12-15-2016

Isolation of Pharmaceutical-Degrading Bacteria from Lake Sediments Associated with Wastewater Effluents

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by:

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B.S., SUNY Geneseo, 2014

A thesis submitted in partial fulfillment of
the requirements for the degree of
Master of Science in Environmental Science

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College of Science

Rochester Institute of Technology
Rochester, NY
December 15, 2016

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Table of Contents

Acknowledgements

First and foremost, I would like to express my sincere gratitude to my advisor, Dr. Jeffrey Lodge, for his continuous support of my thesis research. His patience, motivation, and immense knowledge of the topic helped me throughout this entire process. The mentoring he provided has improved my knowledge, confidence, and passion for the environmental sciences. I could not have imagined a better advisor and mentor. I would also like to thank my thesis committee, Dr. Christina Tyler and Dr. Corey Ptak, for all the time, consideration, and cooperation they put into this project. Their feedback, participation, and encouragement generally enhanced my experience at RIT. I would also like to thank the Student-Faculty Research Committee for funding part of my project.

Finally, thank you to my mom and dad, for providing me with encouragement and support in not only my thesis work, but in everything I do. Last but not least, thank you to my brothers and sister for the continuous support and love. I could not have done this without you all. I would like to dedicate this work to my late best friend Brendan Englert (1992-2013). I know you were with me throughout this entire journey. Shine on my friend.

List of Tables

Tables

Supplemental Tables

List of Figures

Figures

Supplemental Figures

Abstract

Many wastewater treatment plants (WWTPs) are not properly equipped for the removal of various compounds, including non-steroidal anti-inflammatory drugs (NSAIDs), analgesics, and hormones. These compounds are continually discharged into surface waters, which has become an emerging issue for environmental and public health. Microorganisms in the natural environment may play a crucial role in ecosystem self-purification processes such as contaminant degradation. The aim of this research was to determine if there were microorganisms from water and sediment samples located near wastewater effluent outfalls in Central and Western New York capable of degrading ibuprofen, naproxen, acetaminophen, and 17β-estradiol, and if the degradation capability of microorganisms varied by sampling site. An isolation approach was developed using serial enrichment in mineral medium containing each individual pharmaceutical as the sole carbon source available to heterotrophs. After four weeks of enrichment, bacteria were isolated and the growth of each isolate on its selected pharmaceutical source was measured. The biodegradation of pharmaceuticals was then examined with the isolates that showed the most consistent growth. Results from the various enrichment experiments have led to the isolation of several heterotrophic bacteria capable of utilizing the compounds as their sole carbon sources. An isolate cultured from Payne Beach had the ability to remove up to 40.1% $\pm$ 3.9% of acetaminophen, 23.2% $\pm$ 5.7% of ibuprofen, and 18.6% $\pm$ 5.3% of 17β-estradiol and an isolate cultured from Charlotte Beach had the ability to remove up to 23.4% $\pm$ 3.5% of ibuprofen, 32.2% $\pm$ 2.5% of naproxen, and 29.1% $\pm$ 1.9% of 17β-estradiol. The data suggests that there are endogenous heterotrophs located near wastewater outfalls that can degrade various pharmaceuticals, and that the degradation capability of microorganisms on certain compounds may be site specific.

Introduction

Emerging contaminants are increasingly being detected at low levels in surface waters and have been gaining the attention of both the scientific community and the public as a result. Pharmaceuticals are a large portion of this new class of contaminants (Daughton, 2002), which originate from both human and agricultural use (Risen, 2012). Emerging contaminants are explicitly designed to be bioactive at low concentrations, making them distinct from conventional contaminants (Caracciolo et al., 2015). The bioactivity of pharmaceuticals and their metabolites possess potentially harmful effects to the environment and human health through the consumption of water and food containing pharmaceutical residues. This presents a major concern for public health since little is known regarding the potential interactive effects that may occur from a complex mixture of these compounds; therefore, both research and public action are required to reduce their presence in the environment (Risen, 2012).

WWTPs are the largest contributor of emerging contaminants into our aquatic environment because they are not properly equipped to handle these compounds. Therefore, NSAIDs such as ibuprofen (Figure 1a) and naproxen (Figure 1b), analgesics such as acetaminophen (Figure 1c), and hormones such as 17β-estradiol (Figure 1d) are continually discharged into our surface waters. However, microorganisms in the natural environment may be able to compensate for their release as these organisms are involved in ecosystem "self-purification" processes (Caracciolo et al., 2015). To date, there is a lack of knowledge regarding the biodegradation capability of microorganisms and the overall effects on ecological processes, emphasizing the importance of investigating further the degradative capability of microorganisms that are naturally occurring in surface waters and sediments (Caracciolo et al., 2015). Therefore, the aim of my research was to determine if there were microorganisms in freshwater sediments associated with wastewater effluents that can degrade ibuprofen, naproxen, acetaminophen, and 17β-estradiol.

Presence of Emerging Contaminants in Aquatic Environments

High quantities of pharmaceutical compounds are consumed annually across the globe. For example, in one study, it was determined that 345 tons of ibuprofen were consumed in Germany in 2001 and 35 tons of naproxen were consumed in England in 2000 (Nikolaou et al., 2007). As pharmaceutical consumption increases, the risk of these compounds entering our water systems has become more concerning. Hence, numerous projects have been undertaken across the globe to measure the concentrations of these compounds in the environment (Risen, 2012). The United States Geological Survey (USGS) has led the way by sampling various waters across the country and giving us insight in to the widespread nature of the contamination (Barnes et al., 2008; Focazio et al., 2008; Kolpin et al., 2002). For example, a 1999 and 2000 study implemented by the USGS found some presence of pharmaceuticals in 80% of the waters sampled from a large network of streams in 30 states (Kolpin et al., 2002). Although most pharmaceutical compounds and/or their metabolites are found at concentrations of ng/L in surface waters (Kim et al., 2007), concentrations of some compounds can reach into the low µg/L level (Kolpin et al., 2002).

Among the most commonly prescribed drugs in modern history, NSAIDs are the most frequently detected pain killers in our surface waters (Caracciolo et al., 2015). Various reports have detected noteworthy concentrations of both ibuprofen and naproxen in our natural waters. This may be due to the fact that up to 30% of ibuprofen and 40% of naproxen can pass through WWTPs unaltered (Carballa et al., 2007). In one study, effluent samples from Back River WWTP in Baltimore, Maryland contained ibuprofen and naproxen at significant concentrations of 250 ng/L and 380 ng/L, respectively (Yu et al., 2006). However, the detection of several other compounds in natural waters has varied significantly. Acetaminophen was detected in concentrations ranging from 14 to 1600 ng/L in surface waters nearby a WWTP (Lin et al., 2010), whereas concentrations were found to be up to 10 µg/L in numerous waters throughout the U.S. (Kolpin et al., 2002). Lastly, natural and synthetic hormones are often detected in the ng/L range (Chimchirian et al., 2007). For example, 17β-estradiol was found to be in the range of 1 to 50 ng/L from WWTP effluents in Britain (Desbrow et al., 1998). These reports are significant because they show that emerging contaminants are ubiquitous in surface waters, and

require direct attention to determine what effects they may be having and how to limit their concentrations in the environment (Risen, 2012).

Sources of Emerging Contaminants

WWTPs are not specifically designed to remove emerging contaminants and are the major source of these compounds into surface waters. Several studies have determined that the partial removal of emerging contaminants in WWTPs often takes days to weeks (Almeida et al., 2013; Carballa et al., 2007; Salgado et al., 2012), and depends on the type of treatment processes occurring at the plant and the time of year. Removal rates tend to be highest in the summer months since the degradative activity of bacteria is greater at warmer temperatures (Castiglioni et al., 2006). However, it has been suggested that the most important factor in eliminating these types of contaminants is the amount of time allowed for degradation (Quintana et al., 2005; Radjenovic et al., 2008). To ensure the greatest removal of these contaminants, longer hydraulic retention times and solid retention times are essential. Several studies in particular have suggested that hydraulic retention times of 12 hours and solids retention times of 10 days are successful at removing a majority of parent compounds (Clara et al., 2005; Metcalfe et al., 2003; Miege et al., 2008). However, a heavy precipitation event has the potential to interrupt treatment processes (Risen, 2012) and cause the release of partially treated wastewater into surface waters (Buerge et al., 2006). For example, 7 million gallons of raw sewage were recently released into Onondaga Lake following 21 straight hours of rain (Coin, 2016). Therefore, in order to handle emerging contaminants, WWTPs need to be upgraded to manage higher flow with longer retention times (Risen, 2012); however, doing so would be a costly affair for each plant.

Pharmaceuticals are transported to WWTPs via two major routes. First off, most pharmaceuticals that are consumed by individuals are not completely eliminated in the body and are excreted in feces and urine (Heberer, 2002). In one study, it was found that approximately 58-68% of acetaminophen is excreted from the body during use (Muir et al. 1997). This is of concern since, after excretion, most pharmaceutical compounds are still biologically active as they are only slightly transformed or unchanged conjugated polar molecules (Heberer, 2002). Second, unused or expired drugs may be improperly disposed of by individual households, hospitals, and nursing homes (Heberer, 2002). These drugs may be flushed down the septic

system or thrown in the trash, in which they will eventually runoff into landfill leachate and be transported through a WWTP. Pharmaceuticals may also be released directly into surface waters via runoff. Wastes generated on stock-raising farms often contain pharmaceutical residues, which are released in runoff during precipitation events (Topp et al., 2008; Heberer., 2002). For example, naproxen is given to horses to treat pain, and is often found in water sources that are in close proximity to farms (Topp et al., 2008).

Consequences to Aquatic Organisms

Aquatic organisms are at risk of having continuous, multigenerational exposure to emerging contaminants when they are released into surface waters. Organisms may develop various malformations during their growth (Ragugnetti et al., 2011); however, it is important to note that the effects of exposure during the early stages of life may not be perceived until adulthood (EPA, 2016a). Although, many of these contaminants exhibit low acute toxicity; emerging contaminants can cause significant effects at very low levels of exposure (EPA, 2016a). Thus far, various studies have focused primarily on the effects of ibuprofen on various organisms since it is frequently detected in our natural waters at significant concentrations. That being said, sublethal effects have been reported in the µg/L to ng/L range (Risen, 2012). For example, a dose dependent response was observed in Java-medaka (*Oryzias latipes*) during chronic exposure to ibuprofen concentrations of 1 to 100 µg/L, which induced a decrease in cyclooxygenase activity and longer days between spawning (Flippin et al., 2007). Other studies have also found that ng/L concentrations of ibuprofen can inhibit eicosanoid synthesis in Daphnia magna (Hayashi 2008) and significantly lower the levels of micronuclei frequencies in Nile Tilapia (*Oreochromis niloticus*) (Ragugnetti et al., 2011). These examples of low dose effects reflect carefully chosen endpoints which mirror the intended effects of ibuprofen (Risen, 2012).

Many emerging contaminants also act as endocrine disrupters, which alter the normal functions of hormones. Exposure to endocrine disrupters can lead to a variety of health effects for aquatic organisms, including growth and reproductive effects. In one study, the effects of 17β-estradiol on the reproduction of Java-medaka were tested (Imai et al. 2005). After six months of exposure to 17β-estradiol, several key findings were reported including reduced

fecundity, inhibition of secondary sexual characteristics, elevated vitellogenin production, and feminizing effects (Imai et al. 2005). While certain species may be more sensitive than others to endocrine effects (Risen, 2012), there are clear risks associated with hormone release into our waters.

Concern for Public Health

Pharmaceuticals in drinking water pose significant risks to humans and ecosystem health as well. The reported levels of pharmaceuticals in water may be significantly lower than those applied during general use; however, the potential health effects associated with long term exposure to trace levels cannot be ignored with respect to water reuse purposes (Kümmerer et al. 2001). There is also a lack of knowledge regarding the fate of various compounds (Murdoch et al., 2015) and their biotransformations in the natural environment. As stated previously, many of these compounds and/or their metabolites are unchanged, and thus biologically active in the environment (Wojcieszyńska et al., 2014). Therefore, reactions may occur in the human body that differ from the intended purpose of the compounds. Humans may be at risk through cumulative effects from long-term exposure to very low levels of the compounds since they have the potential to bioaccumulate in the body's tissues (Nikolaou et al., 2007). Endocrine disruption is also a warranted concern as endogenous chemicals have the capability of triggering gene expression cascades (Casals-Casas et al., 2011). However, more research in all of these areas is required to develop a better understanding of what these compounds have the potential to do in the human body, especially long-term.

Elimination of Emerging Contaminants

Scientists have taken many approaches to investigating the elimination and removal of emerging contaminants in the natural environment. Recent studies have looked at pure strains tied to co-metabolism to degrade pharmaceutical compounds. In one study, a low level of naproxen removal accompanied by a decrease in *Stenotrophomonas maltophilia* KB2 was detected in a monoculture experiment (Wojcieszyńska, et al., 2014). However, when glucose and phenol were added individually, high levels of naproxen removal were detected (Wojcieszyńska,

et al., 2014). Another study used a pure isolate from activated sludge and co-metabolism to degrade ibuprofen and found that the isolate was more successful at degrading ibuprofen in the presence of other carbon sources (Almeida et al., 2013). The issue with using co-metabolism is that researchers are often skeptical about adding alternative carbon sources to treat water, especially phenol. Therefore, microorganisms in natural ecosystems should be explored in more detail since these organisms are being exposed to pharmaceutical compounds in their natural habitat. Four 17β-estradiol-degrading bacterial strains were isolated from natural sources (Zhou et al., 2013). A different study reported that acetaminophen has the potential to be biodegraded by 80% in a natural water system (Yamamoto et al., 2009). These studies emphasize the importance of investigating further the degradation capability of microorganisms that are naturally occurring in surface waters and sediments to help support an integrated strategy for the protection of ecosystems and human health (Nikolaou et al., 2007), and provide a better understanding of the bacteria that are degrading pharmaceuticals in the natural environment.

