion, and overseeding pastures with legumes is pushed to aid P removal (United State. Department of Agriculture).

As erosion is a predominant means in which P is released into the environment (Bowman 2009), measures should be taken to decreased erosion. After planting, manure should be applied as soon as possible (Ritter and Shirmohammadi 2001). However, it should not be applied at rates that exceed the infiltration rate nor bring the soil to holding capacity. After crop harvest, normally in the fall, farmers often apply more manure to the fields. In fact, over two-thirds of swine and dairy manures from large operations are applied in the fall after harvest has taken place. In Ohio, the most manure violations relate to this time of year when most liquid manure is applied, with the worst months for violations being October, November, and April. Liquid manure applied to the soil without a cover crop to absorb nutrients moves more rapidly through drained land to surrounding water ways (Hoorman et al. 2008). Therefore, this practice should be avoided as much as possible, as there is evidence that fall application increases the risk of nutrient loss (Jamieson et al. 2002).

Rate of application is extremely important. Rates should be closely tied to nutrient requirements and available holding capacity of the soil. Application rates should not exceed the lower of the nutrient restrictions, available holding capacity of the soil, or 13,000 gallons/acre. Figure 27, below, gives more descriptive information on the water holding capacity of different soils and the recommended application rates. Concerning liquid manure, smaller multiple applications allow the soil to absorb it better than one application. Some tillage may be required to improve infiltration and absorption. (Hoorman et al. 2008). Manure should always be applied at a known rate and uniformly over the land.

Maximum Available Water Holding Capacity of Soils

Table 1: Available Water Capacity (AWC) Practical Soil Moisture Interpretations for Various Soils Textures and Conditions to Determine Liquid Waste Volume Applications not to exceed AWC.

This table shall be used to determine the AWC (upper 8 inches) at the time of application and the liquid volume in gallons that can be applied not to exceed the AWC. To determine the AWC in the upper 8 inches use a soil probe or similar device to evaluate the soil to a depth of 8 inches. The manure application rates should be less than AWC to reduce the potential for contaminated runoff.

Available Moisture in the Soil	Sands and Loamy Sands	Sandy Loam and Fine Sandy Loam	Very Fine Sandy Loam, Loam, Silt Loam, Silty Clay Loam, Clay Loam, Sandy Clay Loam	Sandy Clay, Silty Clay, Clay
< 25% Soil Moisture	Dry, loose and single-grained; flows through fingers.	Dry and loose; flows through fingers.	Powdery dry; in some places slightly crusted but breaks down easily into powder.	Hard, baked and cracked; has loose crumbs on surface in some places.
Amount to Reach AWC	20,000 gallons/acre	27,000 gallons/acre	40,000 gallons/acre	27,000 gallons/acre
25–50% or Less Soil Moisture	Appears to be dry; does not form a ball under pressure.	Appears to be dry; does not form a ball under pressure.	Somewhat crumbly but holds together under pressure.	Somewhat pliable; balls under pressure.
Amount to Reach AWC	15,000 gallons/acre	20,000 gallons/acre	30,000 gallons/acre	20,000 gallons/acre
50–75% Soil Moisture	Appears to be dry; does not form a ball under pressure.	Balls under pressure but seldom holds together.	Forms a ball under pressure; somewhat plastic; sticks slightly under pressure.	Forms a ball; ribbons out between thumb and forefinger.
Amount to Reach AWC	10,000 gallons/acre	13,000 gallons/acre	20,000 gallons/acre	13,000 gallons/acre
75% to Field Capacity	Sticks together slightly; may form a weak ball under pressure.	Forms a weak ball that breaks easily, does not stick.	Forms ball; very pliable, sticks readily if relatively high in clay.	Ribbons out between fingers easily; has a slick feeling.
Amount to Reach AWC	5,000 gallons/acre	7,000 gallons/acre	11,000 gallons/acre	7,000 gallons/acre
100% Field Capacity	On squeezing, no free water appears on soil, but wet outline of ball on hand.	On squeezing, no free water appears on soil, but wet outline of ball on hand.	On squeezing, no free water appears on soil, but wet outline of ball on hand.	On squeezing, no free water appears on soil, but wet outline of ball on hand.
Above Field Capacity	Free water appears when soil is bounced in hand.	Free water is released with kneading.	Free water can be squeezed out.	Puddles: free water forms on surface

Ohio-NRCS Conservation Practice Standard 633.

Figure 27: Maximum Available Water Holding Capacity of Soils (Hoorman et al. 2008)

Weather events should strongly be taken into consideration when it comes to the timing of manure application. In general, applying manure when rainfall or snowmelt is predicted or right after a rainfall or snowmelt should be

avoided. Manure should also not be applied to frozen ground. A proposed practice to reduce runoff nutrient loss is to increase the time between manure application and the first rain-runoff event after application (Vadas et al. 2011). Another recommendation is that applicators should note the flow condition within the tiles prior to application, as well as the likelihood of precipitation within three weeks after application (Jamieson et al. 2002). A weather journal can be kept, maintaining a log of forecasts and actual weather conditions before and after a manure application event. Liquid manure should not be applied to soils that are prone to flooding.

In terms of ditch maintenance, buffer strips and riparian zones, such as those seen in Figure 28 below, can be highly effective at reducing nutrient loads to streams. If fields have subsurface drainage, extra precautions should be taken to avoid pollution. Subsurface drain outlets should be identified, and broken drains should be repaired prior to land application. On-site means of stopping the discharge to ditches from subsurface drains are useful to have in place. If manure flow occurs, all effluent should be captured. Outlets should be monitored before, during, and after application for potential manure discharge. Liquid manure should not be applied to tile drained land if the drains are already flowing (Hoorman et al. 2008)

Figure 28: Riparian Buffer (Korleski 2010)

In terms of managing for pathogens, water contamination should be a top concern, and management systems should be built around avoiding it. Many of the recommendations above, including watching the weather to avoid land application when snow or precipitation is called for and providing buffer strips around ditches, will reduce contamination. The risk of fecal contamination should always be acknowledged. Also helpful is keeping livestock out of streams, and using feeds that reduce the excretion of pathogens (Bowman 2009). New technologies are also available for farmers to utilize for the purpose of pathogen control. For instance, industrial composting facilities can be used on dairy farms. Composting animal waste so that it generates a sustained temperature of 50 degrees Celsius will kill pathogens before the manure is applied to fields (Bowman 2009). Processes used to treat manure are processes that have been used in the wastewater industry to treat human waste. The processes have been adapted to

use on farms, but have often been largely modified for cost reasons, and much of the processing that is done have little or nothing to do with controlling pathogens (Bowman 2009). Figures 29a and 29b lists advantages and disadvantages to various on-farm manure processing systems. Efficient, cost-effective systems that are addressing the disadvantages for controlling pathogens and nutrients in manure are still being developed. When they do become available, farm operators should use them when possible.

