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Factors Limiting Organic Carbon Degradation by Antibiotic-Resistant Heterotrophic Microorganisms in Wastewater Contaminated Sediments

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FACTORS LIMITING ORGANIC CARBON DEGRADATION BY ANTIBIOTIC-RESISTANT HETEROTROPHIC MICROORGANISMS IN WASTEWATER CONTAMINATED SEDIMENTS

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Abstract

This study aims to assess the interactions between antibiotic resistance and the ability of heterotrophs effected by highly polluted environments to remediate antibiotics from soil *via* biodegradation. These findings would reveal whether a self-cleaning process is occurring in the waters of Western NY. Water and sediment heterotrophs were obtained from wastewater contaminated environments to understand the factors influencing metabolism. A total of 113 isolates from Charlotte, Durand, and Payne Beaches, the Genesee River, and Hemlock Lake were cultured and detected for antibiotic resistance to 24 different antibiotics. Of these, seven robust bacteria from sediments were considered for their ability to use a single antibiotic as their sole carbon source — available carbon limited substrate utilization. Microbes were able to remediate antibiotic substrates from soil between 18.1-42.1% at one-times the concentration, whereas two and three times the original concentration was impotently degraded by 13.4-24.0% and 5.7-18.2%, separately. Heterotrophs also demonstrated unique substrate binding affinity for alternative substrates, which were discovered to be degraded between 1.05% to 72.96%. We found the best alternative substrates with enzyme activity to be tetracycline (70.26-72.96%), chloramphenicol (49.28-66.78%), and trimethoprim (54.51-57.22%). An assessment of antimicrobial susceptibility revealed that the highest resistance was observed to aminoglycoside antibiotics, followed by cephalosporins, beta-lactams, and fluoroquinolones. A robust isolate from Durand Beach was resistant to 19 out of the 24 antibiotics under evaluation. We posit that heterotrophs of highly polluted environments are restricted by carbon-availability, have unique binding affinity for alternative substrates, and are multidrug-resistant.

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List of Abbreviations

ANOVA analysis of variance

AR antibiotic resistant

ARGs antibiotic resistance genes

AU antibiotic usage

CDC Centers for Disease Control and Prevention

CFU/ml colony-forming units per milliliter

CRISPR-Cas9 clustered regularly interspaced short palindromic repeats-CRISPR associated protein 9

DI H_2O deionized water

DMR Discharge Monitoring Report

DNA deoxyribonucleic acid

ECHO Enforcement and Compliance History Online

EPA Environmental Protection Agency

HPC heterotrophic plate count

HSD honest significance test

HUC hydrologic unit code

IBM SPSS International Business Machines Statistical Package for the Social Sciences

m.o. *modus operandi*

MDR multidrug-resistant

NWQ North West Quadrant

PDR pandrug-resistant

PPCPs pharmaceuticals and personal care products

R2A Reasoner's 2A

RG&E Rochester Gas and Electric Corporation

rpm revolutions per minute

SAAR standardized antimicrobial administration ratio

STP sewage treatment plant

U.S. United States

USDA United States Department of Agriculture

UV/Vis ultraviolet−visible spectroscopy

WNY Western New York

WPCF Water Pollution Control Facility

WWTP wastewater treatment plant

WWTPs wastewater treatment plants

XDR extensively drug-resistant

ZOI zone of inhibition

Chapter 1

Introduction and literature review

1.1 Overview

The use of antibiotics has revolutionized medicine while inadvertently making it increasingly complicated to treat disease [1] due to the development of extensively drug-resistant (XDR), multidrug-resistant (MDR), and pandrug-resistant (PDR) pathogens [2], [3]. Since their debut, world-wide yearly antibiotic consumption in 2002 [4] was estimated between 100 to 200 thousand tonnes, over 1 million tonnes in 2010 [5], and now calculated to be between 8.9 to 17.8 million tonnes per year.

The rise of antibiotic resistance has been deemed a public health threat across the globe [1], [6], [7]. Contributing factors to the occurrence of environmental resistance have been attributed to the overuse and misuse of antibiotics in clinical and veterinary settings [1], [7]. The sharing of resistance genes among bacteria in the environment has facilitated human-pathogen acquired resistance [8], through selective pressures leading to natural selection.

Industrial facilities and animal farms act as hot spots for antibiotic resistance genes (ARGs), promoting the evolution of antibiotic resistant (AR) bacteria through selective pressures. Waste from these facilities, containing AR bacteria, ARGs, and antibiotics, is discharged into the environment where it leaches into groundwater and eventually flows into our rivers and oceans [1], [9]–[14].

Drinking water quality standards have been under speculation as chemicals, such as pharmaceuticals, are not regulated [15], and consequently, pharmaceutical levels have been detected in drinking water [16]. Extended exposure to these chemicals through drinking water poses questions about health risks. Thus, pharmaceuticals have been deemed an emerging chemical contaminant, and extensive research has been put towards removal techniques [9], [15]. Remediation of antibiotics from natural waters, sediments, and wastewater can be achieved by use of nanomaterials [17], abiotic and biotic biodegradation [18], and the use

of bioengineered strains [19].

Bioremediation of environmental contaminants involves the use of antibiotic substrates as a carbon source. This study aims to heighten the understanding of the factors that impact the biodegradation of antibiotics in polluted environments. The correlation between antibiotic contaminant removal potential *via* biodegradation and the occurrence of antibiotic resistance in heterotrophs were also under investigation.

1.2 Release of antibiotics into the environment

Heterotrophic microorganisms are ubiquitous in the environment and use organic matter for growth [20]. The release of antibiotics into the environment has been linked to antibiotic resistance of human pathogens, provoking challenges for practitioners when treating disease [21]. Sewage overflow, agricultural runoff, incomplete removal by , and the overuse and misuse of antibiotics are a few of the mechanisms by which antibiotics get in contact with environmental microorganisms.

The Journal of Clinical Infectious Diseases reported that in 2011 there were 60.3 million penicillins, 59.1 million macrolides, 35.6 million cephalosporins, 27.6 million fluoroquinolones, and 21.6 million beta-lactam outpatient prescriptions in the United States [22], inducing a high selective pressure for environmental microbes.

In addition to the use of antibiotics in human medicine, antibiotics are popularly used in farming [23][24] and aquaculture as preventative measures for disease, likely to meet the public demand for meat consumption [1], [7]. Incomplete metabolism of antibiotics in humans and livestock results in excretions containing antibiotic concentrations [1], [11]. Sewage and wastewater contain human excretions which can include trace levels of pharmaceutical antibiotics. Researchers report on the excretion rates of several active substances: 80% ampicillin, 80% streptomycin, 85% cefepime, and 70% levofloxacin (structurally similar to lomefloxacin) [25].

Pharmaceuticals secreted in urine can outflow into water bodies, exposing bacteria to harmful toxins, thereby inducing evolutionary adaptations for survival. Sewage and animal manure runoff into water bodies, eventually making way to inline and drinking water facilities that are not equipped to eliminate antibiotics [13], [14], [26]. Similarly, agricultural soils use biosolids as additives [27]. During precipitation, agricultural soils runoff into water systems. A sewage sludge mesocosm experiment in the United States detected the dry weight concentrations of lomefloxacin at 2.7 μg/kg [27]. The lack of methods by which inline are equipped

to eliminate trace levels of antibiotics from influent pose concerns for the public through the emergence of antibiotic-resistant pathogens.

1.3 Antibiotics under investigation

Within this study, antibiotics used to treat various clinical infections either singly or in combination [28]–[32] (ampicillin, cefepime, lomefloxacin, or streptomycin) were used to select for sediment bacteria capable of substrate degradation. These drugs are of relative significance as they are currently being used in hospital settings for the treatment of disease, and a better understanding of the interactions between antibiotics (synthetic and natural) and microbes in the environment is paramount.

Combination drug therapy is used for the treatment of MDR and XDR. When treating resistant hospital-acquired pneumonia, it is required to treat patients with broad-spectrum antibiotics (i.e., cefepime, levofloxacin) alone or in combination [32], [33]. When MDR is a concern, an aminoglycoside or colistin is added to the mix [32].

In practice, multidrug-resistant urinary infections, neurosurgical procedure infections, and tuberculosis have been treated with lomefloxacin, broad-spectrum antimicrobial therapy (vancomycin, a third or fourth generation cephalosporin, and metronidazole), and streptomycin, respectively [28]–[30]. As more drugs are introduced for the treatment of infections, the ability of microbes to combat these drugs or a combination of these drugs, is imminent.

1.4 Occurrence of antibiotics in the environment

Worldwide reports have detected antibiotics at high concentrations in aquatic environmental ecosystems. Ampicillin has been detected in water, hospital effluent, and wastewater effluent at concentrations of 0.016 μg/ml [34], 0.028-0.0827 μg/ml [25], and 70.6 μg/ml [35], respectively. The other antibiotics under investigation within this study have been detected at levels in the ng/L [36]–[39], but have not been studied as extensively.

1.5 Occurrence of antibiotic resistance in environmental ecosystems

The resistance of antibiotics in the environment has been studied to a great extent across the world, with percentages of resistant cultured microbes ranging drastically by geographic location, signifying a need for site-specific analyses [15]. In aquatic environments, the resistance of water *Escherichia coli* isolates during winter from a river in India demonstrated antibiotic resistance of 39% ampicillin and 10% cefepime [40]. In the same study, the resistance of sediment *E. coli* isolates during winter exhibited 30% resistance to ampicillin and 15% resistance to cefepime [40].

The percent resistance of *E. coli* isolates from a creek in British Columbia were reported as 16% to ampicillin, 8% to streptomycin, 0.5% to ciprofloxacin, and 59% to tetracycline [41]. Significantly, this study found higher antibiotic resistance in biofilms and sediment in contrast to water isolates [41].

1.6 Acquisition of antibiotic resistance genes (ARGs) in environmental microorganisms

Plasmids of microorganisms can contain a wide range of antibiotic resistance genes (ARGs). Many gene cassettes, genetic material encoding for antibiotic resistance can comprise a single integron [42], which then inserts into a transposon [43]. Transposons containing various sets of ARGs can insert into a plasmid to confer antibiotic resistance when it is evolutionarily advantageous for the cell. Additionally, transposable elements can move freely throughout the host genome, inserting sequences into host deoxyribonucleic acid (DNA) or plasmids. Plasmids can be shared between bacterial species through a process called conjugation [44], allowing antibiotic resistance to be widespread within bacterial populations.

Plasmids containing ARGs can be selected for by the exposure of microbes to any selective antibiotic that the plasmid confers resistance to [43]. Consequently, other ARGs existing on the same plasmid would be co-selected through the initial exposure to a single selective antibiotic [43].

In a study by [45], the selection of multiresistance by microbes in freshwater microcosms through enrichment of isolates to toxicant concentrations above levels typically exhibited in the environment was under investigation. The exposure of microorganisms to toxicants was proven to increase the frequency of multiple antibiotic resistance to unrelated antibiotics [45].

1.7 Threat to living organisms

Prior studies examined the connection between environmental antibiotic resistance and human-pathogen acquired resistance. A study in Egypt on Gram-negative bacteria in cancer patients found the resistance of *E. coli* isolates at 100% for ampicillin, 91% for amoxicillin/clavulanic acid, 94% for cefepime, 79% for levofloxacin, and 94% for trimethoprim/sulfamethoxazole, among several other clinically significant antibiotics. Findings of this study are of particular significance as cefepime, penicillins, and third and fourth generation cephalosporins exhibited high microbial resistance in patients [46].

In addition to the effects on public health, anthropogenic environmental toxicants threaten sensitive aquatic ecosystems. Recent studies found pharmaceuticals and personal care products (PPCPs) at concentrations in rivers that are of high or medium risk to living organisms [47]. In particular, the out-competition of naturally occurring microorganisms poses threats for fish and benthic invertebrates that require commensal bacteria for survival. Additionally, higher level organisms undergo bioaccumulation and biomagnification of aquatic pollutants [48], [49]. Research findings demonstrated the bioaccumulation of sulfonamides, trimethoprim, and fluoroquinolones in fish muscle tissue, and macrolide antibiotics in fish liver tissue [50]. The food chain may biomagnify environmental pollutants through trophic levels, and exposure of humans to these toxicants. The pollution of our freshwater bodies impacts the alteration in the balance of the ecosystem and must be monitored and controlled to preserve the environment for future generations to appreciate.

1.8 Existing solutions

In recent years, scientists have pushed the discovery of cutting-edge antibiotics in the fight against antibiotic resistance. In particular, a new class of antibiotics was discovered using revolutionary iChip technology, which cultures microbes outside of the lab [21] — the new antibiotic, teixobactin, aids in the fight against superbugs [51].

Millions of dollars in funding goes toward the fight against antibiotic resistance in the United States. Implementations have been taken to reduce the distribution of antibiotics at the source [52]–[54]. The Centers for Disease Control and Prevention (CDC) implemented a risk-assessment to monitor antibiotic usage (AU), the standardized antimicrobial administration ratio (SAAR), to statistically compare the ratio of antimicrobial therapy measured in days [53].

Despite a wide range of solutions to fight antibiotic resistance, limitations persist, and recommendations propose a better understanding of the toxicology and fate of chemicals in the environment [26]. The methods by which antibiotics disseminate into the environment have been studied extensively, though knowledge gaps remain as data varies geographically [15]. Advanced wastewater removal technologies exist, like, the emerging field of nano-materials which is promising for nano-remediation of chemicals of concern from effluent [26]. Alternative methods, like membrane bioreactors, appear to be effective but have operational drawbacks in terms of cost [26].

Prevention and restrictions on AU in practice assist in reducing the number of antibiotics released into the environment where contact with environmental microorganisms occurs. Appropriate antibiotic usage is a leap towards reducing the development of antibiotic resistance. Studies must be keen on tracking antibiotic resistance in the human microbiome and other organismal reservoirs. With the exposure of environmental microorganisms to high concentrations of synthetic and natural antibiotics, it is natural for organisms to compete for survival, giving rise to the deadliest of bugs [52], [53].

1.9 Objectives

Despite the vast array of scientific data available on microbial resistance, knowledge gaps remain concerning the roles of antibiotic substrate utilization in aquatic ecosystems. This study aims to develop a better understanding of the role of antibiotic resistance and biodegradation of antibiotics as substrates in the environment.

The objective of this study is to evaluate the resistance of microbes to 24 different antibiotics in wastewater contaminated surface water and sediments. The second objective is to heighten the understanding of the impacts of carbon-limitation and substrate availability on microbial isolates cultured from sediment. This study is unique in the way that extremely high antibiotic concentrations (500 μg/ml-25000 μg/ml) were used throughout our experiments.

1.10 Research hypothesis

The fate of antibiotic pollutants has sparked interest more recently as public health continues to deteriorate as a result of human-acquired pathogen resistance; facilitated by lack of removal efficacy by WWTPs and persistence of these compounds in the environment. Prior studies have suggested that some pharmaceutical compounds may be highly degradable [55], indicating a present knowledge gap in understanding the

metabolism of these compounds in the environment.

This study aims to gain a better understanding of antibiotic microbial metabolism from contaminated and uncontaminated sites, while simultaneously addressing antibiotic resistance in robust isolates. By gathering microbiological evidence, this study attempts to confirm *in situ* microbial metabolism by establishing an understanding of carbon-limitation and substrate-specificity in isolates from sampling sites in Western New York (WNY).

Even though the environment contains high concentrations of various antibiotics, we propose that microbial metabolism is limited by antibiotic carbon-availability and maximized by alternate substrates. Results indicate that degradation capacity is weakly substrate-specific and restricted by increases in carbon-availability.

Chapter 2

Materials and methods

2.1 Sampling sites

During summer and fall of 2017, aquatic grab samples within the littoral zone of both surface water and disturbed surface sediments were obtained from Charlotte, Durand, and Payne Beaches, the Genesee River, and Hemlock Lake. Grab samples were obtained using a 500 ml graduated water dipper. Sediment and water samples were taken within 18 meters of where the water meets the shore. Within this study, sampling sites were impacted by flooding and wastewater discharge. Summer samples were obtained on May 23, 2017, and fall samples were obtained on October 29, 2017.

Figure B.4 shows the geographic locations of sampling points. Samples were taken from unusual locations during the summer, as historical flooding was present. Observation of Payne Beach revealed significant flooding. Additionally, Durand Eastman had no beach, Charlotte only had 40% of the beach present, the Hemlock water level was almost up to the road, and there was no difference in the sample location for the Genesee River. In the fall, 20-30% of Charlotte beach and 80% of Durand had receded, Payne Beach was down to the effluent pipe, and Hemlock beach returned to normal levels.

The resistance of heterotrophs in WNY was quantified through the evaluation and comparison of several point sources. The control within this study was Hemlock Lake as it is used as drinking water for the City of Rochester [56]. Wastewater effluent is discharged into three of the other sources, Durand, Charlotte, and Payne Beach. Of which, two of these point sources, Durand and Charlotte, are used by the general public as recreational swimming areas. Samples were also obtained and analyzed from the Genesee River, which is exposed to agricultural runoff through manure used on cornfields.

For Payne Beach, wastewater effluents release from the Northwest Quadrant wastewater treatment plant (WWTP), and there is the potential for runoff from migratory birds as this area is the western region of

Braddock Bay Wildlife Refuge [57], [58]. The Genesee River receives agricultural runoff from nearby fields that apply manure and pesticides to crops which can runoff into the river.

2.2 Environmental pollution

Coordinates of sampling locations were uploaded into ArcGIS Pro as X and Y data. A multipart buffer layer was created including one, five, and ten miles to determine WWTPs near sampling sites that may impact water quality. Using the United States (U.S.) Environmental Protection Agency (EPA) Enforcement and Compliance History Online (ECHO) website, discharge facilities classified as "major facilities" which were located within the hydrologic unit code (HUC) 8 watershed boundaries of each sampling site were added as points to the map [59]. The HUC 8 watershed boundaries were retrieved from the United States Department of Agriculture (USDA) Geospatial Data Gateway [60].

2.3 Bacterial populations

Laboratory methods followed the flow chart as outlined in Figure B.1. Samples obtained at indicated sampling locations (Figure B.4) were enumerated in triplicates. Viable cell count means were calculated using International Business Machines Statistical Package for the Social Sciences (IBM SPSS) computer software for Charlotte, Durand, Genesee, Hemlock, and Payne in summer and fall for both water (Figure 3.4) and sediment (Figure 3.5) samples in order to obtain an understanding of the number of culturable microorganisms present in a particular sample [61]. The enumeration of heterotrophs from freshwater is used as an indicator of water quality changes after treatment [20].

Triplicate serial dilutes of water, and sediment grab samples for each sampling site provided statistical accuracy of data through enumeration of colonies after 10 days of incubation at room temperature for dilutions of 10^{-5}. colony-forming units per milliliter (CFU/ml) for water and sediment were determined by multiplication of the dilution factor.

Water and sediment samples were titered in 1 ml from 10^{-1} to 10^{-5} in 0.9% saline solution. Triplicate serial dilutions of samples were pipetted onto R2A media (100 µL) from 10^{-5} to 10^{-7} using the spread plate method. Samples were incubated at $22 \pm 2°$C and enumerated at 2, 5, 7, and 10-days. Figure B.5 details the serial dilution technique used for water and sediment sample microbial viable cell counts.

0.9% saline solution was prepared and then sterilized with a 0.22 µm filter sterilizer then transferred in 9

ml increments to 15 ml sterile conical tubes. Reasoner's 2A (R2A) media is used to select for and enumerate heterotrophic microbes in potable water. R2A media was prepared using the standard protocol.

2.4 Culture enrichment for the isolation of acclimated bacteria

Through the creation of enrichment cultures (Figure B.6), heterotrophic microorganisms capable of survival with antibiotics from sediment were selected throughout 21-days and growth was measured using spectroscopy. Similar methods, such as the automated checkerboard array, use printer technology to measure the growth of cultures with various levels of antibiotics in synergy [62].

Enrichment cultures consisted of antibiotic-amended culture broth. Following 7 days of incubation, the qualitative turbidity of enrichment culture was observed and recorded (Tables A.12 and A.13). Turbidity was measured by observable cloudiness of the sample as a technique for the determination of microbial presence within the sample. If turbidity was not detected after 7 days of incubation, then the flasks were kept in the incubator for 7 additional days until cloudiness was observed.

Bushnell Haas broth was prepared using the following standard protocol. Into 1 liter of DI H_2O, 3.7 grams of Bushnell Haas broth was suspended and mixed. The pH was adjusted to 7-7.2 when necessary. Sterilization of the broth was either achieved through the use of 0.22-micron filter units or by autoclaving at 121°C for 20 minutes. The sterile broth was stored at room temperature in sealed bottles when not in use.

The enrichment technique includes samples amended with selective antibiotics for a duration of 21-days. Several different antibiotic stock solutions were prepared and added to cultures containing sediment to isolate potential antibiotic degraders. The following antibiotics were used: ampicillin, cefepime, lomefloxacin, and streptomycin. Preparation of antibiotic stocks followed the mass and volumes included in Table A.5. Using the equation for mass concentration (1), where the density (ρ) of a substance is equal to the concentration by dividing the mass of solute (m) by the volume of solvent (V), antibiotic stock concentrations were calculated. Sterilization of stock solutions was performed using 0.22 µm filter sterilizers then transferring the 20 ml stock solution to 50 ml sterile conical centrifuge tubes for storage in the refrigerator when not in use.

$$\rho = \frac{m}{V} \tag{1}$$

Antibiotic stocks were utilized to make three sets of enrichment cultures; in total there were 20 flasks.

Enrichment cultures for summer samples were prepared using sediment source samples. Through the addition of 5 ml of sample sediment with 1 ml of antibiotic stock (ampicillin (7500 μg/ml), cefepime (7500 μg/ml), lomefloxacin (25000 μg/ml), or streptomycin (10000 μg/ml)) and 44 ml Bushnell Haas in a 125 ml Erlenmeyer flask, respectively, enrichment cultures were created. Flasks were incubated for seven days of shaking incubation (120 revolutions per minute (rpm) at room temperature).

Visual detection of turbidity was performed for enrichment one after seven days of incubation. Before subculturing enrichments 2 and 3, at 14 and 21-days, turbidity was assessed. The subculturing process is illustrated in Figure B.6. For the following enrichments, fresh media was used to subculture the cells. Enrichment stocks were incubated and re-incubated shaking at 120 rpm and 22 $\pm$ 2°C. For enrichment 2, at 14 days, the enrichment culture was titered in 0.9% saline from 10^{-1} to 10^{-5} and plated by 100 µl increments onto R2A media from 10^{-4} to 10^{-7} using the spread plate method. The dilution process for enrichments is demonstrated in Figure B.7. Plates were incubated at room temperature and enumerated for 2, 5, 7, and 10-days. Enrichment cultures at 21-days were titered and plated onto R2A media as was conducted with enrichment 2. Fall sample processing followed the same methods as summer enrichment culture.

Further analysis included the collection of all colonies with growth on plates of 10^{-7}at 10-days from enrichment three. Colonies were isolated using a sterile inoculating loop and spread onto R2A media using the T-streak method. Following 48 hours of incubation at room temperature, isolated colonies were inoculated into the sterile nutrient broth to ensure pure cultures. Following 48 hours of shaking incubation of 120 rpm at room temperature in flasks, isolates were re-streaked onto R2A media. Colony morphology was observed after 48 hours of incubation at room temperature and characterized by form, elevation, margin, and color.

2.5 Growth of acclimated bacteria on substrates for the determination of robustness

The ability of selected heterotrophs to thrive in an environment with high concentrations of enriched antibiotic was quantified through absorbance measured at 600 nanometers *via* ultraviolet−visible spectroscopy (UV/Vis). Through growth analysis experimentation, we investigated heterotrophic cellular growth in culture media. Isolated enrichment 3 colonies were observed for the ability to not only co-exist with high antibiotic concentrations but also to multiply and divide in the presence of a single antibiotic of which they were initially enriched.

The growth of enrichment isolates on a particular antibiotic was quantified by UV/Vis spectroscopy.

Microorganisms were isolated from summer and fall enrichment 3 samples (Figures 4.1 and 4.2).

Isolates from the enrichment phase were further inoculated into 125 ml Erlenmeyer flasks containing 10 ml nutrient broth for 48 hours, shaking (120 rpm at room temperature). Isolates were then centrifuged in sterile 15 ml conical tubes for harvesting. Following centrifugation, the supernatant was removed, and a pipette was used to resuspend the pellet of cells into 2 ml of Bushnell Haas media.

In a 125 ml Erlenmeyer flask, 9.6 ml of Bushnell Haas media was added with 0.2 ml resuspended cells, retrieved from enrichment centrifugation and suspension, and 0.2 ml of the antibiotic (ampicillin (7500 μg/ml), cefepime (7500 μg/ml), lomefloxacin (25000 μg/ml), or streptomycin (10000 μg/ml)) of which the sample was enriched for, respectively. The flasks were incubated at room temperature, shaking at 120 rpm. The absorbance of these cultures was measured at 0, 2, 4, 6, 8, and 10-days by retrieving 0.3 ml of respective samples and re-incubating. A quartz cuvette with deionized water was used as a control for the UV/Vis spectrophotometer. With a pipette, 0.3 ml of the respective sample was pipetted into a quartz cuvette along with 1.2 ml of deionized water to measure the absorbance at 600 nanometers.

Utilizing the methods by Gallagher (2016) [63], generation of cells (G) (2) and growth rate (k) (3) were determined using the following calculations where N1 represents the lower absorbance, and N2 equals the higher absorbance measured at 600 nanometers.

$$G = \frac{duration \times \log_{10}(2)}{\log_{10}(N2) - \log_{10}(N1)} \tag{2}$$

$$k = \frac{0.693}{G} \tag{3}$$

2.6 Growth of robust isolates on alternative substrates

Colonies that exhibited a short doubling time and large growth rate from the initial growth analysis with antibiotics used for selection were chosen for further analysis of enrichment on antibiotics from different classes. Table A.14 indicates high capacity isolates that were chosen along with the different enriched antibiotics. Isolation of colonies followed the same procedure as previously mentioned.

In a 125 ml Erlenmeyer flask, 9.6 ml of Bushnell Haas media was added with 0.2 ml resuspended cells (from the 48 hour nutrient broth culture) and 0.2 ml of the antibiotic (6250 μg/ml amoxicillin, 1250 μg/ml chloramphenicol, 12500 μg/ml erythromycin, 25000 μg/ml levofloxacin, 500 μg/ml tetracycline, 2500 μg/ml

trimethoprim) in accordance with Table A.5, respectively. The flasks were incubated at room temperature, shaking at 120 rpm. The absorbance of each sample at 600 nm was measured at 0, 2, 4, 6, 8, and 10-days. A quartz cuvette with deionized water was used as a zero for the UV/Vis spectrophotometer. With a pipette, 0.3 ml of the sample was pipetted into a quartz cuvette to measure the absorbance at 600 nm along with 1.2 ml of Bushnell Haas media. The dilution factor was five.

2.7 Degradation of antibiotics from soil by robust isolates

Robust heterotrophs initially selected for in antibiotic-amended culture were chosen for biodegradation measurements on varying concentrations of the selected pharmaceutical (Tables 4.3 and 4.4). Isolates were inoculated in 10 ml of nutrient medium in flasks in the shaking incubator at 120 rpm and 22 $\pm$ 2° Celsius. Following 72 hours of incubation, 3 ml of cells were aseptically removed from the test tubes and placed into two sterile 1.5 ml microfuge tubes. Cells were centrifuged at 13,000 rpm for 5 minutes. The supernatant was poured off, and the cells were resuspended in the first microfuge tube with 1.0 ml of Bushnell Haas medium. The first microfuge tube was used to resuspend the cells in the second microfuge tube.