Purpose of Study

The overall purpose of this study was to culture bacteria from lake sediments associated with effluent discharges and determine if these isolates had the capability of growing on and degrading selected pharmaceuticals. The objectives were to conduct a variety of experiments to determine the growth and degradation potential of isolates as well as their versatility to grow on and degrade more than one compound. Versatility was an important measure in this study because a variety of pharmaceuticals are being released into surface waters simultaneously. The last objective was to determine if high capacity isolates have the ability to degrade compounds in their natural waters. This would give insight into whether native bacteria are utilizing pharmaceuticals under natural conditions. To the best of my knowledge, this is one of the first studies conducted that has cultured native bacteria from sediments sources and determined their growth and degradation potential on a variety of pharmaceuticals under both laboratory and more natural conditions.

Methods

Overview

Water and sediment samples were collected from three locations on Lake Ontario's south shoreline and one location on Onondaga Lake. Onondaga Lake, Durand Beach, Charlotte Beach, and Payne Beach were all chosen as sampling locations because of their close proximity to WWTP effluent discharges. These locations were sampled during the summer of 2015, the fall of 2015, and the winter of 2016. During each season, an isolation approach was performed using serial enrichment in mineral medium containing 2.5 mg of each individual pharmaceutical as the sole carbon source available to heterotrophs. After four weeks of enrichment, bacteria were isolated and the growth of each isolate on selected pharmaceutical source was measured at 600 nm. The biodegradation of pharmaceuticals was then examined with the isolates that showed the most consistent growth. Lastly, the growth and biodegradation potential of high capacity isolates were measured in their natural waters.

Site Descriptions

Onondaga Lake is located in Syracuse, NY, along the northern side of the city. The lake covers an area of 7.4 kilometers (km) and receives water from a drainage basin of approximately 738 km^2 (EPA, 2016b) as well as effluent discharges from the Metropolitan Syracuse Wastewater Treatment Plant (Onondaga Lake Partnership, 2010).

For 125 years, municipal sewage and industrial waste were dumped into Onondaga Lake, which became heavily contaminated with compounds such as mercury, polychlorinated biphenyls, pesticides, heavy metals, and volatile organic compounds (Onondaga Lake Partnership, 2010). As a result, low oxygen levels and elevated levels of nutrients, disease-causing microorganisms, and toxic contaminants became prevalent throughout the lake (Onondaga Lake Partnership, 2010). Onondaga Lake eventually became an EPA Superfund Site and has recently been remediated. Improvements have included wastewater upgrades and cleanup of the industrial pollution. Since 1990, the WWTP's capacity to treat wastewater has improved through projects such as aeration and digital system upgrades (Onondaga Lake

Partnership, 2010). Lower concentrations of nutrients and contaminants have been reported since 2007, improving the lake's conditions (Onondaga Lake Partnership, 2010). However, stormwater runoff and wind have continued to assist in transporting non-point sources of pollution into the lake. Common sources of pollution are associated with agriculture and urbanization activities, including over-grazed pastures and unstabilized barnyards (Onondaga Lake Partnership, 2010).

Durand Beach lies on Lake Ontario's south shoreline in Irondequoit, NY. The beach receives several outfalls along the shoreline, including effluent discharges from the Van Lare Wastewater Treatment Plant, which is located approximately 0.64 km away. Field investigations done by the Monroe County Health Department have showed that the outfalls are from sewer pipes and natural tributaries that encompass the entire length of the beach (McEntire et al., 2010). Two tributaries in particular, Camp Eastman and Sherry Swamp, contribute high densities of *E. coli* to the near shore waters of the beach, especially during heavy storm events (McEntire et al., 2010). *E. coli* densities often exceeded the 235 colonies/100 mL standard for a single sample (McEntire et al., 2010). During the 2006-2009 Operating Season, Durand Beach was closed for a total of 105 days with water clarity and percentage of bacteria listed as the main reasons for closure (McEntire et al., 2010).

Charlotte Beach lies on the south shoreline of Lake Ontario in Rochester, NY. The beach receives outfalls from both the Van Lare WWTP and the Northwest Quadrant WWTP as well as the Genesee River. The Genesee River Watershed is associated with many water quality concerns stemming from urban and industrial sources, along the northern part of the watershed, and agricultural and other nonpoint sources, along the rural areas of the watershed (NYSDEC, n.d.). Charlotte Beach is often reported as a "repeat offender" by the Natural Resource Defense Council (NRDC) for having persistent contamination problems and high bacterial counts (Dyer, 2014). Charlotte Beach has violated public health standards more than 25 percent of the time for each year spanning from 2009 to 2013 (Dyer, 2014). Decaying algae, typically *Spirogyra* and *Cladophora*, is often cited as a persistent contributor of fecal coliforms to the Charlotte Beach shoreline (Monroe County Department of Health, 2002).

Payne Beach also lies on Lake Ontario's south shoreline in Hilton, NY. The beach receives outfalls from the Northwest Quadrant Treatment Plant, which is located approximately 1.1 km away. There have been no available reports regarding contamination problems at Payne Beach.

Sampling

The Lake Ontario samples were collected in the summer (May 28, 2015), fall (September 22, 2015), and winter (March 8, 2016). The winter sample for Charlotte Beach was collected on March 29, 2016, since both the beach and lakeshore were frozen over on March 8th. The Onondaga Lake samples were collected in the summer (June 3, 2015), fall (September 20, 2015), and winter (February 28, 2016). All of the surface water samples that were used to test the growth of each isolate in their native waters were collected on August 15, 2016. The monthly precipitation rates for both the Rochester and Syracuse regions were recorded to determine if the water levels at each site were normal during sampling (Tables S-1a, S-1b).

The Onondaga Lake samples were collected approximately 4.0 km from an outfall pipe associated with the Metropolitan Syracuse WWTP (Figure 2). The samples were collected near a drainage area next to Onondaga Lake Parkway. The Payne Beach samples were collected near one of the outfalls associated with the Northwest Quadrant Treatment Plant, at the west end of the Braddock Bay Wildlife Refuge (Figures 3a, 3b). The Charlotte Beach samples were collected approximately 0.8 km from the Genesee River outfall and outfall pipes associated with the Van Lare and Northwest Quadrant WWTPs (Figures 3a, 3c). Lastly, the Durand Beach samples were collected directly across from the Van Lare WWTP on Lakeshore Boulevard (Figures 3a, 3d).

A 2 m long graduated dipper with a 500 mL cup was used to collect the samples (Figure S-1). 500 mL of sediment and 500 mL of water were collected at each location. All samples were collected approximately 0.6-0.9 m from the shoreline and 7.6-10.2 cm deep into the sediment. Samples were placed in 1 L HDPE bottles and stored in a refrigerator set at 1.7°C. The temperature, pH, nitrate, and phosphate of all of the surface water samples (August 15, 2016) were taken and recorded (Table S-2).

Enrichment Technique

The enrichment test was carried out in 250 mL culture flasks containing 37 mL of Bushnell-Haas medium (HiMedia Laboratories Pvt. Ltd. India), 4 mL of sediment sample, and a concentration of 60 µg/mL of each individual pharmaceutical. The concentration implemented was based on a previous study in which concentrations of 50 µg/mL to 4500 µg/mL of

acetaminophen were used on pure bacterial strains (Zhang et al., 2013). All flasks were incubated in a shaking incubator (120 rpm) at room temperature for 7 days. Each of the four original samples were serially diluted and plated to R2A agar (BD Difco R2A agar; Sparks, MD, USA). Plates were stored in a room temperature incubator for up to 7 days. This approach was followed for weeks 2, 3, and 4. A diagram of the approach is represented in Figure 4. At week 5, all of the different colony types were picked from the week 4 plates and streaked to R2A plates. Plates were incubated at room temperature for 2-3 days. Isolates were then parafilmed and stored in a cold room (4°C) for further use. Isolates were named with a letter and number designation, and a subscript to represent from which season it was cultured (S represents summer, F represents fall, and W represents winter). Isolates with the same letter and/or number designation from the different seasons do not represent similarity. It is important to note that 17β-estradiol was only tested on the fall and winter samples.

Growth of Isolates on Pharmaceutical Sources

Isolates were inoculated into 10 mL of nutrient broth in 125 mL flasks and incubated for 2 days in a room temperature shaking incubator (120 rpm). After 2 days, the isolates were harvested by centrifugation (15,000 rpm for 5 minutes) and re-suspended into 2 mL of Bushnell-Haas medium. 9.8 mL of Bushnell-Haas medium was added to the 125 mL flask. 0.2 mL of cells were added to the 125 mL flask with a concentration of 750 µg/mL of the selected pharmaceutical. The concentration implemented was increased after performing preliminary studies with 250 µg/mL and 500 µg/mL concentrations. A control series was run for each pharmaceutical tested. Each control consisted of 10 mL of Bushnell-Haas medium and a concentration of 750 µg/mL of the selected pharmaceutical. All cultures were incubated in a shaking incubator (120 rpm) at room temperature for 10 days. Samples were taken at 0, 2, 4, 6, 8, and 10 days. At each time interval, 0.3 mL samples were taken and the absorbance was read at 600 nm. Isolates that grew well on their respective pharmaceuticals were further tested on the other pharmaceuticals and in their natural waters. For the natural water tests, the Bushnell-Haas media was replaced with 9.8 mL of the unfiltered natural water source from which each isolate originated. 0.2 mL of cells and a concentration of 750 µg/mL of the selected pharmaceutical were added to the 125 mL natural water flask. Two control series' were tested: 1) 0.2 mL of cells

with 10 mL of the natural water in which the isolate originated, and 2) a concentration of 750 µg/mL of the selected pharmaceutical with 10 mL of the natural water in which the isolate originated. The same incubation and sampling protocol was followed as outlined.

Growth Analysis

The growth of each isolate on its respective pharmaceutical source were read at 600 nm on a UV/VIS spectrophotometer. The 0.3 mL samples were diluted with 2 mL of Bushnell-Haas media. The absorbance of each sample was multiplied by 5 to account for the dilutions.

The growth rate (k) of each isolate was determined by first calculating the generation time (G) of cells [1]. The duration period of each isolate was considered over a 2-day period of growth (N1 represents the lower absorbance, N2 represents the higher absorbance). The growth rate was then calculated using the generation time that was determined for each isolate [2].

[1] $\mathrm{G} = \frac{duration * log_{10}(2)}{log_{10}(N2) - log_{10}(N1)}$

[2] $k = \frac{0.693}{G}$

Biodegradation of Pharmaceuticals as Measured by Carbon Dioxide

Isolates were inoculated into 10 mL of nutrient broth in 125 mL flasks and incubated for 2 days in a room temperature shaking incubator (120 rpm). After 2 days, the isolates were harvested by centrifugation (15,000 rpm for 5 minutes) and re-suspended into 2 mL of Bushnell-Haas medium. 50 mL of Bushnell-Haas were added to a biometer flask (Figure 5). 1 mL of cells were added to the flasks with 7.5 mg of the selected pharmaceutical. Ascarite, which absorbs carbon dioxide, was added to the flask tower and 10 mL of Potassium hydroxide (KOH) was added to the sidearm. A control series was run for each pharmaceutical tested. Each control consisted of 51 mL of Bushnell-Haas medium and 7.5 mg of the selected pharmaceutical. All flasks were incubated in a non-shaking incubator at room temperature for 10 days. Samples of KOH were taken and carbon dioxide evolution was measured at 2, 4, 6, 8, and 10 days. At each

time interval, the KOH was withdrawn from the sidearm and transferred to a separate 125 mL flask and the sidearm was refilled with 10 mL of fresh 0.2N KOH. 1 mL of saturated barium chloride and 0.1 mL of phenolphthalein were added to the 125 mL flask. Samples were titrated with 0.05N Hydrochloric acid (HCl) until the solutions turned colorless. This was compared to the unexposed KOH sitting at room temperature. Isolates that grew well on the other pharmaceuticals and in their natural waters were further tested. For the natural water tests, the Bushnell-Haas media was replaced with 51 mL of the unfiltered natural water source from which each isolate originated. 1 mL of cells and 7.5 mg of the selected pharmaceutical were added to the biometer flask. Two control series' were tested: 1) 1 mL of cells with 51 mL of the natural water in which the isolate originated, and 2) 7.5 mg of the selected pharmaceutical with 51 mL of the natural water in which the isolate originated. The same incubation and sampling protocol was followed as outlined.

Biodegradation Analysis

The volume of HCl needed to neutralize the KOH was recorded. The volume of HCl needed to neutralize the experimental KOH was then subtracted from the volume needed to neutralize the unexposed KOH and multiplied by 25 to determine the micromoles (µmol) of carbon dioxide evolved [3]. A pharmaceutical control series and triplicates were run for each isolate tested. The average carbon dioxide evolved for each was calculated from the triplicate series'.

The percent biodegradation for each isolate was calculated using the average micromoles of carbon dioxide evolved by each isolate and each pharmaceutical control. The average carbon dioxide evolution from the pharmaceutical control series was subtracted from the average carbon dioxide evolution of each isolate series [4]. The number of micromoles evolved by each isolate was converted into mg [5]. The moles of carbon present in the selected pharmaceuticals was calculated from the molecular weight of each compound and multiplied by 7.5 mg [6]. The mg of carbon evolved by each isolate was then divided by the mg of carbon in each selected pharmaceutical and multiplied by 100 to find the percent biodegradation [7].