Manure management alternative	Advantages	Disadvantages
Daily spreading is being practiced by many farms. Manure and other wastes are spread as they are produced throughout the year.	Capital costs are low. Environmental effects are hidden. Odor problems are minor. Labor and equipment use is steady.	Total costs may be high. Nutrient and pathogen losses during times of saturated soils may provide excessive delivery to waterbodies. Field accessibility may be a problem.
Liquid storage to reduce spreading during high loss and times when fields are inaccessible. About 10% of New York farms have at least 180 days of storage. The CAFO regulations will encourage more storage.	Nutrient management can be easier. Efficiencies in handling can be obtained to keep costs down. Manure can be spread when needed.	Odors are a big problem when spreading. Large liquid-handling equipment needs to be available. Labor and equipment needs peak. Nonearthen storage can be expensive. Catastrophic failure or heavy rainfalls right after spreading can cause peak pollutant discharges.
Odor control of stored liquid manure is a major need. Chemical and biological treatments have been tried and proposed.	Allows spreading of the manure during the growing season and reduces neighbor complaints	In almost all cases, technology has yet to show that it can work effectively without high costs.
Solid separation of the manure solids mechanically can produce a "solid" portion (15 to 30% DM) and a "liquid portion" (4 to 38% DM). About 50 farms in New York have this equipment.	Liquids are easier to handle. Solids can be recovered for bedding, soil amendment, or exported off the farm.	High capital and operating costs. Maintenance of the equipment is a problem. Marketing of the solids may not be successful on all farms.
Composting of the manure by adding bedding or an amendment to produce a biologically decomposed product has had limited success on dairy farms.	Odor reduction is an important advantage of composting. Equipment for solids handling is available on most farms. Storage of solids is safer environmentally than liquid storage. Material may be marketed.	High moisture content of most dairy manure makes conventional composting difficult. Sales may depend on expensive specialized mixing equipment and good management. Composting outside on large areas can create runoff losses.

Figure 29a: Advantages and Disadvantages of On-Farm Manure Processing Systems (Bowman 2010)

Manure management alternative	Advantages	Disadvantages
Biodrying of the manure by recycling dry compost as the amendment in the alleys, and using the heat generated in the aerobic decomposition to dry the manure/compost mix with forced air.	Odor reduction, volume reduction, and weight reduction would occur. Equipment for solids handling is available on most farms. Storage of solids is safer environmentally than liquid storage. Material may be marketed.	Management of drying process is critical. A large roofed area is needed. Costs of operation are high. Material handling may be excessive. Additional amendment is required. Winter operation may require closed buildings.
Plug flow anaerobic digestion takes produced manure and digests it, producing an odorless effluent that has reduced solids content while retaining the nutrients. Methane gas is recovered that can be used to run an engine generator.	Odor reduction and energy recovery will occur. Effluent is reduced in solids content and can be further reduced easily by mechanical solid separation. Demand for the anaerobically digested solids is greater than raw solids.	Management of the digestion process will be critical. Capital costs will be high. Electric utility connections may be difficult.
Mixed anaerobic digestion allows the treatment of higher moisture content material that may settle during plug flow digestion. Mixing keeps the material in suspension and allows the bacteria access to newly introduced food sources.	Odor reduction and energy recovery will occur. Effluent is reduced in solids content and can be further reduced easily by mechanical solid separation. Demand for the anaerobically digested solids is greater than raw solids. Additional material can be added that will increase the biogas production and may bring in substantial tipping fees.	Volume may be greater to handle the additional liquid. A portion of the newly introduced influent will come out as effluent with minimal treatment. The agitators add to the complexity and cost of a mixed system. Management of the digestion process will be critical. Capital costs will be high. Electric utility connections may be difficult.
Lagoon treatment of manure from farms consists of diluting the manure, allowing it to settle in large shallow pools, or mechanically separating the solids; it then flows to a facultative lagoon to be recycled as flush water to dilute more manure. Liquids and solids are periodically removed from the system.	Odors are reduced and solids are separated without mechanical treatment. Works well with a flush system to remove manure from freestall alleys. Solids may be marketed. Liquids can be easily irrigated. Management is relatively easy.	Solids harvesting and dewatering can be difficult. Exposure of large surface areas results in extra water volumes. Impermeable soils on moderately flat terrain are required to keep costs down.

Figure 29b: Advantages and Disadvantages of On-Farm Manure Processing Systems (Bowman 2010)

Recommendations for Watershed Managers and Monitoring

Watershed managers have a big responsibility in helping farmers develop cost-effective, site-specific manure management plans. They must hear concerns from farmers, community members, and policy makers. They must be innovative and stay up to date on the current applicable scientific knowledge. Managers should know that no two watersheds are the same, so effective management plans will vary. They should take a look at the problems the watershed is facing and why they are occurring. They can then take steps to fix the problem or at least decrease the negative effects that it causes. A way that they can do this are to communicate with farmers and the community. For instance, since many farmers in Ohio apply large amounts of manure after fall crop harvest, managers can address this

issue. They can educate farmers about the environmental consequences that come with manure application with no crop on the fields, how it can affect human health, and encourage better behavior. They can hold information and education sections to farmers and community members about the consequences that come with improper manure management, giving visual evidence and explaining why what farmers do on their land can affect everyone downstream. They can then give clear advice on what farmers could do to improve their management practices. For example, they can state that riparian buffer strips can significantly reduce nutrients and sediment in overload flow, improve instream habitat, and in turn improve biotic integrity and ecosystem health (Stone et al. 2005). Best Management Practices should be strongly encouraged, but they will vary depending on the local climate, specific site situations, and the agricultural discharge. Watershed managers can also meet with farmers on an individual basis to develop plans that are specific to each farm and farmer. The risk of fecal contamination of recreational and drinking water sources should always be on the minds of farmer and authorities controlling public water systems.

Besides education, managers could set up programs with the government to encourage good behaviors, such as establishing buffer strips, by giving farmers monetary rewards for doing good. Bad behaviors could also be discouraged by enforcing penalties and fines, but I would not recommend this as small farmers may not be able to pay the fees and would therefore be run out of business by the large animal operations that have more income. Another solution could be to buy land from farmers to aid in the protection or restoration of wetlands, riparian forested buffers, riparian cover, filter areas, and filter strips.