The following reagents were prepared for biometer experiments using standard protocol: 1% phenolphthalein, 0.05 N HCl, 0.2 N KOH, and 0.334 g/ml saturated barium chloride. Preparation of biometer flasks included the addition of 48 ml of Bushnell Haas medium to each flask, 1.0 ml of cells, and then 1.0 ml of antibiotic to the flask. Ascarite was added to the tower, and 10 ml of fresh KOH was added to the sidearm. The flask was incubated at room temperature. Biodegradation analysis of selected antibiotics consisted of the determination of isolate degradation rates of the antibiotic at one, two, and three-fold concentrations (Tables 4.3 and 4.4).

Samples of KOH for measuring carbon dioxide were taken at 2, 4, 7, and 10 days. To determine carbon dioxide evolution rates, the KOH was withdrawn from the sidearm and transferred to a clean flask. Ten ml of fresh KOH was added to the sidearm. To the recovered KOH samples, 1 ml of saturated barium chloride and 0.1 ml of phenolphthalein were added to measure the carbon dioxide trapped in the sidearm KOH. 10 ml of stock KOH was used at each time point as a control. The KOH solutions were titrated with 0.05 N HCl until the solution turned from pink to colorless. The volume of HCl needed to neutralize the retrieved KOH was recorded in milliliters. The following equation from [63] was utilized to determine the milligrams of CO_2

evolved at each time point:

$$CO_2\ (\mu\text{mol}) = (V_C - V_E)\ x\ 25 \tag{4}$$

V_C = volume to titrate control KOH (ml)

V_E = volume to titrate experimental KOH (ml)

$$Amount\ carbon\ (mg)\ =\ CO_2(\mu mol) \times \frac{10^{-6} mol}{1\ \mu mol} \times \frac{12.01\ g\ C}{1\ mol} \times 1000\ mg\ \times\ 0.27 \tag{5}$$

For data collection, milligrams of carbon dioxide evolved versus time was determined for each sediment culture. CO_2 evolution is significant to ascertain if microorganisms are utilizing the antibiotic substrate as their sole carbon source. The amount of carbon dioxide evolved was determined at each time point of potassium hydroxide retrieval and titration using equation (4). The amount of carbon was calculated using equation (5). For each substrate, the percent carbon was calculated by dividing the molecular weight of the substrate by the molecular weight of all carbons in the compound (6).

$$Percent\ Carbon\ of\ Substrate = \frac{MW\ of\ Substrate}{(Number\ of\ Carbons \times MW\ of\ Carbon)} \tag{6}$$

$$MW = Molecular\ Weight\ (grams/mole)$$

The amount of available carbon for carbon dioxide evolution was computed for each concentration of substrate by multiplying the percent carbon by the concentration of substrate using equation (7).

$$Milligrams\ Available\ Carbon = Percent\ Carbon \times Concentration\ of\ Substrate \tag{7}$$

The percent of carbon evolved was calculated for each time point by dividing the amount of carbon dioxide evolved by the milligrams of available carbon and multiplying by 100 using equation (8).

$$Percent\ Carbon\ Evolved = \frac{Amount\ Carbon}{Milligrams\ Available\ Carbon} \times 100 \quad (8)$$

Isolates enriched on lomefloxacin did not pass the growth analysis experiment; therefore these isolates were not utilized to measure biodegradation (Figure 4.1, 4.2). Isolates from Hemlock Lake did not pass the growth analysis.

2.8 Degradation of alternative substrates from sediment by robust isolates

Microbial isolates from selective antibiotic cultures were further analyzed for the ability to use alternative substrates as a carbon source. Biodegradation methods as mentioned previously were applied in alternative substrate analysis. Though, rather than measuring isolate CO_2 evolution of selective antibiotic, varying classes of antibiotics were analyzed for microbial biodegradation capacity by microbes enriched on selective antibiotics.

Bioassays were created by the addition of 1.0 ml of resuspended cells and 1.0 ml antibiotic stock (6250 μg/ml amoxicillin, 1250 μg/ml chloramphenicol, and 500 μg/ml tetracycline) to the biometer flask with 48 ml of Bushnell Haas. For erythromycin and levofloxacin, 1.0 ml of resuspended cells, 3.0 ml antibiotic stock (12500 μg/ml and 25000 μg/ml), and 46 ml of Bushnell Haas were combined, resulting in concentrations of 37500 μg/ml and 75000 μg/ml, respectively. Flasks for trimethoprim degradation involved the addition of 2.0 ml of antibiotic stock (2500 μg/ml) and 1.0 ml of resuspended cells into the flask containing 47 ml of Bushnell Haas, giving a resulting concentration of 5000 μg/ml.

At 2, 4, 7, and 10-days, KOH was recovered from the sidearm with a syringe and titrated in a new flask with HCl. Fresh KOH was added to the biometer flask and re-incubated at room temperature until the next time point.

2.9 Antibiotic susceptibility testing

Enriched sediment isolates and unenriched water and sediment colonies were isolated by T-streak and then inoculated into 125 ml Erlenmeyer flasks containing 10 ml nutrient broth for 48 hours shaking at 120 rpm, room temperature, respectively. After 48 hours, isolates were swabbed onto Mueller Hinton agar using sterile

cotton swabs, and the antibiotics were dispensed onto the surface. In total, 24 different antibiotics were tested using the disc diffusion method. Table A.15 lists the 24 antibiotics used. Plates were incubated at room temperature for 48 hours, and the diameter of the zone of inhibition (ZOI) was measured. Antimicrobial susceptibility was determined by comparing ZOI values with Table A.15 [64].

$$\frac{N_R}{N_C \times N_A} \times 100 = \%\ resis\tan ce\ of\ colonies\ and\ antibiotics$$

$$\begin{aligned} N_R &= count\ of\ resis\tan ce\ observed\ over\ multiple\ isolates\ and\ antibiotics\ in\ class \\ N_C &= number\ of\ colonies\ considered \\ N_A &= total\ number\ of\ antibiotics\ in\ class \end{aligned} \tag{9}$$

Equation (9) was used for the analysis of antibiotic resistance. Each antibiotic was grouped into their respective class as indicated in Table A.7. The resistance of varying counts of multiple colonies was observed for each site. The count of observed resistance for a single sampling site was totaled including all isolates and all antibiotics within each class (N_R). This count was divided by the number of observed isolates (N_C) and multiplied by the number of antibiotics considered for resistance (N_A). This value was multiplied by 100 to obtain the percent resistance of colonies to various antibiotics within each class from each sampling site.

2.10 Classification of robust heterotrophs

Unknown samples 1-7 were streaked onto R2A nutrient media, incubated for 48 hours at $22 \pm 2^{\circ}$C, then evaluated for colony morphology and pigmentation. Gram's method was performed using the usual *modus operandi* (m.o.) to classify unknown bacteria.

The following m.o. was followed to perform Gram's method. A coin-sized circle was applied to the glass microscope slide using a wax crayon. Next, a loopful of deionized water (DI H_2O) was retrieved with a sterile inoculating loop and placed inside the circle on the microscope slide. With a sterile disposable inoculating loop, a small amount of the isolated colony was smeared evenly onto the glass slide. Following air drying of the isolate and DI H_2O, the slide was heat fixed with a Bunsen burner. Crystal violet was then applied

to the slide for 60 seconds, then rinsed off with DI H_2O. Next, Gram's iodine was poured over the slide for an additional 60 seconds, then rinsed again with water. Ethanol then flooded the slide for 5-10 seconds and was rewashed with water. Finally, safranin was applied to the slide for 40-60 seconds, rinsed with water, and then blotted with bibulous paper. Following the gram-stain procedure, slides were examined under 10x, 40x, and 100x objective lenses using a Bausch & Lomb Galen III binocular compound microscope. Isolates were photographed under 1000x immersion oil.

2.11 Statistical analysis

A two-way analysis of variance (ANOVA) was used to evaluate if the number of culturable heterotrophic microorganisms were statistically different between the sampling site and season. The response was either water or sediment viable cell counts (CFU/ml) totaling a set of 30 samples. The fixed factors for the evaluation were sampling location with five levels (Charlotte, Durand, Genesee, Hemlock, and Payne) and season with two levels (summer and fall). Sampling size was equal to 3 (n=3) for triplicates performed from each sample location. Statistical analyses were performed using IBM SPSS software.

Chapter 3

Site comparison

3.1 Overview

In an attempt to gather microbiological information about the environment by which bacteria capable of biodegradation originate from, isolation and enumeration of heterotrophic plate count (HPC) bacteria from contaminated and uncontaminated sampling sites was performed. Heterotrophic bacteria are widely used as a measure of drinking water quality [20].

We found that season and sampling site significantly impacted the amount of HPC bacteria in water and sediment samples. We propose the negative effect effluent discharge has on water quality and the importance of water body volume in contaminant risk assessment.

3.2 Results

3.2.1 Geospatial analysis of WWTPs

Figure 3.1 shows the proximity of major DMR facilities within 1, 5, and 10-mile buffers around the Charlotte sampling site. Within the 5 mile buffer, Charlotte receives wastewater discharge from Eastman Business Park and Frank E Van Lare WWTP. Just outside the 5-mile buffer is the RG&E Beebee Station. It is worth mentioning that Eastman Business Park and the RG&E Beebee Station are in the HUC 8 watershed where the Charlotte samples were obtained, therefore draining of these facilities would have a direct impact on Charlotte Beach. Of all the sites within this study, Charlotte Beach likely receives the highest amount of wastewater discharge pollution. Wastewater information was collected using the U.S. EPA ECHO pollutant loading tool for 2017 Discharge Monitoring Report (DMR) [59] (Table A.3).

Within the 1 mile buffer of Durand Beach is the Frank E Van Lare WWTP (Figure 3.1). Durand Beach is

also significantly impacted by Eastman Business Park (5 miles) and Rochester Gas and Electric Corporation (RG&E) Beebee Station effluent between 5 and 10 miles from the site. In addition to the Van Lare WWTP, the HUC 8 region (04140101) also contains Walter W Bradley Water Pollution Control Facility (WPCF) just outside the 10-mile buffer region.

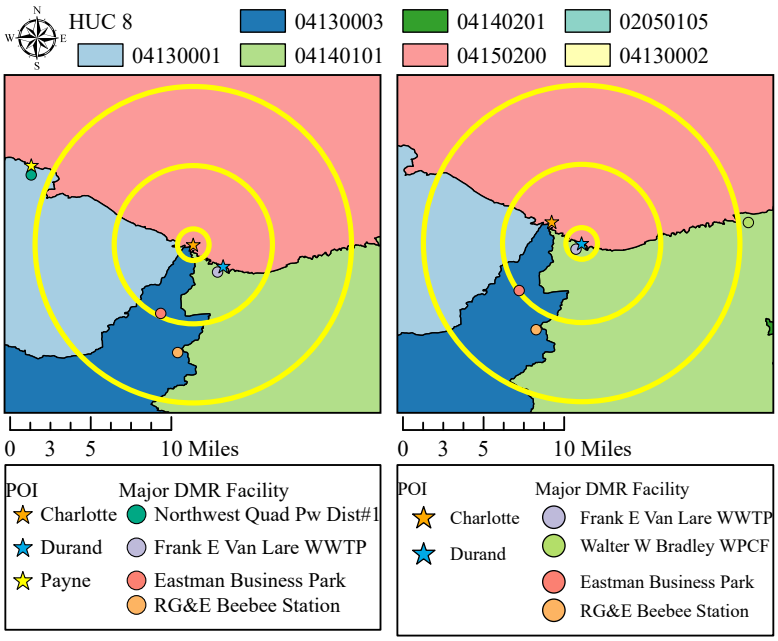


Figure 3.1: Major WWTPs within 10 miles from Charlotte (left) and Durand (right)

Wastewater discharge facilities do not as substantially impact the Genesee River as the other sampling locations. Just beyond the 5-mile buffer and within the HUC 8 watershed (04130003) is the Avon sewage treatment plant (STP) (Figure 3.2). It is notable that the Genesee River flows from South to North into Lake Ontario.

Hemlock Lake is used as a drinking water source and at approximately 10 miles out is Conesus Lake County Sewer District (Figure 3.2), within the HUC 8 watershed boundary (04130003).

Payne Beach is near Braddock Bay Bird Observatory. This sampling site is directly where the North West Quadrant (NWQ) WWTP effluent pipe is situated. In addition to the NWQ WWTP located within 1 mile of the site, Spencerport WWTP and Eastman Business Park are located just outside the 10-mile buffer as shown in Figure 3.3.

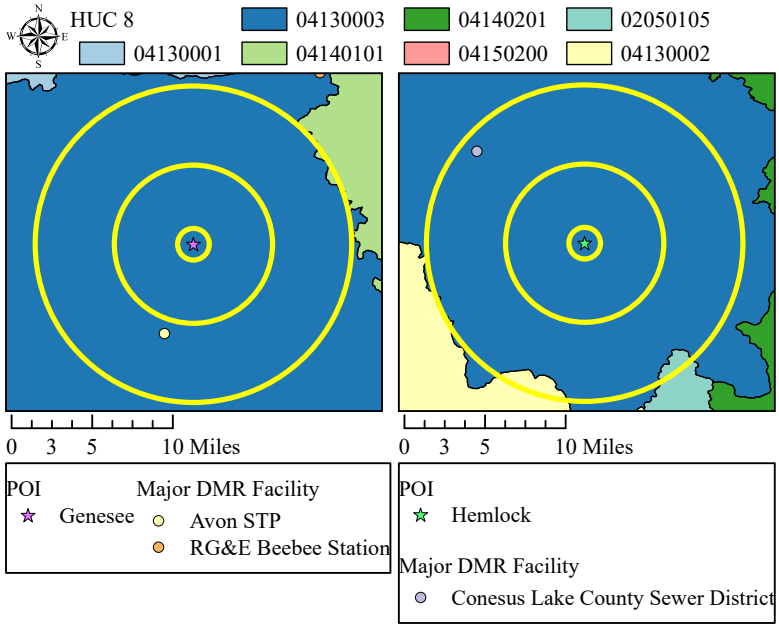


Figure 3.2: Major WWTPs within 10 miles from Genesee (left) and Hemlock (right)

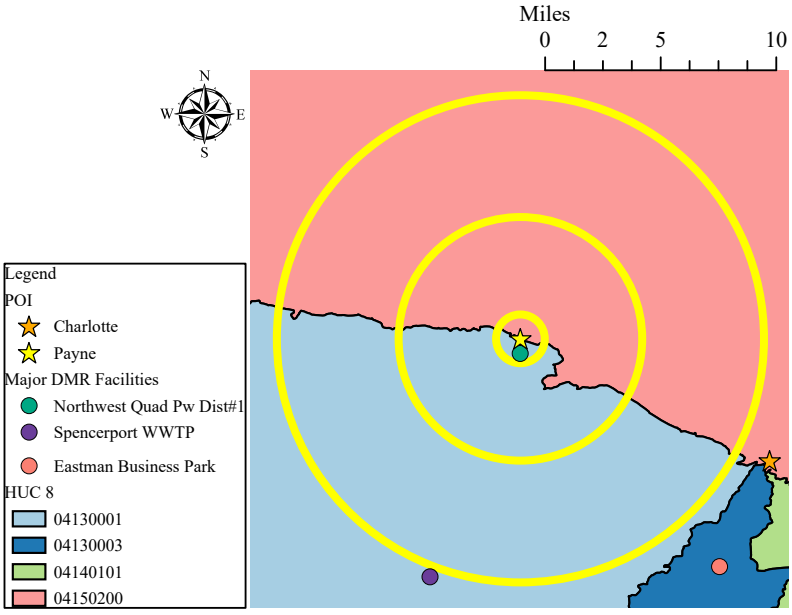


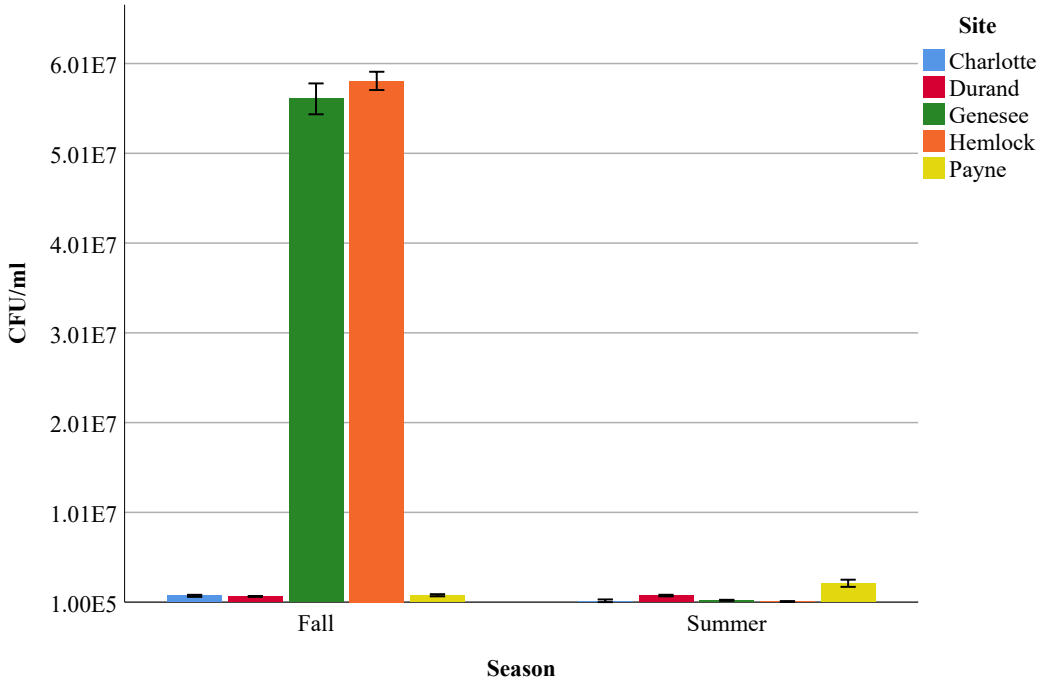
Figure 3.3: Major WWTPs within 10 miles from Payne Beach

3.2.2 Bacterial populations

The highest summer water sample cell counts were from Durand ($8.33x10^5$) and Payne ($2.20x10^6$) (Table A.1). The lowest summer water sample viable cell counts were from Hemlock ($1.67x10^5$), Charlotte ($2.00x10^5$), and Genesee ($3.00x10^5$) (Figure 3.4 and Table 3.1a). In contrast, fall water samples were highest from Genesee ($5.62x10^7$) and Hemlock ($5.82x10^7$). The lowest fall water viable cell counts were cultured from Durand ($7.33x10^5$), Charlotte ($8.00x10^5$), and Payne ($8.67x10^5$).

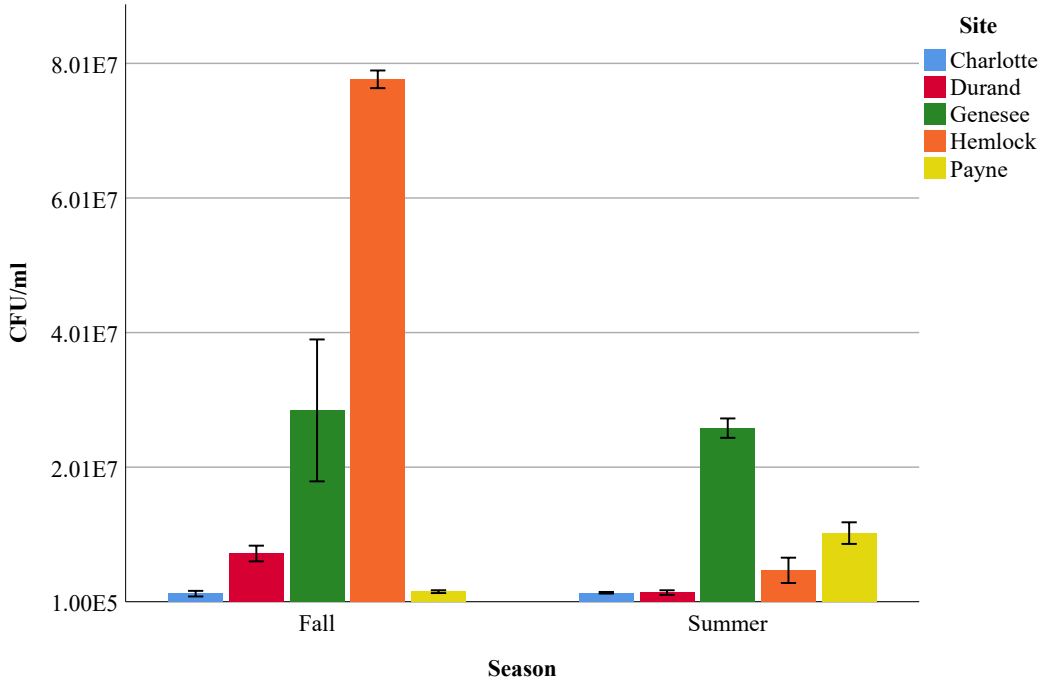
Summer sediment mean CFU/ml was highest from Genesee ($2.59x10^7$) and Payne ($1.03x10^7$) (Figure 3.5 and Table 3.1b). The lowest summer sediment viable cell counts were observed in samples obtained from Charlotte ($1.43x10^6$), Durand ($1.47x10^6$), and Hemlock ($4.77x10^6$). Similar in comparison to the highest mean viable cell counts among triplicates from fall water samples, fall sediment CFU/ml was highest from Genesee ($2.85x10^7$) and Hemlock ($7.77x10^7$). Among the sampling sites with the lowest fall sediment CFU/ml were Charlotte ($1.30x10^6$), Payne ($1.60x10^6$), and Durand ($7.27x10^6$).

Figure 3.4: Viable cell counts of water cultures



The above figure presents the triplicate means of culturable water isolates measured in colony forming units at ten days. Error bars: ±1 SE.

Figure 3.5: Viable cell counts of sediment cultures



The above figure presents the triplicate means of culturable sediment isolates measured in colony forming units at ten days. Error bars: ±1 SE.

Table 3.1: Viable cell counts of water and sediment

(a) Water

Site	Fall	Summer
Charlotte	$8.00x10^5$	$2.00x10^5$
Durand	$7.33x10^5$	$8.33x10^5$
Genesee	$5.62x10^7$	$3.00x10^5$
Hemlock	$5.82x10^7$	$1.67x10^5$
Payne	$8.67x10^5$	$2.20x10^6$

(b) Sediment

Site	Fall	Summer
Charlotte	$1.30x10^6$	$1.43x10^6$
Durand	$7.27x10^6$	$1.47x10^6$
Genesee	$2.85x10^7$	$2.59x10^7$
Hemlock	$7.77x10^7$	$4.77x10^6$
Payne	$1.60x10^6$	$1.03x10^7$

3.2.3 Effect of sampling site and season on water bacterial populations

A two-way ANOVA analysis was conducted on viable cell counts from water samples to determine if it was season or site that was impacting CFU/ml or both of these factors. Season, fall and summer, has a significant effect on culturable heterotrophs in water (Table A.1). The F distribution of season was calculated to be: $F(1, 20) = 3020.5$, and with 95% ($p < 0.05$) confidence, we reject the null hypothesis that season will have no significant effect on water counts as the F-value is far greater than the critical value of the distribution.

Moreover, the F distribution of sampling site ($F(4,20) = 1094.4$) allowed the rejection of the null hypothesis that the site will have no significant effect on counts. For season and sampling site ($F(4, 20) = 1161.9$, $p < 0.05$), we accept the alternative hypothesis that season and site interaction have a significant effect on heterotrophic, culturable colony forming units per milliliter in water.

3.2.4 Effect of sampling site and season on sediment bacterial populations

Sediment heterotroph colony counts were also analyzed for the impacts of season and site on counts, both separately and together. The analysis of our results indicate that with 95% confidence, sampling site has a significant effect ($F(4, 20) = 49.4$, $p < 0.05$) on culturable microbes in sediment (Table A.2). The F distribution of season ($F(1, 20) = 42.9$, $p < 0.05$) by which samples were obtained and further cultured from, indicates that this factor has a significant effect on CFU/ml in sediments of Western New York. Jointly, both site and season have a significant impact on sediment counts. F-values indicate that, for sediment samples, the site has a greater impact on colony counts than that of season and site together or season alone.

For water viable cell counts (CFU/ml), a Tukey honest significance test (HSD) test revealed that Genesee and Hemlock presented significantly higher cell counts than Charlotte, Durand, and Payne, respectively (Table A.10). For sediment HPC analysis, a Tukey HSD test illustrated that with 95% confidence, Hemlock varied significantly from Genesee, Payne, Durand, and Charlotte (Table A.11). We conclude that there is a huge variability of water and sediment colony counts across space and time.

3.3 Discussion

3.3.1 Importance of water body volume when assessing effluent discharge

Between 2015 to 2017, Charlotte Beach received 36.3, 67.2, and 3.88 million, totaling approximately 107 million, pounds of pollutants in wastewater effluent from the Frank E. Van Lare WWTP, Kodak, and the NWQ WWTP, respectively (Table A.3). These facilities exceeded effluent guidelines by over 1.6 million pounds. During the same period, Durand Beach received the highest amount of wastewater effluent, of over 115 million pounds from the nearest facilities: Van Lare, Walter Bradley, Kodak, and Xerox. The third highly polluted sampling site with wastewater effluent was Payne Beach, which was calculated to receive about 71 million pounds of pollutants from the NWQ WWTP and Kodak. Hemlock Lake acquired approximately 206 thousand pounds of contaminants from Consensus Lake Sewer District and Hemlock Water Filtration Plant,

whereas the Genesee River was calculated to have the least amount of effluent pollution at just 55 thousand pounds from Avon STP and Leroy WWTP.

Effluent discharge between 2015 and 2017 suggests that water quality should descend in condition from Genesee > Hemlock > Payne > Durand > Charlotte. The counts of heterotrophs are used as a measure of water quality [20]. Our studies indicate that there is an overlooked factor influencing water quality at these sampling sites.

One would expect the water quality at Charlotte to be lesser than that of Hemlock, due to observations in effluent discharge from the adjacent facilities. Though, this is not the case, as we recorded a lower quantity of heterotrophs from Charlotte Beach (Lake Ontario) than Hemlock Lake. Our results indicate that water volume plays a significant role in water quality as wastewater effluent and other water pollutants would theoretically be more diluted in higher volumes of water.

One of the Great Lakes, Lake Ontario, has a maximum depth of 244 meters and the volume was computed to be 392.23 miles^3 (1.64x10^{15} liters) by using information on the water area and average depth [65]. On the other hand, one of the Finger Lakes, Hemlock Lake has a maximum depth of 28 meters [66] with an estimated water volume of 0.025 miles^3 (1.04x10^{11} liters).

We observed elevated HPCs from Hemlock Lake, likely as a result of higher pressure exerted by wastewater effluent due to lesser water volume. Our study indicates that when determining impacts on water quality, water body volume is an essential factor that must not be ignored.