[3] $CO_2(\mu mol) = \left(Volume(mL)_{(unexposed)} - Volume(mL)_{(experimental)}\right) x\ 25$

[4] $\bar{x}CO_2(\mu mol) = \bar{x}CO_2(\mu mol)_{(isolate)} - \bar{x}CO_2(\mu mol)_{(pharmaceutical\ control)}$

[5] $Amount\ Carbon\ (mg)_{(isolate)} = \bar{x}CO_2(\mu mol)\ x\ \frac{10^{-6}\ mol}{\mu mol} x\ \frac{12\ g\ C}{1\ mol}\ x\ 1000\ mg\ x\ 0.27$

[6] $Amount\ Carbon\ (mg)_{(pharmaceutical)} = \frac{Amount\ Carbon\ (\frac{g}{mol})}{MW\ of\ pharmaceutical\ (\frac{g}{mol})}\ x\ 7.5\ mg$

[7] $\%\ Biodegradation = \frac{Amount\ Carbon\ (mg)_{(isolate)}}{Amount\ Carbon\ (mg)_{(pharmaceutical)}}\ x\ 100$

****It is important to note that these calculations do assume complete degradation of contaminants and do not take into account the potential metabolic products.*

Statistical Analysis

Using sampling season as the replicate (n = 3 seasons), the number of colony-forming units (cfu/mL) and colony diversity for each pharmaceutical were evaluated using one-way ANOVA with sampling site as the fixed factor. The amount of CO_2 evolved by isolates under natural conditions were evaluated using one-way ANOVA with specific measure (experimental or control test) as the fixed factor. Following these analyses, a post-hoc Tukey HSD and a Fisher LSD test were performed to identify sample means that were significantly different from one another.

Results

Cell Enrichment

The average cfu/mL and diversity of heterotrophs were determined for each sampling site. A one-way ANOVA test indicated that the average cfu/mL growing on acetaminophen significantly differed between sites ($p < 0.05$) (Table S-3b). A post-hoc Tukey HSD test determined that Durand Beach had a significantly greater number of cfu/mL growing on acetaminophen than both Payne Beach and Onondaga Lake (Table S-3a). The average number of cfu/mL from Durand Beach utilizing acetaminophen was $1.3x10^8$ after four weeks of enrichment (Table S-3a). The post-hoc test also indicated that the number of cfu/mL from Charlotte Beach growing on acetaminophen could not be significantly differentiated from the three other sites (Table S-3a). A one-way ANOVA test indicated that the average cfu/mL growing on ibuprofen also significantly differed between sites ($p < 0.05$) (Table S-3b). Although significance was determined, a post-hoc Tukey test failed to indicate which sites differed. Therefore, a Fisher LSD test was performed, and it was determined that Durand Beach had a significantly greater number of cfu/mL growing on ibuprofen than both Payne Beach and Onondaga Lake (Table S-3a). However, this test also indicated that the number of cfu/mL from Charlotte Beach growing on ibuprofen could not be differentiated from the three other sites (Table S-3a). One-way ANOVA and post-hoc tests indicated that there were no significant differences between the sites and the number of cfu/mL growing on both naproxen and 17β-estradiol ($p > 0.05$) (Table S-3a, S-3b). One-way ANOVA and post-hoc tests determined that there were no significant differences between the sampling sites and the diversity of colonies growing on the selected pharmaceuticals ($p > 0.05$) (Table S-4a, S-4b).

Growth and Biodegradation of Acetaminophen by Initial Isolates

The growth of isolates initially enriched on acetaminophen was assessed for each sampling site. There were a number of isolates from each site that exhibited no growth, long lag phases, or rapid die-off when acetaminophen was administered (Table 1). These isolates were not further studied as a result. However, a number of bacteria from each site were capable of

growing on acetaminophen. Isolate $K4_W$, cultured from Durand Beach, exhibited the highest rate of growth on acetaminophen, with a growth rate of 1.55/day (Table 1). A number of isolates cultured from Payne Beach demonstrated high rates of growth on acetaminophen (Table 1). $N1_S$ and $E1_W$ were two isolates in particular that sustained growth rates of 1.22/day (Table 1). Isolates that displayed consistent rates of growth and absorbance on acetaminophen were further studied. A growth curve of isolates utilizing acetaminophen is represented in Figure 6. In several instances, the mixture in the flask turned a dark brown color over the test period, suggesting that a number of isolates oxidized the acetaminophen when administered (Figure 7).

The rates of degradation by isolates initially enriched on acetaminophen were then assessed. There were a number of isolates from each site that displayed variable rates of growth on acetaminophen (Table 2). These isolates were not further studied because of their lack of consistency. However, there were a number of bacteria that were capable of degrading acetaminophen (Table 2). A majority of these isolates were cultured from Payne Beach. $N1_S$ and $L1_F$ were two isolates in particular that could degrade acetaminophen by approximately 40% over the 10 days (Table 2). Isolates that exhibited consistent rates of degradation were further studied. The degradation of acetaminophen by various isolates is represented in Figure 8. Isolate $N1_S$ was capable of oxidizing acetaminophen, as shown in Figure 9.

Growth and Biodegradation of Acetaminophen by Alternative Isolates

Isolates that were capable of utilizing ibuprofen, naproxen, or 17β-estradiol were also assessed on acetaminophen. Isolates $N3_F$ and $S2_F$, cultured from Payne Beach and Durand Beach respectively, were the only two alternative isolates that were capable of growing on acetaminophen (Table 1). In terms of degradation potential, isolate $N3_F$ was capable of degrading acetaminophen by approximately 28% and $S2_F$ was capable of degrading acetaminophen by approximately 29% (Table 2).

Growth and Biodegradation of Ibuprofen by Initial Isolates

The growth of isolates initially enriched on ibuprofen was assessed for each sampling site. There were a number of isolates from each site that exhibited no growth, long lag phases, or

rapid die-off when ibuprofen was administered (Table 3). These isolates were not further studied as a result. However, a number of bacteria from each site were capable of growing on ibuprofen. Isolate $L4_W$, cultured from Durand Beach, exhibited the highest rate of growth on ibuprofen, with a growth rate of 1.52/day (Table 3). A number of isolates cultured from Onondaga Lake demonstrated consistent rates of growth on ibuprofen (Table 3). $G1_F$ and $G4_F$ were two isolates in particular that sustained growth rates of 0.58/day and 0.27/day, respectively (Table 3). Isolates that displayed consistent rates of growth and absorbance on ibuprofen were further studied.

The rates of degradation by isolates initially enriched on ibuprofen were then assessed. There were a number of isolates from each site that displayed variable rates of growth on ibuprofen (Table 4). These isolates were not further studied because of their lack of consistency. However, a number of bacteria that were capable of degrading ibuprofen (Table 4). Isolate $L4_W$ was capable of degrading ibuprofen by approximately 25%, which was the highest percent degradation of ibuprofen found in this study (Table 4). Isolate $B1_W$, cultured from Charlotte Beach, degraded ibuprofen by approximately 23% (Table 4). Isolates that exhibited consistent rates of degradation on ibuprofen were further studied.

Growth and Biodegradation of Ibuprofen by Alternative Isolates

Isolates that were capable of utilizing acetaminophen, naproxen, or 17β-estradiol were also assessed on ibuprofen. A number of isolates were capable of growing on ibuprofen (Table 3). A majority of these isolates were cultured from both Payne Beach and Charlotte Beach. $D1_W$ and $B1_F$ were two isolates in particular that sustained growth rates of 0.57/day and 0.42/day, respectively (Table 3). Isolate $N2_W$, cultured from Durand Beach, had the highest rate of growth on ibuprofen, with a growth rate of 1.18/day (Table 3). The degradation of ibuprofen by alternative isolates was also assessed. $N2_W$ was capable of degrading ibuprofen by approximately 20% (Table 4). Isolate $N1_S$ had the highest degradation potential on ibuprofen, and degraded the compound by approximately 22% (Table 4).

Growth and Biodegradation of Naproxen by Initial Isolates

The growth of isolates initially enriched on naproxen was assessed for each sampling site. There were a number of isolates from each site that exhibited no growth, long lag phases, or rapid die-off when naproxen was administered (Table 5). These isolates were not further studied as a result. However, a number of bacteria from Payne Beach and Durand Beach were capable of growing on naproxen. Isolates $O2_F$ and $O3_F$, cultured from Payne Beach, exhibited the highest rates of growth on naproxen, with growth rates of 0.72/day and 0.79/day (Table 5). Isolates that displayed consistent rates of growth and absorbance on naproxen were further studied.

The rates of degradation by isolates initially enriched on naproxen were then assessed. There were a select few isolates that displayed variable rates of growth on naproxen (Table 6). These isolates were not further studied because of their lack of consistency. However, there were a number of bacteria that were capable of degrading naproxen (Table 6). Isolate $N1_W$ was capable of degrading naproxen by approximately 27%, which was the highest percent degradation of naproxen found in this study by an isolate initially enriched on the compound (Table 6). Isolates that exhibited consistent rates of degradation on naproxen were further studied.

Growth and Biodegradation of Naproxen by Alternative Isolates

Isolates that were capable of consistently utilizing acetaminophen, ibuprofen, or 17β-estradiol were also assessed on naproxen. A number of isolates were capable of growing on naproxen (Table 5). A majority of these isolates were cultured from Durand Beach. $L1_S$, $S2_F$, $L4_W$, and $N2_W$ were all isolates in particular that displayed consistent growth on naproxen over the 10-day period (Table 5). Isolate $N2_W$, cultured from Durand Beach, had the highest rate of growth on naproxen, with a growth rate of 1.09/day (Table 5). The degradation of naproxen by alternative isolates was also assessed. Isolates $L1_S$, $S2_F$, $L4_W$, and $N2_W$ were all capable of degrading naproxen (Table 6). Isolate $L4_W$ had the highest degradation potential on naproxen, and degraded the compound by approximately 35% (Table 6).

Growth and Biodegradation of 17β-estradiol by Initial Isolates

The growth of isolates initially enriched on 17β-estradiol was assessed for each sampling site. There were a number of isolates from each site that exhibited no growth, long lag phases, or rapid die-off when 17β-estradiol was administered (Table 7). These isolates were not further studied as a result. However, a number of bacteria from Durand Beach were capable of growing on 17β-estradiol. Isolates $S2_F$ and $T2_F$ were two isolates in particular that sustained growth rates of 0.32/day and 0.38/day (Table 7). Isolates that displayed consistent rates of growth and absorbance on 17β-estradiol were further studied.

The rates of degradation by isolates initially enriched on 17β-estradiol were then assessed. There were a select few of isolates that displayed variable rates of growth on 17β-estradiol (Table 8). These isolates were not further studied because of their lack of consistency. However, there were a number of bacteria that were capable of degrading 17β-estradiol (Table 8). Isolate $N2_W$, cultured from Durand Beach, was capable of degrading 17β-estradiol by approximately 27%, which was the highest percent degradation of 17β-estradiol found in this study by an isolate initially enriched on the compound (Table 8). Isolates that exhibited consistent rates of degradation on 17β-estradiol were further studied.

Growth and Biodegradation of 17β-estradiol by Alternative Isolates

Isolates that were capable of consistently utilizing acetaminophen, ibuprofen, or naproxen were also assessed on 17β-estradiol. A number of isolates were capable of growing on 17β-estradiol (Table 7). $B1_W$ and $L4_W$, cultured from Charlotte Beach and Durand Beach respectively, were two isolates in particular that displayed consistent growth on 17β-estradiol over the 10-day period (Table 7). Isolate $L4_W$ had the highest rate of growth on 17β-estradiol, with a growth rate of 0.45/day (Table 7). The degradation of 17β-estradiol by alternative isolates was also assessed. Isolates $B1_W$ and $L4_W$ were both capable of degrading 17β-estradiol by approximately 29% (Table 8).

Growth of High Capacity Isolates in Natural Water Systems

The growth of high capacity isolates was tested on pharmaceuticals in their natural waters. High capacity isolates were those that were able to grow on and degrade more than one compound. There were a number of isolates from each site that exhibited no growth, long lag phases, or rapid die-off when the selected pharmaceuticals were administered in their natural waters (Table 9). These isolates were not further studied as a result. No isolates cultured from Onondaga Lake were capable of utilizing pharmaceuticals in their natural waters. However, a number of bacteria from the three other sites were capable of growing on the compounds (Table 9). $S2_F$, cultured from Durand Beach, was one isolate in particular that was capable of growing on both acetaminophen and ibuprofen in its natural water (Table 9). The isolate also exhibited higher overall growth rates than those of the tested controls (Table 9). Isolate $E1_W$, cultured from Payne Beach, sustained a growth rate of 0.80/day on acetaminophen in its natural water (Table 9). However, isolate $E1_W$ was unable to grow on 17β-estradiol in its natural water (Table 9). In fact, no isolate was capable of growing on 17β-estradiol in its natural water.

Biodegradation of Pharmaceuticals by High Capacity Isolates in Natural Water

Following growth analysis, the degradation of pharmaceuticals by high capacity isolates in natural waters was assessed. The degradation of naproxen by isolate $L1_S$ and its associated controls is represented in Figure 11. A majority of isolates tested were incapable of degrading the selected pharmaceuticals to significant levels ($p > 0.05$) (Table 10, Figure 10). However, there were a select few that were capable of degrading the selected pharmaceuticals to significant levels ($p < 0.05$) (Table 10, Figure 10). $B1_W$, $E1_W$, and $N2_W$ were three isolates in particular (Table 10, Figure 10). However, the mean micromoles of CO_2 evolved by these isolates on their selected pharmaceuticals only significantly differed from one of the tested controls (Figure 10). That being said, the total degradation of naproxen by isolate $B1_W$ was significantly different from naproxen in Durand Beach water solely, but was similar to $B1_W$ in Durand Beach water solely (Figure 10). The total degradation of acetaminophen by isolate $E1_W$ was significantly different from acetaminophen in Payne Beach water solely, but was similar to $E1_W$ in Payne Beach water solely (Figure 10). Lastly, the total degradation of ibuprofen by $N2_W$ was

significantly different from $N2_W$ in Durand Beach water solely, but was similar to ibuprofen in Durand Beach water solely (Figure 10).