After plans of action are established and better management begins, monitoring will need to become a priority. Watershed managers and farmers can work together to inspect tiled fields prior to manure application on an annual basis for broken tiles or blow holes. Anything broken should be prepared before land application. Field runoff should be closely monitored to make sure manure laden flow does not occur. If it does, plans should be made to capture the effluent or make sure it does not continue to occur. Waters should also be monitored on an annual basis to make sure they meet required standards. If they don't, there should be a plan in place to identify the reason and fix the problems. Water quality in Lake Erie should be monitored closely for severity of algal blooms, coliforms, and eutrophication. If these problems stay the same or increase in severity, managers and scientists may need to enforce more strict rules and come up with different solutions. To help with monitoring efforts, managers can establish volunteer programs. They can train volunteers to collect water samples, soil samples, and perform simple monitoring tasks, which would cut down on their labor and monitoring costs.

Information and planning can only go so far. The upfront and maintenance costs of these improved practices will be a concern of the farmers. They may not have the funds available to pay for better management or take land out of production to use as buffer strips, for example. Obtaining the necessary funds is not always easy. A couple suggestions are that watershed managers and farmers can do fundraising to cover the costs, or a new watershed-wide tax could be implemented. This issue can be further discussed with policy managers.

Recommendations for Policy Makers

Most agricultural manure production, application, and disposal is unregulated. Program dollars are also too broadly dispersed to get water quality results (Korleski 2010). This needs to be changed to protect ditch, stream, river, and lake habitat, as well as water quality. Policy makers and watershed managers should work hand-in-hand to

develop policies and regulations that will be effective for the particular regions, and to allocate funds to specific programs. They must know the manure management issue well.

Policies that address methods, amount, form, placement, timing, and incorporation of manure application will be beneficial (Korleski 2010). For instance, a new rule may be that nutrients be injected into the soil, if the equipment is available, as they are then less likely to be mobile. A practical way must be figured out and established to allow farmers to utilize Best Management Practices, and they must be tailored to particular sites because no two sites are the same. An example in which this was done comes from the Grand Lake/Wabash Watershed Alliance, part of the Mercer Soil and Water Conservation District. In the "New Rules" document they published, rules that were established for the region were passed by the Joint Committee on Agency Rule Review. Very specific rules and regulations were established to improve poor water quality conditions caused primarily by agricultural runoff in the Grand Lake St. Marys Watershed (*New Rules*). This example can be followed by other watersheds in Northwest Ohio for the protection of Lake Erie and its tributaries. Monitoring must be required and standards of water quality should be adopted and continually updated.

Future Studies

There is room for and a high need for more research to be done on a variety of issues surrounding manure management. One potential topic is how pathogens travel from animal excretions into the water supply, as little information is currently available (Bowman 2009). Research topics can also involve soil stratification relating to P buildup in the upper few inches of the soil. Future studies could focus on the extent of stratification and its potential role in DRP in runoff from agricultural fields. Further study could also investigate what is causing the increasing DRP in the Lake Erie basin (Korleski 2010). Relating to habitat quality, using macroinvertebrates as indicators of ditch, river, and lake environmental quality may be useful when evaluating current water quality and habitat status, in addition to microbiological and chemical analyses.

Future studies related to antibiotic resistance should also be explored. A more comprehensive understanding of the development and emergence of ampicillin resistance in feedlots (Mirzaagha et al. 2011) and other livestock operations is needed, as is its possible transmission to other animals in the herd. Studies have shown that resistance to antibiotics may be related to other environmental factors, such as diet and environmental stressors (Alexander et al. 2008), but the findings should gain support from additional research.

Conclusion

My results allow me to conclude that the most nutrient and pathogen pollution occurs after large rainstorm events, especially after manure has been applied to land for months with no precipitation events, and after manure application on frozen ground. These results support the findings from similar studies. I can also generalize that many of the soils from the field sites that I collected from had buildups of phosphorus, which likely contributed to the high concentrations of phosphorus in the runoff samples that I collected. This result shows that farmers should be aware of the high levels of nutrients already in the soils from their fields, and should refrain from adding additional nutrients that will end up running off of the fields and polluting nearby waterways. I can also conclude that the manure pathogens that I examined for antibiotic resistance were resistant to high levels of ampicillin. This result further supports the severity of antibiotic resistance and the negative health effects and environmental effects that they can

cause. More research should be done on this topic and what can be done about resistance of manure pathogens to antibiotics administered to farm animals.

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Tables

Table 1: Crops on Fields of Interest For 2012 and 2013

Crops on the Fields of Interest					
Field	Size	Season	Crop	Yield per Acre (lbs)	Total Yield (lbs)
8	15 acres	2012	Wheat	3900	~58,500
8	15 acres	2013	Alfalfa	8000-9000	~120,000-135,000
9	18 acres	2012	Corn	4200	~75,600
9	18 acres	2013	Soybeans	1800	~32,400
22	22 acres	2012	Rye	2000	~44,000
22	22 acres	2013	Soybeans	1800	~39,600
23	15 acres	2012	Alfalfa	8000-9000	~120,000-135,000
23	15 acres	2013	Alfalfa	8000-9000	~120,000-135,000
14	12 acres	2012	Corn	4200	~50,400
14	12 acres	2013	Corn	4200	~50,400
15	18 acres	2012	Red clover	4000	~72,000
15	18 acres	2013	Soybeans	1800	~32,400
16	18 acres	2012	Corn	4200	~75,600
16	18 acres	2013	Wheat	3900	~70,200
29	12 acres	2012	Wheat	3900	~46,800
29	12 acres	2013	Red clover	4000	~48,000
30	11.5 acres	2012	Corn	4200	~48,300
30	11.5 acres	2013	Wheat	3900	~44,850
31	12 acres	2012	Corn	4200	~50,400
31	12 acres	2013	Corn	4200	~50,400
32	14 acres	2012	Red clover	4000	~56,000
32	14 acres	2013	Corn	4200	~58,800
1	16 acres	2012	Alfalfa	8000-9000	~128,000-144,000
1	16 acres	2013	Alfalfa	8000-9000	~128,000-144,000
5	18 acres	2012	Soybeans	1800	~32,400
5	18 acres	2013	Corn	4200	~75,600
6	13 acres	2012	Wheat	3900	~50,700
6	13 acres	2013	Red clover	4000	~52,000