3.4 Conclusion

Firstly, we found that HPC bacteria varied by season in both water and sediment samples. This notion is supported by a study from the Missouri River, where water HPC bacteria were found to be significantly higher in the summer in comparison with the winter [67]. This study supports their findings, and as [67] indicate, we also suggest measuring drinking water contamination by HPC counts under low-temperature conditions as the season is proven to have a dominant effect on indicator bacteria. We hypothesize the negative, dominant effect wastewater effluents exert on freshwater, natural ecosystems of Western New York and the significance of water volume when determining water quality.

Chapter 4

Biodegradation

4.1 Overview

Heterotrophs were isolated from the waters of Western New York in an attempt to heighten the understanding of their metabolic characteristics. Microbial acclimation to antibiotic substrates was examined in terms of 1) carbon supply, and 2) alternative substrates. We propose that microbial degradation capacity is limited by organic carbon substrate availability and maximized by alternative substrate presence, the latter of which, binding affinities were augmented.

4.2 Results

4.2.1 Enumeration of enrichment cultures

Enrichment 3 was enumerated in an attempt to culture heterotrophic microorganisms capable of thriving in high concentrations of a particular pharmaceutical. Quite a few summer sediment enrichments were observed with no growth when plated onto R2A media (Figure 4.1), despite turbidity presence in enrichment 3 (Table A.12). These bacteria were cultured from Hemlock, Genesee, and Charlotte enriched on ampicillin; Hemlock, Charlotte, and Durand enriched on streptomycin; and Genesee enriched on lomefloxacin (Table 4.1). In contrast, fall sediment enrichment viable cell counts only exhibited no growth for Durand culture enriched on lomefloxacin (Table 4.2), of which, visual qualifications of turbidity can be observed in Table A.13. It must be considered that dead cells can make a culture appear turbid [68].

Of the summer enrichment cultures, particular sampling locations were ranked with the highest colony counts for particular pharmaceuticals. Out of all other sampling locations, Durand was detected to have the highest viable cell counts when enriched on ampicillin (2.60×10^8) and cefepime (1.00×10^8), respectively.

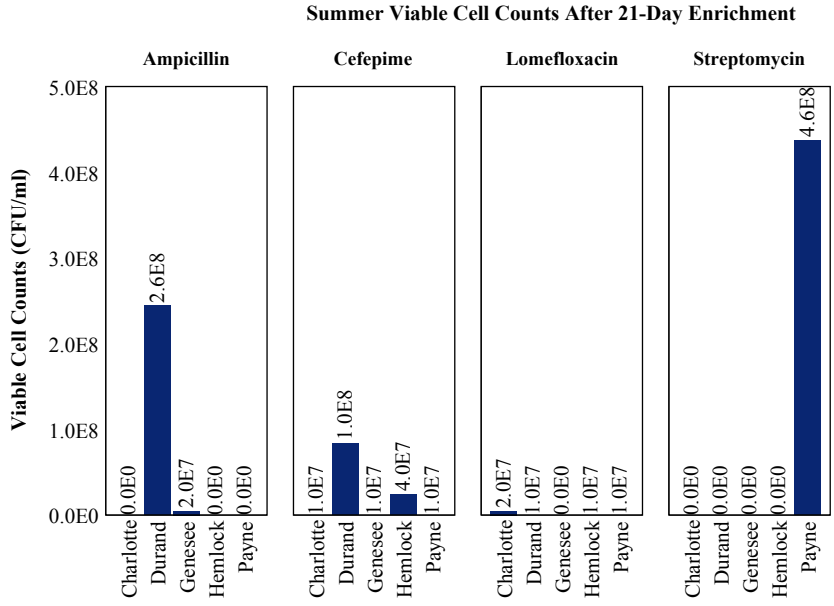


Figure 4.1: Summer viable cell counts after 21-day enrichment

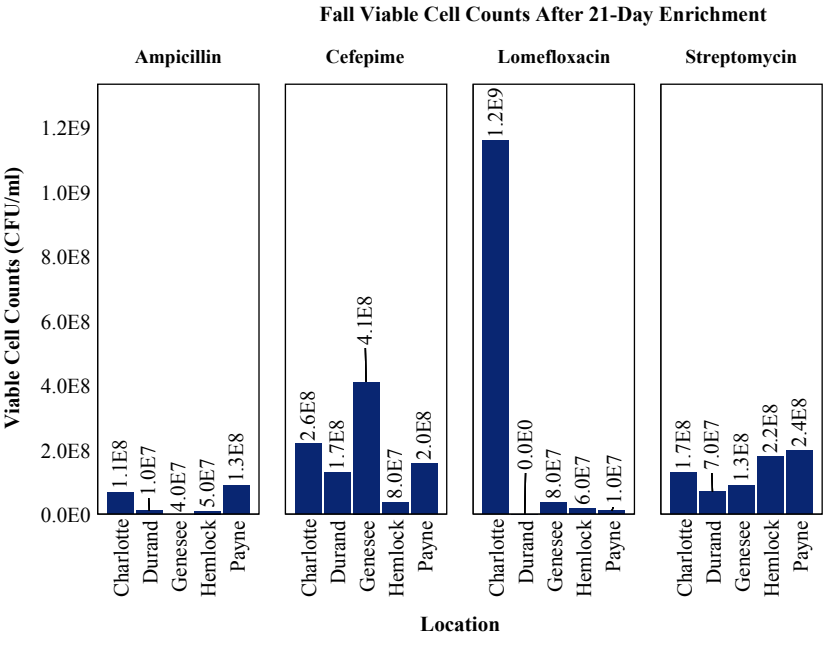


Figure 4.2: Fall viable cell counts after 21-day enrichment

Lomefloxacin enrichment counts were the highest from Charlotte ($2.00x10^7$) and streptomycin enrichment counts were only documented from Payne ($4.60x10^8$) (Figure 4.1).

Charlotte sediment culture enriched on lomefloxacin ($1.20x10^9$) was predicted to have the highest viable cell counts for fall enrichments (Figure 4.2). Out of all other sampling locations from fall enrichments on ampicillin, Payne displayed the highest viable cell counts ($1.30x10^8$). Fall enrichments on cefepime produced the most significant quantity of bacteria from Genesee culture ($4.10x10^8$). Similar to summer enrichment viable cell counts, fall enrichment on lomefloxacin from Charlotte had the highest counts ($1.20x10^9$) out of all sampling location lomefloxacin enrichments. Similar to summer enrichment counts, the fall enrichment on streptomycin was the highest from Payne sediment culture ($2.40x10^8$).

Table 4.1: Growth of isolates on substrates used for selection

(a) Summer

Growth substrate	Unknown	k (days^{-1})
Ampicillin	6	0.22
	23	NG
	24	NG
Cefepime	7	0.16
	25	NG
	26	NG
Lomefloxacin	27	NG
	28	NG
	29	NG
	30	NG
Streptomycin	5	0.17
	31	NG
	32	NG
	33	NG
	34	NG
	35	0.06

(b) Fall

Growth substrate	Unknown	k (days^{-1})
Ampicillin	1	0.21
	8	0.14
	9	0.21
	10	0.18
Cefepime	2	0.19
	3	0.25
	4	0.25
	11	0.11
	12	0.06
	13	0.08
	14	NG
	15	0.16
Lomefloxacin	16	NG
	17	NG
	18	0.07
Streptomycin	19	0.16
	20	0.03
	21	0.20
	22	0.21

4.2.2 Growth of acclimated bacteria on substrates for the determination of robustness

The growth rate (k) of enrichment summer isolates can be observed in Table 4.1a. Isolates formerly enriched on cefepime from Durand and Charlotte Beaches were capable of rapid cell division in the presence of cefepime. In the presence of cefepime, unknown 2 had a growth rate of 0.19 day^{-1}, whereas unknown 4 was able to divide at a rate of 0.25 day^{-1}. Unknown 3 isolate from Charlotte was capable of growing as fast as 0.25 day^{-1}. Durand unknown 1 isolate, initially enriched on ampicillin, was able to grow at a rate of 0.21 day^{-1}.

Fall growth rates from enrichment isolates are organized in Table 4.1b. Unknown 5 from Durand grew at a rate of 0.17 day^{-1} on streptomycin, unknown 6 from Payne grew at 0.22 day^{-1} on ampicillin, and unknown 7 from the Genesee River grew at 0.16 day^{-1} on cefepime. Isolates with high growth rates and short doubling times were deemed robust and chosen for further experimentation.

4.2.3 Growth of robust isolates on alternative substrates

Table 4.2 illustrates the growth rate (k) of robust fall and summer unknowns on alternative antibiotic substrates. These selected isolates were originally enriched on a single antibiotic and chosen for further analysis with different classes of antibiotics.

Unknown	Alternative substrate	k (days^{-1})
1	Ampicillin	0.10
	Cefepime	0.07
	Chloramphenicol†	0.25
	Tetracycline	0.16
2	Amoxicillin†	0.18
	Cefepime†	0.18
	Chloramphenicol	0.11
	Trimethoprim	0.20
3	Cefepime	0.24
	Erythromycin†	0.28
	Levofloxacin	0.24
	Tetracycline	0.10
4	Cefepime	0.10
	Erythromycin	0.08
	Levofloxacin	NG
	Tetracycline†	0.27
5	Amoxicillin†	0.15
	Erythromycin	0.12
	Levofloxacin	NG
	Streptomycin	0.12
6	Ampicillin†	0.24
	Erythromycin	NG
	Levofloxacin	NG
	Trimethoprim	0.23
7	Cefepime	NG
	Erythromycin	NG
	Levofloxacin	NG
	Trimethoprim†	0.02

k, growth rate; †, best substrate.

Table 4.2: Growth of robust isolates on alternative substrates

Each unknown summer isolate used for further alternative substrate growth analysis grew best on a particular pharmaceutical. Unknown 1 further enriched on chloramphenicol had a growth rate of 0.25 day^{-1} (Table 4.2). Unknown 2 had the highest growth rates on amoxicillin and cefepime, each at a rate of 0.18 day^{-1}. Unknown 3 grew best on erythromycin, with a growth rate of 0.28 day^{-1}. Lastly, unknown 4 achieved the highest growth rate on tetracycline at 0.27 day^{-1}.

Unknown 5, formerly enriched on streptomycin, had the highest growth rate on amoxicillin at 0.15 day^{-1}. Unknown 6, initially enriched on ampicillin accomplished the highest growth rate on the antibiotic it was initially enriched on, ampicillin at 0.24 day^{-1} and secondly on trimethoprim at 0.23 day^{-1}. Unknown 7, formerly enriched on cefepime, only attained a growth rate with trimethoprim at 0.02 day^{-1}, but no growth rate was detected for the other enriched antibiotics.

Unknown 3 was the strongest isolate capable of co-existing with cefepime at 0.24 day^{-1}, erythromycin at 0.28 day^{-1}, and levofloxacin at 0.24 day^{-1}. The next strongest isolate was unknown 6, being the best unknown to co-exist with ampicillin at 0.24 day^{-1} and trimethoprim at 0.23 day^{-1}. Furthermore, optimal co-existing between unknown and antibiotic was observed as follows: unknown 1 with chloramphenicol at 0.25 day^{-1}; unknown 2 with amoxicillin at 0.18 day^{-1}; unknown 4 with tetracycline at 0.27 day^{-1}; and unknown 5 with streptomycin at 0.12 day^{-1}.

4.2.4 Degradation of antibiotics from soil by robust isolates

The biodegradation of ampicillin at one, two, and three-fold concentrations by unknown 1 was calculated to be 33.3%, 16.3%, and 13.0%, respectively (Table 4.3, Figure B.10). Unknown 6 attained a similar degradation pattern of ampicillin at 27.0% of 7.5 mg, 13.4% of 15 mg, and 11.9% of 22.5 mg (Table 4.4, Figure B.15). Ampicillin biodegradation measured during the summer and fall at 7.5 mg, 15 mg, and 22.5 milligrams showed similar patterns of degradation as did cefepime.

Cefepime biodegradation was assessed with unknowns 2, 3, 4, and 7. Unknown 2 was capable of remediating 7.5, 15, and 22.5 milligrams of cefepime from sediment by 42.1%, 24.0%, and 15.9%, independently (Table 4.3, Figure B.11). Cefepime degradation by unknown 4 exhibited similar patterns as other unknowns with degradation capacity being 20.6% of 7.5 mg, 18.9% of 15 mg, and 5.7% of 22.5 mg (Figure B.13). Streptomycin biodegradation of 10 mg, 20 mg, and 30 milligrams achieved similar patterns of degradation as did ampicillin and cefepime. Unknown 5 was capable of degrading 18.1% of 10 mg, 17.8% of 20 mg, and

Unknown	Original Substrate	Mol Wt	ρ	C avail.	CO_2 evol.	Deg. (%)
1	Ampicillin	349.4				
			7.5	4.12	1.37	33.3
			15	8.25	1.35	16.3
			22.5	12.38	1.61	13.0
2	Cefepime	480.6				
			7.5	3.56	1.50	42.1
			15	7.05	1.69	24.0
			22.5	10.58	1.68	15.9
3	Cefepime	480.6				
			7.5	3.56	1.09	30.6
			15	7.05	1.01	14.3
			22.5	10.58	1.93	18.2
4	Cefepime	480.6				
			7.5	3.56	0.74	20.6
			15	7.05	1.33	18.9
			22.5	10.58	0.61	5.7

ρ, concentration of antibiotic substrate in mg/ml; CO_2 evol., total cumulative carbon dioxide evolved at 10 days; C avail., available carbon in sample; Deg. (%), total degradation percentage.

Table 4.3: CO_2 evolution of summer unknowns with substrates used in selection

13.8% of 30 mg streptomycin (Table 4.4, Figure B.14).

It must be mentioned that there were some instances where increasing the antibiotic concentration past a certain threshold proved to stimulate antibiotic degradation, suggesting an optimal concentration at which antibiotics are more readily biodegraded in sediments. For instance, Charlotte summer isolate (unknown 3) degraded 30.6% of 7.5 mg, 14.3% of 15 mg, and 18.2% of 22.5 mg (Table 4.3, Figure B.12). Similarly, degradation of cefepime was optimized in fall Genesee isolate originally enriched on cefepime as the microbe degraded 18.7% of 7.5 milligrams, 14.9% of 15 milligrams, and 17.4% of 22.5 milligrams (Table 4.4, Figure B.16).

4.2.5 Degradation of alternative substrates from soil by robust isolates

Robust isolates selected for in antibiotic amended cultures were further analyzed for metabolism of alternative antibiotic substrates. Unknown 1 was able to degrade 28.65% of 3.56 mg cefepime, 49.28% of 0.51 mg chloramphenicol, and 70.26% of 0.30 mg tetracycline (Table 4.5, Figure B.17). Unknown 2 was able to degrade 40.16% of 3.29 mg amoxicillin, 66.76% of 0.51 mg chloramphenicol, and 55.94% of 5.0 mg

Unknown	Original Substrate	Mol Wt	ρ	C avail.	CO_2 evol.	Deg. (%)
5	Streptomycin	581.8				
			10	4.3	0.8	18.1
			20	8.7	1.5	17.8
			30	13	1.8	13.8
6	Ampicillin	349.4				
			7.5	4.1	1.1	27.0
			15	8.3	1.1	13.4
			22.5	12.4	1.5	11.9
7	Cefepime	480.6				
			7.5	3.6	0.7	18.7
			15	7.1	1.1	14.9
			22.5	10.6	1.8	17.4

ρ, concentration of antibiotic substrate in mg/ml; CO_2 evol., total cumulative carbon dioxide evolved at 10 days; C avail., available carbon in sample; Deg. (%), total degradation percentage.

Table 4.4: CO_2 evolution of fall unknowns with substrates used in selection

trimethoprim (Figure B.18). Unknown 3 was able to degrade 2.42% of 22.7 mg erythromycin and 1.38% of 44.87 mg levofloxacin (Figure B.19). Unknown 4 was able to degrade 6.13% of 22.7 mg erythromycin, 1.13% of 44.87 mg levofloxacin, and 72.96% of 0.30 tetracycline (Figure B.20).

Unknown 5 degraded 18.25% of 3.29 mg amoxicillin, 9.39% of 22.7 mg erythromycin, and 1.05% of 44.87 mg levofloxacin (Table 4.6, Figure B.21). Unknown 6, originally enriched on ampicillin, was able to degrade 6.39% of 22.7 mg erythromycin, 1.25% of 44.87 mg levofloxacin, and 57.22% of 2.90 mg trimethoprim (Figure B.22). Unknown 7, originally enriched on cefepime, was able to degrade 9.75% of 22.7 mg erythromycin, 2.46% of 44.87 mg levofloxacin, and 54.51% of 2.90 mg trimethoprim (Figure B.23).

4.3 Discussion

4.3.1 Out-competition of native bacteria by culture enrichment

The enrichment of sediments on a particular antibiotic was shown to promote the growth of heterotrophs in summer and fall. Through the growth of sediments with a selective antibiotic, microbes that did not confer resistance were selected against thereby allowing resistant microbes to thrive in the culture. This is confirmed through the comparison of viable cell counts in sediment (Figure 3.5) with the enrichment viable cell counts (Figure 4.1, 4.2). It is noticeable that fall viable cell counts were much higher after enrichment than that of

Unk.	Original Substrate	Alternative Substrate	Mol Wt	ρ	CO_2 evol.	C avail.	Deg. (%)
1	Ampicillin						
		Cefepime	480.6	6.25	1.02	3.56	28.65
		Chloramphenicol	323.1	1.25	0.25	0.51	49.28
		Tetracycline	444.4	0.50	0.21	0.30	70.26
2	Cefepime						
		Amoxicillin	365.4	6.25	1.32	3.29	40.16
		Chloramphenicol	323.1	1.25	0.34	0.51	66.76
		Trimethoprim	290.3	2.90	1.62	5.0	55.94
3	Cefepime						
		Erythromycin	733.9	37.5	0.55	22.7	2.42
		Levofloxacin	361.4	75.0	0.62	44.87	1.38
4	Cefepime						
		Erythromycin	733.9	37.5	1.39	22.7	6.13
		Levofloxacin	361.4	75.0	0.51	44.87	1.13
		Tetracycline	444.4	0.50	0.22	0.30	72.96

Unk., unknown isolate; ρ, concentration of antibiotic substrate in mg/ml; CO_2 evol., total cumulative carbon dioxide evolved at 10 days; C avail., available carbon in sample; Deg. (%), total degradation percentage.

Table 4.5: CO_2 evolution of summer isolates with alternative substrates

Unk.	Original Substrate	Alternative Substrate	Mol Wt	ρ	CO_2 evol.	C avail.	Deg. (%)
5	Streptomycin						
		Amoxicillin	365.4	6.25	0.60	3.29	18.25
		Erythromycin	733.9	37.5	1.91	22.7	9.39
		Levofloxacin	361.4	75	0.47	44.87	1.05
6	Ampicillin						
		Erythromycin	733.9	37.5	1.45	22.7	6.39
		Levofloxacin	361.4	75	0.56	44.87	1.25
		Trimethoprim	290.3	5	1.66	2.90	57.22
7	Cefepime						
		Erythromycin	733.9	37.5	2.21	22.7	9.75
		Levofloxacin	361.4	75	1.10	44.87	2.46
		Trimethoprim	290.3	5	1.58	2.90	54.51

Unk., unknown isolate; ρ, concentration of antibiotic substrate in mg/ml; CO_2 evol., total cumulative carbon dioxide evolved at 10 days; C avail., available carbon in sample; Deg. (%), total degradation percentage.

Table 4.6: CO_2 evolution of fall isolates with alternative substrates

summer.

Enumeration of sediment enrichments with ampicillin, cefepime, lomefloxacin, or streptomycin illustrated that the exposure of natural water sources to high concentrations of pharmaceutical induces the selection of microbes with the ability to survive in these highly polluted environments through natural selection. In some instances, enrichment of culture resulted in higher viable cell counts than that of un-amended cultures. This result suggests these sampling sites receive high concentrations of specific pharmaceuticals from wastewater effluents as microbes within the sample were capable of survival following exposure.

As anticipated, summer cell counts of Payne Beach increased from $1.03x10^7$ in un-amended cultures to $4.6x10^8$ CFU/ml in streptomycin enrichment cultures (Figure 3.5; Figure 4.1). On the other hand, the enrichment of Payne Beach sediment to cefepime and lomefloxacin resulted in approximately the same viable cell counts in enriched and un-amended cultures for summer, while ampicillin enrichments resulted in a complete loss of colony counts. This outcome suggests that there may be little to no ampicillin being released into Payne Beach or that there is a microbial self-cleansing process at work within these sediments. Payne Beach receives wastewater effluent from the Northwest Quadrant WWTP and droppings from migratory birds that find refuge at Braddock Bay Bird Observatory [57], [58].

It was surprising that Hemlock Lake sediments presented the highest amount of viable cells ($7.77x10^7$ CFU/ml) in fall and the third highest ($4.77x10^6$) in summer, as this site is used as drinking water (Figure 3.5). Despite the high number of cells present on R2A media, enumeration of enrichment cultures revealed that Hemlock likely does not receive high concentrations of pharmaceuticals as naturally occurring colonies were unable to survive and were not out-competed by the exposure to high concentrations (Figures 4.1 and 4.2). It is important to note that summer enrichment of culture on cefepime resulted in approximately the same amount of culturable microbes as un-amended cultures. This case suggests that these microbes can survive in the presence of cefepime due to resistance genes occurring from possible metal or antibiotic exposure through effluents. The exposure of natural environments to heavy metals has been observed in prior studies to facilitate the resistance of antibiotics [69]. Hemlock Lake may receive high concentrations of cefepime from Consensus Lake County Sewer District which is located within 10 miles from the water body.

It is notable to mention that in fall, the exposure of Charlotte Beach sediments to ampicillin, cefepime, lomefloxacin, and streptomycin, respectively, over 21-days resulted in a significant increase in viable cell counts from $1.3x10^6$ CFU/ml in un-enriched sediment cultures (Figure 3.5) to $1.1x10^8$, $2.6x10^8$, $1.2x10^9$,

and 1.7x10^8 CFU/ml in enriched cultures, respectively (Figure 4.2). These results suggest that Charlotte Beach receives high concentrations of ampicillin, cefepime, lomefloxacin, and streptomycin as there were microbes present in the culture that was able to withstand extremely high concentrations of antibiotic. Charlotte Beach receives wastewater that is deposited into the Genesee River which flows from south to north into Lake Ontario. Charlotte Beach is located directly to the west of the Genesee River mouth. Before outflowing into Lake Ontario, the Genesee receives medical wastewater effluents from Rochester Regional Health and Highland Hospital. Additionally, Charlotte receives effluents from Van Lare WWTP, Kodak (Eastman Business Park), and RG&E Beebee Station. There may be a significant release of ampicillin, cefepime, lomefloxacin, and streptomycin into Charlotte Beach, resulting in high amounts of bacteria capable of using these compounds in sediment.

The enrichment of culture sampled from Durand Beach with ampicillin, cefepime, or lomefloxacin increased the number of heterotrophs in comparison with un-enriched culture (1.47x10^6) in summer and (7.27x10^6) fall (Figures 3.5, 4.1, and 4.2). These results suggest that Durand Beach receives significantly higher concentrations of ampicillin, cefepime, and lomefloxacin than the other sampling sites. It is possible that Durand may receive elevated concentrations of these pharmaceuticals from wastewater effluents on the southeastern coast of Lake Ontario, such as Walter W. Bradley WPCF. Additionally, Durand receives effluents from Van Lare WWTP and may be impacted by Kodak and RG&E effluents that outflow into Charlotte Beach and may make way to Durand which is located to the east. The observation of the variability between seasons in the number of microorganisms able to survive after enrichment suggests that microbes in the environment can restore themselves to normal over many generations (Figures 4.1 and 4.2). This assumption suggests that microbes may undergo loss of ARGs over time if antibiotic concentrations are reduced in the environment either through biodegradation or lessened environmental exposure through effluents. These results may point to the solution that increased efficiency of removal of pharmaceuticals from effluents may quickly reverse antibiotic resistance observed in the environment.

4.3.2 Growth promotion with antibiotic substrates

Colonies were isolated from enriched cultures, and the growth on pharmaceuticals used for selection and on alternative antibiotics was assessed. The growth analysis was performed to narrow down microbes with the potential to use specific antibiotics as their sole carbon source. The growth of summer isolates on selected

antibiotics was significantly more successful than fall enriched isolates, suggesting differences in the microbial composition of sampling sites. This difference may be attributed to the fact that summer samples were obtained in odd locations as regular sites were inaccessible due to flooding. A wide variety of heterotrophs were able to withstand growth on selected antibiotics and alternative antibiotics. This observation suggests that the exposure to high concentrations of a single pharmaceutical compound would inevitably enable the survivability and growth of microbes with alternative antibiotics. The ability of microbes to grow with antibiotics may have a direct link with high concentrations of antibiotics in wastewater effluents released into surface waters that make way into sediments by the settling of compounds. Isolates with a short doubling time and high growth rate were chosen for further analysis on their ability to remove these substrates from the environment through degradation experiments.

4.3.3 Limitation of available carbon on biodegradation

Prior studies indicate that environmental exposure to chemicals may induce the biodegradability of these compounds due to adaptation techniques for survival [70]. Microbes capable of sufficient growth with antibiotics from Charlotte, Durand, Genesee, and Payne were used for degradation experiments. Isolates from Hemlock Lake did not grow well with the selected antibiotics, which is likely a result of little to no effluent containing antibiotics being emitted into the lake. Conversely, the successful growth of microbes with antibiotics from the other four sampling sites suggests that pharmaceuticals in wastewater effluents may play a role in the ability of microbes to survive in highly polluted environments and further their ability to degrade such compounds.

In particular, several isolates from Durand Eastman Beach were able to degrade ampicillin, cefepime, and streptomycin. This finding suggests that wastewater effluents containing high antibiotic concentrations may induce degradation capabilities of microbes in freshwater environments. Furthermore, the degradation capacity was limited by available carbon. Despite the three-fold increase in the amount of available carbon for use, heterotrophic bacteria were not able to use more (Tables 4.3 and 4.4). A prior study of dose-response relationships by Topp, et al. 2012, observed accelerated antibiotic biodegradation in soil samples exposed to 0.01 μg/ml in comparison with 1.0 x 10^{-4} μg/ml of sulfamethazine [71]. Our findings suggest that the microbial degradation of antibiotics is optimized at a unique concentration and varies for each microbe-chemical relationship.

Findings suggest a correlation existing between wastewater effluents and degradation abilities of heterotrophs in the waters of Western New York. Prior studies found that isolated heterotrophs from effluent receiving water bodies in this region were able to degrade acetaminophen and ibuprofen [63]. Moreover, a study found the removal efficiency of ARGs between 33.30 and 97.56% [23]. This study supports those findings while simultaneously reporting the ability of these microbes to degrade antibiotics.

Results presented in this study suggest that bacteria in highly polluted ecosystems may have the ability to maintain homeostasis of their environment by utilizing pollutants as energy sources. These results also indicate that antibiotic-degrading bacteria are present in freshwater sediments *ex situ*, though further studies must focus on the analysis of this phenomenon *in situ* and then apply these findings to the treatment of wastewater.

Metabolism of antibiotic substrates by heterotrophs was shown to be limited by available carbon biomass concentration. Increases in total available carbon biomass from antibiotic substrates did not prove to stimulate microbial metabolism; therefore we conclude that enzyme synthesis is dependent on the amount of antibiotic in cultures. This finding is corroborated by prior findings from Lodge, 1980, that the assimilation of iron was controlled by concentration [72].