Discussion

Enrichment of Bacteria from Natural Environments

The removal of emerging contaminants from surface waters is of great practical significance. In this study, microorganisms capable of using various pharmaceuticals as their sole carbon sources were readily isolated from lake sediments associated with wastewater effluent discharges. This was of no surprise given that pharmaceuticals are increasingly being detected in surface waters at higher rates. The enrichment studies of this experiment illustrate that all four sampling sites are able to sustain large bacterial communities as indicated by the average cfu/mL counts (Tables S-3a), consisting of a mix of gram negative and gram positive bacteria (Table 11, Figure 12, Figure S-2(a-c)). As a result, a variety of bacteria were assessed on the selected pharmaceuticals during this study.

By sampling different seasons, it was revealed that the number of colony-forming units and the overall diversity of the colonies growing on the selected pharmaceuticals did not significantly differ between seasons ($p > 0.05$). This suggests that the number and diversity of bacteria in the four sampling sites are consistent over the three seasons. However, by sampling different sites, it was revealed that the number of cfu/mL did significantly change between sites, depending on the pharmaceutical being tested ($p < 0.05$) (Table S-3a, S-3b). That being said, the number of cfu/mL on both acetaminophen and ibuprofen individually, were found to be significantly different at Durand Beach as compared to Onondaga Lake and Payne Beach (Table S-3a). This data suggests that there may be more acetaminophen and ibuprofen being released into Durand Beach than both Onondaga Lake and Payne Beach. Durand Beach receives several outfalls along its shoreline, including those from sewer pipes and the Van Lare WWTP, which may be leading to an increased release of both acetaminophen and ibuprofen. This may be causing a significant increase in the number of bacteria present at Durand Beach capable of utilizing these two compounds. However, it was revealed that the diversity of bacteria did not change between sites ($p > 0.05$) (Table S-4a, S-4b). This suggests that there are similar types of bacteria present at each of the four locations, which was of no surprise given that three of the four sampling locations lie on Lake Ontario's south shoreline.

Growth of Isolates on Selected Pharmaceuticals

Following the enrichment tests, the growth of isolated bacteria on pharmaceuticals was assessed. This was a way to eliminate isolates that demonstrated little potential of utilizing the pharmaceuticals as their sole carbon sources. Several isolates, both those that were initially enriched on specific pharmaceuticals or those that were tested on alternative pharmaceuticals, were unable to sustain growth on administered compounds. These isolates may have been able to survive the enrichment technique, in which a concentration of 60 µg/mL of pharmaceuticals were used, but were unable to survive during the growth tests, in which higher concentrations of pharmaceuticals were implemented. Overall, the growth experiments in this study revealed a variety of isolates from the four sampling sites that were capable of growing on the selected pharmaceuticals under both laboratory conditions and more natural conditions. This was of no surprise given the increased prevalence of pharmaceuticals in surface waters and the malleability of microbes. As a result, the isolates that were capable of growing on specific pharmaceuticals were assessed and their overall ability to remove the compounds was determined.

Biodegradation of Selected Pharmaceuticals Under Laboratory Conditions

It has been suggested that the biodegradability toward certain compounds could be a result of the adaptation of microbial communities to chemical contamination in the past or present (Nishihara et al., 1997). Generally speaking, the release of pharmaceuticals in effluents may play a factor in the degradation capability of bacteria present at specific locations. In this part of the study, the degradation of pharmaceuticals by isolates from each site were assessed under laboratory conditions. A large number of bacteria from all four sampling sites were capable of degrading the selected compounds.

Durand Beach sustained a large number of bacteria that were capable of degrading the pharmaceuticals tested. This suggests that the release of pharmaceuticals into the environment may be capable of inducing a high number of pharmaceutical-degrading bacteria. Isolate $L4_W$ was capable of degrading 17β-estradiol by 29%, ibuprofen by 25%, and naproxen by 35% and isolate $N2_W$ was capable of degrading ibuprofen by 20%, 17β-estradiol by 27%, and naproxen by 26%. Payne Beach sustained a large number of bacteria that were capable of degrading

acetaminophen. Both isolates $N1_S$ and $L1_F$ were capable of degrading acetaminophen by approximately 40% whereas $N3_F$ was capable of degrading acetaminophen by approximately 28%. Isolate $B1_W$, cultured from Charlotte Beach, was capable of degrading ibuprofen by 23%, naproxen by 32%, and 17β-estradiol by 29%. Onondaga Lake did not sustain as many isolates that were capable of degrading the pharmaceuticals; however, $G1_F$ was capable of degrading ibuprofen by 20% and $G4_F$ was capable of degrading both ibuprofen and naproxen by 18% and 28%, respectively.

The results of these experiments indicate that pharmaceutical-degrading bacteria are present in lake sediments, and can be used to degrade pharmaceuticals under laboratory conditions. However, testing their capability to utilize pharmaceuticals under more realistic conditions is practical to determine how they are playing a role in the removal of these compounds in the environment.

Biodegradation of Selected Pharmaceuticals Under More Realistic Conditions

In order to translate the laboratory results into more realistic results, the degradation of selected pharmaceuticals under more natural conditions was assessed. To date, there are a lack of studies that incorporate laboratory-scale biodegradation tests with natural water (Yamamoto et al., 2009). In this part of the study, the only bacteria assessed were those that showed consistent degradation rates on pharmaceuticals and versatility under laboratory conditions. Although a number of isolates were capable of degrading selected compounds under laboratory conditions, the overall removal efficiencies of compounds by the majority of isolates tested under natural conditions were not significant in relation to the controls ($p > 0.05$) (Table 10, Figure 10). This suggests that certain types of bacteria may not be as adaptive in utilizing pharmaceuticals in natural sources and are finding easier ways to obtain carbon. However, three isolates were able to significantly degrade the selected compounds ($p < 0.05$) (Table 10, Figure 10). However, these results also brought about skepticism. Although isolate $N2_W$ evolved significantly more micromoles of CO_2 on ibuprofen than the $N2_W$ and water control, $N2_W$ did not evolve significantly more CO_2 on ibuprofen than the ibuprofen and water control (Figure 10). This suggests that the activity of $N2_W$ may have been enhanced when ibuprofen was administered, or that there are other bacteria in the water that are compensating for the ibuprofen release. More

studies would have to be implemented in order to determine the exact result. In relation to this, although isolate $E1_W$ evolved significantly more micromoles of CO_2 on acetaminophen than the acetaminophen and water control, $E1_W$ did not evolve significantly more CO_2 on acetaminophen than the $E1_W$ and water control (Figure 10). However, that being said, it is likely that isolate $E1_W$ does have the capability of removing acetaminophen in its natural waters because the acetaminophen and $E1_W$ mixture turned a dark brown color during the experimental test whereas the $E1_W$ and water control did not. These results indicate that $E1_W$ has the capability of oxidizing acetaminophen as well as utilizing other carbon sources when acetaminophen is not present. Lastly, although isolate $B1_W$ evolved significantly more micromoles of CO_2 on naproxen than the naproxen and water control, $B1_W$ did not evolve significantly more CO_2 on naproxen than the $B1_W$ and water control (Figure 10). This suggests that $B1_W$ may have the capability of utilizing naproxen as well as other carbon sources when in its natural environment; however, more studies would have to be implemented in order to determine the exact result.

Durand Beach, Payne Beach, and Charlotte Beach likely have bacteria present that are helping to compensate for the release of pharmaceuticals into their environments. The release of pharmaceuticals into these environments may be influencing the community structure of organisms that live on these particular contaminants. Given that the number of colonies growing on ibuprofen was significant at Durand Beach, it can be assumed that a significant amount of ibuprofen is being released into the environment along Durand's shoreline. The increased load of ibuprofen may be selecting for certain types of bacterial communities. Isolate $N2_W$ was one isolate in particular that was capable of utilizing ibuprofen under natural conditions. This suggests that isolate $N2_W$ may be aiding in the removal of ibuprofen from Durand Beach. Isolate $B1_W$ may be a promising for Charlotte Beach as it is capable of degrading naproxen to lower loads than applied. The Genesee River drains into Charlotte Beach, which may be releasing pharmaceutical residues, especially naproxen, into the beach area. No isolates from Onondaga Lake were capable of degrading selected pharmaceuticals under natural conditions. This may be due to a variety of environmental factors or to the location in which the water was sampled. The history of Onondaga Lake may also explain the differences, since the chemistry of the lake may not have yet bounced back. Lastly, 17β-estradiol was not utilized by any of the bacterial isolates during the implemented water tests, which may be due to its complexity as a compound. It is also

important to note that all of the selected compounds may change composition in natural habits and/or form microbial byproducts, which are often harder for bacteria to utilize.

Overall, the degradations of ibuprofen, naproxen, and acetaminophen were lower than those found under laboratory conditions. This was expected and likely due to the introduction of environmental factors that were essentially void under the laboratory tests. In natural environments, bacteria are often limited by the extremes of pH and temperature, the lack of nutrients, and the toxicity of some compounds (USGS, 2007). pH variations in natural systems can significantly impact the activity of bacteria. Therefore, the environmental characteristics of the sampled water were evaluated to see if the water's conditions may have played a factor on the behavior of isolated bacteria in this study. The pH of each sampling site was between 8.25 and 8.61 (Table S-2). Onondaga Lake and Charlotte Beach both had pH's of 8.61, which may have been due to runoff and/or discharges into the edges of these surface waters. Considering a bacterium from Charlotte Beach ($B1_W$) was able to degrade naproxen to lower levels than applied, it is predicted that the bacteria present at this site may be adapted to higher pH levels. However, a bacterium from Onondaga Lake ($G4_F$) was incapable of utilizing both ibuprofen and naproxen in water, which may be a result of the pH level. As for temperature, seasonal variations can often influence the nature of microbial communities. It has been suggested that the ideal temperatures for bacterial activity are in the range of 25°C to 35°C (Metcalf and Eddy, 2003). In this study, the temperatures of all four sampling sites were within the optimum temperature range of bacterial activity. Lastly, both nitrate and phosphate levels were measured to be low in these systems, which should not have had a significant impact on microbial activity.

Characteristics of Promising Isolates

A handful of bacteria from previous studies have been identified that have the capability of degrading a variety of pharmaceuticals. In fact, several sphingomonads have been found to degrade xenobiotics including ibuprofen (Murdoch and Hay, 2005) and 17β-estradiol (Kurisu et al., 2010; Yu et al., 2007). Sphingomonas sp and Sphingobium sp are gram negative rods. In this study, several bacteria from each site capable of degrading ibuprofen were identified as gram negative rods, including $J2_S$, $B1_F$ (Figure 12), and $B1_W$. Along with sphingomonads, several other species have been isolated from effluents that can degrade 17β-estradiol, including Isoptericola

sp (gram positive, rods or cocci), Nubsella sp (yellow-pigmented, gram negative, rods), and Rhodococcus sp (gram positive, rods) (Zhou et al. 2013), and Acinetobacter sp (gram negative, bacilli) (Pauwels et al., 2008). Most of the bacteria capable of degrading 17β-estradiol in this study were gram negative rods. One isolate in particular was $S2_F$ (Figure S-2b), which was a yellow-pigmented, gram negative rod. Based off of these characteristics, $S2_F$ is likely to be a Nubsella sp. Sphingomonas sp have also been shown to degrade naproxen (Zhou et al., 2013). In this study, a mix of gram negative and gram positive bacteria were found to degrade naproxen. For example, $N3_F$, $B1_W$, and $L4_W$ were all examples of gram negative rods that were capable of degrading naproxen to various levels and $N1_W$ was a gram positive rod. Lastly, some pseudomonas sp (gram negative, rods) have the capability of degrading acetaminophen (Hu et al., 2013). Again, a mix of gram negative and gram positive bacteria were found that were capable of degrading acetaminophen. The top degraders of acetaminophen were a gram positive cocci ($N1_S$, (Figure 12)) and gram negative rods ($L1_F$ and $E1_W$).

Conclusion

Pharmaceuticals and their metabolites are increasingly being detected in the environment since the common WWTP is insufficient in completely eliminating these drugs. It has been suggested that the rate at which pharmaceuticals are removed from the environment depends on the presence of natural microbial populations able to degrade them (Caracciolo et al., 2015). In fact, repeated exposure of microbial populations to such compounds may enhance the activity of microbes and reduce the persistence of compounds (Caracciolo et al., 2015). Our results demonstrate that under laboratory conditions, there are a variety of bacteria that are capable of utilizing ibuprofen, naproxen, acetaminophen, and 17β-estradiol as their sole carbon sources; thus, reducing these compounds to lower levels than administered. Under more realistic conditions, a select number of bacteria were also capable of degrading ibuprofen, naproxen, and acetaminophen in their natural environments. This suggests that the release of pharmaceuticals into the sampling sites may be influencing the community structure of organisms that live on these particular contaminants. Thus, going forward, a variety of studies could be implemented to better understand the environmental fate of emergent compounds and their overall risks to the environment. Determining the metabolic products of parent compounds is important, since metabolites could be more persistent and present in concentrations even higher than their parent compounds (Radjenović et al., 2008). Many pharmaceuticals are present in the environment simultaneously as well (Nikolaou et al., 2007); therefore, studies should also be implemented to determine how microbial communities may manage a mixture of such compounds. That being said, a consortium of bacterial isolates should be tested with pharmaceuticals to determine if cooperative mechanisms are involved in the degradation of these products, and if byproducts are produced in return. Molecular analysis of isolated strains should also be determined to identify which bacteria may be induced by pharmaceutical presence in the environment.