Table 2: Precipitation Records Obtained from KOHKALD2 Weather Station

Precipitation Records from the Kalida Local	
Schools Weather Station (KOHKALD2) on	
www.wunderground.com	
Date	**Precipitation (inches)**
1/3/2013	0.01
1/4/2013	0.01
1/10/2013	0.01
1/11/2013	0.69
1/12/2013	1.83
1/13/2013	0.52
1/14/2013	0.55
1/17/2013	0.01
1/18/2013	0.05
1/19/2013	0.2
1/20/2013	0.2
1/28/2013	0.02
1/29/2013	0.03
1/30/2013	0.01
1/31/2013	0.01
2/5/2013	0.02
2/6/2013	0.03
2/7/2013	0.05
2/8/2013	0.02
2/9/2013	0.01
2/10/2013	0.66
2/11/2013	0.69
2/12/2013	0.11
2/19/2013	0.03
2/22/2013	0.2
2/23/2013	0.01
2/24/2013	0.01
2/26/2013	1.18
2/27/2013	0.36
2/28/2013	0.13

3/1/2013	0.13
3/6/2013	0.01
3/7/2013	0.01
3/10/2013	0.01
3/11/2013	0.35
3/12/2013	0.35
3/15/2013	0.18
3/16/2013	0.03
3/17/2013	0.04
3/18/2013	0.03
3/19/2013	0.03
3/25/2013	0.05
3/26/2013	0.05
3/31/2013	0.01
4/1/2013	0.01
4/8/2013	0.01
4/9/2013	0.01
4/10/2013	3.03
4/11/2013	0.66
4/12/2013	0.05
4/13/2013	0.01
4/14/2013	0.01
4/16/2013	0.63
4/17/2013	0.65
4/18/2013	0.18
4/19/2013	0.02
4/20/2013	0.02
4/23/2013	0.19
4/24/2013	1.34
4/25/2013	1.34
4/28/2013	0.61
4/29/2013	0.61
5/8/2013	0.03
5/9/2013	0.04
5/10/2013	0.02
5/11/2013	0.03
5/12/2013	0.01
5/13/2013	0.01
5/22/2013	0.14

5/23/2013	0.03
5/24/2013	0.03
5/27/2013	1.4
5/28/2013	0.04
5/29/2013	0.09
5/30/2013	0.01
5/31/2013	0.24
6/1/2013	0.1
6/2/2013	0.1
6/9/2013	0.04
6/10/2013	0.48
6/11/2013	0.48
6/13/2013	1.61
6/16/2013	0.03
6/17/2013	0.03
6/25/2013	0.01
6/26/2013	0.12
6/27/2013	0.14
6/28/2013	0.42
6/29/2013	0.29
6/30/2013	0.01
7/1/2013	1.91
7/2/2013	1.14
7/3/2013	1.14
7/4/2013	0.01
7/5/2013	0.15
7/6/2013	0.07
7/7/2013	0.11
7/8/2013	0.7
7/9/2013	0.01
7/10/2013	0.55
7/11/2013	0.55
7/22/2013	0.02
7/23/2013	0.03
7/27/2013	0.13
7/31/2013	0.3
8/1/2013	0.19
8/2/2013	2.2

8/3/2013	3.8
8/12/2013	0.03
8/13/2013	0.03
8/22/2013	0.01
8/23/2013	0.02
8/29/2013	0.01
9/12/2013	0.02
9/13/2013	0.02
9/15/2013	0.5
9/16/2013	0.5
9/19/2013	0.11
9/20/2013	0.74
9/21/2013	1.27
9/26/2013	0.03
9/29/2013	0.96
9/30/2013	0.01
10/1/2013	0.04
10/3/2013	0.73
10/4/2013	2.34
10/5/2013	0.44
10/6/2013	0.36
10/7/2013	0.36
10/8/2013	0.01
10/9/2013	0.01
10/12/2013	0.01
10/15/2013	0.11
10/16/2013	0.11
10/17/2013	0.14
10/18/2013	0.14
10/19/2013	0.57
10/20/2013	0.57
10/21/2013	0.01
10/22/2013	0.06
10/23/2013	0.09
10/24/2013	0.01
10/25/2013	0.01
10/31/2013	1.26
11/1/2013	1.26

11/2/2013	0.19
11/3/2013	0.43
11/6/2013	0.19
11/7/2013	0.19
11/11/2013	0.26
11/12/2013	0.01
11/13/2013	0.01
11/17/2013	0.54
11/18/2013	0.54
11/21/2013	0.39
11/22/2013	0.09
11/23/2013	0.09
12/2/2013	0.12
12/3/2013	0.12
12/19/2013	0.09
12/20/2013	0.08
12/21/2013	5.21
12/22/2013	0.02
12/23/2013	0.02
1/5/2014	0.1
1/6/2014	0.1
1/10/2014	0.57
1/11/2014	0.12
1/12/2014	0.12
1/14/2014	0.01
1/15/2014	0.01
1/20/2014	0.01
1/21/2014	0.01
2/1/2014	1.78
2/2/2014	1.78
2/3/2014	0.03
2/4/2014	0.03
2/13/2014	0.01
2/14/2014	0.01
2/18/2014	0.17
2/19/2014	0.18
2/20/2014	0.66
2/21/2014	0.01

Table 3: Precipitation Records Obtained from Farmers

Precipitation Records from the Farmers	
Date	Precipitation (inches)
Jan-13	Records Not Available
Feb-13	Records Not Available

Mar-13	Records Not Available
Apr-13	Records Not Available
5/26/2013	0.7
5/30/2013	0.4

6/1/2013	1.9
6/2/2013	0.1
6/10/2013	0.4
6/12/2013	0.9
6/15/2013	0.1
6/26/2013	0.2
6/28/2013	1.5
7/1/2013	1.5
7/2/2013	0.5
7/7/2013	1.5
7/9/2013	0.3
7/19/2013	1.3
7/22/2013	0.3
7/29/2013	2.5

13-Aug	No rain records
9/10/2013	0.2
9/12/2013	0.2
9/14/2013	0.4
10/2/2013	0.06
10/5/2013	0.03
10/6/2013	0.04
10/30-31/2013	1.5