We propose that increased carbon biomass limits microbial metabolism in heterotrophs cultured from the sediment of highly polluted environments. This notion is supported by results from [73], where scientists indicate that oxygen flux is limited by carbon biomass concentration in heterotrophs. For example, in contrast to the low biomass concentration of 0.2 mg cm^{-3} Fe(III)-oxide, high biomass concentrations of 2.0 mg cm^{-3} were proven to decrease the net community growth rate.

4.3.4 Biodegradation augmentation using alternative substrates

Bacteria isolated from Charlotte, Durand, Genesee, and Payne was capable of growth on alternative substrates and was further studied for their ability to remove these compounds. Alternative antibiotic substrates of various antibiotic classes were chosen based upon known molecular differences in mechanisms of action on the cell. Isolates selected initially for using a single antibiotic presented successful growth on other classes of antibiotics from these four sampling sites, suggesting that the exposure of sediments to a single pharmaceutical will enhance the survival and removal abilities in the presence of structurally different chemicals.

An isolate from Durand Beach was able to degrade approximately the same percentage of streptomycin,

an aminoglycoside, as amoxicillin, a beta-lactam. Alternatively, isolates from the Genesee River and Payne Beach showed increased removal abilities of the alternative antibiotic substrate, trimethoprim, in comparison with the initially enriched substrate. These results suggest alternative substrates have an affinity for binding to the enzyme involved in metabolism. Isolates cultured from Payne Beach and the Genesee River were enriched on a beta-lactam and a cephalosporin, respectively, which are structurally similar as these compounds interfere with bacterial cell wall synthesis. Jayakody, Johnson, Whitman, et al. (2018) reported similar findings as we present here, as an engineered microbe was able to metabolize waste carbon and bind to various substrates [19]. These findings have significant applications in terms of valorization or reuse of toxic waste [19].

The assimilation of other antibiotic substrates by selected heterotrophs significantly stimulates microbial metabolism. Similarly, prior studies have indicated that various iron nutrients stimulate heterotrophic growth [74]. Isolates exhibit heightened binding affinity to various substrates, as metabolism was limited with the selected antibiotic substrate. Therefore, we conclude that the assimilation of antibiotics in wastewater contaminated environments by heterotrophs is controlled by substrate availability.

4.4 Conclusion

While antibiotics are abundant in the environment, microbial metabolism is limited by carbon-availability and maximized by the use of alternative substrates. In other words, microbes showed expanded substrate utilization. Prior studies have used bioengineered strains of *P. putida* to detoxify thermochemical (TC) wastewater using aerobic catabolic pathways of metabolism [19]. We provide evidence of these pathways in heterotrophs exposed to industrially-relevant drug levels.

We conclude that microbial assimilation of antibiotics in the environment is carbon-limited and weakly substrate-specific. Increases in total available carbon biomass from antibiotic substrates did not stimulate microbial metabolism. Metabolism of antibiotic substrates by heterotrophs is shown to be limited by available carbon biomass concentration and maximized by alternative substrates. These results suggest these microbes possess unusual metabolic capabilities as isolates exhibit high binding affinity to various substrates and the enzymes involved in this process are not immensely substrate-specific.

Chapter 5

Antibiotic resistance

5.1 Overview

Samples from sites with point source pollution (Charlotte, Durand, Genesee, and Payne) were compared to a site with used as a drinking water source (Hemlock). Firstly, water and sediment isolates were analyzed for antibiotic resistance. Secondly, highly polluted environments were modeled in sediment samples through 21-day exposure enrichments, after which, isolates were cultured and examined for antibiotic resistance. Most significantly, unknown isolates that were used to measure biodegradation were further analyzed for antimicrobial susceptibility.

This study reports the antibiotic resistance of heterotrophic microorganisms isolated from these sites which were enriched and unenriched to 24 different antibiotics. We posit that there may be a link between antibiotic resistance and biodegradation in sediment heterotrophs of polluted freshwater environments and that wastewater pollution increase bacterial antibiotic resistance in freshwater and sediment.

5.2 Results

5.2.1 Antibiotic resistance of water and sediment isolates

Tables 5.3 and 5.2 show the resistance of culturable heterotrophic surface water and sediment isolates. Overall, surface water and sediment isolates cultured in summer and fall from sites with point source pollution (Charlotte, Durand, Genesee, and Payne) exhibited significantly higher percent antibiotic resistance across all antibiotic classes when compared to Hemlock Lake.

Point source pollution for Charlotte is the most varied among all sources studied as it receives wastewater from Monroe County Water Authority, Van Lare, Kodak, Highland Hospital, and Rochester Regional Health.

Braddock Bay Wildlife Refuge is also near to Charlotte (as well as Payne). Durand Eastman Beach is approximately 250 feet from Van Lare and also would receive pollutants from Webster Wastewater Treatment and Brighton Landfill. Samples obtained from Payne Beach are directly at the location of a sewage pipe from the NWQ Public Works. The sampling site along the Genesee River is significantly further from point sources, such as Leroy WWTP (13 miles), Mill Seat Landfill (11.0 miles), and Water Treatment by Culligan.

Antibiotic class	Charlotte	Durand	Genesee	Hemlock	Payne
Surface water					
Cephalosporin	43.3	ND	9.5	0.0	58.3
Beta-lactam	37.5	ND	8.9	0.0	34.4
Fluoroquinolone	6.7	ND	9.5	0.0	16.7
Aminoglycoside	5.0	ND	3.6	0.0	12.5
Sediment					
Cephalosporin	60.0	79.2	91.7	5.6	54.2
Beta-lactam	37.5	46.9	65.6	4.2	34.4
Fluoroquinolone	0.0	0.0	25.0	22.2	0.0
Aminoglycoside	95.0	25.0	37.5	0.0	12.5

Values represent the percentage of colonies resistant to antibiotics within each class, as specified in Table A.7. Equation (9) was used to obtain percentages. ND, not determined as no isolates were cultured.

Table 5.1: Fall surface water and sediment antibiotic resistance

5.2.2 Evaluation of resistance in surface water heterotrophs

Colonies isolated in the summer from Durand Beach exhibited 100% resistance to all four classes of antibiotics within this study. Resistance for isolates from Charlotte and Genesee was not determined as colonies did not grow well on Bushnell Haas media. Payne Beach presented the highest resistance to cephalosporins (53.3%), followed by aminoglycosides (30.0%), beta-lactams (22.5%), and fluoroquinolones (20.0%). Hemlock surface water heterotrophs exhibited resistance to cephalosporins (20.8%) and beta-lactams (6.3%), but no resistance to fluoroquinolone (0.0%) or aminoglycoside (0.0%) antibiotics.

Colonies from Durand Beach cultured during the fall did not grow well on Bushnell Haas media; therefore resistance was not determined for this site. Overall, the most resistance was observed to cephalosporin antibiotics. Charlotte isolates presented the most resistance to cephalosporin (43.3%) and beta-lactam (37.5%) antibiotics, followed by fluoroquinolones (6.7%) and aminoglycosides (5.0%) (Table 5.3). Similarly, Payne Beach isolates displayed high frequencies of resistance to the cephalosporin (58.3%) and beta-lactam (34.4%)

antibiotics, with lower resistance to fluoroquinolone (16.7%) and aminoglycoside (12.5%) antibiotics. As anticipated, the drinking water source (Hemlock) did not exhibit resistance to any of the four classes of antibiotics studied. Similarly, Genesee presented low resistance to the cephalosporin (9.5%), fluoroquinolone (9.5%), beta-lactam (8.9%), and aminoglycoside (3.6%) antibiotics.

Antibiotic class	Charlotte	Durand	Genesee	Hemlock	Payne
Surface water					
Cephalosporin	ND	100.0	ND	20.8	53.3
Beta-lactam	ND	100.0	ND	6.3	22.5
Fluoroquinolone	ND	100.0	ND	0.0	20.0
Aminoglycoside	ND	100.0	ND	0.0	30.0
Sediment					
Cephalosporin	72.9	56.7	38.9	21.4	50.0
Beta-lactam	73.4	50.0	8.3	21.4	39.2
Fluoroquinolone	72.9	56.7	38.9	21.4	50.0
Aminoglycoside	59.4	50.0	33.3	32.1	35.0

Values represent the percentage of colonies resistant to antibiotics within each class, as specified in Table A.7. Equation (9) was used to obtain percentages. ND, not determined as no isolates were cultured.

Table 5.2: Summer surface water and sediment antibiotic resistance

5.2.3 Evaluation of resistance in sediment heterotrophs

Heterotrophs cultured during the summer from sediment at Charlotte displayed high resistance in comparison with the other sampling sites to the cephalosporin (72.9%), beta-lactam (73.4%), fluoroquinolone (72.9%), and aminoglycoside (59.4%) antibiotics. Durand Beach displayed resistance to cephalosporin (56.7%), fluoroquinolone (56.7%), beta-lactam (50.0%), and aminoglycoside (50.0%) antibiotics. Of the Payne Beach colonies isolated during the summer from sediment, there was 50% resistance to cephalosporin antibiotics, as well as resistance to fluoroquinolone (50.0%), beta-lactam (39.2%), and aminoglycoside (35.0%) antibiotics. Genesee displayed the highest resistance to cephalosporins (38.9%) and fluoroquinolones (38.9%), followed by aminoglycoside (33.3%) and beta-lactam (8.3%) antibiotics. Hemlock displayed high resistance to aminoglycoside (32.1%) antibiotics, followed by cephalosporin (21.4%), beta-lactam (21.4%), and fluoroquinolone (21.4%) antibiotics.

Isolates cultured from fall sediment exhibited different resistance. Genesee demonstrated the highest resistance to cephalosporin (91.7%) antibiotics, followed by beta-lactam (65.6%), aminoglycoside (37.5%),

and fluoroquinolone (25.0%) antibiotics. Durand Beach displayed high resistance to the cephalosporin (79.2%), beta-lactam (46.9%), and aminoglycoside (25.0%) antibiotics, but not resistant to fluoroquinolones. Charlotte isolates displayed the highest resistance to aminoglycoside (95.0%) antibiotics, followed with the cephalosporin (60.0), and beta-lactam (37.5%) antibiotics, but there was no resistance to fluoroquinolone antibiotics. Durand Beach presented the highest resistance to cephalosporin (79.2%) antibiotics, followed by resistance to beta-lactam (46.9%) and aminoglycoside (25.0%) antibiotics, with no resistance to fluoroquinolones. Payne Beach isolates were resistant to cephalosporin (54.2%), beta-lactam (34.4%), and aminoglycoside (12.5%) antibiotics, but no resistance (0.0%) to fluoroquinolone antibiotics. Hemlock Lake isolates displayed resistance to fluoroquinolones (22.2%), followed by cephalosporin (5.6%), and beta-lactam (4.2%) antibiotics, but no resistance to aminoglycoside antibiotics.

Antibiotic class	Charlotte	Durand	Genesee	Hemlock	Payne
Surface water					
Cephalosporin	43.3	ND	9.5	0.0	58.3
Beta-lactam	37.5	ND	8.9	0.0	34.4
Fluoroquinolone	6.7	ND	9.5	0.0	16.7
Aminoglycoside	5.0	ND	3.6	0.0	12.5
Sediment					
Cephalosporin	60.0	79.2	91.7	5.6	54.2
Beta-lactam	37.5	46.9	65.6	4.2	34.4
Fluoroquinolone	0.0	0.0	25.0	22.2	0.0
Aminoglycoside	95.0	25.0	37.5	0.0	12.5

Values represent the percentage of colonies resistant to antibiotics within each class, as specified in Table A.7. Equation (9) was used to obtain percentages. ND, not determined as no isolates were cultured.

Table 5.3: Fall surface water and sediment antibiotic resistance

Microbes isolated from fall dilutions enriched on cefepime from Charlotte were 55.6% resistant to cephalosporins, 25.0% resistant to beta-lactams, 0.0% resistant to fluoroquinolones, and 0.0% resistant to aminoglycosides. Durand isolates enriched on cefepime were 16.7% resistant to cephalosporins, 12.5% resistant to beta-lactams, 66.7% resistant to fluoroquinolones, and 25.0% resistant to aminoglycosides. Microbes cultured from Genesee displayed 33.3% resistance to cephalosporins, 25.0% to beta-lactams, 33.3% to fluoroquinolones, and 50.0% to aminoglycosides (Table 5.4).

Heterotrophs enriched on ampicillin from Charlotte were 41.7% resistant to cephalosporins, 31.3% resistant to beta-lactams, 16.7% resistant to fluoroquinolones, and 25.0% resistant to aminoglycosides. Genesee

isolates were 33.3% resistant to cephalosporins, 12.5% resistant to beta-lactams, 16.7% resistant to fluoroquinolones, and 25.0% resistant to aminoglycosides. Payne Beach isolates presented resistance of 50.0% to cephalosporins, 12.5% to beta-lactams, 22.2% to fluoroquinolones, and 16.7% to aminoglycosides.

Microbes enriched on streptomycin from Charlotte were 100.0% resistant to cephalosporins, 100.0% resistant to beta-lactams, 66.7% resistant to fluoroquinolones, and 25.0% resistant to aminoglycosides. Isolates from Durand were documented with the resistance of 66.7% to cephalosporins, 62.5% to beta-lactams, 0.0% to fluoroquinolones, and 100.0% to aminoglycosides. Of Genesee isolates, 61.1% were resistant to cephalosporins, 16.7% to beta-lactams, 33.3% to fluoroquinolones, and 16.7% aminoglycosides. Isolates from Hemlock presented 50.0% resistance to cephalosporins, 43.8% to beta-lactams, 100.0% to fluoroquinolones, and 50.0% to aminoglycosides. Payne isolates attained resistance of 72.2% to cephalosporins, 70.8% to beta-lactams, 66.7% to fluoroquinolones, and 8.3% to aminoglycosides.

Heterotrophic microbes enriched on lomefloxacin from Charlotte were 88.9% resistant to cephalosporins, 62.5% to beta-lactams, 33.3% to fluoroquinolones, and 75.0% to aminoglycosides. Isolates from Hemlock presented resistance of 33.3% to cephalosporins, 12.5% to beta-lactams, 0.0% resistance to fluoroquinolones, and 0.0% resistance to aminoglycosides. Payne Beach heterotrophs presented resistance of 16.7% to cephalosporins, 25.0% to beta-lactams, 66.7% to fluoroquinolones, and 50.0% to aminoglycosides.

5.2.4 Calculated resistance in acclimated bacteria

Summer isolates enriched on the cephalosporin, cefepime, presented increased resistance to cephalosporin antibiotics cultured from Charlotte (83.3%), Durand (83.3%), Genesee (100.0%), Hemlock (88.9%), and Payne (83.3%). Beta-lactam resistance of cefepime enriched isolates was observed at 62.5% of antibiotics from Charlotte, 87.5% of Durand, 62.5% of Genesee, 54.2% of Hemlock, and 75.0% of Payne. Fluoroquinolone resistance by these isolates was seen to 0.0% of antibiotics from Charlotte, 0.0% of Durand, 33.3% of Genesee, 55.6% of Hemlock, and 33.3% of Payne. Heterotrophic microbes enriched on cefepime displayed aminoglycoside resistance in 50.0% of Charlotte, 81.3% of Durand, 25.0% of Genesee, 58.3% of Hemlock, and 25.0% of Payne (Table 5.5).

Isolates enriched on the beta-lactam, ampicillin, presented heightened antibiotic resistance to all classes of antibiotics. Durand demonstrated resistance of 95.8% to cephalosporins, 75.0% to beta-lactam, 75.0% to fluoroquinolone, and 68.8% to aminoglycoside antibiotics. Genesee exhibited a resistance of 91.7% to

Antibiotic class	Enriched on cefepime				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	55.6	16.7	33.3	ND	ND
Beta-lactam	25.0	12.5	25.0	ND	ND
Fluoroquinolone	0.0	66.7	33.3	ND	ND
Aminoglycoside	0.0	25.0	50.0	ND	ND
	Enriched on ampicillin				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	41.7	ND	33.3	ND	50.0
Beta-lactam	31.3	ND	12.5	ND	12.5
Fluoroquinolone	16.7	ND	16.7	ND	22.2
Aminoglycoside	25.0	ND	25.0	ND	16.7
	Enriched on streptomycin				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	100.0	66.7	61.1	50.0	72.2
Beta-lactam	100.0	62.5	16.7	43.8	70.8
Fluoroquinolone	66.7	0.0	33.3	100.0	66.7
Aminoglycoside	25.0	100.0	16.7	50.0	8.3
	Enriched on lomefloxacin				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	88.9	ND	ND	33.3	16.7
Beta-lactam	62.5	ND	ND	12.5	25.0
Fluoroquinolone	33.3	ND	ND	0.0	66.7
Aminoglycoside	75.0	ND	ND	0.0	50.0

Values represent the percentage of colonies resistant to antibiotics within each class, as specified in Table A.7. Equation (9) was used to obtain percentages. ND, not determined as no isolates were cultured.

Table 5.4: Resistance of enriched fall isolates

cephalosporins, 81.3% to beta-lactams, 33.3% to fluoroquinolones, and 87.5% to aminoglycosides. Colony resistance from Charlotte, Hemlock, and Payne enriched on ampicillin were not determined.

Colonies enriched on the aminoglycoside, streptomycin, from Charlotte were 100.0% resistant to cephalosporins, 37.5% resistant to beta-lactams, 33.3% resistant to fluoroquinolones, and 75.0% resistant to aminoglycosides. Payne isolates enriched on streptomycin were 83.3% resistant to cephalosporins, 58.3% resistant to beta-lactams, 88.9% resistant to fluoroquinolones, and 33.3% resistant to aminoglycosides.

Heterotrophic microbes enriched on lomefloxacin, a fluoroquinolone antibiotic, from Charlotte were 100.0% resistant to cephalosporins, 87.5% resistant to beta-lactams, 100.0% resistant to fluoroquinolones, and 100.0% resistant to aminoglycosides. Microbes from Durand were 83.3% resistant to cephalosporins, 37.5% resistant to beta-lactams, 66.7% resistant to fluoroquinolones, and 0.0% resistant to aminoglycosides.

Colonies from Payne were 83.3% resistant to cephalosporins, 75.0% resistant to beta-lactams, 0.0% resistant to fluoroquinolones, and 25.0% resistant to aminoglycosides.

Antibiotic class	Enriched on cefepime				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	83.3	83.3	100.0	88.9	83.3
Beta-lactam	62.5	87.5	62.5	54.2	75.0
Fluoroquinolone	0.0	0.0	33.3	55.6	33.3
Aminoglycoside	50.0	81.3	25.0	58.3	25.0
	Enriched on ampicillin				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	ND	95.8	91.7	ND	ND
Beta-lactam	ND	75.0	81.3	ND	ND
Fluoroquinolone	ND	75.0	33.3	ND	ND
Aminoglycoside	ND	68.8	87.5	ND	ND
	Enriched on streptomycin				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	100.0	ND	ND	ND	83.3
Beta-lactam	37.5	ND	ND	ND	58.3
Fluoroquinolone	33.3	ND	ND	ND	88.9
Aminoglycoside	75.0	ND	ND	ND	33.3
	Enriched on lomefloxacin				
	Charlotte	Durand	Genesee	Hemlock	Payne
Cephalosporins	100.0	83.3	ND	ND	83.3
Beta-lactam	87.5	37.5	ND	ND	75.0
Fluoroquinolone	100.0	66.7	ND	ND	0.0
Aminoglycoside	100.0	0.0	ND	ND	25.0

Values represent percentage of colonies resistant to antibiotics within each class, as specified in Table A.7. Equation (9) was used to obtain percentages. ND, not determined as no isolates were cultured.

Table 5.5: Resistance of enriched summer isolates

5.2.5 Susceptibility of robust sediment isolates

Antibiotic susceptibility of robust isolates is shown in Table 5.6. Unknown 1, cultured from Durand Eastman Beach in the summer and enriched on ampicillin, was resistant to a total of 19 out of 24 (79.2%) antibiotics tested within this study. Unknown 2, also from Durand Beach cultured in the summer, was enriched on cefepime and displayed resistance to 16 (66.7%) different antibiotics. The Charlotte heterotroph (unknown 3) cultured during the summer and enriched on cefepime was resistant to 12 (50.0%) of the antibiotics tested. Unknown 4, cultured from Durand Beach during the summer and enriched on cefepime was resistant to 15

(62.5%) antibiotics.

Microbes (unknowns 5-7) were used for degradation studies and cultured during the fall. Unknown 5, from Durand and enriched on streptomycin, was resistant to 14 (58.3%) antibiotics under investigation. Payne Beach isolate enriched on ampicillin demonstrated resistance to 7 (29.2%) antibiotics. Similarly, the Genesee isolate enriched on cefepime was resistant to 7 (29.2%) of a total of 24 different antibiotics assessed for microbial susceptibility using the Kirby-Bauer disc diffusion method.

Antibiotic	Unknown							N_R
	1	2	3	4	5	6	7	
Amikacin	R	R	S	S	R	I	R	4
Ampicillin	R	R	R	R	R	S	S	5
Carbenicillin	R	R	R	R	R	S	R	6
Cefazolin	R	R	R	R	R	R	S	6
Cefepime	R	S	R	S	S	S	S	2
Cefixime	R	R	R	R	R	R	R	7
Cefoxitin	R	I	R	R	R	S	R	5
Ceftriaxone	S	R	S	R	I	R	S	3
Cephalothin	R	R	R	R	R	S	S	5
Chloramphenicol	R	R	S	R	R	R	S	5
Ciprofloxacin	R	S	S	S	S	S	R	2
Co-amoxiclav	S	R	S	R	S	S	S	2
Co-trimoxazole	R	I	S	S	S	S	S	1
Doxycycline	R	S	S	S	S	S	S	1
Gentamicin	R	R	S	S	R	S	S	3
Imipenem	S	S	S	S	S	S	S	0
Levofloxacin	S	S	S	S	S	S	S	0
Lomefloxacin	S	I	S	S	S	S	S	0
Mezlocillin	R	R	R	R	R	S	S	5
Oxacillin	R	R	R	R	R	R	R	7
Piperacillin	R	R	R	R	I	S	S	4
Streptomycin	R	R	R	R	R	R	R	7
Ticarcillin	R	R	S	R	R	S	S	4
Tobramycin	R	R	R	R	R	R	S	6
N_R	19	16	12	15	14	7	7	90

Antibiotic resistance of robust unknown isolates. Refer to Table A.14 for unknowns. The following unknowns were originally enriched on the corresponding antibiotics: 1 and 6 (ampicillin), 2-4 and 7 (cefepime), 5 (streptomycin). N_R, total occurrence of resistance; co-amoxiclav, amoxicillin/ clavulanic acid; co-trimoxazole, trimethoprim/ sulfamethoxazole.

Table 5.6: Antimicrobial susceptibility of robust isolates

It is notable to mention that all of the unknowns cultured were resistant to cefixime, oxacillin, and streptomycin. On the other hand, resistance to imipenem, levofloxacin, and lomefloxacin was not observed in these high capacity isolates. Furthermore, isolates cultured from Durand (1, 2, 4, 5) were resistant to ampicillin, carbenicillin, cefazolin, cefixime, cephalothin, chloramphenicol, mezlocillin, oxacillin, streptomycin, ticarcillin, and tobramycin.

Table 5.7 presents the resistance of colonies to antibiotics grouped by class by high capacity unknown isolates enriched on various antibiotics. Unknowns enriched on ampicillin (1, 6) were resistant to 75.0% of aminoglycoside, 43.8% of beta-lactam, 66.7% of cephalosporin, and 16.7% of fluoroquinolone antibiotics. Microorganisms enriched on cefepime (2-4, 7) were resistant to 62.5% of aminoglycoside, 65.6% of beta-lactam, 66.7% of cephalosporin, and 8.3% of fluoroquinolone antibiotics. Unknown 5, enriched on streptomycin, was resistant to 100.0% of aminoglycoside, 62.5% of beta-lactam, 66.7% of cephalosporin, and 0.0% of fluoroquinolone antibiotics.

$N_{antibiotics}$	Antibiotic class	Enriched substrate		
		Ampicillin	Cefepime	Streptomycin
4	Aminoglycoside	6 (75.0)	10 (62.5)	4 (100.0)
8	Beta-lactam	7 (43.8)	21 (65.6)	5 (62.5)
6	Cephalosporin	8 (66.7)	16 (66.7)	4 (66.7)
3	Fluoroquinolone	1 (16.7)	1 (8.3)	0 (0.0)

Antibiotic resistance of robust unknown isolates grouped by enriched substrate. $N_{antibiotics}$, number of antibiotics tested in each antibiotic class.

Table 5.7: Resistance of robust isolates, grouped by enriched substrate, to antibiotic classes

5.2.6 Classification of robust heterotrophs

Robust isolates were further assessed by observing colony morphology, Gram stain, and appearance on culture media (Tables A.8 and A.9). Unknown 1, cultured from Durand sediment and formerly enriched on ampicillin, appeared Gram-negative with short bacilli under immersion oil, and on culture media demonstrated mustard yellow pigmentation with punctiform, flat, and entire morphology. Unknown 2, cultured from Durand sediment and formerly enriched on cefepime, had Gram-negative cocci chains greater than five and pale yellow pigmentation on culture media with circular, convex, and entire morphology. Unknown 3, cultured from Charlotte sediment and enriched on cefepime, was observed to be Gram-negative with chain

forming, short bacilli greater than five, and on media appeared translucent white with circular, raised, and entire morphology. Unknown 3, cultured from Durand sediment and enriched on cefepime, appeared to be Gram-negative with short bacilli and on media appeared beige with circular, raised, and entire morphology (Figure B.8).

Unknown 5, cultured from Durand fall sediment, and initially enriched on streptomycin, was phenotypically observed to be circular, convex, and entire with pigmentation characterized as off-white. Furthermore, unknown 5, when observed under immersion oil using light microscopy, appeared Gram-negative with cocci chains less than five. Unknown 6, cultured from Payne Beach sediment and enriched on ampicillin, was determined to be Gram-negative with diplococci and on culture media appeared marigold with circular, raised, and entire morphology. Unknown 7, cultured from Genesee sediment and initially enriched on cefepime, was Gram-variable with short and long bacilli that appeared to have terminal endospores, and was vivid white with circular, flat, and undulate morphology on culture media (Figure B.9).

5.3 Discussion

5.3.1 Antibiotic resistance heightened by wastewater pollution

The resistance of microorganisms in the environment is of great concern as microbes are gaining antibiotic resistance faster than new drugs can be developed [21]. In this study, heterotrophs from five sampling sites in Western New York were resistant to varying combinations of 24 different antibiotics. We found that sites with direct wastewater effluent pollution presented a clear association with antibiotic resistance. These findings are justifiable as prior studies have observed increased antibiotic resistance of sediment coliforms downstream of an effluent pipe in comparison with upstream [75], [76].

As predicted, the antibiotic resistance of sediment bacteria was increased in Charlotte, Durand, and Payne Beaches and the Genesee River, which receive wastewater effluents. In contrast, antibiotic resistance by microbes isolated from Hemlock Lake was significantly lower. These results are consistent with the notion of a correlation existing between wastewater effluents and antibiotic resistance.

In particular, all of the summer Durand surface water isolates were resistant to every antibiotic under investigation. These results suggest that Durand Beach is heavily polluted with high antibiotic loads from Van Lare WWTP and surrounding industrial and municipal facilities, such as Walter W Bradley WPCF, on the southeastern coast of Lake Ontario within the HUC region 04140101.