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Tables

Table 1. Growth of isolates on acetaminophen. The final absorbance and growth rate were determined at the 10-day mark. The final absorbance was taken at 600 nm. The growth rate represents days^{-1}. Not determined (N/D) = No growth and/or immediate die-off, † = Longer than 4-day lag phase, ‡ = Growth, but die-off period(s), * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Isolate Origin	Final Absorbance	Growth Rate
Summer			
Control		0.15	N/D
$^{*}B1_S$	Charlotte	0.055	N/D
$^{*}B2_S$	Charlotte	0.235	0.18†
$^{*}C1_S$	Charlotte	0.85	0.24†
$^{*}F1_S$	Onondaga	1.49	1.36
$^{*}F2_S$	Onondaga	0.115	N/D
$^{*}G1_S$	Onondaga	5.28	1.17
$^{*}J1_S$	Durand	5.78	0.91
$^{*}N1_S$	Payne	1.6	1.22
$^{*}N2_S$	Payne	0.07	N/D
$^{*}O1_S$	Payne	3.97	0.98
$^{*}O2_S$	Payne	1.28	0.45
$^{*}R2_S$	Onondaga	1.17	0.64
$^{*}S2_S$	Charlotte	0.815	0.34
$J2_S$	Durand	0.13	N/D
$L1_S$	Durand	0.26	0.21‡
$L2_S$	Payne	0.98	0.17†
Fall			
Control		0.025	N/D
$^{*}A1_F$	Charlotte	5.855	0.97
$^{*}A3_F$	Charlotte	8.44	1.21
$^{*}A4_F$	Charlotte	2.41	0.65‡
$^{*}B1_F$	Charlotte	5.235	1.18
$^{*}E2_F$	Onondaga	0.29	0.19†
$^{*}F2_F$	Onondaga	0.27	0.26
$^{*}I1_F$	Durand	0.08	N/D
$^{*}I2_F$	Durand	0.16	0.13†
$^{*}L1_F$	Payne	6.545	0.72
$^{*}L3_F$	Payne	0.62	N/D
$^{*}M1_F$	Payne	0.3	0.39†
$^{*}M2_F$	Payne	6.69	0.87
$G1_F$	Onondaga	0.25	0.61†
$G4_F$	Onondaga	0.32	0.39†
$N3_F$	Payne	4.5	1.16
$S2_F$	Durand	1.82	1.03

Table 1. (Continued)

Winter			
Control		0.055	N/D
$^{*}A1_W$	Charlotte	0.44	0.44†
$^{*}A2_W$	Charlotte	0.225	0.63†
$^{*}D4_W$	Payne	0.2	0.54†
$^{*}E1_W$	Payne	5.92	1.16
$^{*}E2_W$	Payne	0.87	0.93‡
$^{*}E3_W$	Payne	1.47	1.22
$^{*}G3_W$	Onondaga	0.04	N/D
$^{*}G4_W$	Onondaga	0.03	N/D
$^{*}K3_W$	Durand	0.11	0.65‡
$^{*}K4_W$	Durand	6.24	1.55
$B1_W$	Charlotte	0.055	0.35‡
$D1_W$	Charlotte	0.2	0.56‡
$L4_W$	Durand	0.46	0.40‡
$N1_W$	Durand	0.235	0.65‡
$N2_W$	Durand	0.08	1.05‡

Table 2. Biodegradation of acetaminophen by isolates. The total micromoles and total degradation were determined at the 10-day mark. * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Total Micromoles	Total Degradation
Summer		
*$F1_S$	455.0 ± 12.6	30.9% ± 0.9%
*$G1_S$	534.0 ± 109.2	36.3% ± 7.4%
*$J1_S$	460.9 ± 128.7	31.3% ± 8.8%
*$N1_S$	587.5 ± 24.6	40.0% ± 1.7%
*$O1_S$	466.7 ± 52.1	31.7% ± 3.6%
*$O2_S$	513.4 ± 4.4	34.9% ± 0.3%
*$R2_S$	464.2 ± 31.7	31.6% ± 2.2%
*$S2_S$	511.7 ± 25.1	34.8% ± 1.7%
Fall		
*$A1_F$	330.9 ± 210.5	22.5% ± 14.3%
*$A3_F$	421.7 ± 33.6	28.7% ± 2.3%
*$B1_F$	550.0 ± 35.5	37.3% ± 2.4%
*$L1_F$	590.0 ± 56.3	40.1% ± 3.9%
*$M2_F$	504.2 ± 58.7	34.3% ± 4.0%
$N3_F$	407.5 ± 33.0	27.7% ± 2.3%
$S2_F$	424.2 ± 45.1	28.9% ± 3.1%
Winter		
*$E1_W$	486.7 ± 77.2	33.1% ± 5.3%
*$E3_W$	481.7 ± 79.1	32.8% ± 5.4%
*$K4_W$	430.9 ± 80.9	29.3% ± 5.5%

Table 3. Growth of isolates on ibuprofen. The final absorbance and growth rate were determined at the 10-day mark. The final absorbance was taken at 600 nm. The growth rate represents days^{-1}. Not determined (N/D) = No growth and/or immediate die-off, † = Longer than 4-day lag phase, ‡ = Growth, but die-off period(s), * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Isolate Origin	Final Absorbance	Growth Rate
Summer			
Control		0.005	N/D
$^{*}H1_S$	Onondaga	0.235	N/D
$^{*}H2_S$	Onondaga	0.365	0.07
$^{*}J2_S$	Durand	0.22	0.33
$^{*}L1_S$	Durand	0.35	0.17
$^{*}P1_S$	Payne	0.275	N/D
$^{*}P2_S$	Payne	0.22	0.08
$^{*}Q1_S$	Payne	0.39	0.10†
$^{*}U1_S$	Charlotte	0.21	0.18†
$L2_S$	Payne	0.86	0.19
$N1_S$	Payne	1.005	0.11
$O2_S$	Payne	0.485	0.06
$R2_S$	Onondaga	0.685	N/D
$S2_S$	Charlotte	0.52	0.37
Fall			
Control		0.005	N/D
$^{*}B3_F$	Charlotte	0.24	0.68
$^{*}B4_F$	Charlotte	0.13	0.29†
$^{*}C1_F$	Charlotte	0.36	N/D
$^{*}C2_F$	Charlotte	0.105	0.25‡
$^{*}C3_F$	Charlotte	0.17	0.09
$^{*}G1_F$	Onondaga	0.32	0.58
$^{*}G4_F$	Onondaga	0.53	0.27
$^{*}J1_F$	Durand	0.42	0.14
$^{*}J2_F$	Durand	0.26	0.57
$^{*}M3_F$	Payne	0.295	0.28‡
$^{*}N1_F$	Payne	0.19	N/D
$B1_F$	Charlotte	0.265	0.42
$L1_F$	Payne	0.335	0.39
$M2_F$	Payne	0.28	0.19†
$N3_F$	Payne	0.195	N/D
$S2_F$	Durand	0.605	0.36

Table 3. (Continued)

Winter			
Control		0.005	N/D
$^{*}A4_W$	Charlotte	0.14	N/D
$^{*}B1_W$	Charlotte	0.13	0.24
$^{*}B2_W$	Charlotte	0.205	0.24‡
$^{*}E4_W$	Payne	0.005	N/D
$^{*}F2_W$	Payne	0.14	0.9
$^{*}H2_W$	Onondaga	0.11	1.15
$^{*}H3_W$	Onondaga	0.14	N/D
$^{*}L2_W$	Durand	0.04	0.51‡
$^{*}L3_W$	Durand	0.1	0.19†
$^{*}L4_W$	Durand	0.1	1.52
$D1_W$	Charlotte	0.21	0.57
$E1_W$	Payne	0.015	N/D
$E3_W$	Payne	0.1	N/D
$N1_W$	Durand	0.09	0.64‡
$N2_W$	Durand	0.15	1.18

Table 4. Biodegradation of ibuprofen by isolates. The total micromoles and total degradation were determined at the 10-day mark. * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Total Micromoles	Total Degradation
Summer		
$^{*}J2_S$	357.5 ± 9.5	20.4% ± 0.6%
$^{*}L1_S$	351.7 ± 13.3	20.1% ± 1.5%
$L2_S$	176.7 ± 17.5	10.1% ± 1.0%
$N1_S$	385.9 ± 44.9	22.0% ± 2.6%
$S2_S$	373.4 ± 43.1	21.3% ± 2.5%
Fall		
$^{*}B3_F$	286.7 ± 6.6	16.4% ± 0.4%
$^{*}G1_F$	353.4 ± 33.3	20.2% ± 1.9%
$^{*}G4_F$	314.2 ± 15.0	18.0% ± 0.9%
$^{*}J2_F$	277.5 ± 14.5	15.9% ± 0.8%
$B1_F$	266.7 ± 75.5	15.2% ± 4.3%
$L1_F$	406.7 ± 99.0	23.2% ± 5.7%
$S2_F$	334.2 ± 5.0	19.1% ± 0.3%
Winter		
$^{*}B1_W$	409.2 ± 60.7	23.4% ± 3.5%
$^{*}H2_W$	301.7 ± 26.5	17.2% ± 1.5%
$^{*}L4_W$	440.0 ± 49.7	25.1% ± 2.9%
$D1_W$	330.8 ± 7.7	18.9% ± 0.5%
$N2_W$	345.9 ± 18.8	19.8% ± 1.1%

Table 5. Growth of isolates on naproxen. The final absorbance and growth rate were determined at the 10-day mark. The final absorbance was taken at 600 nm. The growth rate represents days^{-1}. Not determined (N/D) = No growth and/or immediate die-off, † = Longer than 4-day lag phase, ‡ = Growth, but die-off period(s), * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Isolate Origin	Final Absorbance	Growth Rate
Summer			
Control		0	N/D
*$D1_S$	Charlotte	0.535	N/D
*$D2_S$	Charlotte	0.18	N/D
*$L2_S$	Payne	0.9	0.17
$J2_S$	Durand	0.027	N/D
$L1_S$	Durand	0.325	0.21
$N1_S$	Payne	0.61	N/D
$O2_S$	Payne	0.44	0.05†
$R2_S$	Onondaga	0.495	N/D
$S2_S$	Charlotte	0.255	N/D
Fall			
Control		0.005	N/D
*$D4_F$	Charlotte	0.105	N/D
*$H3_F$	Onondaga	0.305	0.25‡
*$H4_F$	Onondaga	0.535	0.07
*$K3_F$	Durand	0.51	0.16
*$K4_F$	Durand	0.555	0.11
*$N3_F$	Payne	0.33	0.45
*$N4_F$	Payne	0.15	0.23†
*$O1_F$	Payne	0.12	N/D
*$O2_F$	Payne	0.28	0.72
*$O3_F$	Payne	0.255	0.79
*$P1_F$	Payne	0.36	0.09
$B1_F$	Charlotte	0.275	0.25†
$G1_F$	Onondaga	0.26	0.58
$G4_F$	Onondaga	0.38	0.49
$L1_F$	Payne	0.315	0.38†
$M2_F$	Payne	0.37	0.13‡
$S2_F$	Durand	0.475	0.17
Winter			
Control		0.005	N/D
*$B3_W$	Charlotte	0.01	0.27‡
*$B4_W$	Charlotte	0.2	N/D
*$F4_W$	Payne	0.17	0.08‡
*$F3_W$	Payne	0.12	0.14‡

Table 5. (Continued)

*$I2_W$	Onondaga	0.05	0.05‡
*$M3_W$	Durand	0.025	N/D
*$M4_W$	Durand	0.02	0.24‡
*$N1_W$	Durand	0.19	0.16
$B1_W$	Charlotte	0.355	0.44
$D1_W$	Charlotte	0.075	N/D
$E1_W$	Payne	0.03	N/D
$E3_W$	Payne	0.16	0.32‡
$L4_W$	Durand	0.33	0.49
$N2_W$	Durand	0.145	1.09

Table 6. Biodegradation of naproxen by isolates. The total micromoles and total degradation were determined at the 10-day mark. * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Total Micromoles	Total Degradation
Summer		
$^{*}L2_{S}$	331.7 ± 10.4	19.6% ± 0.6%
$L1_{S}$	421.7 ± 20.8	25.5% ± 1.3%
Fall		
$^{*}N3_{F}$	336.7 ± 7.2	19.9% ± 0.5%
$^{*}O2_{F}$	392.5 ± 79.0	23.2% ± 4.7%
$^{*}O3_{F}$	380.0 ± 28.9	22.5% ± 1.7%
$G1_{F}$	304.2 ± 56.8	18.0% ± 3.4%
$G4_{F}$	477.5 ± 5.0	28.3% ± 0.3%
$S2_{F}$	426.7 ± 33.0	25.3% ± 2.0%
Winter		
$^{*}N1_{W}$	449.2 ± 7.7	26.6% ± 0.5%
$B1_{W}$	544.2 ± 42.0	32.2% ± 2.5%
$L4_{W}$	589.2 ± 25.3	34.9% ± 1.5%
$N2_{W}$	439.2 ± 40.5	26.0% ± 2.4%

Table 7. Growth of isolates on 17β-estradiol. The final absorbance and growth rate were determined at the 10-day mark. The final absorbance was taken at 600 nm. The growth rate represents days^{-1}. Not determined (N/D) = No growth and/or immediate die-off, † = Longer than 4-day lag phase, ‡ = Growth, but die-off period(s), * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Isolate Origin	Final Absorbance	Growth Rate
Fall			
Control		0.075	N/D
$^{*}P2_F$	Charlotte	0.22	0.22†
$^{*}Q1_F$	Charlotte	0.3	0.17‡
$^{*}Q2_F$	Onondaga	0.335	0.06
$^{*}R1_F$	Payne	0.075	N/D
$^{*}R2_F$	Payne	0.22	N/D
$^{*}S1_F$	Payne	0.105	0.08
$^{*}S2_F$	Durand	0.765	0.32
$^{*}T1_F$	Durand	0.26	N/D
$^{*}T2_F$	Durand	0.28	0.38
$^{*}U1_F$	Durand	0.345	0.21
$B1_F$	Charlotte	0.28	0.38
$G1_F$	Onondaga	0.29	0.51‡
$G4_F$	Onondaga	0.12	0.28‡
$L1_F$	Payne	0.355	0.40
$M2_F$	Payne	0.295	0.24
$N3_F$	Payne	0.075	0.25†
Winter			
Control		0.075	N/D
$^{*}D1_W$	Charlotte	0.3	0.33
$^{*}D2_W$	Charlotte	0.25	0.05
$^{*}G1_W$	Payne	0.2	N/D
$^{*}G2_W$	Payne	0.32	N/D
$^{*}J3_W$	Onondaga	0.1	0.10‡
$^{*}N2_W$	Durand	0.35	0.24
$^{*}N3_W$	Durand	0.51	0.12‡
$^{*}O1_W$	Durand	0.01	N/D
$B1_W$	Charlotte	0.335	0.27
$E1_W$	Payne	0.255	0.38
$E3_W$	Payne	0.22	0.44‡
$L4_W$	Durand	0.35	0.45
$N1_W$	Durand	0.18	0.35‡

Table 8. Biodegradation of 17β-estradiol by isolates. The total micromoles and total degradation were determined at the 10-day mark. * = Initial pharmaceutical particular isolates were enriched on. The isolates in gray were chosen for further testing.