11/6/2013	little rain
11/17/2013	1
11/21/2013	little rain
12/20-22/13	3.5

Table 4: Manure Application

	Manure Application Records		
			1 load= 221-321 cubic feet=1654.19-2402.69 gallons
Date	Field	Acres	Amount Hauled
2/11/2013	15	18	12 loads (1654.19-2402.69 gallons/acre) ,solids (strawpack), not tilled in
3/4-8/2013	15	18	10 loads (919-1334.8 gallons/acre), solids (strawpack),not tilled in
3/4-8/2-13	14	12	12 loads (1654.19-2402.69 gallons/acre), solids (strawpack), not tilled in
3/4-8/2013	32	14	30 loads (3544.7-5148.6 gallons/acre),solids (not strawpack), not tilled in
3/21/2013	32	14	7 loads (827.1-1201.3 gallons/acre), solids, not tilled in
3/21/2013	24	4.5	3 loads (1102.8-1601.8 gallons/acre), solids, not tilled in
3/22/2013	14	12	10 loads, (1378.5-2002.2 gallons/acre), solids, not tilled in
5/21-24/13	1	16	8,600 gallons/acre, liquid, not tilled in
5/21-24/13	23	15	13,300 gallons/acre, liquid, not tilled in
5/21-24/13	22	22	24,000 gallons/acre, liquid, not tilled in
8/17/2013	30	11.5	10 loads (1438.4-2089.3 gallons/acre), solids (strawpack)
8/27/2013	8	15	18 loads (1985-2883.2 gallons/acre), solids (strawpack)
8/29/2013	6	13	15 loads (1908.7-2772.3 gallons/acre), solids (strawpack)
10/16/2013	8	15	20-25 loads (2205.5-3203.6 to 2757-4004.5 gallons/acre), solids, not tilled in
11/8/2013	6	13	3-4 loads (381.7-554.5 to 509-739.3 gallons/acre), solids, not tilled in
11/12-14/13	6	13	10-12 loads (1272.5-1848.2 to 1526.9-2217.9 gallons/acre) , solids, not tilled in
11/29/2013	23	15	13,300 gallons/acre, liquid, not tilled in
12/2/2013	6	13	8 loads (1018-1478.6 gallons/acre), solids, not tilled in
12/4/2013	8	15	1-2 loads (110.3-160.2 to 220.6-320.6 gallons/acre), solids, not tilled in
12/19/2013	6	13	7-8 loads (890.7-1293.8 to 1018-1478.6 gallons/acre), solids, not tilled in
1/25/2014	6	13	4 loads of frozen liquids (had to spread liquids) (509-739.3 gallons/acre), not tilled in
1/27/2014	6	13	4 loads of frozen liquids (had to spread liquids) (509-739.3 gallons/acre), not tilled in
1/28/2014	6	13	12 loads of frozen liquids (had to spread liquids) (1526.8-2217.9 gallons/acre), not tilled in
1/30/2014	8	15	4 loads of frozen liquids (had to spread liquids) (441.1-640.7 gallons/acre), not tilled in
2/14/2014	6	13	5 loads (636.2-924.1 gallons/acre), solids
2/17/2014	8	15	13 loads (1433.6-2082.3 gallons/acre), solids (strawpack)

Table 5: Collection Information

Date Collected	Date Sampled	Site	Weather	Flow	Notes	More Notes
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	1A	below freezing	light cover of ice	few inches deep	took more upstream sample; liquids, surface
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	2A	below freezing	light cover of ice	higher slope than 1a	took more downstream sample; liquids; both
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	1B	below freezing, windy	ice covered	water low; lots of sediment	upstream sample; solid; surface
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	2B	below freezing, windy	1/2 inch thick of ice	20 feet wide, 1 foot deep; middle of channel sampled	took at site; solid; surface
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	3B	below freezing, windy	totally frozen, 3/4 inch thick	app. 7 inches deep, got it about 8 feet upstream and 4 feet from the edge; 20 feet wide	upstream sample; solid; surface
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	4B	below freezing, windy	3/4 inch thick	cracked ice to get to 3 inch deep surface drainage, 1 foot wide	downstream; liquids (but downstream of solids); surface
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	1C	below freezing, windy	1/8 inch thick ice	took 3 feet upstream; 4 foot wide	upstream (downstream of farm, pasture, fields); liquids; both
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	2C	below freezing, windy	1/4 inch thick ice	took 2 feet from the edge, 2 feet downstream; 10 feet wide	at site but more downstream; liquids; both
1/20/13, 1/21/13	1/22/13 (put in inc. bio+streaked EMB +Mac), 1/24/13 (counted EMB+ Mac), 1/28/2013 (chem)	1D	below freezing, windy		took 3 feet upstream; 7 feet wide, 9 iinches deep	upstream; none; both
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	2D	below freezing, windy		took 3 feet upstream; 7 feet wide, 9 iinches deep	downstream; none; both
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	1E	below freezing, windy	1/8 inch thick ice	2 1/2 feet wide, under culvert; 3 inches deep; steep slope	downstream; maybe solids/none; both
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	2E	below freezing, windy	1/4 inch thick ice	3 feet wide; 3 inches deep; steep slope	upstream; maybe solids/none; both
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	2F	below freezing, windy	1/2 inch thick of ice	took in the middle of the ditch; 3 1/2 feet wide	
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	1G	below freezing, windy	flowing	took in the middle of the ditch; 3 feet wide; 5 inches deep	both (surface+subsurface)
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	2G	below freezing, windy	flowing	took in the middle of the ditch; 3 feet wide; 5 inches deep	both (surface+subsurface)
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	1J	below freezing, windy	flowing	took at the edge; 20 feet away from field start point, at the culvert	at site, but more upstream; solid/strawpack; both
1/20/13, 1/21/13	1/22/13 (put in inc.bio+ streaked EMB + Mac), 1/23/2014 (chem), 1/24/13 (counted EMB + Mac)	2J	below freezing, windy	flowing	took at the edge of the ditch; 5 inches deep	downstream; solid/strawpack; both
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	9C		running a lot		
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	6C		surface water flowing fast		
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	4C		tile flowing	draingage from field 6	water up high and mixing of field and ditch water (so not accurate)
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	1D		surface water flowing		
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	2D		tile flowing		water mixing with pond water, and other field and ditch water (so not accurate)
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	8C		tile running a lot		
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	5C		surface drainage; light flow		
3/10/2013	3/11/2013 (chem+ put in inc. bio+ streaked EMB), 3/13/13 (counted EMB),	3C		tile flowing		
3/26/2013	3/27/13 (chem+put in inc. bio+streaked EMB), 3/29/13 (counted EMB)	1D		water not flowing, but water in deep pools	took water from deep, stationary pools	
3/26/2013	3/27/13 (chem+put in inc. bio+streaked EMB), 3/29/13 (counted EMB)	2D		flowing	water under pipe	
3/26/2013	3/27/13 (chem+put in inc. bio+streaked EMB), 3/29/13 (counted EMB)	7C		surface, not flowing, so took water from small pools	took water from small, stationary pools	
12/21/2013	12/26/13 (put in inc. bio), 12/31 /13 (streaked EMB),12/26/13 (chem ammonia), 1/3/14 (chem N, P), 1/2/14 and 1/3/14 (counted colonies)	6C		flowing	Farmer got these samples for me	goes by canal; got it before it went into ditch
12/21/2013	12/26/13 (put in inc. bio), 12/31 /13 (streaked EMB),12/26/13 (chem ammonia), 1/3/14 (chem N, P), 1/2/14 and 1/3/14 (counted colonies)	1C		surface, flowing	Farmer got these samples for me	drains field 6
12/21/2013	12/26/13 (put in inc. bio), 12/31 /13 (streaked EMB),12/26/13 (chem ammonia), 1/3/14 (chem N, P), 1/2/14 and 1/3/14 (counted colonies)	5C		surface, flowing	Farmer got these samples for me	drains fields 1, 5, 6
12/21/2013	12/26/13 (put in inc. bio), 12/31 /13 (streaked EMB),12/26/13 (chem ammonia), 1/3/14 (chem N, P), 1/2/14 and 1/3/14 (counted colonies)	1B		surface, flowing	Farmer got these samples for me	before field 8
12/21/2013	12/26/13 (put in inc. bio), 12/31 /13 (streaked EMB),12/26/13 (chem ammonia), 1/3/14 (chem N, P), 1/2/14 and 1/3/14 (counted colonies)	2B		surface, flowing	Farmer got these samples for me	after field 8
2/21/2014	2/22/14 (chem + put in inc. bio), 2/24/14 (streaked EMB plates), 2/27/14 (counted colonies)	1C	very windy	slushy water		
2/21/2014	2/22/14 (chem + put in inc. bio), 2/24/14 (streaked EMB plates), 2/27/14 (counted colonies)	5C	very windy	slushy water		
2/21/2014	2/22/14 (chem + put in inc. bio), 2/24/14 (streaked EMB plates), 2/27/14 (counted colonies)	1B	very windy	slushy water, wind blowing water opposite way	had to get this sample a few feet before the spot because the wind was blowing the water from 2 B into it	
2/21/2014	2/22/14 (chem + put in inc. bio), 2/24/14 (streaked EMB plates), 2/27/14 (counted colonies)	2B	very windy	slushy water, wind blowing water opposite way		