Charlotte Beach may have high effluent concentrations of heavy metals from Kodak and RG&E Beebee Station, which may induce selection of ARGs. Additionally, Charlotte Beach is the receiving water body of the Genesee River that is subject to hospital effluent from Rochester Regional Health and Highland Hospital, which may have increased beta-lactam, cephalosporin, fluoroquinolone, and aminoglycoside antibiotic levels as suggested through observations of antibiotic resistance by heterotrophs in this study.

Surprisingly, Hemlock Lake sediment isolates presented moderate resistance to cephalosporin, beta-lactam, fluoroquinolone, and aminoglycoside antibiotics, suggesting that a small amount of Consensus Lake County Sewer District effluent may be seeping into Hemlock Lake despite it being located approximately 10 miles away. Other discharge may also be influencing the properties of heterotrophs in Hemlock Lake. It is worthy of mentioning that Hemlock is stocked with fish [77], which may unintentionally cause microbes to gain resistance. Heavy metal discharge has been observed to carry along antibiotic resistance genes [69]. Alternatively, this observed resistance may have been due to human error, like, contamination of grab samples or culture.

Within this study, cephalosporins appeared to be the antibiotic class with the most observed antibiotic resistance across all sampling sites, followed by beta-lactams, aminoglycosides, and fluoroquinolones. These results are likely due to the interactions between the following factors: bioaccumulation of toxicants in water and sediments over time, the number of antibiotics prescribed and discharged, and persistence of chemical compounds in the environment.

Antibiotic resistance observed in surface waters followed the same pattern in terms of the influence of effluent. Prior studies have noted that antibiotic resistance in surface waters was observed to be lower than that of sediments [40], which is likely due to increased settling out of pollutants into the sediment. In this study, antibiotic resistance of surface water heterotrophs from Durand Beach was significantly increased in comparison to sediments. These results suggest that effluent containing high antibiotic concentrations may have recently been discharged to the water or that microbes in the sediment are reversing the process of antibiotic resistance through a self-cleansing process.

Prior studies on the exposure of microorganisms to high levels of antibiotics have proven that this allows for the selection of ARGs for the survival of organisms [78]. Therefore, high amounts of antibiotic resistance may suggest specific indications in highly polluted environments. Results are indicative of observed antibiotic resistance as a direct impact of wastewater effluents through the selective pressure of highly polluted

environments.

5.3.2 Resistance induced by short-term and high-concentration exposure

The resistance of microbes to various classes of antibiotics following the extended exposure to a single antibiotic is of great importance as multi-resistant human pathogens are resulting in high morbidity and calling for a worldwide public health crisis [21], [79], [80]. We found that across five sampling sites, regardless of wastewater effluent presence in the environment, the exposure of isolates to cefepime, ampicillin, streptomycin, and lomefloxacin separately induced the occurrence of antibiotic resistance of sediment microbes *in vitro*.

The enrichment of sediment isolates on cefepime resulted in high observed antibiotic resistance to cephalosporins, beta-lactams, and aminoglycosides in isolates from all five sampling sites. Cefepime enrichment also increased the prevalence of fluoroquinolone resistance in heterotrophs from Genesee, Hemlock, and Payne. It was surprising that Hemlock Lake presented high amounts of antibiotic resistance because un-amended sediment isolates were not as resistant to antibiotics, which may be due to the lack of effluent discharged into the lake. These results suggest that duration as short as a 21-day exposure to elevated antibiotic levels may be enough to induce antibiotic resistance to a wide array of compounds in environments regardless of prior environmental pollution.

The enrichment of sediments on ampicillin, a beta-lactam antibiotic, appeared to increase antibiotic resistance to all 24 antibiotics by heterotroph isolates from Durand and Genesee. Interestingly, the enrichment of ampicillin seemed to induce beta-lactam antibiotic resistance.

Similarly, the enrichment on an aminoglycoside antibiotic, streptomycin, increased microbial resistance to aminoglycosides of isolates cultured from Charlotte Beach when compared with isolates that were enriched on another antibiotic. In comparison, Payne Beach isolates resistance to aminoglycosides increased slightly, but was not as high as those observations from Charlotte. Surprisingly, the enrichment on streptomycin appeared to influence the likelihood of microbes to survive in the presence of other antibiotics.

The enrichment of cultures with lomefloxacin, a fluoroquinolone, appeared to influence and increase fluoroquinolone resistance when compared to isolates enriched with other antibiotics as with Charlotte and Durand isolates. It is important to mention that this was not the case with Hemlock isolates enriched on lomefloxacin.

These results suggest that short duration and high exposure of heterotrophs to antibiotics, as observed in highly polluted environments, may increase the likelihood of microbial survival and resistance to alternative antibiotics in sediments.

5.3.3 Robust isolate resistance assessment

The resistance of unknown microbial isolates used for the removal of various pharmaceuticals is of particular significance as there may be a relationship between antibiotic resistance and biodegradation. Durand Beach isolate, unknown 1 enriched on ampicillin, was resistant to 79% of antibiotics examined within this study. It is important to mention that unknown 1 was also the isolate that degraded the majority of available carbon from ampicillin during degradation studies. These results suggest that there may be a common denominator between antibiotic resistance and degradation in environmental heterotrophs of highly polluted environments in Western New York that must be studied in greater depth.

Alternatively, recent studies have hypothesized that the formation of biofilms in conjunction with nutrient limitation assists in the survival of microbes with antibiotics [81]. These findings are relevant in this context as, despite their degradation capabilities, unknowns 1, 2, 3, 4, and 5 were 85.7%, 66.7%, 83.3%, 83.3%, and 100.0% resistant to antibiotics in the same class (Table A.6) by which they were selected for, respectively, suggesting that their survival may be due to biofilm formation and nutrient-limitation *ex situ*. Tables A.12 and A.13 indicate visual detection of a mass of cells, most possibly a biofilm, in the culture following incubation. Qualitative observations of growth signify that unknowns 2, 4, and 7 presented visual detection of biofilm formation, therefore, the resistance observed should be carefully considered.

5.4 Conclusion

Point source pollution from industrial and municipal wastewater induces selective pressure on environmental microorganisms, thereby favoring resistance traits for survival. This notion above is supported in this study as heightened antibiotic resistance to cephalosporin, beta-lactam, fluoroquinolone, and aminoglycoside antibiotics were observed at sampling sites located in water bodies that receive wastewater directly in comparison with water used as a drinking source. Furthermore, antibiotic resistance was induced in drinking-water source sediment cultures exposed to extremely high concentrations of antibiotic which dramatically heightened the

survival of microbes with antibiotic resistance in the culture. We postulate that wastewater pollution promotes resistance within the gene pool, which may be suppressed under certain conditions. Moreover, the degradation capabilities of heterotrophs to specific substrates in contaminated sediments is observed in parallel with antibiotic resistance of these substrates, suggesting proximity and selective advantage of genes for multiresistance and biodegradation. We hypothesize that wastewater pollution increases antibiotic resistance of heterotrophs in freshwater and soil environments.

Chapter 6

Recommendations for future research

It is essential to understand metabolic functions that improve biodegradation. To implement these technologies, a basic understanding of the factors that control biodegradation efficiency is necessary. The metabolic factors of biodegradation can be applied to several applications of soil remediation [82]. In this study, we identify key factors impacting the metabolism of antibiotics in soil heterotrophs. An understanding of the genes involved in biodegradation can be useful for the improvement of phytoremediation in polluted environments [82].

For bioremediation of antibiotics in contaminated soil environments to be improved, future research must focus on:

- The use of transcriptomics to induce the expression and alteration of biodegradation genes under contaminated conditions [82].
- Proteomics can be used to identify changes in protein expression in response to stress [82].
- Metabolomics technology can be used to understand biodegradation under contaminated conditions [82].
- Metabolic engineering of microbes [83].

Future research should identify critical genes involved in biodegradation to improve removal of toxic substances in contaminated soil environments. The regulation of specific genes involved in this process can initiate bioremediation in the absence of environmental stressors of contaminated environments by targeting gene expression [82].

The use of riboswitch technology is an intriguing idea that can be applied using the knowledge within this study. Riboswitch technology has critical applications for the bioremediation of contaminated soils. With

an understanding of the physiological regulations of gene expression, riboswitches can be genetically engineered to turn genes on or off for the translation of key enzymatic proteins involved in biodegradation [82]. For instance, we found that under high concentrations of a primary substrate, the metabolism of alternative substrates is enhanced.

Successful manipulation of metabolic pathways has been performed using clustered regularly interspaced short palindromic repeats-CRISPR associated protein 9 (CRISPR-Cas9) tools. Moreover, studies have identified key enzymes involved in contaminant degradation. Gene editing tools can be used to overexpress these genes to clean contaminants from soil [82]. These genes can be identified by the use of the conditions of this study, then apply that knowledge to improve the bioremediation of antibiotics in soil.

Bibliography

[1] A. D. McEachran, B. R. Blackwell, J. D. Hanson, K. J. Wooten, G. D. Mayer, S. B. Cox, and P. N. Smith, "Antibiotics, bacteria, and antibiotic resistance genes: Aerial transport from cattle feed yards via particulate matter," *Environmental Health Perspectives*, vol. 123, no. 4, pp. 337–343, 2015, ISSN: 15529924. DOI: 10.1289/ehp.1408555.

[2] D. E. Karageorgopoulos and M. E. Falagas, "Pandrug Resistance (PDR), Extensive Drug Resistance (XDR), and Multidrug Resistance (MDR) among Gram-Negative Bacilli: Need for International Harmonization in Terminology," *Clinical Infectious Diseases*, vol. 46, no. 7, pp. 1121–1122, Apr. 2008, ISSN: 1058-4838. DOI: 10.1086/528867. [Online]. Available: https://doi.org/10.1086/528867.

[3] A. P. Magiorakos, A. Srinivasan, R. B. Carey, Y. Carmeli, M. E. Falagas, C. G. Giske, S. Harbarth, J. F. Hindler, G. Kahlmeter, B. Olsson-Liljequist, D. L. Paterson, L. B. Rice, J. Stelling, M. J. Struelens, A. Vatopoulos, J. T. Weber, and D. L. Monnet, "Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance," *Clinical Microbiology and Infection*, vol. 18, no. 3, pp. 268–281, 2012, ISSN: 14690691. DOI: 10.1111/j.1469-0691.2011.03570.x.

[4] R. Wise, "Antimicrobial resistance: priorities for action," *Journal of Antimicrobial Chemotherapy*, vol. 49, no. 4, pp. 585–586, Apr. 2002, ISSN: 0305-7453. DOI: 10.1093/jac/49.4.585. [Online]. Available: https://doi.org/10.1093/jac/49.4.585.

[5] D. I. Andersson and D. Hughes, "Antibiotic resistance and its cost: is it possible to reverse resistance?" *Nature Reviews Microbiology*, pp. 260–271, Mar. 2010. [Online]. Available: https://doi.org/10.1038/nrmicro2319http://10.0.4.14/nrmicro2319.

[6] A. Pruden, D. G. Joakim Larsson, A. Amézquita, P. Collignon, K. K. Brandt, D. W. Graham, J. M. Lazorchak, S. Suzuki, P. Silley, J. R. Snape, E. Topp, T. Zhang, and Y. G. Zhu, "Management options for

reducing the release of antibiotics and antibiotic resistance genes to the environment," *Environmental Health Perspectives*, vol. 121, no. 8, pp. 878–885, 2013, ISSN: 00916765. DOI: 10.1289/ehp.1206446.

[7] S. Mishra, R. Singh, B. Das, R. Kaur, C. H. Brower, M. Sran, S. Hayer, R. Laxminarayan, J. Gill, S. J. Patel, L. Chatterjee, A. Zehra, P. Singh, and S. Mandal, "The Prevalence of Extended-Spectrum Beta-Lactamase-Producing Multidrug-Resistant Escherichia Coli in Poultry Chickens and Variation According to Farming Practices in Punjab, India," *Environmental Health Perspectives*, vol. 125, no. 7, p. 077 015, 2017, ISSN: 0091-6765. DOI: 10.1289/ehp292.

[8] M. Madigan, K. Bender, D. Buckley, W. Sattley, and D. Stahl, *Brock Biology of Microorganisms*, 15th ed. 2018.

[9] J. Penuelas, P. Simonet, M. Gillings, S. Banwart, Y. G. Zhu, and D. Stekel, "Human dissemination of genes and microorganisms in Earth's Critical Zone," *Global Change Biology*, vol. 24, no. 4, pp. 1488–1499, Apr. 2017, ISSN: 13541013. DOI: 10.1111/gcb.14003.

[10] D. Barcelo and J. Bennett, "Antibiotic Resistance in the Environment," *Science of the Total Environment (Virtual Special Issue)*, 2018. [Online]. Available: https://www.sciencedirect.com/journal/science-of-the-total-environment/special-issue/10TRKQGXGGN.

[11] K. Kümmerer, "Drugs in the environment: emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources - a review," *Chemosphere*, vol. 45, pp. 957–969, 2001. [Online]. Available: www.elsevier.com/locate/chemosphere.

[12] K Kümmerer, A Al-Ahmad, and V Mersch-Sundermann, "Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test," *Chemosphere*, vol. 40, pp. 701–710, 2000, ISSN: 00456535. DOI: 10.1016/S0045-6535(99)00439-7.

[13] D. Larsson, "Pollution from drug manufacturing: review and perspectives," *Philosophical Transactions of the Royal Society B*, vol. 369, 2014, ISSN: 15334406 00284793. DOI: 20130571.

[14] D Larsson, "Antibiotics in the environment," *Upsala Journal of Medical Sciences*, vol. 119, no. 2, pp. 108–112, 2014, ISSN: 03009734. DOI: 10.3109/03009734.2014.896438.

[15] C. M. Villanueva, M. Kogevinas, S. Cordier, M. R. Templeton, R. Vermeulen, J. R. Nuckols, M. J. Nieuwenhuijsen, and P. Levallois, "Assessing Exposure and Health Consequences of Chemicals in

Drinking Water: Current State of Knowledge and Research Needs," *Environmental Health Perspectives*, vol. 122, no. 3, pp. 213–221, Mar. 2014, ISSN: 0091-6765. DOI: 10.1289/ehp.1206229.

[16] T. Ternes, "The occurrence of micopollutants in the aquatic environment: a new challenge for water management.," *Water Science and Technology*, vol. 55, no. 12, 2007, ISSN: 0273-1223.

[17] M. M. Khin, A. S. Nair, V. J. Babu, R. Murugan, and S. Ramakrishna, "A review on nanomaterials for environmental remediation," *Energy and Environmental Science*, vol. 5, no. 8, pp. 8075–8109, 2012, ISSN: 17545706. DOI: 10.1039/c2ee21818f.

[18] M. Kumar, S. Jaiswal, K. K. Sodhi, P. Shree, D. K. Singh, P. K. Agrawal, and P. Shukla, "Antibiotics bioremediation: Perspectives on its ecotoxicity and resistance," *Environment International*, pp. 448–461, 2019, ISSN: 18736750. DOI: 10.1016/j.envint.2018.12.065.

[19] L. N. Jayakody, C. W. Johnson, J. M. Whitham, R. J. Giannone, B. A. Black, N. S. Cleveland, D. M. Klingeman, W. E. Michener, J. L. Olstad, D. R. Vardon, R. C. Brown, S. D. Brown, R. L. Hettich, A. M. Guss, and G. T. Beckham, "Thermochemical wastewater valorization via enhanced microbial toxicity tolerance," *Energy and Environmental Science*, vol. 11, no. 6, pp. 1625–1638, 2018, ISSN: 17545706. DOI: 10.1039/c8ee00460a.

[20] M. J. Allen, S. C. Edberg, and D. J. Reasoner, "Heterotrophic plate count bacteria - What is their significance in drinking water?" *International Journal of Food Microbiology*, vol. 92, no. 3, pp. 265–274, 2004, ISSN: 01681605. DOI: 10.1016/j.ijfoodmicro.2003.08.017.

[21] L. L. Ling, T. Schneider, A. J. Peoples, A. L. Spoering, I. Engels, B. P. Conlon, A. Mueller, T. F. Schäberle, D. E. Hughes, S. Epstein, M. Jones, L. Lazarides, V. A. Steadman, D. R. Cohen, C. R. Felix, K. A. Fetterman, W. P. Millett, A. G. Nitti, A. M. Zullo, C. Chen, and K. Lewis, "A new antibiotic kills pathogens without detectable resistance," *Nature*, vol. 517, no. 7535, pp. 455–459, 2015, ISSN: 14764687. DOI: 10.1038/nature14098.

[22] L. A. Hicks, M. G. Bartoces, R. M. Roberts, K. J. Suda, R. J. Hunkler, T. H. Taylor, and S. J. Schrag, "US outpatient antibiotic prescribing variation according to geography, patient population, and provider specialty in 2011," *Clinical Infectious Diseases*, vol. 60, no. 9, pp. 1308–1316, 2015, ISSN: 15376591. DOI: 10.1093/cid/civ076.

[23] C. W. Tao, B. M. Hsu, W. T. Ji, T. K. Hsu, P. M. Kao, C. P. Hsu, S. M. Shen, T. Y. Shen, T. J. Wan, and Y. L. Huang, "Evaluation of five antibiotic resistance genes in wastewater treatment systems of swine farms by real-time PCR," *Science of the Total Environment*, vol. 496, pp. 116–121, 2014, ISSN: 18791026. DOI: 10.1016/j.scitotenv.2014.07.024. [Online]. Available: http://dx.doi.org/10.1016/j.scitotenv.2014.07.024.

[24] Y. Zhang, J. Xu, Z. Zhong, C. Guo, L. Li, Y. He, W. Fan, and Y. Chen, "Degradation of sulfonamides antibiotics in lake water and sediment," *Environmental Science and Pollution Research*, vol. 20, no. 4, pp. 2372–2380, 2013, ISSN: 09441344. DOI: 10.1007/s11356-012-1121-8.

[25] K. Kümmerer, "Promoting resistance by the emission of antibiotics from hospitals and households into effluent," *Clinical Microbiology and Infection*, vol. 9, no. 12, pp. 1203–1214, 2003, ISSN: 1198743X. DOI: 10.1111/j.1469-0691.2003.00739.x.

[26] R. A. Hamza, O. T. Iorhemen, and J. H. Tay, "Occurrence, impacts and removal of emerging substances of concern from wastewater," *Environmental Technology & Innovation*, vol. 5, pp. 161–175, 2016, ISSN: 2352-1864. DOI: https://doi.org/10.1016/j.eti.2016.02.003. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S2352186416300104.

[27] E. Walters, K. McClellan, and R. U. Halden, "Occurrence and loss over three years of 72 pharmaceuticals and personal care products from biosolids-soil mixtures in outdoor mesocosms," *Water Research*, vol. 44, no. 20, pp. 6011–6020, 2011. DOI: 10.1016/j.watres.2010.07.051.Occurrence.

[28] D. Hooper and J. Wolfson, "Fluorquinolone Antimicrobial Agents," *New England Journal of Medicine*, vol. 324, no. 6, pp. 384–394, 1991, ISSN: 0960-8788. DOI: 10.1080/09608789308570876.

[29] M. C. Brouwer, A. R. Tunkel, G. M. McKhann, and D. van de Beek, "Brain Abscess," *New England Journal of Medicine*, vol. 371, no. 5, pp. 447–456, 2014, ISSN: 0028-4793. DOI: 10.1056/NEJMra1301635. [Online]. Available: http://www.nejm.org/doi/10.1056/NEJMra1301635.

[30] D. E. Snider, G. M. Cauthen, L. S. Farer, G. D. Kelly, J. O. Kilburn, R. C. Good., and S. W. Dooley, "Drug-Resistant Tuberculosis," *American Review of Respiratory Disease*, vol. 144, no. 3_pt_1, p. 732, Sep. 1991, ISSN: 0003-0805. DOI: 10.1164/ajrccm/144.3{_}Pt{_}1.732. [Online]. Available: https://doi.org/10.1164/ajrccm/144.3_Pt_1.732.

[31] J. S. Wolfson and D. C. Hooper, "Bacterial Resistance to Quinolones: Mechanisms and Clinical Importance," *Reviews of Infectious Diseases*, vol. 11, S960–S968, 1989, ISSN: 01620886. [Online]. Available: http://www.jstor.org/stable/4455043.

[32] L. A. Mandell and M. S. Niederman, "Aspiration Pneumonia," *New England Journal of Medicine*, vol. 380, no. 7, pp. 651–663, Feb. 2019, ISSN: 0028-4793. DOI: 10.1056/NEJMra1714562. [Online]. Available: https://doi.org/10.1056/NEJMra1714562.

[33] A. C. Kalil, M. L. Metersky, M. Klompas, J. Muscedere, D. A. Sweeney, L. B. Palmer, L. M. Napolitano, N. P. O'Grady, J. G. Bartlett, J. Carratalà, A. A. El Solh, S. Ewig, P. D. Fey, T. M. File, M. I. Restrepo, J. A. Roberts, G. W. Waterer, P. Cruse, S. L. Knight, and J. L. Brozek, "Management of Adults With Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society," *Clinical Infectious Diseases*, vol. 63, no. 5, e61–e111, Sep. 2016, ISSN: 1058-4838. DOI: 10.1093/cid/ciw353. [Online]. Available: https://academic.oup.com/cid/article-lookup/doi/10.1093/cid/ciw353.

[34] F. O. Agunbiade and B. Moodley, "Pharmaceuticals as emerging organic contaminants in Umgeni River water system, KwaZulu-Natal, South Africa," *Environmental Monitoring and Assessment*, vol. 186, no. 11, pp. 7273–7291, 2014, ISSN: 15732959. DOI: 10.1007/s10661-014-3926-z.

[35] S. I. Abou-Elela and M. A. El-Khateeb, "Performance evaluation of activated sludge process for treating pharmaceutical wastewater contaminated with β-lactam antibiotics," *Journal of Industrial Pollution Control*, vol. 31, no. 1, pp. 1–5, 2015, ISSN: 09702083.

[36] H. Färber, "Antibiotika im Krankenhausabwasser (Antibiotics in the hospital sewage)," *Hyg. Med.*, vol. 27, 2002.

[37] K. Kümmerer, "Antibiotics in the aquatic environment - A review - Part I," *Chemosphere*, vol. 75, no. 4, pp. 417–434, 2009, ISSN: 00456535. DOI: 10.1016/j.chemosphere.2008.11.086. [Online]. Available: http://dx.doi.org/10.1016/j.chemosphere.2008.11.086.

[38] ——, "Antibiotics in the aquatic environment - A review - Part II," *Chemosphere*, vol. 75, no. 4, pp. 435–441, 2009, ISSN: 00456535. DOI: 10.1016/j.chemosphere.2008.12.006. [Online]. Available: http://dx.doi.org/10.1016/j.chemosphere.2008.12.006.

[39] L. Tahrani, J. Van Loco, H. B. Mansour, and T. Reyns, "Occurrence of antibiotics in pharmaceutical industrial wastewater, wastewater treatment plant and sea waters in Tunisia," *Journal of Water and Health*, vol. 14, no. 2, pp. 208–213, 2016, ISSN: 14778920. DOI: 10.2166/wh.2015.224.

[40] V. Diwan, N. Hanna, M. Purohit, S. Chandran, E. Riggi, V. Parashar, A. J. Tamhankar, and C. Stålsby Lundborg, "Seasonal variations in water-quality, antibiotic residues, resistant bacteria and antibiotic resistance genes of Escherichia coli isolates from water and sediments of the Kshipra River in Central India," *International Journal of Environmental Research and Public Health*, vol. 15, no. 6, pp. 1–16, 2018, ISSN: 16604601. DOI: 10.3390/ijerph15061281.

[41] R. Maal-Bared, K. H. Bartlett, W. R. Bowie, and E. R. Hall, "Phenotypic antibiotic resistance of Escherichia coli and E. coli O157 isolated from water, sediment and biofilms in an agricultural watershed in British Columbia," *Science of the Total Environment*, vol. 443, pp. 315–323, 2013, ISSN: 00489697. DOI: 10.1016/j.scitotenv.2012.10.106. [Online]. Available: http://dx.doi.org/10.1016/j.scitotenv.2012.10.106.

[42] C. Levesque, L. Piche, C. Larose, and P. H. Roy, "PCR mapping of integrons reveals several novel combinations of resistance genes," *Antimicrobial Agents and Chemotherapy*, vol. 39, no. 1, pp. 185–191, 1995, ISSN: 00664804. DOI: 10.1128/AAC.39.1.185.

[43] M. R. Gillings and H. W. Stokes, "Are humans increasing bacterial evolvability?" *Trends in Ecology and Evolution*, vol. 27, no. 6, pp. 346–352, 2012, ISSN: 01695347. DOI: 10.1016/j.tree.2012.02.006. [Online]. Available: http://dx.doi.org/10.1016/j.tree.2012.02.006.

[44] B. G. Spratt, "Hybrid penicillin-binding proteins in penicillin-resistant strains of Neisseria gonorrhoeae," *Nature*, vol. 332, no. 6160, pp. 173–176, 1988, ISSN: 1476-4687. DOI: 10.1038/332173a0. [Online]. Available: https://doi.org/10.1038/332173a0.

[45] C. Baker-Austin, M. S. Wright, R. Stepanauskas, and J. V. McArthur, "Co-selection of antibiotic and metal resistance," *Trends in Microbiology*, vol. 14, no. 4, pp. 176–182, 2006, ISSN: 0966842X. DOI: 10.1016/j.tim.2006.02.006.

[46] S. T. Tohamy, K. M. Aboshanab, H. A. El-Mahallawy, M. R. El-Ansary, and S. S. Afifi, "Prevalence of multidrug-resistant Gram-negative pathogens isolated from febrile neutropenic cancer patients with

bloodstream infections in Egypt and new synergistic antibiotic combinations," *Infection and Drug Resistance*, vol. 11, pp. 791–803, 2018, ISSN: 11786973. DOI: 10.2147/IDR.S163293.

[47] S. Tewari, R. Jindal, Y. L. Kho, S. Eo, and K. Choi, "Major pharmaceutical residues in wastewater treatment plants and receiving waters in Bangkok, Thailand, and associated ecological risks," *Chemosphere*, vol. 91, no. 5, pp. 697–704, 2013, ISSN: 00456535. DOI: 10.1016/j.chemosphere.2012.12.042.

[48] J. Borucinska, "The Rasputin Effect: When Commensals and Symbionts Become Parasitic," in *Advances in Environmental Microbiology: The Rasputin Effect: When Commensals and Symbionts Become Parasitic*, ser. Advances in Environmental Microbiology, vol. 3, Springer International Publishing, 2016, ISBN: 978-3-319-28168-1. DOI: 10.1007/978-3-319-28170-4. [Online]. Available: http://link.springer.com/10.1007/978-3-319-28170-4.

[49] C. Liu, V. Nanaboina, G. V. Korshin, and W. Jiang, "Spectroscopic study of degradation products of ciprofloxacin, norfloxacin and lomefloxacin formed in ozonated wastewater," *Water Research*, vol. 46, no. 16, pp. 5235–5246, 2012, ISSN: 00431354. DOI: 10.1016/j.watres.2012.07.005. [Online]. Available: http://dx.doi.org/10.1016/j.watres.2012.07.005.