Isolate	Total Micromoles	Total Degradation
Fall		
$^{*}S2_{F}$	435.0 ± 33.1	23.7% ± 1.8%
$^{*}T2_{F}$	469.2 ± 134.0	25.6% ± 7.3%
$^{*}U1_{F}$	425.0 ± 7.5	23.2% ± 0.4%
$B1_{F}$	400.9 ± 38.3	21.8% ± 2.1%
$L1_{F}$	341.7 ± 96.3	18.6% ± 5.3%
$M2_{F}$	292.5 ± 85.3	15.9% ± 4.7%
Winter		
$^{*}D1_{W}$	450.0 ± 50.2	24.5% ± 2.8%
$^{*}N2_{W}$	496.7 ± 51.3	27.1% ± 2.8%
$B1_{W}$	534.2 ± 34.7	29.1% ± 1.9%
$E1_{W}$	501.7 ± 38.9	27.3% ± 2.1%
$L4_{W}$	535.9 ± 43.1	29.2% ± 2.4%

Table 9. Growth of high capacity isolates on contaminants in natural water sources. The final absorbance and growth rate were determined at the 10-day mark. The final absorbance was taken at 600 nm. The growth rate represents days^{-1}. Not determined (N/D) = No growth and/or immediate die-off, † = Longer than 4-day lag phase, ‡ = Growth, but die-off period(s). The isolates in gray were chosen for further testing.

Isolate & Source on Waters	Final Absorbance	Growth Rate
$L1_S$		
$L1_S$ Durand	0.15	0.07‡
Durand Ibuprofen	0.009	N/D
$L1_S$ Durand Ibuprofen	0.06	N/D
Durand Naproxen	0.002	0.13†
$L1_S$ Durand Naproxen	0.22	0.09
$N1_S$		
$N1_S$ Payne	0.1	0.11†
Payne Acetaminophen	1.61	0.78†
$N1_S$ Payne Acetaminophen	1.48	0.78
Payne Ibuprofen	0.085	1.04‡
$N1_S$ Payne Ibuprofen	0.04	N/D
$S2_S$		
$S2_S$ Charlotte	0.09	0.17‡
Charlotte Acetaminophen	1.25	0.45†
$S2_S$ Charlotte Acetaminophen	0.98	0.46
Charlotte Ibuprofen	0.05	N/D
$S2_S$ Charlotte Ibuprofen	0.26	0.15
$B1_F$		
$B1_F$ Charlotte	0.03	N/D
Charlotte Acetaminophen	3.65	0.99
$B1_F$ Charlotte Acetaminophen	5.255	1.23
Charlotte 17β-Estradiol	0.08	N/D
$B1_F$ Charlotte 17β-Estradiol	0.05	N/D
$G4_F$		
$G4_F$ Onondaga	0.03	N/D
Onondaga Ibuprofen	0.02	1.02‡
$G4_F$ Onondaga Ibuprofen	0.075	0.12‡
Onondaga Naproxen	0.06	N/D
$G4_F$ Onondaga Naproxen	0.05	N/D
$N3_F$		
$N3_F$ Payne	0.02	N/D
Payne Acetaminophen	0.085	0.61†
$N3_F$ Payne Acetaminophen	3.725	0.82
Payne Naproxen	0.03	N/D
$N3_F$ Payne Naproxen	0.13	N/D

Table 9. (Continued)

$S2_F$		
$S2_F$ Durand	0.005	N/D
Durand Acetaminophen	1.865	0.46†
$S2_F$ Durand Acetaminophen	3.295	1.80
Durand Ibuprofen	0.025	N/D
$S2_F$ Durand Ibuprofen	0.14	0.28
Durand Naproxen	0.025	N/D
$S2_F$ Durand Naproxen	0.02	N/D
Durand 17β-Estradiol	0.055	N/D
$S2_F$ Durand 17β-Estradiol	0.015	N/D
$B1_W$		
$B1_W$ Charlotte	0.025	N/D
Charlotte Ibuprofen	0.035	0.46
$B1_W$ Charlotte Ibuprofen	0.035	0.12‡
Charlotte Naproxen	0.16	0.03
$B1_W$ Charlotte Naproxen	0.235	0.06
Charlotte 17β-Estradiol	0.08	N/D
$B1_W$ Charlotte 17β-Estradiol	0.09	0.16‡
$D1_W$		
$D1_W$ Charlotte	0.03	N/D
Charlotte Ibuprofen	0.035	0.46
$D1_W$ Charlotte Ibuprofen	0.075	N/D
Charlotte 17β-Estradiol	0.08	N/D
$D1_W$ Charlotte 17β-Estradiol	0.055	0.13‡
$E1_W$		
$E1_W$ Payne	0.03	N/D
Payne Acetaminophen	0.085	N/D
$E1_W$ Payne Acetaminophen	1.595	0.80
Payne 17β-Estradiol	0.06	N/D
$E1_W$ Payne 17β-Estradiol	0.07	N/D
$L4_W$		
$L4_W$ Durand	0.03	N/D
Durand Ibuprofen	0.025	N/D
$L4_W$ Durand Ibuprofen	0.095	N/D
Durand Naproxen	0.025	N/D
$L4_W$ Durand Naproxen	0.035	N/D
Durand 17β-Estradiol	0.055	N/D
$L4_W$ Durand 17β-Estradiol	0.025	N/D

Table 9. (Continued)

N2w		
N2W Durand	0.03	N/D
Durand Ibuprofen	0.025	0.08‡
N2W Durand Ibuprofen	0.13	0.05
Durand Naproxen	0.025	N/D
N2W Durand Naproxen	0.16	0.10
Durand 17β-Estradiol	0.055	N/D
N2W Durand 17β-Estradiol	0.055	N/D

Table 10. One-way ANOVA comparing the biodegradation of pharmaceuticals by isolates in natural water ($p \leq 0.05$). Significant isolates are highlighted in gray.

Isolate	Source	Site	P-value	F-value	Total df
$N1_S$	Acetaminophen	Payne	0.152	3.77	8
$S2_S$	Acetaminophen	Charlotte	0.736	0.34	8
$N3_F$	Acetaminophen	Payne	0.384	1.34	8
$B1_F$	Acetaminophen	Charlotte	0.506	0.86	8
$S2_F$	Acetaminophen	Durand	0.274	2.05	8
$E1_W$	Acetaminophen	Payne	0.019	19.53	8
$S2_S$	Ibuprofen	Charlotte	0.680	0.44	8
$S2_F$	Ibuprofen	Durand	0.587	0.64	8
$N2_W$	Ibuprofen	Durand	0.034	12.67	8
$L1_S$	Naproxen	Durand	0.315	1.74	8
$B1_W$	Naproxen	Charlotte	0.032	13.53	8
$N2_W$	Naproxen	Durand	0.165	3.48	8

Table 11. Characteristics of isolates from the summer, fall, and winter cultures.

Isolate	Pigment	Gram Characteristics
Summer		
$C1_S$	Pale yellow	Negative rods
$F1_S$	Orange	Negative rods
$G1_S$	Pale Yellow	Negative rods
$J1_S$	Off-white	Positive rods
$J2_S$	Red	Negative rods
$L1_S$	Off-white	Positive rods
$L2_S$	Fluorescent	Positive cocci
$N1_S$	Yellow	Positive cocci
$O1_S$	Orange	Negative rods
$O2_S$	White	Negative rods
$R2_S$	Off-white	Positive rods
$S2_S$	Pale Orange	Positive cocci
Fall		
$A1_F$	White	Positive rods
$A3_F$	Orange	Positive cocci
$B1_F$	Yellow	Negative rods
$B3_F$	White	Negative rods
$G1_F$`	Off-white	Negative rods
$G4_F$	White	Positive rods
$J2_F$	Off-white	Negative rods
$L1_F$	Off-white	Negative rods
$M2_F$	White	Negative rods
$N3_F$	Pale Orange	Negative rods
$O2_F$	Bright Orange	Positive rods
$O3_F$	Mustard Yellow	Negative cocci
$S2_F$	Yellow	Negative rods
$T2_F$	White	Negative rods
$U1_F$	Pink	Negative rods
Winter		
$B1_W$	Off-white	Negative rods
$D1_W$	Pale Orange	Positive cocci
$E1_W$	White	Negative rods
$E3_W$	Pale yellow	Positive rods
$F2_W$	White	Negative cocci
$H2_W$	Pale yellow	Negative rods
$K4_W$	White	Negative cocci
$L4_W$	Off-White	Negative rods
$N1_W$	White	Positive rods
$N2_W$	Orange	Negative rods

Figures

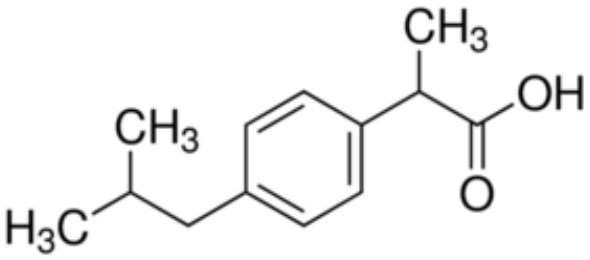

Figure 1a. Structure of ibuprofen.

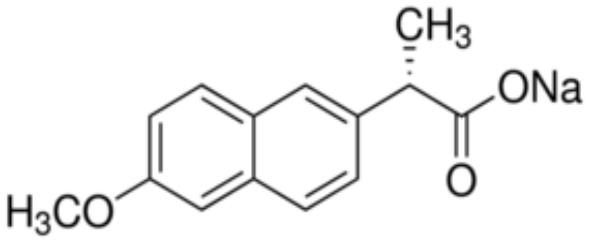

Figure 1b. Structure of naproxen.

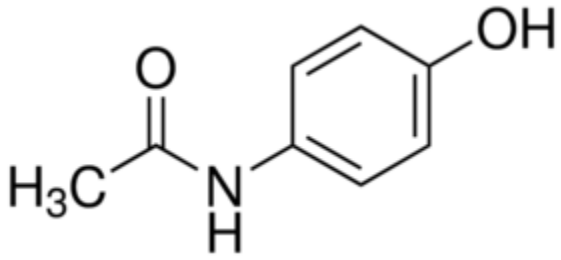

Figure 1c. Structure of acetaminophen.

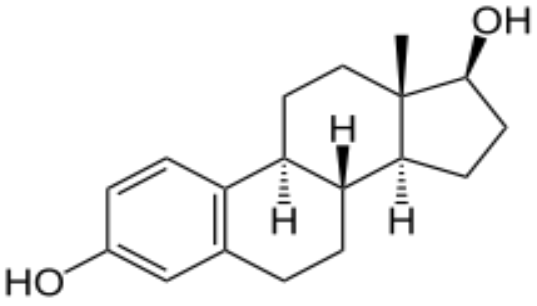

Figure 1d. Structure of 17β-estradiol.