Table 6: Nutrient and Biological Data from Dr. Midden's Lab

Date Collected	Site	Ammonia (NH3)	Nitrate (Nox)	Phosphate (oP)	Total Coliform, Midden lab, undiluted, MPN/100 ml	Total Coliform, Midden lab, diluted, MPN/100 ml	E. Coli, undiluted, MPN/100 ml	E. Coli, diluted, MPN/100 ml
1/20/13, 1/21/13	1A	0.05865	0.71865	0.231	>2419.6	1413.6	29.2	6.3
1/20/13, 1/21/13	2A	-0.0266	2.09605	0.1011	>2419.6	>2419.6	26.2	2
1/20/13, 1/21/13	1B	1.07155	-0.32085	1.48985	>2419.6	>2419.6	344.8	24.6
1/20/13, 1/21/13	2B	0.0717	-0.4245	0.5001	>2419.6	290.9	6.3	<1.0
1/20/13, 1/21/13	3B	0.30575	-0.38675	0.56965	>2419.6	>2419.6	125.9	6.3
1/20/13, 1/21/13	4B	-0.02405	-0.407	0.31075	>2419.6	387.3	30.1	6.3
1/20/13, 1/21/13	1C	0.04465	2.6186	0.10205	>2419.6	866.4	77.2	6.3
1/20/13, 1/21/13	2C	0.05645	2.7577	0.139	>2419.6	613.1	166.4	20.1
1/20/13, 1/21/13	1D	0.07535	3.471	0.183	>2419.6	755.6	1553.1	114.5
1/20/13, 1/21/13	2D	0.2104	3.94815	0.25215	1011.2	2419.6	360.9	47.3
1/20/13, 1/21/13	1E	0.4934	2.71225	0.22935	>2419.6	648.8	19.9	5.2
1/20/13, 1/21/13	2E	0.4907	9.0175	0.24685	2419.6	224.7	13.1	1
1/20/13, 1/21/13	2F	0.16935	2.8951	0.26975	>2419.6	1203.3	201.4	17.1
1/20/13, 1/21/13	1G	0.2624	4.82445	0.1141	>2419.6	547.5	135.4	12
1/20/13, 1/21/13	2G	0.27645	3.55415	0.1272	1011.2	547.5	225.4	21.3
1/20/13, 1/21/13	1J	0.0115	4.7791	0.05915	691	2419.6	141.4	26.2
1/20/13, 1/21/13	2J	0.01495	4.45485	0.0829	1011.2	2419.6	185	32.3
3/10/2013	9C	0.8618	4.31235	0.0972	1011.2	>2419.6	10.9	2
3/10/2013	6C	0.06835	4.34405	0.5105	1011.2	>2419.6	36.8	20
3/10/2013	4C	-0.15255	12.5246	0.46085	>2419.6	>2419.6	141.4	14.6
3/10/2013	1D	1.0703	3.98185	0.27415	>2419.6	1986.3	206.4	27.9
3/10/2013	2D	1.25685	5.87435	0.6712	>2419.6	>2419.6	410.6	33.7
3/10/2013	8C	-0.0058	2.3583	0.3837	>2419.6	>2419.6	4.1	<1.0
3/10/2013	5C	-0.0997	0.75395	3.79365	>2419.6	1732.9	24.3	1
3/10/2013	3C	-0.0093	5.4341	0.41295	1011.2	>2419.6	104.6	14.8
3/26/2013	1D	0.34765	2.77725	0.1123	>2419.6	>2419.6	1	<1.0
3/26/2013	2D	0.30385	11.35995	0.2355	>2419.6	>2419.6	488.4	52.9
3/26/2013	7C	0.9219	4.554	0.734	>2419.6	>2419.6	>2419.6	1203.3
12/21/2013	6C	3.6054	10.31145	6.2421	1011.2	>2419.6	913.9	260.3
12/21/2013	1C	0.7943	2.1787	3.68865	533.5	>2419.6	533.5	>2419.6
12/21/2013	5C	0.1831	0.94475	1.74615	533.5	>2419.6	533.5	410.6
12/21/2013	1B	0.72905	5.6025	5.3699	437.4	>2419.6	437.4	>2419.6
12/21/2013	2B	0.06365	4.5077	4.2693	691	>2419.6	419.8	21.6
2/21/2014	1C	0.6267	1.9932	0.45385	>2419.6	>2419.6	547.5	71.2
2/21/2014	5C	7.305	1.2426	2.62965	>2419.6	>2419.6	307.6	36.4
2/21/2014	1B	1.04855	1.8058	0.4165	>2419.6	1553.1	>2419.6	325.5
2/21/2014	2B	17.3086	1.24275	6.72225	>2419.6	>2419.6	>2419.6	>2419.6