[50] S. Liu, T. G. Bekele, H. Zhao, X. Cai, and J. Chen, "Bioaccumulation and tissue distribution of antibiotics in wild marine fish from Laizhou Bay, North China," *Science of the Total Environment*, vol. 631-632, pp. 1398–1405, 2018, ISSN: 18791026. DOI: 10.1016/j.scitotenv.2018.03.139. [Online]. Available: https://doi.org/10.1016/j.scitotenv.2018.03.139.

[51] I. Singh, "Simplified teixobactin analogues to target superbugs," *Future Medicinal Chemistry*, vol. 10, no. 2, pp. 133–134, 2018, ISSN: 17568927. DOI: 10.4155/fmc-2017-0256.

[52] CDC, "Antibiotic Use in the United States, 2017: Progress and Opportunities," Tech. Rep., 2017. [Online]. Available: https://www.cdc.gov/antibiotic-use/stewardship-report/outpatient.html.

[53] K. L. Van Santen, J. R. Edwards, A. K. Webb, L. A. Pollack, E. O'Leary, M. M. Neuhauser, A. Srinivasan, and D. A. Pollock, "The standardized antimicrobial administration ratio: A new metric for measuring and comparing antibiotic use," *Clinical Infectious Diseases*, vol. 67, no. 2, pp. 179–185, 2018, ISSN: 15376591. DOI: 10.1093/cid/ciy075.

[54] T. Frieden, "Antibiotic Resistance Threats in the United States, 2013," CDC, Tech. Rep., 2013, p. 112. [Online]. Available: https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.

[55] M. S. Fram and K. Belitz, "Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California," *Science of The Total Environment*, vol. 409, no. 18, pp. 3409–3417, 2011, ISSN: 0048-9697. DOI: https://doi.org/10.1016/j.scitotenv.2011.05.053. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0048969711005778.

[56] J. Halfman and K. O'Neill, "Water Quality of the Finger Lakes: 2005-2008," Finger Lakes Institute, Hobart & William Smith Colleges, Geneva, NY, Tech. Rep., 2009, pp. 1–33.

[57] M. Oberkircher, *Seasonal Variation in Chronic Stress and Energetic Condition in Three Species of Migratory Birds (MSc thesis)*. 2015. [Online]. Available: https://scholarworks.rit.edu/theses/8773/.

[58] M. Rubaiyat, *Monitoring the Natural Processing of Organic Carbon and Phenols in Selective Wetlands within Monroe County, NY (MSc thesis)*. 2013. [Online]. Available: https://scholarworks.rit.edu/theses/4055/.

[59] United States Environmental Protection Agency, *Enforcement and Compliance History Online*, 2017. [Online]. Available: https://echo.epa.gov/facilities/facility-search.

[60] United States Department of Agriculture, *Geospatial Data Gateway*, 2018. [Online]. Available: https://datagateway.nrcs.usda.gov/.

[61] IBM Corp., *IBM SPSS Statistics for Macintosh*, Armonk, NY, 2017.

[62] T. Brennan-Krohn and J. Kirby, "Antimicrobial Synergy Testing by the Inkjet Printer-assisted Automated Checkerboard Array and the Manual Time-kill Method,"

[63] N. A. Gallagher, *Isolation of Pharmaceutical-Degrading Bacteria from Lake Sediments Associated with Wastewater Effluents (MSc thesis)*. 2016. [Online]. Available: https://scholarworks.rit.edu/theses/9296/.

[64] Becton Dickinson and Company, "BBL Sensi-Disc Antimicrobial Susceptibility Test Discs," Tech. Rep., 2011, p. 4. [Online]. Available: http://legacy.bd.com/ds/technicalCenter/inserts/8840621(201107).pdf.

[65] United States Environmental Protection Agency and Government of Canada, *The Great Lakes: An Environmental Atlas and Resource Book*, Third edition. 1995, ISBN: 0-662-23441-3.

[66] "Hemlock-Candice Unit Management Plan," NYS Department of Environmental Conservation, Tech. Rep., 2015, p. 257.

[67] B. J. Brazos and J. T. O'Connor, "Seasonal Effects on Generation of Particle-Associated Bacteria during Distribution," *Journal of Environmental Engineering*, vol. 122, no. 12, pp. 1050–1057, 2002, ISSN: 0733-9372. DOI: 10.1061/(asce)0733-9372(1996)122:12(1050).

[68] I. Pepper and C. Gerba, *Environmental microbiology: A Laboratory Manual*, 2nd, E. Loya, Ed. Elsevier Inc., 2004, pp. 1–197, ISBN: 0-12-550656-2.

[69] A. A. Al-Gheethi, J. Lalung, E. A. Noman, J. D. Bala, and I. Norli, "Removal of heavy metals and antibiotics from treated sewage effluent by bacteria," *Clean Technologies and Environmental Policy*, vol. 17, no. 8, pp. 2101–2123, 2015, ISSN: 16189558. DOI: 10.1007/s10098-015-0968-z.

[70] T. Nishihara, S. Hasebe, J. Nishikawa, and M. Kondo, "Biodegradation of aniline, anthracene, chlornitrophen, fenitrothion and linear alkylbenzene sulphonate in pond water," Tech. Rep., 1997, pp. 441–447.

[71] E. Topp, R. Chapman, M. Devers-Lamrani, A. Hartmann, R. Marti, F. Martin-Laurent, L. Sabourin, A. Scott, and M. Sumarah, "Accelerated Biodegradation of Veterinary Antibiotics in Agricultural Soil following Long-Term Exposure, and Isolation of a Sulfamethazine-degrading Microbacterium sp.," English, *Journal of Environmental Quality*, vol. 42, pp. 173–178, 2013. DOI: 10.2134/jeq2012.0162. [Online]. Available: http://dx.doi.org/10.2134/jeq2012.0162.

[72] J. Lodge, C. Gaines, J. Arceneaux, and B. Byers, "Non-hydrolytic release of iron from ferrienterobactin analogs by extracts of bacillus subtilis," *Biochemical and biophysical research communications*, vol. 97, no. 4, pp. 1291–1295, 1980.

[73] K. A. Hunt, R. M. Jennings, W. P. Inskeep, and R. P. Carlson, "Multiscale analysis of autotroph-heterotroph interactions in a high-temperature microbial community," *PLoS Computational Biology*, 2018, ISSN: 15537358. DOI: 10.1371/journal.pcbi.1006431.

[74] J. S. Lodge, C. G. Gaines, J. E. L. Arceneaux, and B. R. Byers, "Ferrisiderophore reductase activity in Agrobacterium tumefaciens," *Journal of Bacteriology*, 1982, ISSN: 00219193.

[75] T. Akiyama and M. C. Savin, "Populations of antibiotic-resistant coliform bacteria change rapidly in a wastewater effluent dominated stream," *Science of the Total Environment*, 2010, ISSN: 00489697. DOI: 10.1016/j.scitotenv.2010.08.055.

[76] T. Lopes, I. Costa, F. Periotto, and A. Pletsch, "Antibiotic resistance in E. coli isolated in effluent from a wastewater treatment plant and sediments in receiver body," *International Journal of River Basin Management*, vol. 14, no. 4, pp. 441–445, 2016. DOI: 10.1080/15715124.2016.1201094.

[77] P. Austerman, "Bureau of Fisheries Technical Brief # 2018046 Hemlock Lake Standard Lake Trout Netting Survey (Survey #: 818018)," Department of Environmental Conservation, Tech. Rep., 2018.

[78] S. Kim, Z. Yun, U. H. Ha, S. Lee, H. Park, E. E. Kwon, Y. Cho, S. Choung, J. Oh, C. A. Medriano, and K. Chandran, "Transfer of antibiotic resistance plasmids in pure and activated sludge cultures in the presence of environmentally representative micro-contaminant concentrations," *Science of the Total Environment*, vol. 468-469, pp. 813–820, 2014, ISSN: 18791026. DOI: 10.1016/j.scitotenv.2013.08.100. [Online]. Available: http://dx.doi.org/10.1016/j.scitotenv.2013.08.100.

[79] R. Subramani, M. Narayanasamy, and K. D. Feussner, *Plant-derived antimicrobials to fight against multi-drug-resistant human pathogens*, 2017. DOI: 10.1007/s13205-017-0848-9.

[80] A. Pruden, D. G. Joakim Larsson, A. Amézquita, P. Collignon, K. K. Brandt, D. W. Graham, J. M. Lazorchak, S. Suzuki, P. Silley, J. R. Snape, E. Topp, T. Zhang, and Y. G. Zhu, "Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment," *Environmental Health Perspectives*, vol. 121, no. 8, pp. 878–885, 2013, ISSN: 00916765. DOI: 10.1289/ehp.1206446.

[81] J. N. Anderl, J. Zahller, F. Roe, and P. S. Stewart, "Role of Nutrient Limitation and Stationary-Phase Existence in Klebsiella pneumoniae Biofilm Resistance to Ampicillin and Ciprofloxacin," *Antimicrobial Agents and Chemotherapy*, vol. 47, no. 4, pp. 1251–1256, 2003, ISSN: 0066-4804. DOI: 10.1128/AAC.47.4.1251-1256.2003.

[82] S. Basu, R. C. Rabara, S. Negi, and P. Shukla, "Engineering PGPMOs through Gene Editing and Systems Biology: A Solution for Phytoremediation?" *Trends in Biotechnology*, vol. 36, no. 5, pp. 499–510, 2018, ISSN: 18793096. DOI: 10.1016/j.tibtech.2018.01.011. [Online]. Available: http://dx.doi.org/10.1016/j.tibtech.2018.01.011.

[83] A. K. Dangi, B. Sharma, R. T. Hill, and P. Shukla, "Bioremediation through microbes: systems biology and metabolic engineering approach," *Critical Reviews in Biotechnology*, vol. 39, no. 1, pp. 79–98, 2019, ISSN: 15497801. DOI: 10.1080/07388551.2018.1500997.

[84] C. Walsh, *Antibiotics : actions, origins, resistance*. 2003, pp. 1–329, ISBN: 1555812546.

[85] M. K. Bhattacharjee, "Introduction to Antibiotics," in *Chemistry of Antibiotics and Related Drugs*, Cham: Springer International Publishing, 2016, pp. 1–25, ISBN: 9783319407449. DOI: 10.1007/978-3-319-40746-3. [Online]. Available: http://link.springer.com/10.1007/978-3-319-40746-3_1.

[86] L. Jackson, O. Kroukamp, and G. Wolfaardt, "Effect of carbon on whole-biofilm metabolic response to high doses of streptomycin," *Frontiers in Microbiology*, vol. 6, p. 953, 2015, ISSN: 1664-302X. DOI: 10.3389/fmicb.2015.00953. [Online]. Available: https://www.frontiersin.org/article/10.3389/fmicb.2015.00953.

[87] *iChemLabs*, 2018.

Appendix A

Supplemental tables

Tests of Between-Subjects Effects

Dependent Variable: Water Cell Counts

Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	$1.53x10^{16a}$	9	$1.698x10^{15}$	1338.392	0.000
Intercept	$4.351x10^{15}$	1	$4.351x10^{15}$	3428.886	0.000
Season	$3.833x10^{15}$	1	$3.833x10^{15}$	3020.457	0.000
Site	$5.555x10^{15}$	4	$1.389x10^{15}$	1094.351	0.000
Season * Site	$5.898x10^{15}$	4	$1.474x10^{15}$	1161.916	0.000
Error	$2.538x10^{13}$	20	$1.269x10^{12}$		
Total	$1.966x10^{16}$	30			
Corrected Total	$1.531x10^{16}$	29			

a. R Squared = 0.998 (Adjusted R Squared = 0.998)
SS, sum of squares.

Table A.1: Effect of sampling location and season on concentrations of culturable microorganisms in water

Tests of Between-Subjects Effects

Dependent Variable: Sediment Cell Counts

Source	Type III SS	df	Mean Square	F	Sig.
Corrected Model	$1.54x10^{16a}$	9	$1.716x10^{15}$	46.557	0.000
Intercept	$7.709x10^{15}$	1	$7.709x10^{15}$	209.155	0.000
Season	$7.283x10^{15}$	4	$1.821x10^{15}$	49.401	0.000
Site	$1.580x10^{15}$	1	$1.580x10^{15}$	42.862	0.000
Season * Site	$6.581x10^{15}$	4	$1.645x10^{15}$	44.638	0.000
Error	$7.371x10^{14}$	20	$3.686x10^{13}$		
Total	$2.389x10^{16}$	30			
Corrected Total	$1.618x10^{16}$	29			

a. R Squared = 0.954 (Adjusted R Squared = 0.934)
SS, sum of squares.

Table A.2: Effect of sampling location and season on concentrations of culturable microorganisms in sediment

Facility name	T (lb)	LOL (lb)	HUC 8
Avon STP	$3.01x10^{5}$	0	04130003
Bradley WPCF	$2.16x10^{6}$	0	04140101
Consensus Sewer	$1.53x10^{5}$	0	04130003
Hemlock WFP	$5.36x10^{4}$	$6.19x10^{3}$	04130003
Honeoye WWTP	$6.35x10^{4}$	$6.32x10^{2}$	04130003
Kodak	$6.72x10^{7}$	$2.23x10^{-3}$	04130003
Leroy WWTP	$5.24x10^{4}$	0	04130003
NWQ WWTP	$3.88x10^{6}$	$4.26x10^{3}$	04130001
Van Lare WWTP	$3.63x10^{7}$	$1.61x10^{6}$	04140101
Xerox	$1.03x10^{7}$	$2.36x10^{3}$	04140101

Refer to Table A.4 for sampling sites within each watershed boundary. T, total pounds of pollutants discharged from the facility between 2015 and 2017; LOL, total load over limit for numeric permits; Avon STP, Avon Sewage Treatment Plant; Bradley WPCF, Walter W. Bradley Water Pollution Control Facility; Consensus Sewer, Consensus Lake County Sewer District; Hemlock WFP, Hemlock Water Filtration Plant; Honeoye WWTP, Honeoye Falls WWTP; Kodak, Eastman Kodak Company; NWQ WWTP, Northwest Quadrant Pure Waters District No. 1; Xerox, Xerox Joseph C Wilson Center For Technology.

Table A.3: Facilities with effluent limit exceedances (2015-2017)

Site	HUC 8
Charlotte	04130003
Durand	04140101
Genesee	04130003
Hemlock	04130003
Payne	04130001

Table A.4: HUC 8 region of sampling sites

Antibiotic	m	V	ρ (g/l)	ρ (μg/ml)
Amoxicillin	125	20	6.25	6250
Ampicillin*	150	20	7.50	7500
Cefepime*	150	20	7.50	7500
Chloramphenicol	25	20	1.30	1300
Erythromycin	250	20	12.5	12500
Levofloxacin	500	20	25.0	25000
Lomefloxacin*	500	20	25.0	25000
Streptomycin*	200	20	10.0	10000
Tetracycline	10	20	0.50	500
Trimethoprim	50	20	2.50	2500

ρ, mass concentration of substrate; m, mass of antibiotic substrate in milligrams; V, volume of Bushnell Haas in milliliters; *, antibiotics used in original enrichments.

Table A.5: Preparation of antibiotic stock solutions

Unknown	Enriched substrate	Antibiotic class	% resistance
1	Ampicillin	Beta-lactam	85.7
2	Cefepime	Cephalosporin	66.7
3	Cefepime	Cephalosporin	83.3
4	Cefepime	Cephalosporin	83.3
5	Streptomycin	Aminoglycoside	100.0
6	Ampicillin	Beta-lactam	14.3
7	Cefepime	Cephalosporin	33.3

% resistance, percent resistance too all antibiotics considered for each class. Refer to Table A.7 for groupings of antibiotics.

Table A.6: Percent resistance of unknown isolates to selected antibiotic class

Aminoglycoside	Beta-lactam	Cephalosporin	Fluoroquinolone
Amikacin	Co-amoxiclav	Cefazolin	Ciprofloxacin
Gentamicin	Ampicillin	Cefepime	Levofloxacin
Streptomycin	Carbenicillin	Cefixime	Lomefoxacin
Tobramycin	Imipenem	Cefoxitin	
	Mezlocillin	Cephalothin	
	Oxacillin	Ceftriaxone	
	Piperacillin		
	Ticarcillin		

Co-amoxiclav, amoxicillin/ clavulanic acid.

Table A.7: Antibiotics split into classes

Unknown	Location	Gram reaction	Morphology
1	Durand	Negative	Short bacilli
2	Durand	Negative	Cocci chains >5
3	Charlotte	Negative	Short bacilli >5, chain forming
4	Durand	Negative	Short bacilli
5	Durand	Negative	Cocci chain <5
6	Payne	Negative	Diplococci
7	Genesee	Variable	Short and long bacilli

Table A.8: Unknown isolate characteristics

Unknown	Location	Form, elevation, margin	Pigmentation
1	Durand	Punctiform, flat, entire	Mustard yellow
2	Durand	Circular, convex, entire	Pale yellow
3	Charlotte	Circular, raised, entire	Translucent white
4	Durand	Circular, raised, entire	Beige
5	Durand	Circular, convex, entire	Off-white
6	Payne	Circular, raised, entire	Marigold
7	Genesee	Circular, flat, undulate	Vivid white

Table A.9: Unknown isolate appearance

Tukey HSD[a,b]

		Subset	
Site	N	1	2
Charlotte	6	$5.0x10^5$	
Durand	6	$7.8x10^5$	
Payne	6	$1.5x10^6$	
Genesee	6		$2.8x10^7$
Hemlock	6		$2.9x10^7$
Sig.		0.521	0.613

Means for groups in homogeneous subsets are displayed. Based on observed means.
a. Uses Harmonic Mean Sample Size = 6.000.
b. Alpha = 0.05.

Table A.10: Post-Hoc Tukey test for water cell counts

Tukey HSD[a,b]

		Subset		
Site	N	1	2	3
Charlotte	6	$1.37x10^6$		
Durand	6	$4.37x10^6$		
Payne	6	$5.95x10^6$		
Genesee	6		$2.72x10^7$	
Hemlock	6			$4.13x10^7$
Sig.		0.690	1.000	1.000

Means for groups in homogeneous subsets are displayed.
Based on observed means.
a. Uses Harmonic Mean Sample Size = 6.000.
b. Alpha = 0.05.

Table A.11: Post-Hoc Tukey test for sediment cell counts

Site	Ampicillin	Cefepime	Lomefloxacin	Streptomycin
Charlotte	(+)	(+)	(+)	(+)
Durand	(+/-)	(+/-)	(+/-)	(+/-)
Genesee	(++)	(++)	(++)	(++)
Hemlock	(+)	(+)	(+)	(+)
Payne	(+)	(+)	(+)	(+)

(a) Enrichment 1

Site	Ampicillin	Cefepime	Lomefloxacin	Streptomycin
Charlotte	(+)	(+/-)	(+)	(+)
Durand	(+)	(+)	(+)	(+)
Genesee	(++)	(++)	(++)	(++)
Hemlock	(+)	(+/-)	(+/-)	(+)
Payne	(+)	(+/-)	(+/-)	(+)

(b) Enrichment 2

Site	Ampicillin	Cefepime	Lomefloxacin	Streptomycin
Charlotte	(++)*	(+)†	(++)	(+)*
Durand	(+)†	(++)*†	(++)*	(+)*
Genesee	(++)	(++)	(+)	(+)
Hemlock	(++)	(++)*	(++)*	(+)
Payne	(+)	(++)*	(+)	(+)*

(c) Enrichment 3

(+), regular growth/ turbidity; (++), ample growth/ turbidity; (-), no growth/ turbidity; (+/-), some growth/ turbidity; *, enrichment 3 cultures with floaters/ mass of clumpy growth; †, flask used to culture unknown high capacity isolates.

Table A.12: Qualitative growth of heterotrophs from sediments during summer enrichments

Site	Ampicillin	Cefepime	Lomefloxacin	Streptomycin
Charlotte	(+/-)	(+)	(+/-)	(+)
Durand	(+)	(+)	(+)	(+)
Genesee	(++)	(++)	(++)	(++)
Hemlock	(++)	(++)	(++)	(++)
Payne	(+)	(+)	(+)	(+)

(a) Enrichment 1

Site	Ampicillin	Cefepime	Lomefloxacin	Streptomycin
Charlotte	(+/-)	(+)	(+)	(+)
Durand	(+/-)	(+)	(+)	(+)
Genesee	(++)	(++)	(++)	(+/-)
Hemlock	(++)	(++)	(++)	(++)
Payne	(+)	(+)	(+)	(+/-)

(b) Enrichment 2

Site	Ampicillin	Cefepime	Lomefloxacin	Streptomycin
Charlotte	(+)	(+)*	(+)*	(+/-)*
Durand	(+/-)	(+)*	(+)*	(++)†
Genesee	(+)	(+)*†	(+)	(+)*
Hemlock	(++)	(++)	(++)	(++)*
Payne	(+)†	(+)*	(++)*	(+)

(c) Enrichment 3

(+), regular growth/ turbidity; (++), a lot of growth/ turbidity; (-), no growth/ turbidity; (+/-), some growth/ turbidity; *, enrichment 3 cultures with floaters/ mass of clumpy growth; †, flask used to culture unknown high capacity isolates.

Table A.13: Qualitative growth of heterotrophs from sediments during fall enrichments

Unknown	Sampling site	Enriched on	Season
1	Durand	Ampicillin	Summer
2	Durand	Cefepime	Summer
3	Charlotte	Cefepime	Summer
4	Durand	Cefepime	Summer
5	Durand	Streptomycin	Fall
6	Payne	Ampicillin	Fall
7	Genesee	Cefepime	Fall
8	Durand	Ampicillin	Summer
9	Durand	Ampicillin	Summer
10	Genesee	Ampicillin	Summer
11	Durand	Cefepime	Summer
12	Durand	Cefepime	Summer
13	Genesee	Cefepime	Summer
14	Hemlock	Cefepime	Summer
15	Payne	Cefepime	Summer
16	Charlotte	Lomefloxacin	Summer
17	Durand	Lomefloxacin	Summer
18	Payne	Lomefloxacin	Summer
19	Charlotte	Streptomycin	Summer
20	Payne	Streptomycin	Summer
21	Payne	Streptomycin	Summer
22	Payne	Streptomycin	Summer
23	Charlotte	Ampicillin	Fall
24	Genesee	Ampicillin	Fall
25	Charlotte	Cefepime	Fall
26	Charlotte	Cefepime	Fall
27	Charlotte	Lomefloxacin	Fall
28	Charlotte	Lomefloxacin	Fall
29	Genesee	Lomefloxacin	Fall
30	Hemlock	Lomefloxacin	Fall
31	Genesee	Streptomycin	Fall
32	Genesee	Streptomycin	Fall
33	Hemlock	Streptomycin	Fall
34	Hemlock	Streptomycin	Fall
35	Payne	Streptomycin	Fall

Unknowns 1 to 7 were chosen for further analyses based upon high growth rate and short doubling times during the growth analysis phase.

Table A.14: Identification of unknowns used within this study

			Zone standards (mm)		
Antimicrobial Agent	Code	Disc Potency	R	I	S
Amikacin	AN-30	30 μg	$\leq$14	15-16	$\geq$17
Co-amoxiclav	AmC-30	20/10 μg	$\leq$13	15-16	$\geq$17
Ampicillin	AM-10	10 μg	$\leq$13	14-16	$\geq$17
Carbenicillin	CB-100	100 μg	$\leq$19	20-22	$\geq$23
Cefazolin	CZ-30	30 μg	$\leq$14	15-17	$\geq$18
Cefepime	FEP-30	30 μg	$\leq$14	15-17	$\geq$18
Cefixime	CFM-5	5 μg	$\leq$15	16-18	$\geq$19
Cefoxitin	FOX-30	30 μg	$\leq$14	15-17	$\geq$18
Cephalothin	CF-30	30 μg	$\leq$14	15-17	$\geq$18
Chloramphenicol	C-30	30 μg	$\leq$12	13-17	$\geq$18
Ceftriaxone	CRO-30	30 μg	$\leq$13	14-20	$\geq$21
Ciprofloxacin	CIP-5	5 μg	$\leq$15	16-20	$\geq$21
Doxycycline	D-30	30 μg	$\leq$12	13-15	$\geq$16
Gentamicin	GM-120	120 μg	$\leq$12	13-14	$\geq$15
Imipenem	IPM-10	10 μg	$\leq$13	14-15	$\geq$16
Levofloxacin	LVX-5	5 μg	$\leq$13	14-16	$\geq$17
Lomefloxacin	LOM-10	10 μg	$\leq$18	19-21	$\geq$22
Mezlocillin	MZ-75	75 μg	$\leq$17	18-20	$\geq$21
Oxacillin	OX-1	1 μg	$\leq$10	11-12	$\geq$13
Piperacillin	PIP-100	100 μg	$\leq$17	18-20	$\geq$21
Streptomycin	S-300	300 μg	$\leq$11	12-14	$\geq$15
Ticarcillin	TIC-75	75 μg	$\leq$14	15-19	$\geq$20
Tobramycin	NN-10	10 μg	$\leq$12	13-14	$\geq$15
Co-trimoxazole	SXT	1.25 μg	$\leq$10	11-15	$\geq$16
		23.75 μg			

Zone standards (mm); zone diameter standards in millimeters; co-amoxiclav, amoxicillin/ clavulanic acid; co-trimoxazole, trimethoprim/ sulfamethoxazole. The table was adapted using the reference sheet from Becton, Dickinson and Company, 2011 BD [64].