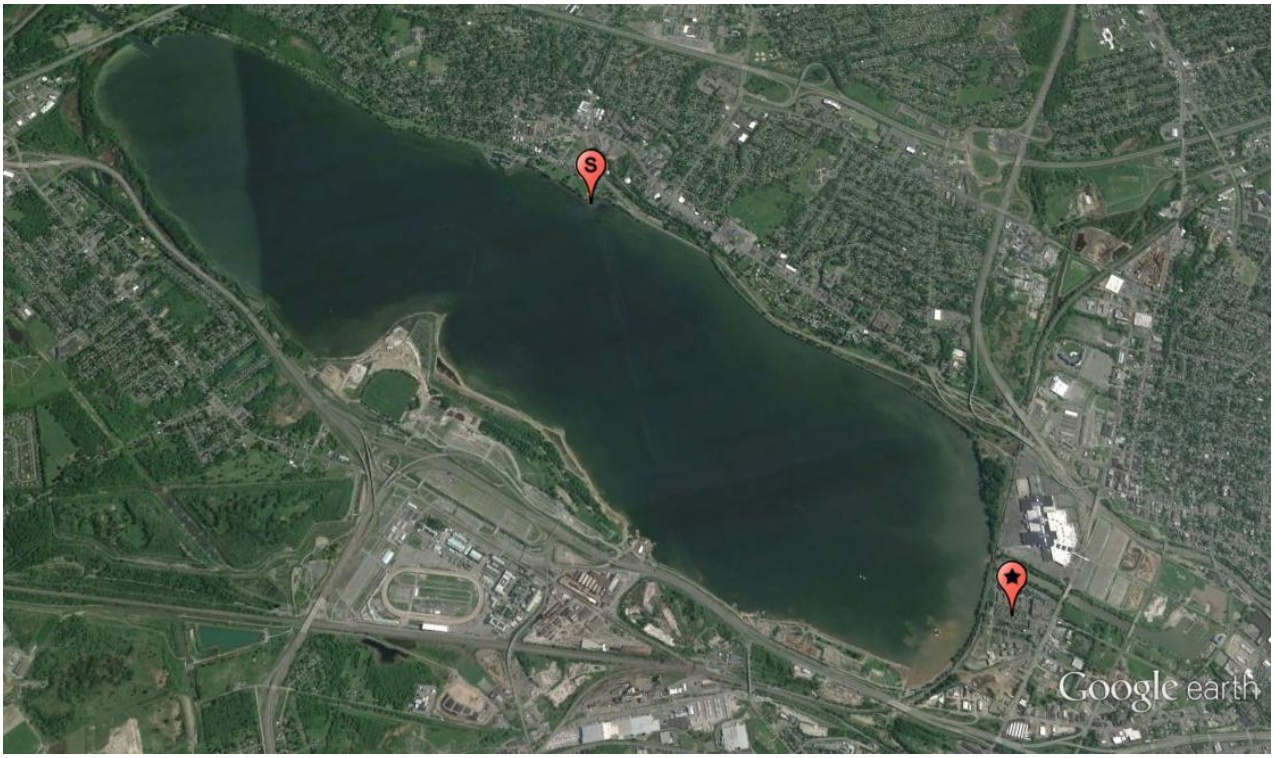

Figure 2. Google Earth image of Onondaga Lake. The red placemark with the S signifies the sampling location. The red placemark with the star signifies the Metropolitan Syracuse WWTP.

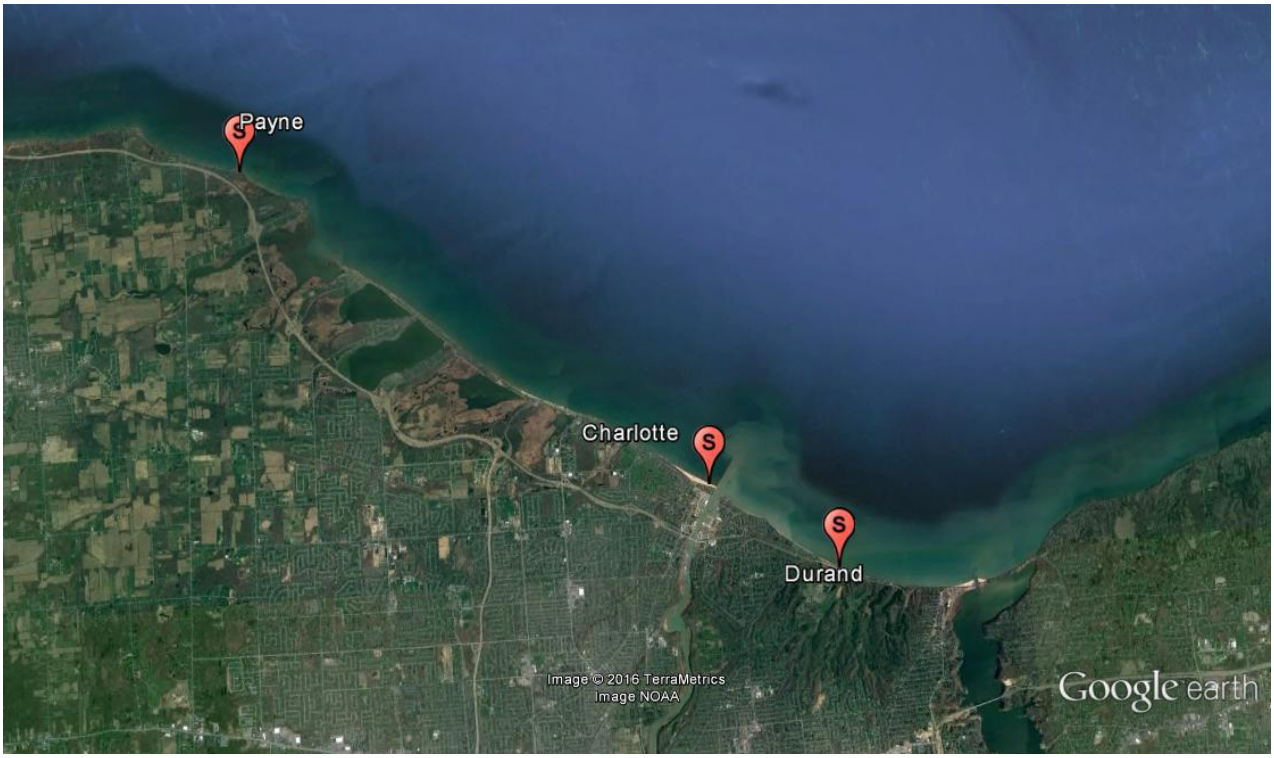

Figure 3a. Google Earth image of the Lake Ontario sampling sites. The red placemarks signify the sampling locations of each beach.

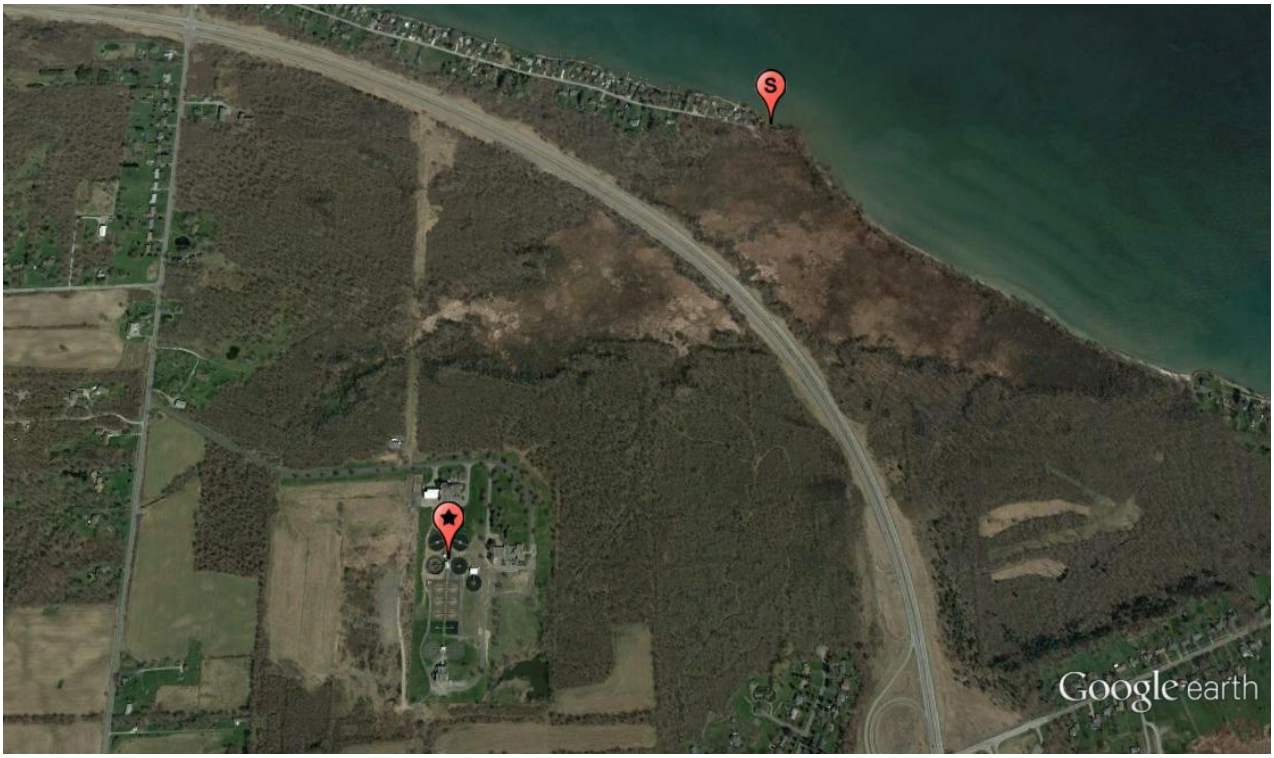

Figure 3b. Google Earth image of Payne Beach. The red placemark with the S signifies the sampling location. The red placemark with the star signifies the Northwest Quadrant Treatment Plant.

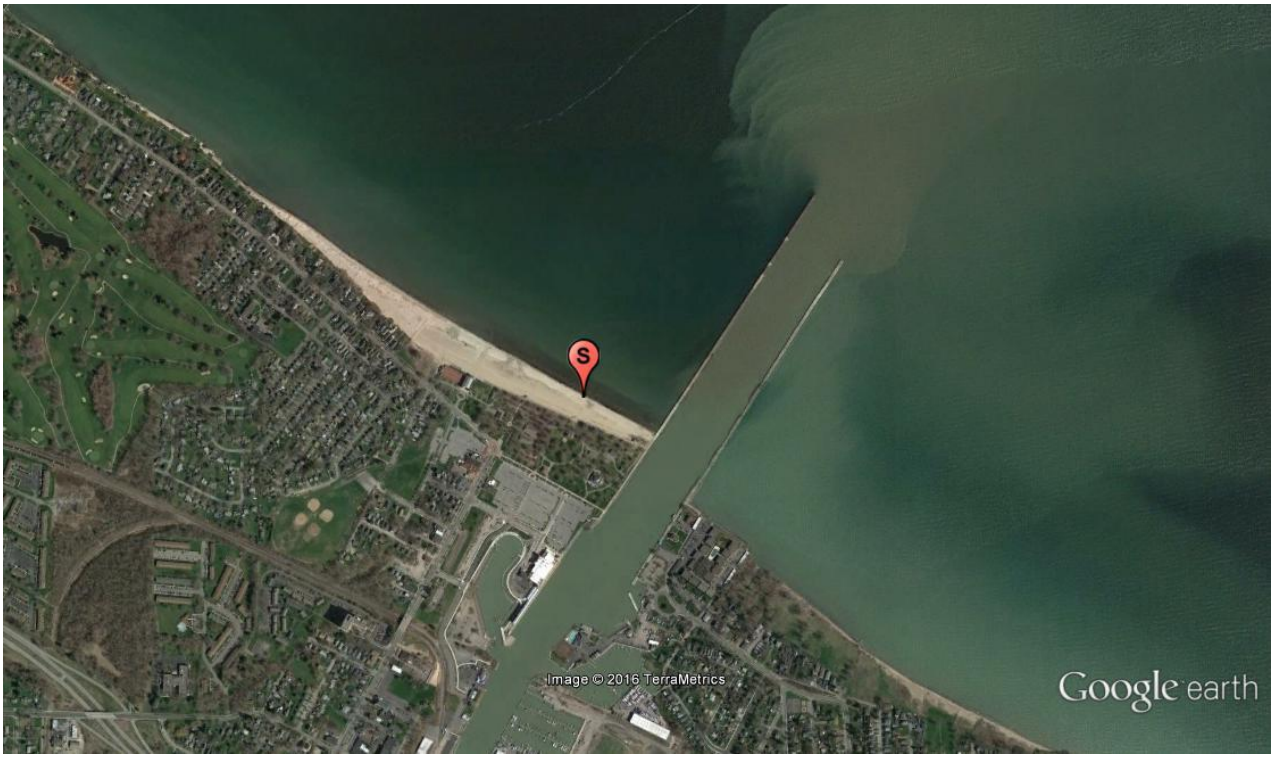

Figure 3c. Google Earth image of Charlotte Beach. The red placemark with the S signifies the sampling location. The water being discharged into the lake is from the Genesee River.

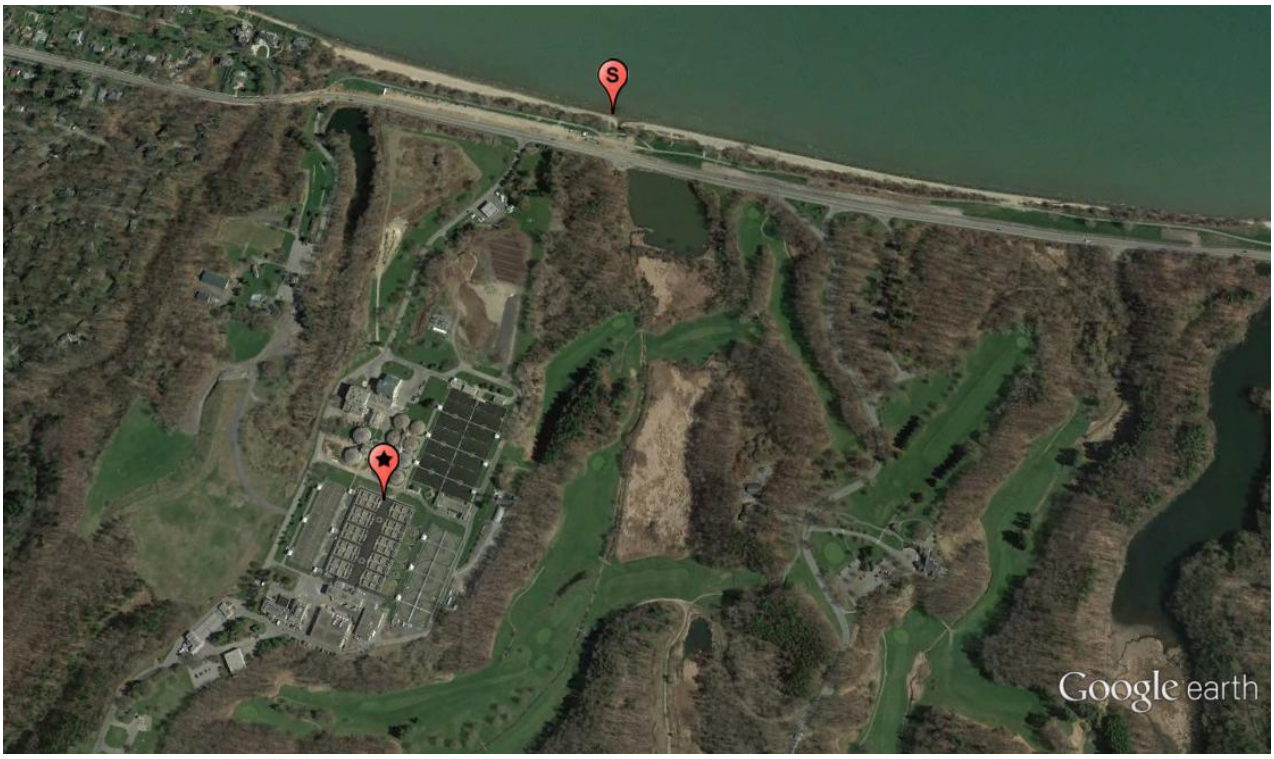

Figure 3d. Google Earth image of Durand Beach. The red placemark with the S signifies the sampling location. The red placemark with the star signifies the Van Lare WWTP.