Table 7: Concentrations of Nutrient Results

Key:
Ammonia:
Normal
High
Nitrate:
Low
Low-Moderate
Moderately High
High
Phosphate:
Low
Moderate
Moderately High
High

Date Collected	Site	Ammonia (NH3)	Nitrate (Nox)	Phosphate (oP)
1/20/13, 1/21/13	1A	0.05865	0.71865	0.231
1/20/13, 1/21/13	2A	-0.0266	2.09605	0.1011
1/20/13, 1/21/13	1B	1.07155	-0.32085	1.48985
1/20/13, 1/21/13	2B	0.0717	-0.4245	0.5001
1/20/13, 1/21/13	3B	0.30575	-0.38675	0.56965
1/20/13, 1/21/13	4B	-0.02405	-0.407	0.31075
1/20/13, 1/21/13	1C	0.04465	2.6186	0.10205
1/20/13, 1/21/13	2C	0.05645	2.7577	0.139
1/20/13, 1/21/13	1D	0.07535	3.471	0.183
1/20/13, 1/21/13	2D	0.2104	3.94815	0.25215
1/20/13, 1/21/13	1E	0.4934	2.71225	0.22935
1/20/13, 1/21/13	2E	0.4907	9.0175	0.24685
1/20/13, 1/21/13	2F	0.16935	2.8951	0.26975
1/20/13, 1/21/13	1G	0.2624	4.82445	0.1141
1/20/13, 1/21/13	2G	0.27645	3.55415	0.1272
1/20/13, 1/21/13	1J	0.0115	4.7791	0.05915
1/20/13, 1/21/13	2J	0.01495	4.45485	0.0829
3/10/2013	9C	0.8618	4.31235	0.0972
3/10/2013	6C	0.06835	4.34405	0.5105
3/10/2013	4C	-0.15255	12.5246	0.46085
3/10/2013	1D	1.0703	3.98185	0.27415
3/10/2013	2D	1.25685	5.87435	0.6712
3/10/2013	8C	-0.0058	2.3583	0.3837
3/10/2013	5C	-0.0997	0.75395	3.79365
3/10/2013	3C	-0.0093	5.4341	0.41295
3/26/2013	1D	0.34765	2.77725	0.1123
3/26/2013	2D	0.30385	11.35995	0.2355
3/26/2013	7C	0.9219	4.554	0.734
12/21/2013	6C	3.6054	10.31145	6.2421
12/21/2013	1C	0.7943	2.1787	3.68865
12/21/2013	5C	0.1831	0.94475	1.74615
12/21/2013	1B	0.72905	5.6025	5.3699
12/21/2013	2B	0.06365	4.5077	4.2693
2/21/2014	1C	0.6267	1.9932	0.45385
2/21/2014	5C	7.305	1.2426	2.62965
2/21/2014	1B	1.04855	1.8058	0.4165
2/21/2014	2B	17.3086	1.24275	6.72225

Table 8: Final Colilert Water Tray Coliform and E.coli Results

Key for E.coli:	Date Collected	Site	Total Coliform, Midden lab, MPN/100 ml (CFU/100 ml)	Mean E.coli, Midden lab, MPN/100 ml (CFU/100 ml)
	1/20/13, 1/21/13	1A		
Below 235 CFU/100 ml	1/20/13, 1/21/13	2A		
Between 235 and 1,000 CFU/100 ml	1/20/13, 1/21/13	1B	>2419.6	184.7
Above 1,000 CFU/100 ml	1/20/13, 1/21/13	2B	290.9	3.65
	1/20/13, 1/21/13	3B		
	1/20/13, 1/21/13	4B		
	1/20/13, 1/21/13	1C	866.4	41.75
	1/20/13, 1/21/13	2C		
	1/20/13, 1/21/13	1D	755.6	833.8
	1/20/13, 1/21/13	2D	1715.4 (1 empty well)	204.1
	1/20/13, 1/21/13	1E		
	1/20/13, 1/21/13	2E		
	1/20/13, 1/21/13	2F		
	1/20/13, 1/21/13	1G		
	1/20/13, 1/21/13	2G		
	1/20/13, 1/21/13	1J		
	1/20/13, 1/21/13	2J		
	3/10/2013	9C		
	3/10/2013	6C	>1715.4 (1 empty well)	19.4
	3/10/2013	4C		
	3/10/2013	1D	1986.3	117.15
	3/10/2013	2D	>2419.6	222.15
	3/10/2013	8C		
	3/10/2013	5C	1732.9	12.65
	3/10/2013	3C		
	3/26/2013	1D	>2419.6	<1.0
	3/26/2013	2D	>2419.6	270.65
	3/26/2013	7C		
	12/21/2013	6C	>1715.4 (1 empty well); 1:10 dilution was >2419.6	587.1
	12/21/2013	1C	>1476.55 (3 empty wells); 1:10 dilution was >2419.6	1476.55
	12/21/2013	5C	>1476.55 (3 empty wells); 1:10 dilution was >2419.7	472.05
	12/21/2013	1B	>1428.5 (4 empty wells); 1:10 dilution was >2419.6	>1428.5
	12/21/2013	2B	>1555.3 (2 empty wells); 1:10 dilution was >2419.6	220.7
	2/21/2014	1C	>2419.6	309.35
	2/21/2014	5C	>2419.6	172
	2/21/2014	1B	>1553.1	1372.55
	2/21/2014	2B	>2419.6	>2419.6