Table A.15: Kirby-Bauer zone standards for determining bacterial susceptibility

Appendix B

Supplemental figures

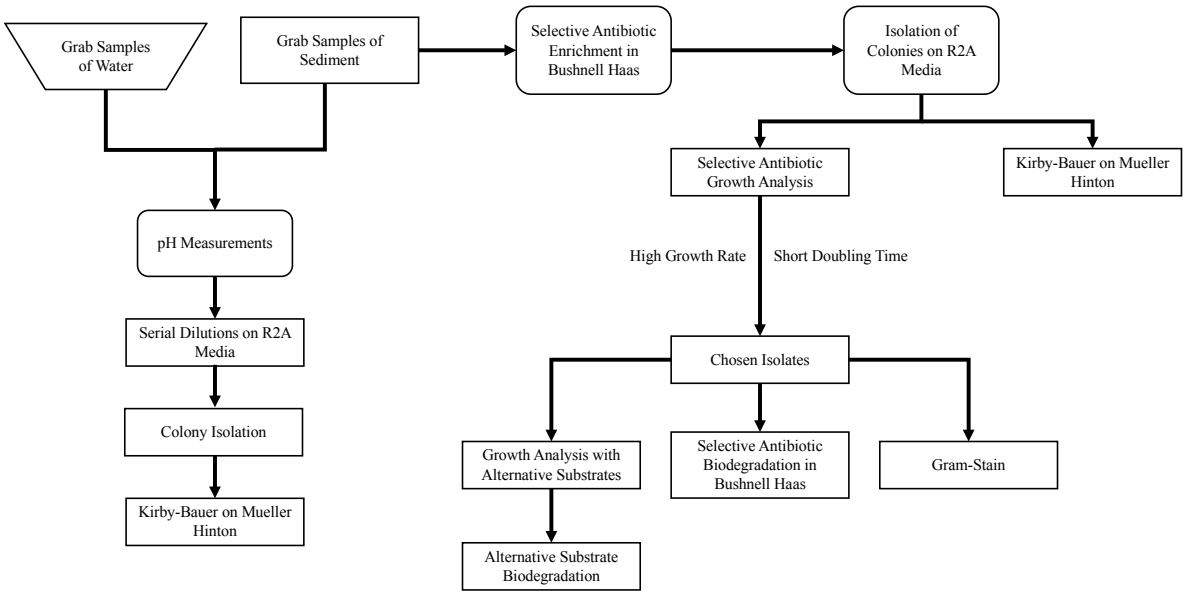
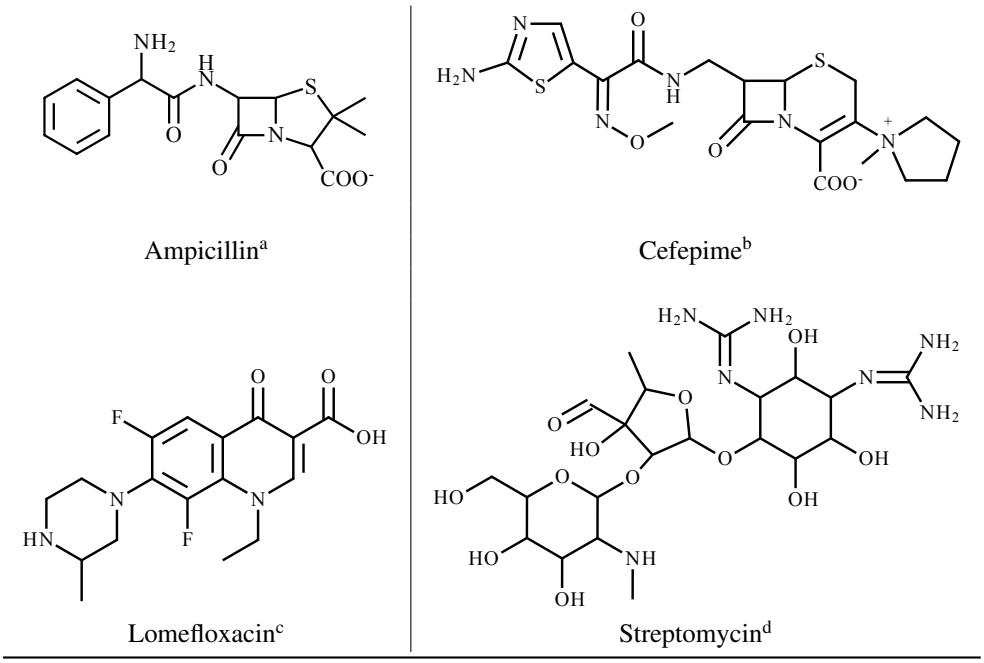
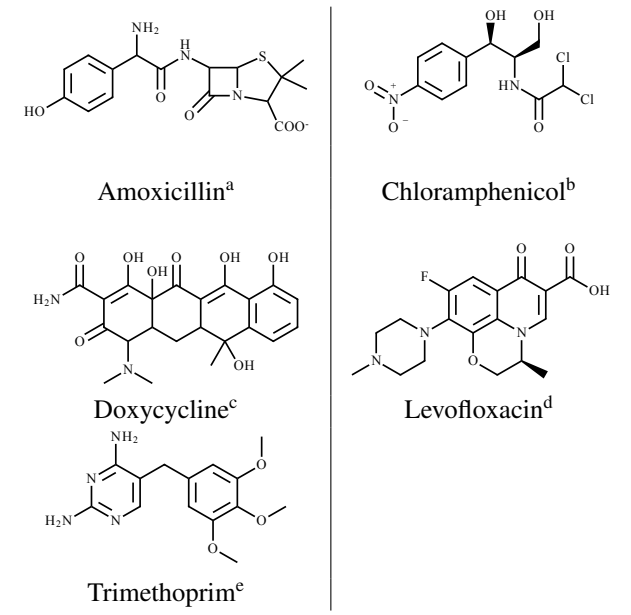


Figure B.1: Flow chart of methodology



[a]beta-lactam, targets envelope; [b]cephalosporin, targets envelope; [c]fluoroquinolone, targets DNA gyrase [31]; [d]aminoglycoside, inhibits protein synthesis [84], [85] and biofilms [86]. Chemical structures were generated using iChemLabs (2018) software [87].

Figure B.2: Chemical structures of antibiotics used in initial nutrient acclimation



[a]beta-lactam; [b]chloramphenicol; [c]macrolide; [d]fluoroquinolone; [e]trimethoprim [84], [85]. Chemical structures were generated using iChemLabs (2018) software [87].

Figure B.3: Chemical structures of antibiotics used for alternate substrate metabolism

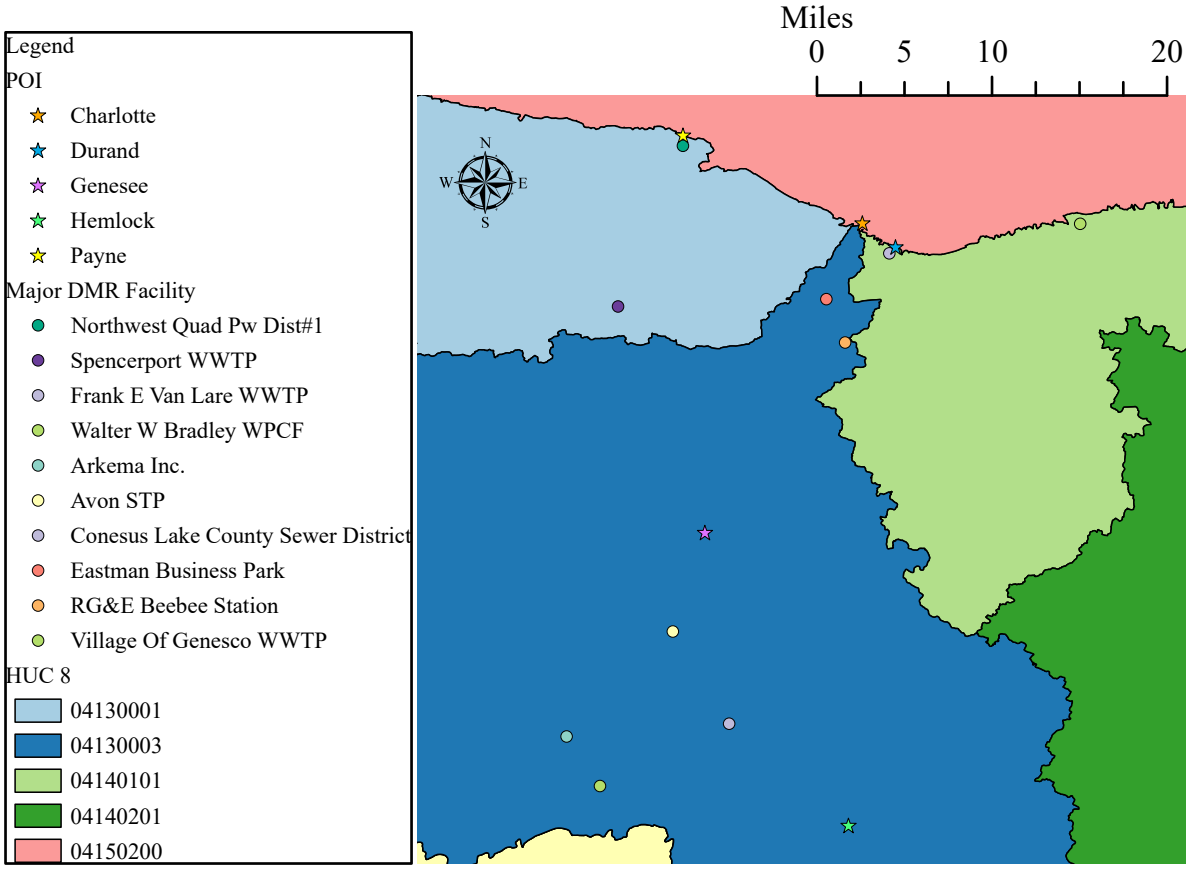


Figure B.4: Sampling locations in Western New York

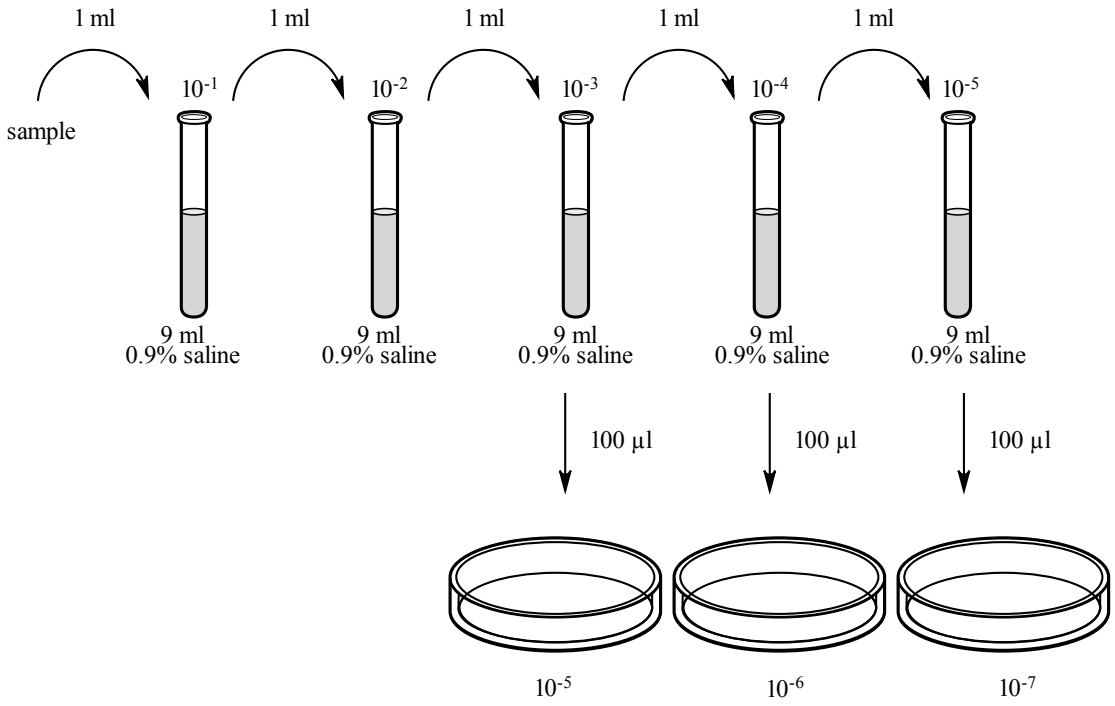


Figure was generated using iChemLabs (2018) software [87].

Figure B.5: Serial dilutions technique for water and sediment

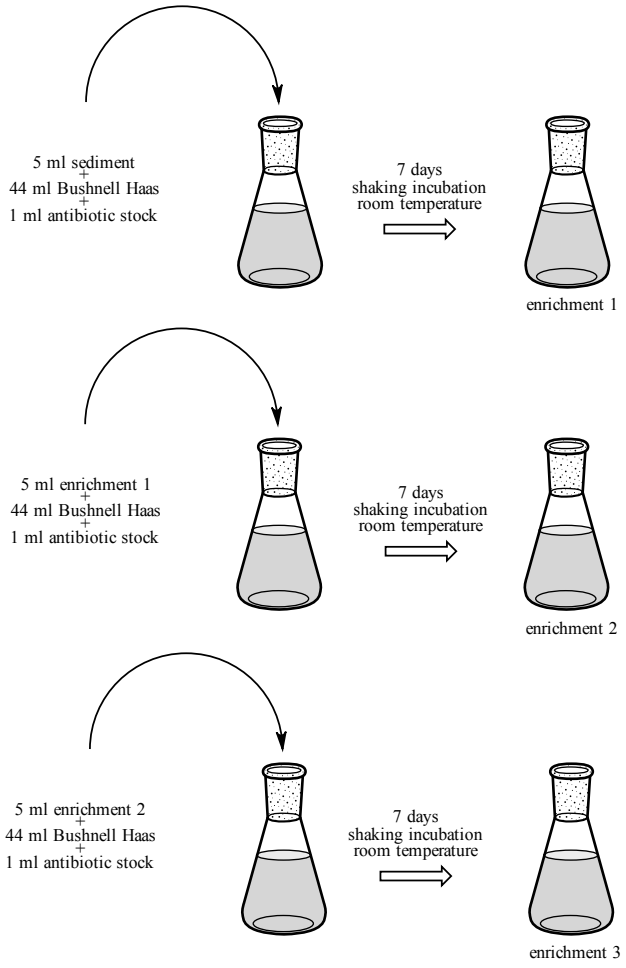


Figure was generated using iChemLabs (2018) software [87].

Figure B.6: Enrichment technique

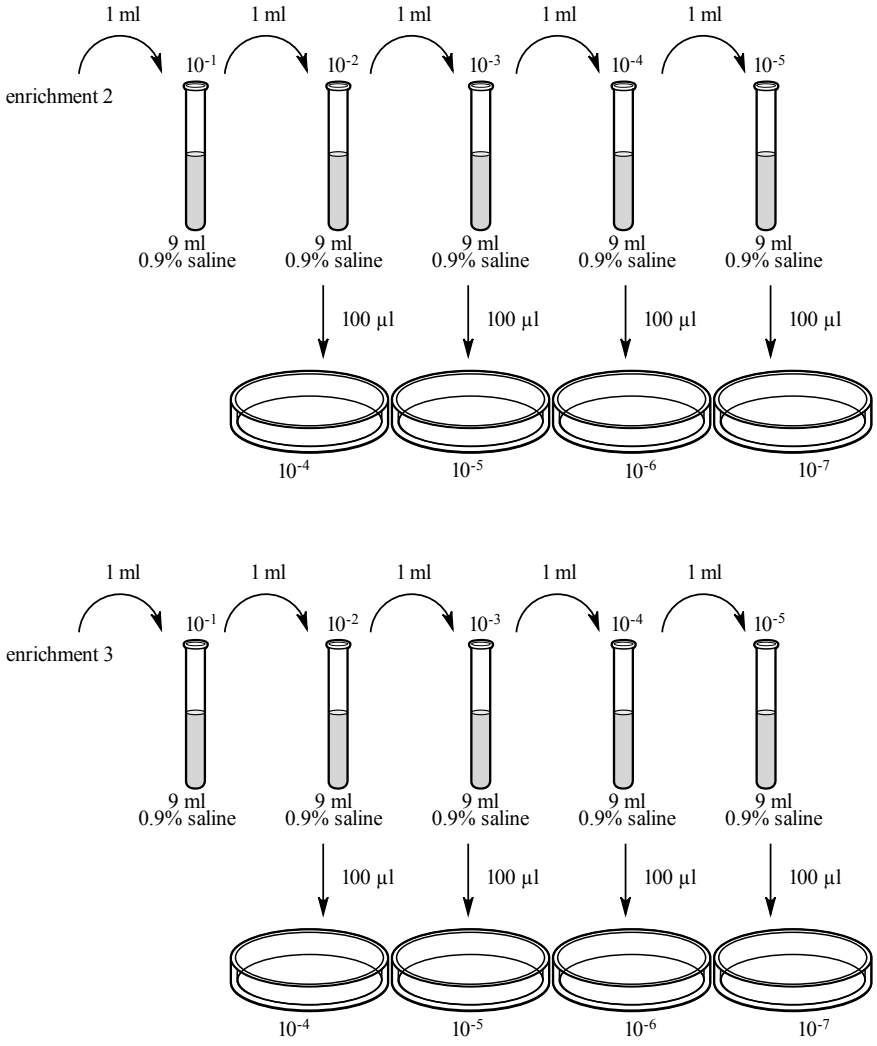
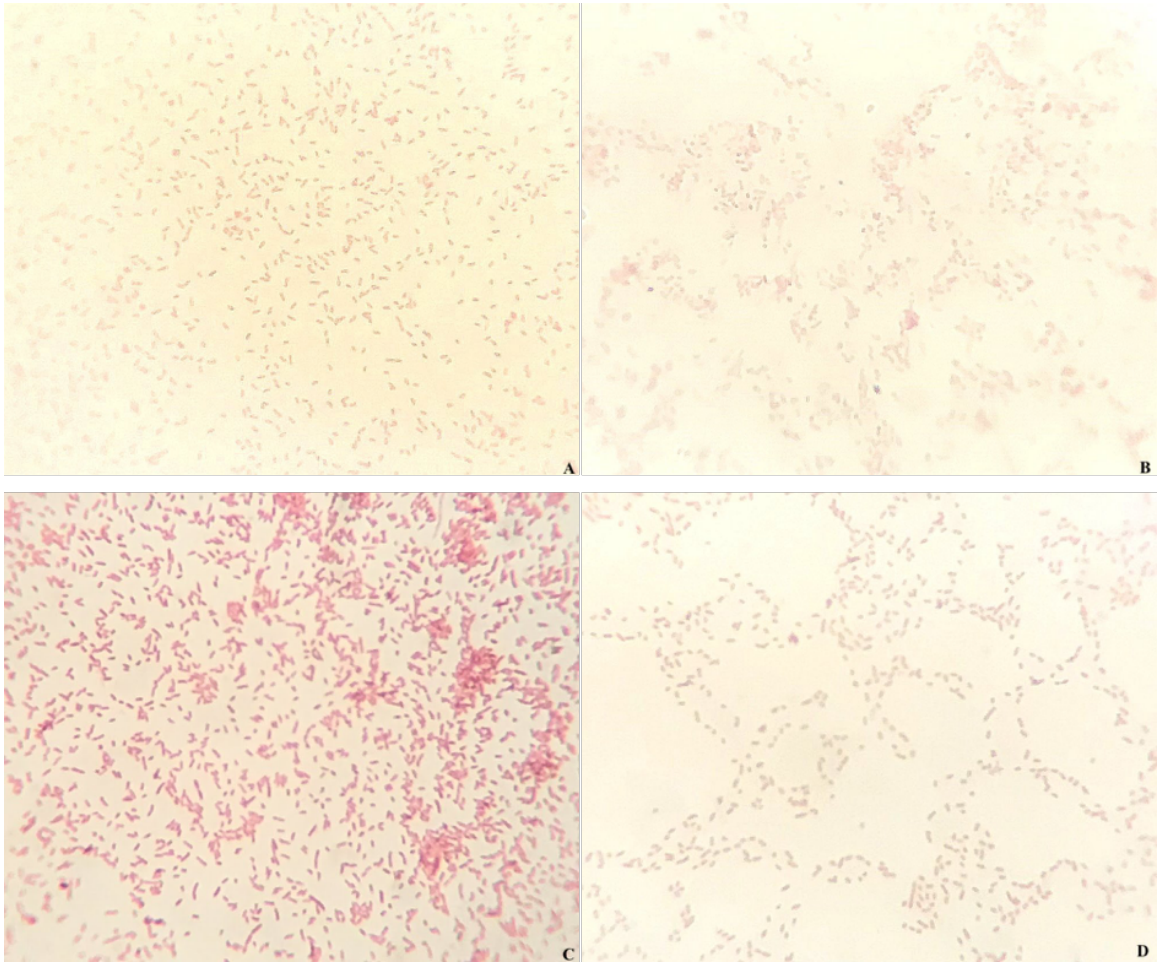


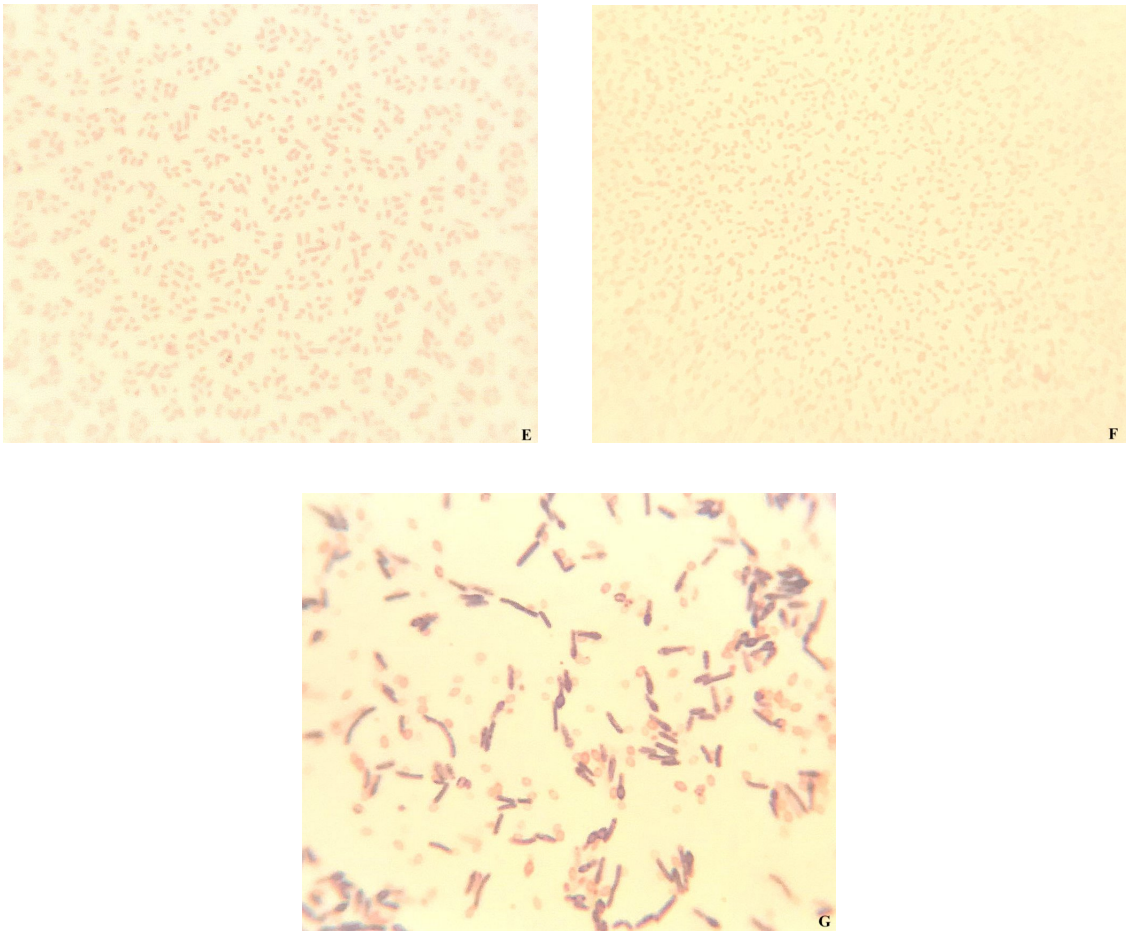
Figure was generated using iChemLabs (2018) software [87].

Figure B.7: Enrichment technique



A, unknown 1 from Durand Beach and enriched on ampicillin; B, unknown 2 from Durand Beach and enriched on cefepime; C, unknown 3 from Charlotte Beach and enriched on cefepime; D, unknown 4 from Durand Beach and enriched on cefepime.

Figure B.8: Gram stain of unknowns 1-4



E, unknown 5 from Durand Beach and enriched on streptomycin; F, unknown 6 from Payne Beach and enriched on ampicillin; G, unknown 7 from the Genesee River and enriched on cefepime.