Table 9: Biological Data from Dr. Bullerjahn's Lab

Date Collected	Site	Gram Negative Colonies, MacConkey (Mac), Bullerjahn lab, CFU/100ml	Gram Negative Colonies, Eosin Methylene Blue (EMB), Bullerjahn lab, CFU/100ml	MacConkey, Lactose + Coliform (Bullerjahn), CFU/100ml	EMB, Lactose + Colonies (Bullerjahn), CFU/ 100ml
1/20/13, 1/21/13	1A	1,300,000	2,100,000	200	<100
1/20/13, 1/21/13	2A	500,000	400,000	100	<100
1/20/13, 1/21/13	1B	4,400,000	14,800,000	200	2,000
1/20/13, 1/21/13	2B	300,000	400,000	<100	<100
1/20/13, 1/21/13	3B	500,000	1,600,000	100	<100
1/20/13, 1/21/13	4B	100,000	600,000	<100	<100
1/20/13, 1/21/13	1C	300,000	400,000	<100	<100
1/20/13, 1/21/13	2C	200,000	400,000	<100	<100
1/20/13, 1/21/13	1D	100,000	100,000	100	<100
1/20/13, 1/21/13	2D	300,000	400,000	<100	<100
1/20/13, 1/21/13	1E	<100,000	200,000	<100	<100
1/20/13, 1/21/13	2E	<100,000	<100,000	<100	<100
1/20/13, 1/21/13	2F	<100,000	700,000	<100	200
1/20/13, 1/21/13	1G	100,000	200,000	100	<100
1/20/13, 1/21/13	2G	100,000	100,000	<100	<100
1/20/13, 1/21/13	1J	100,000	200,000	100	<100
1/20/13, 1/21/13	2J	100,000	700,000	100	<100
3/10/2013	9C	N/A	1,700,000	N/A	100
3/10/2013	6C	N/A	12,100,000	N/A	100
3/10/2013	4C	N/A	193,000	N/A	200
3/10/2013	1D	N/A	23,000	N/A	200
3/10/2013	2D	N/A	2,100,000	N/A	100
3/10/2013	8C	N/A	7,600,000	N/A	300
3/10/2013	5C	N/A	6,400,000	N/A	<100
3/10/2013	3C	N/A	13,300,000	N/A	400
3/26/2013	1D	N/A	2,000,000	N/A	<100
3/26/2013	2D	N/A	26,600,000	N/A	1,000
3/26/2013	7C	N/A	app. 110,000,000	N/A	100
		EMB, total coliform, 1:1000		EMB,lactose +, 1:1000	
12/21/2013	6C	1: <1,000,000 2: <1,00,00 03: <1,000,000 (Average=<1,000,000)	1: <100,000 2: 100,000 3: <100,000 (Average=<100,000)	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
12/21/2013	1C	1: 1,000,000 2: 1,000,000 3: 4,000,000 (Average=2,000,000)	1: 800,000 2: <100,000 3: 1,600,000 (Average=<833,333)	1: 1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: 100 2: <100 3: <100 (Average=<100)
12/21/2013	5C	1: <1,000,000 2:2,000,000 3: <1,000,000 (Average=<1,333,333)	1: <100,000 2: 400,000 3: 900,000 (Average=<466,666)	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
12/21/2013	1B	1: <1,000,000 2: 1,000,000 3: <1,000,000 (Average=<1,000,000)	1: <100,000 2: <100,000 3: 300,000 (Average=<166,666)	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
12/21/2013	2B	1: <1,000,000 2: <1,000,000 3: 1,000,000 (Average=<1,000,000)	1: 500,000 2: <100,000 3: 100,000 (Average=<350,000)	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
2/21/2014	1C	N/A	1: 1,700,000 2: 500,000 (Average=1,100,000)	N/A	1: 1,000 2: 300 (Average=650)
2/21/2014	5C	N/A	1: 6,900,000 2: 2,600,000 (Average=4,750,000)	N/A	1: 2,700 2: 800 (Average=1750)
2/21/2014	1B	N/A	1: 300,000 2: 400,000 (Average=350,000)	N/A	1: <1002: <100 (Average=<100)
2/21/2014	2B	N/A	1: 4,900,000 2: 3,800,000 (Average=4,350,000)	N/A	1: 2,700 2:1,900 (Average=2300)

Table 10: Levels of Lactose Positive Contamination

Key:	Date Collected	Site	MacConkey, Lactose + Coliform (Bullerjahn), CFU/100ml	EMB, Lactose + Colonies (Bullerjahn), CFU/ 100ml
	1/20/13, 1/21/13	1A	200	<100
Below 235 CFU/100 ml	1/20/13, 1/21/13	2A	100	<100
Between 235 and 1,000 CFU/100 ml	1/20/13, 1/21/13	1B	200	2,000
Above 1,000 CFU/100 ml	1/20/13, 1/21/13	2B	<100	<100
	1/20/13, 1/21/13	3B	100	<100
	1/20/13, 1/21/13	4B	<100	<100
	1/20/13, 1/21/13	1C	<100	<100
	1/20/13, 1/21/13	2C	<100	<100
	1/20/13, 1/21/13	1D	100	<100
	1/20/13, 1/21/13	2D	<100	<100
	1/20/13, 1/21/13	1E	<100	<100
	1/20/13, 1/21/13	2E	<100	<100
	1/20/13, 1/21/13	2F	<100	200
	1/20/13, 1/21/13	1G	100	<100
	1/20/13, 1/21/13	2G	<100	<100
	1/20/13, 1/21/13	1J	100	<100
	1/20/13, 1/21/13	2J	100	<100
	3/10/2013	9C	N/A	100
	3/10/2013	6C	N/A	100
	3/10/2013	4C	N/A	200
	3/10/2013	1D	N/A	200
	3/10/2013	2D	N/A	100
	3/10/2013	8C	N/A	300
	3/10/2013	5C	N/A	<100
	3/10/2013	3C	N/A	400
	3/26/2013	1D	N/A	<100
	3/26/2013	2D	N/A	1,000
	3/26/2013	7C	N/A	100
			EMB,lactose +, 1:1000	
	12/21/2013	6C	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
	12/21/2013	1C	1: 1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: 100 2: <100 3: <100 (Average=<100)
	12/21/2013	5C	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
	12/21/2013	1B	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
	12/21/2013	2B	1: <1,000 2: <1,000 3: <1,000 (Average=<1,000)	1: <100 2: <100 3: <100 (Average=<100)
	2/21/2014	1C	N/A	1: 1,000 2: 300 (Average=650)
	2/21/2014	5C	N/A	1: 2,700 2: 800 (Average=1750)
	2/21/2014	1B	N/A	1: <100 2: <100 (Average=<100)
	2/21/2014	2B	N/A	1: 2,700 2: 1,900 (Average=2300)

Table 11: Summary of Ampicillin Resistance Results Showing How Resistant the Community Is

Percentage of Total Colonies Grown on AMP Plates That Were Ampicillin Resistant:	Percentage of Total Colonies Grown on AMP Plates That Were Lactose +:	Percentage of Lactose + Colonies Grown on AMP Plates That Were Ampicillin Resistant:
60.28%	29.63%	56.58%

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