Figure B.9: Gram stain of unknowns 5-7

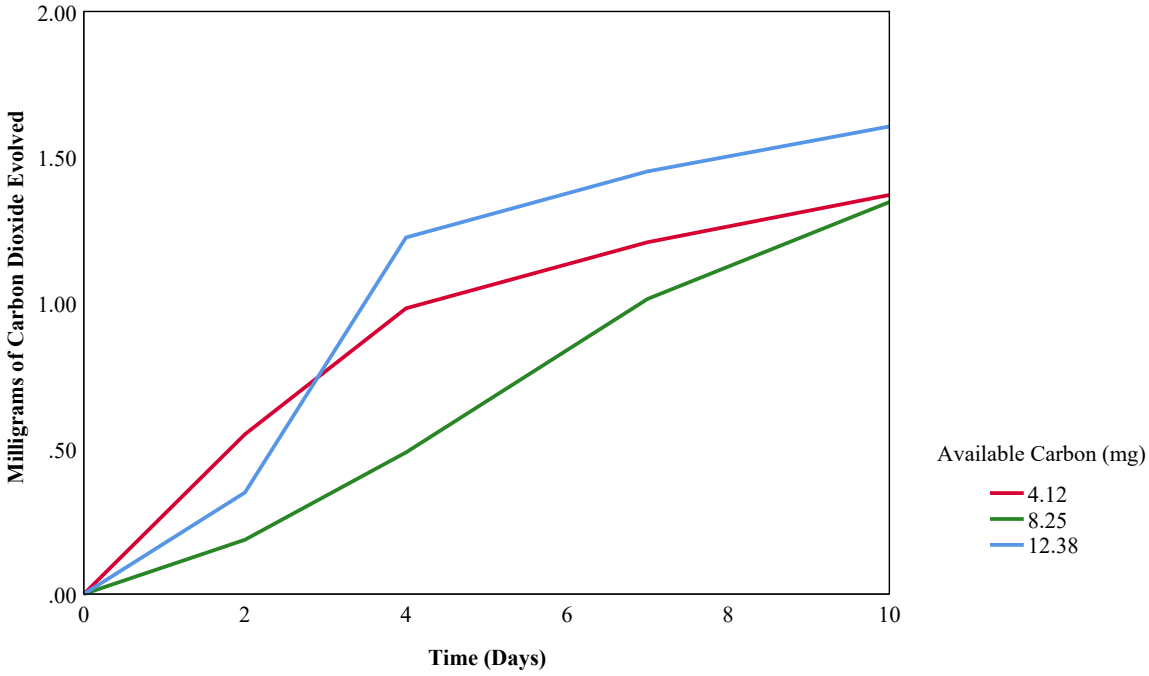


Figure B.10: Degradation of ampicillin by unknown 1 from Durand Beach

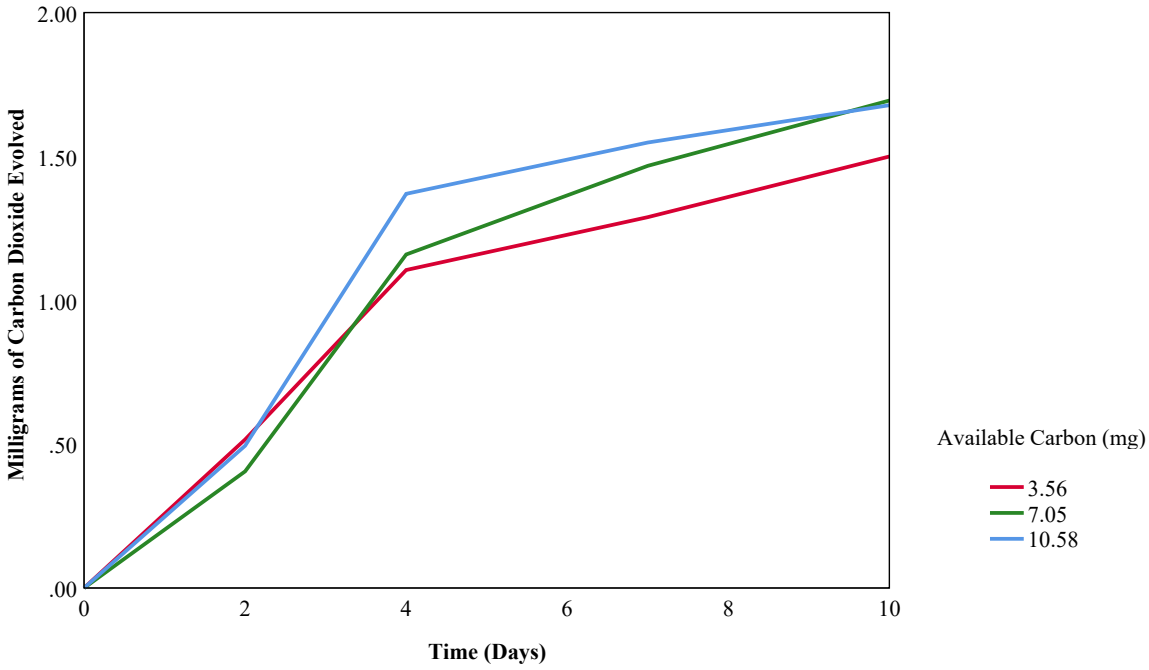


Figure B.11: Degradation of cefepime by unknown 2 from Durand Beach

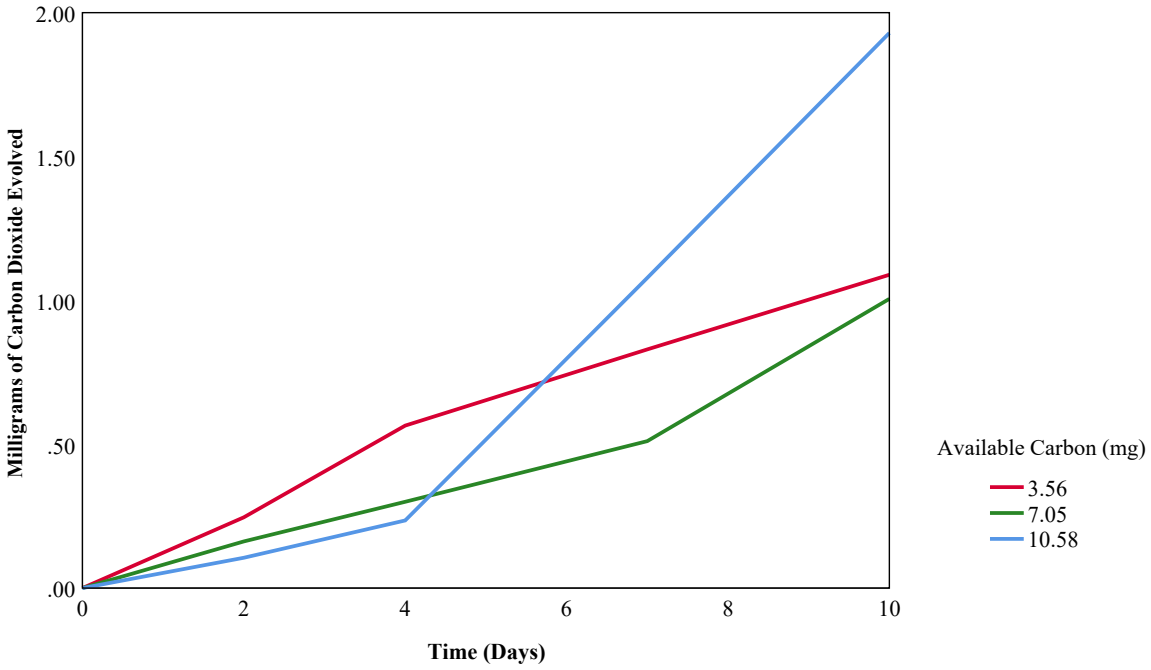


Figure B.12: Degradation of cefepime by unknown 3 from Charlotte Beach

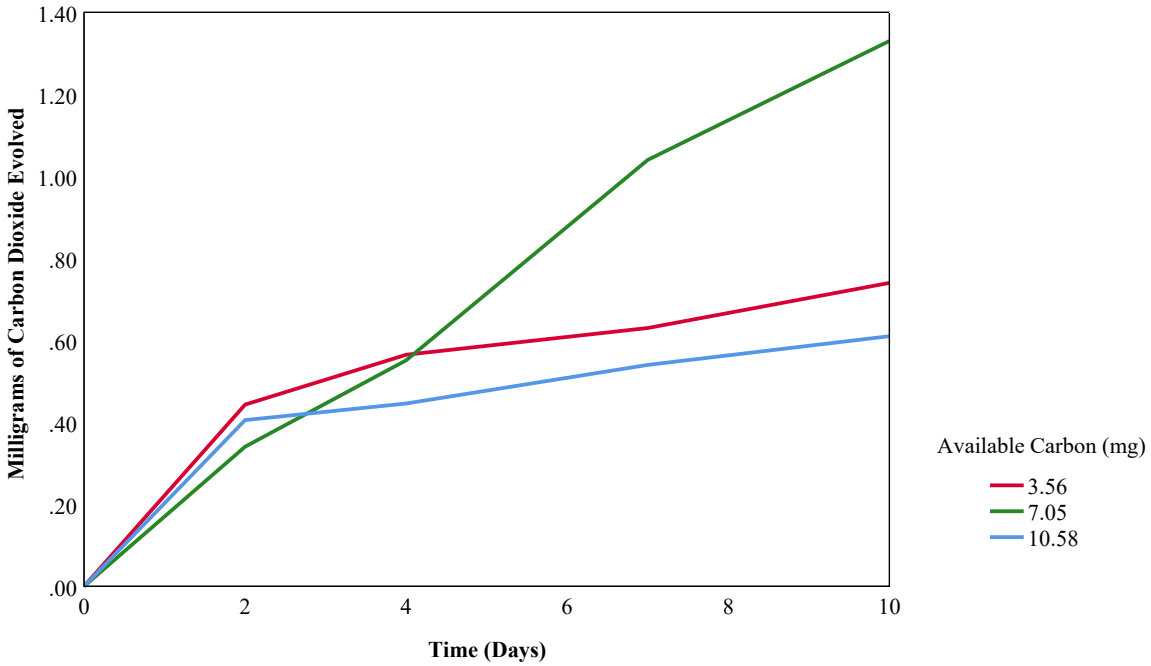


Figure B.13: Degradation of cefepime by unknown 4 from Durand Beach

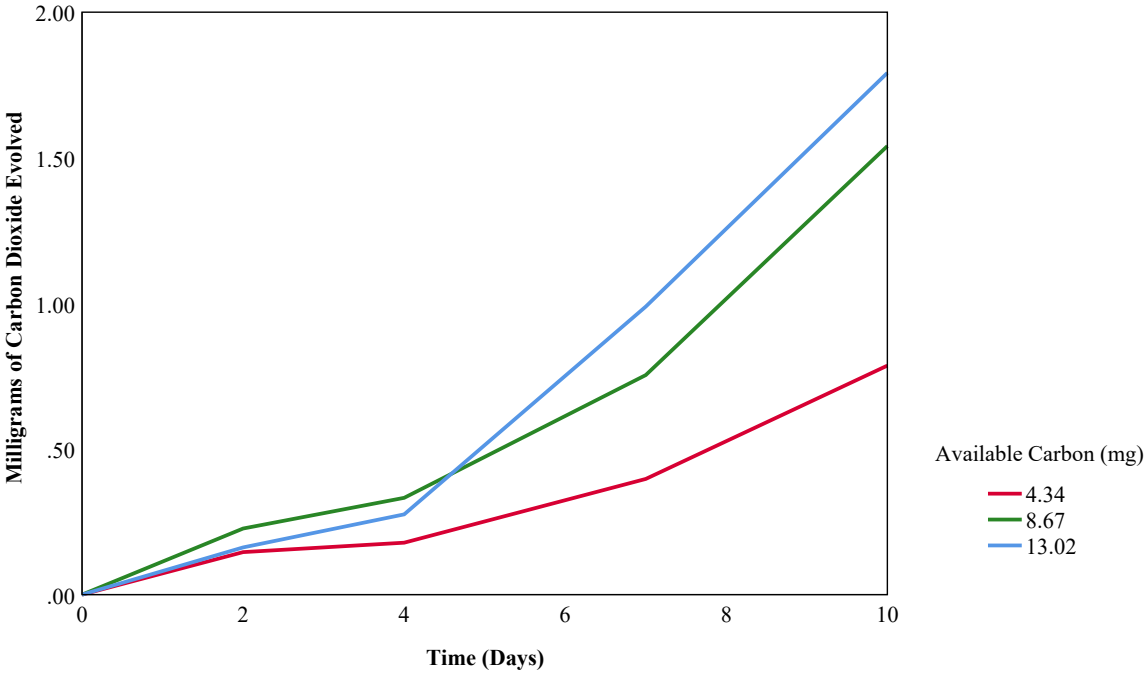


Figure B.14: Degradation of streptomycin by unknown 5 from Durand Beach

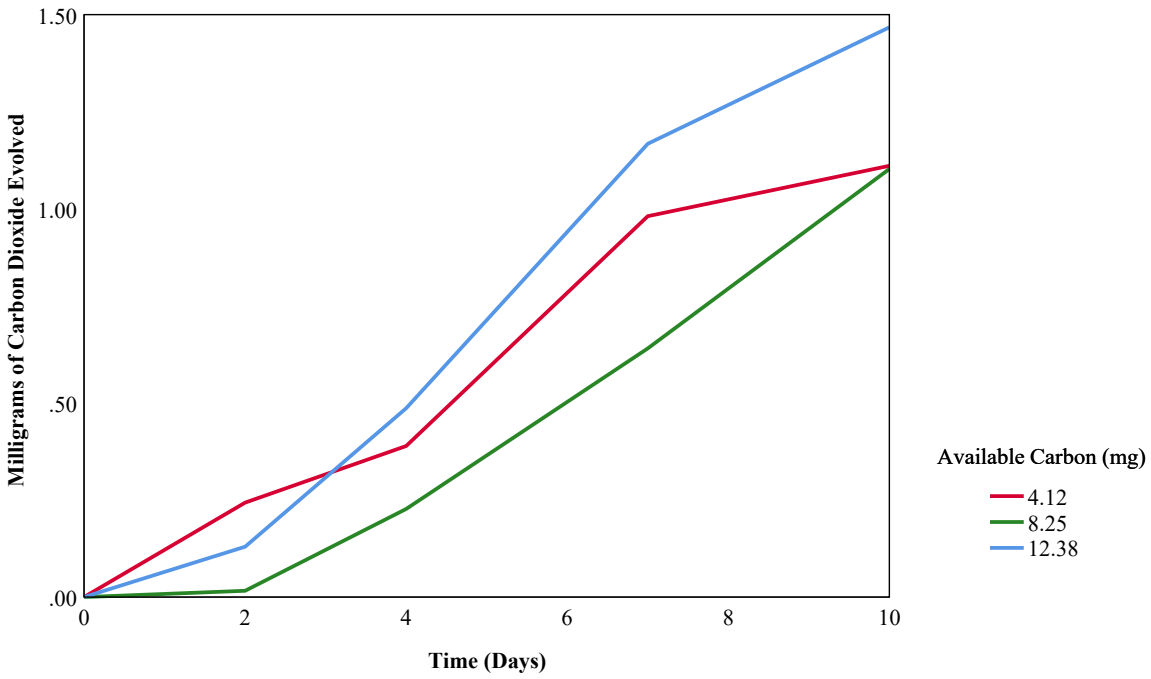


Figure B.15: Degradation of ampicillin by unknown 6 from Payne Beach

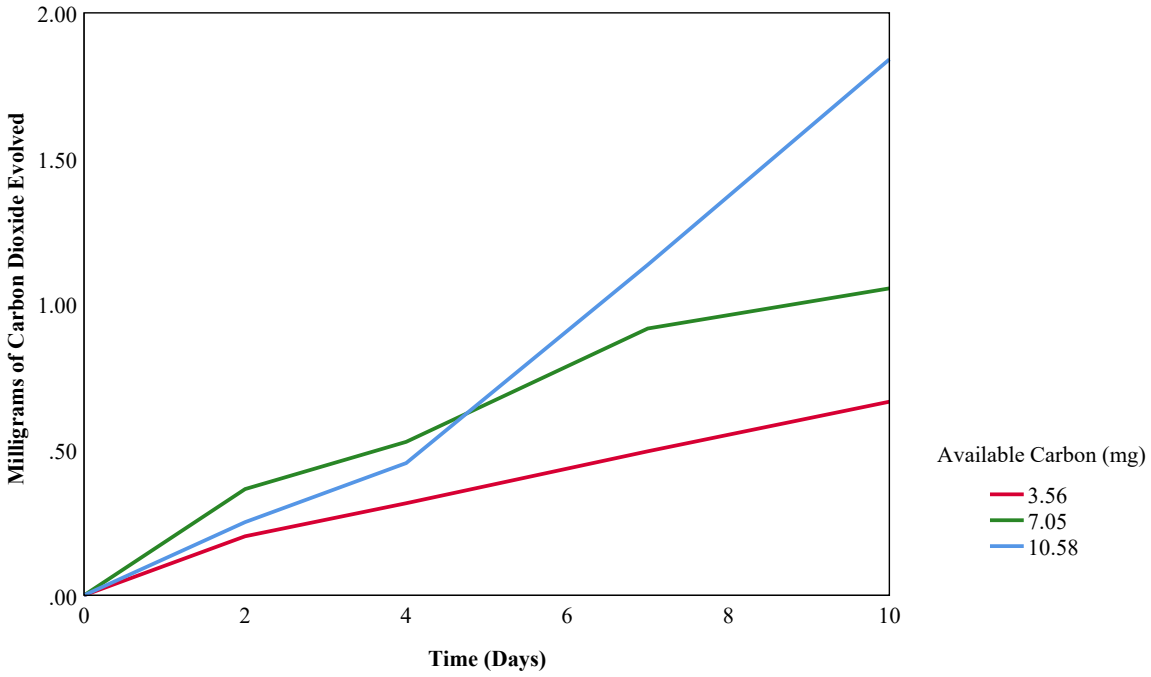
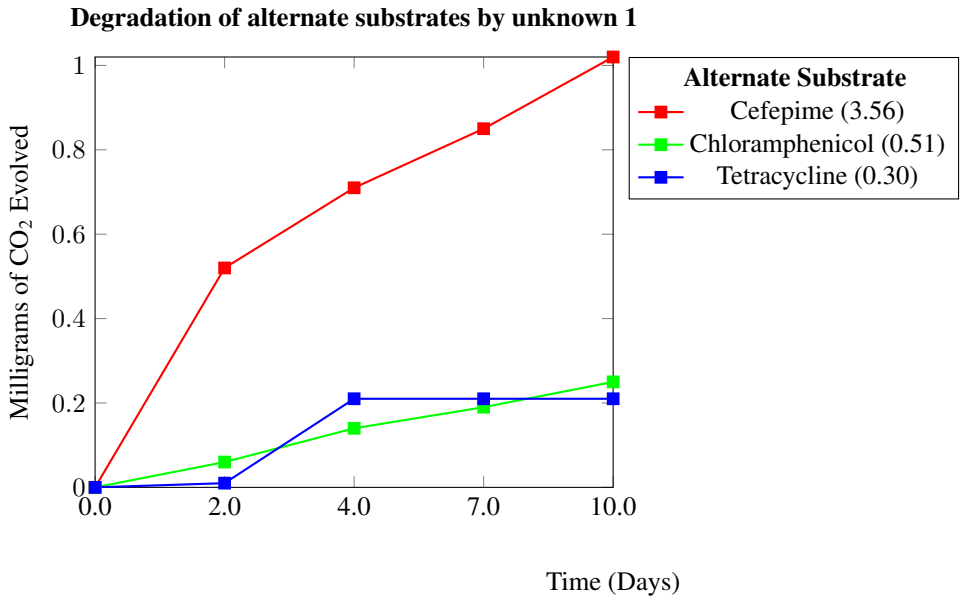
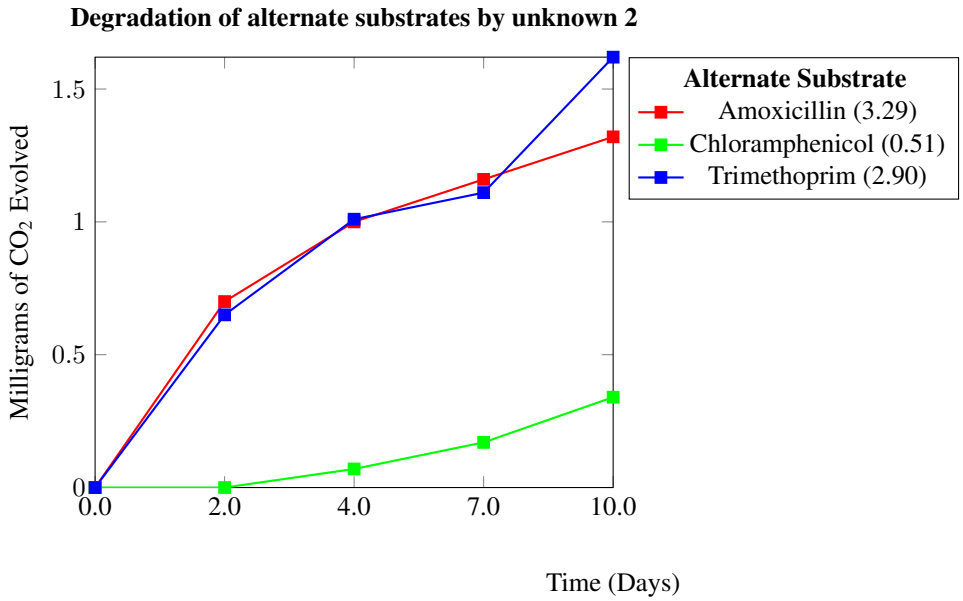


Figure B.16: Degradation of cefepime by unknown 7 from the Genesee River



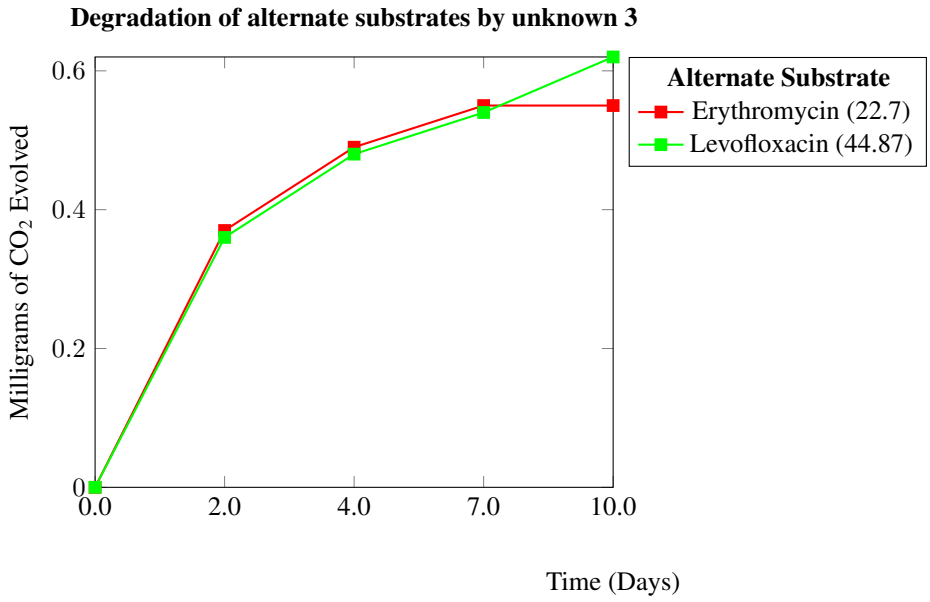
The total milligrams of carbon available for a particular substrate are indicated in the legend.

Figure B.17: Degradation of alternate substrates by unknown 1



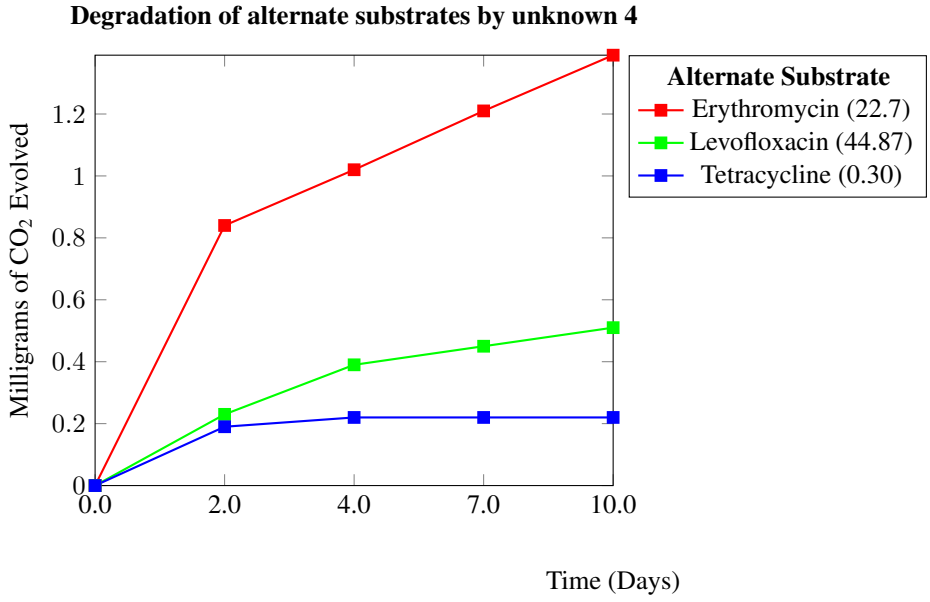
The total milligrams of carbon available for a particular substrate are indicated in the legend.

Figure B.18: Degradation of alternate substrates by unknown 2



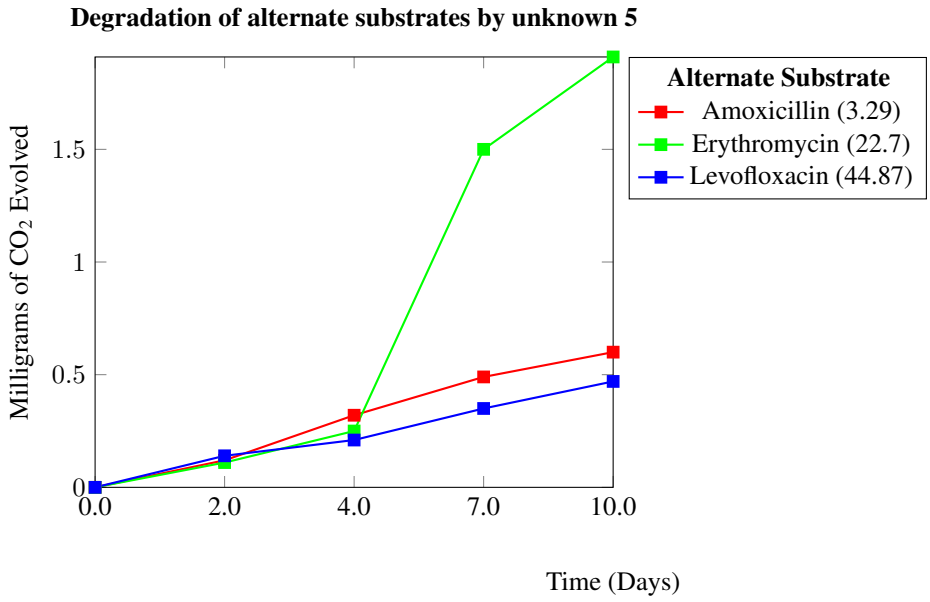
The total milligrams of carbon available for a particular substrate are indicated in the legend.

Figure B.19: Degradation of alternate substrates by unknown 3



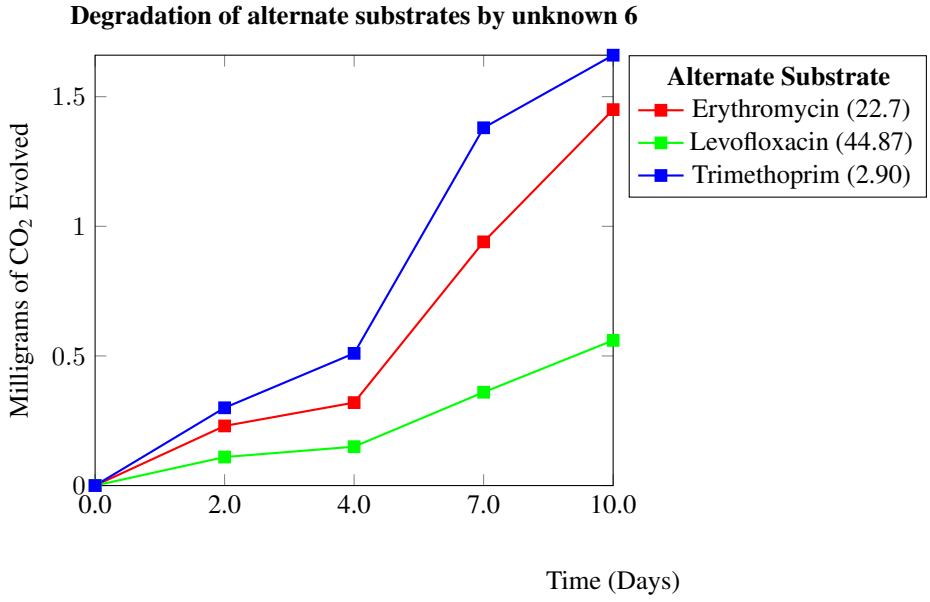
The total milligrams of carbon available for a particular substrate are indicated in the legend.

Figure B.20: Degradation of alternate substrates by unknown 4



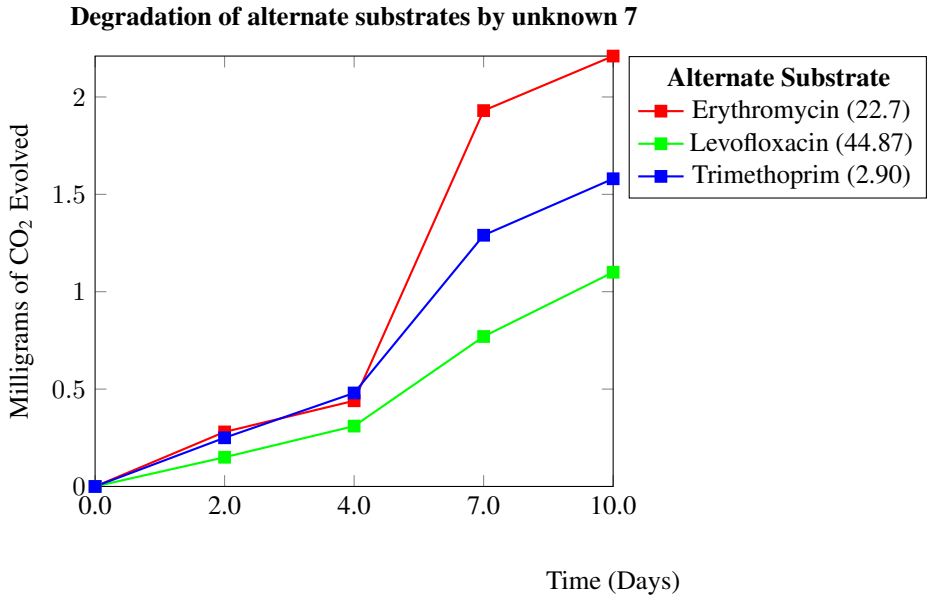
The total milligrams of carbon available for a particular substrate are indicated in the legend.

Figure B.21: Degradation of alternate substrates by unknown 5



The total milligrams of carbon available for a particular substrate are indicated in the legend.

Figure B.22: Degradation of alternate substrates by unknown 6



The total milligrams of carbon available for a particular substrate are indicated in the legend.

Figure B.23: Degradation of alternate substrates by unknown 7