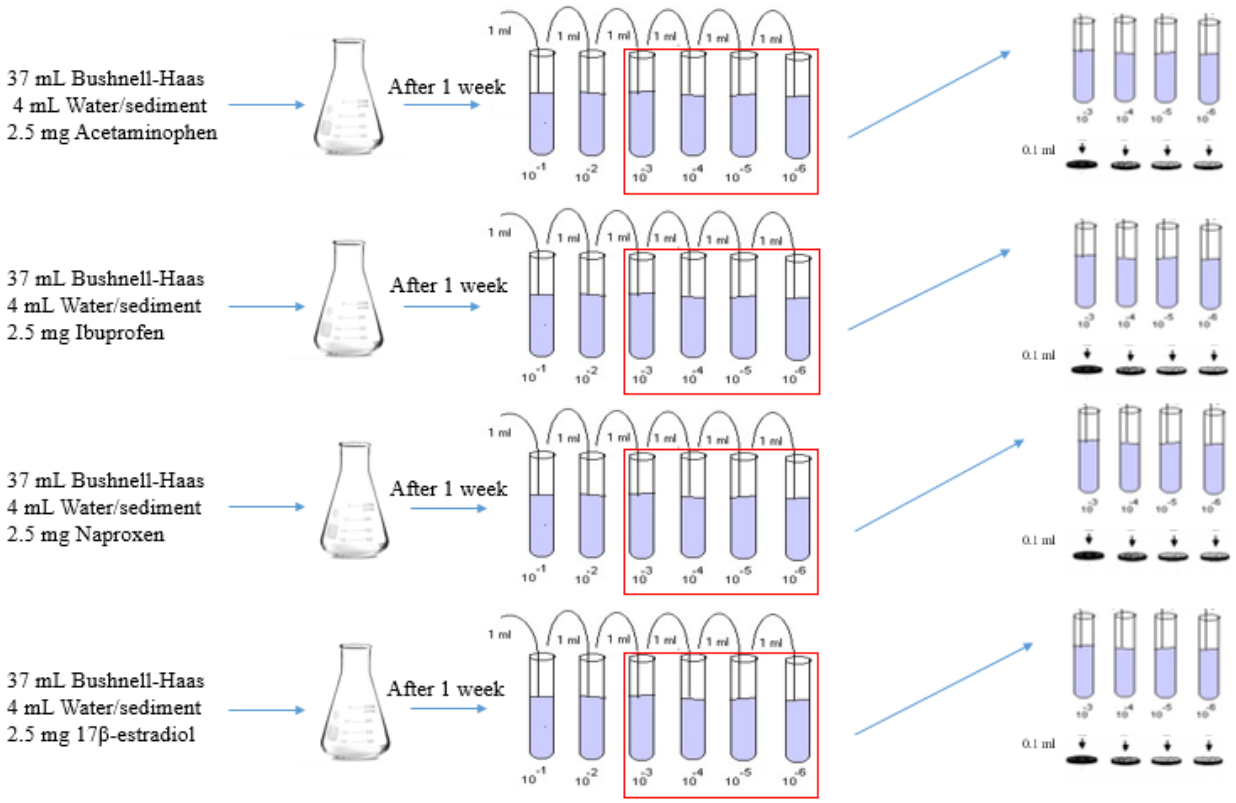

Figure 4. Enrichment technique after 1 week of growth in culturing flasks. Weeks 2, 3, and 4 all following the same general protocol except that 4 mL of solution are taken out of each flask. Water/sediment from the stock bottle is not added after initial enrichment.

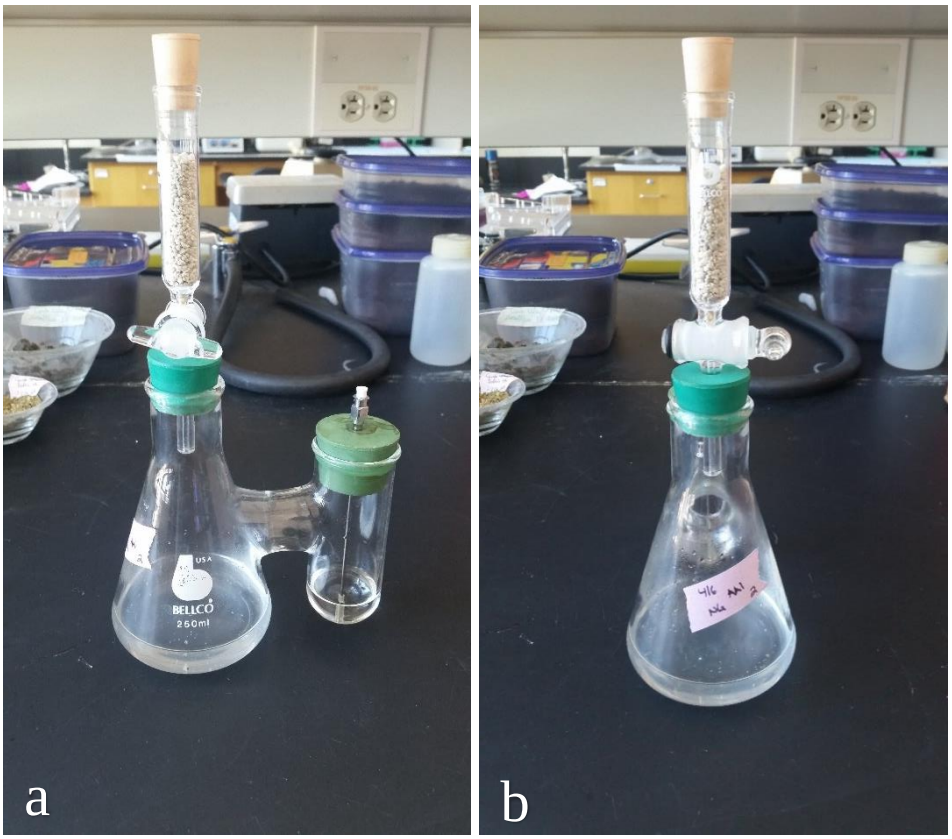

Figure 5. Biometer flask setup. Bushnell-Haas medium, isolate of choice, and selected pharmaceutical are placed in the base. KOH is placed in the sidearm and ascarite is placed in the top; a) side view, and b) front view.

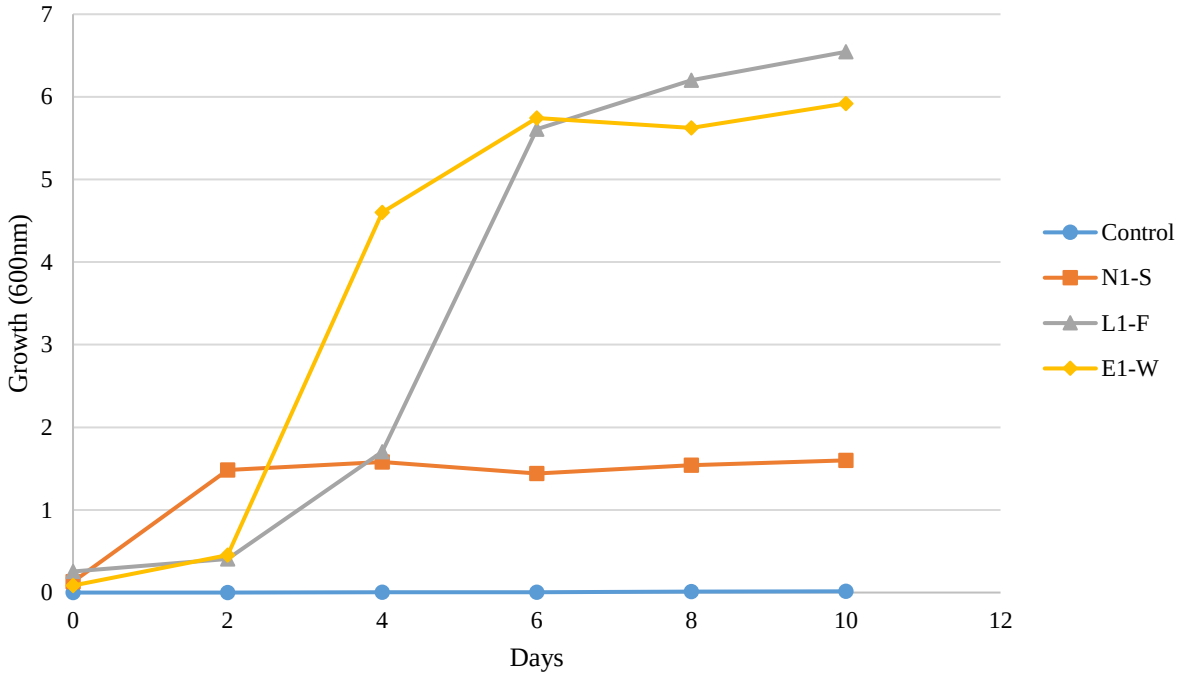

Figure 6. Growth of isolates on acetaminophen. N1-S (summer), L1-F (fall), E1-W (winter).

Figure 7. a) Control, b) $N1_S$, c) $L1_F$, and d) $E1_W$ after 10 days of growth on acetaminophen.

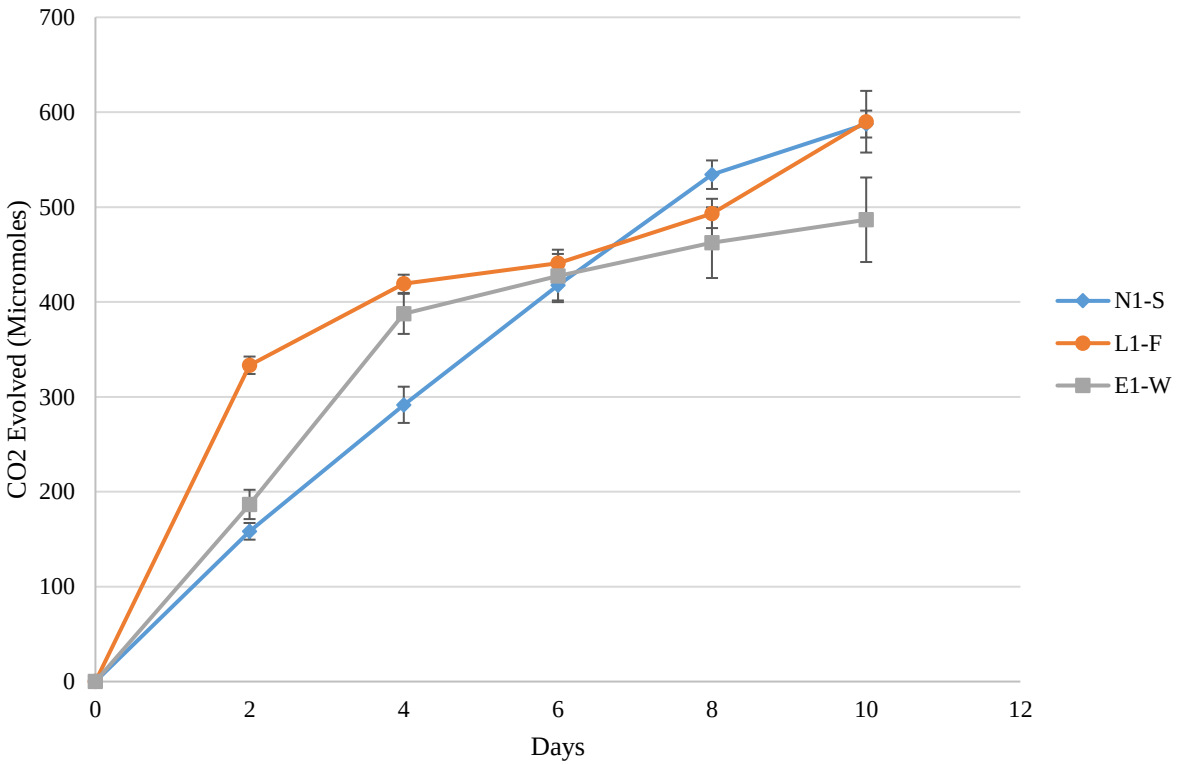

Figure 8. Degradation of acetaminophen by isolates. Standard error bars are representative of each isolate's three trials. N1-S (summer), L1-F (fall), E1-W (winter).

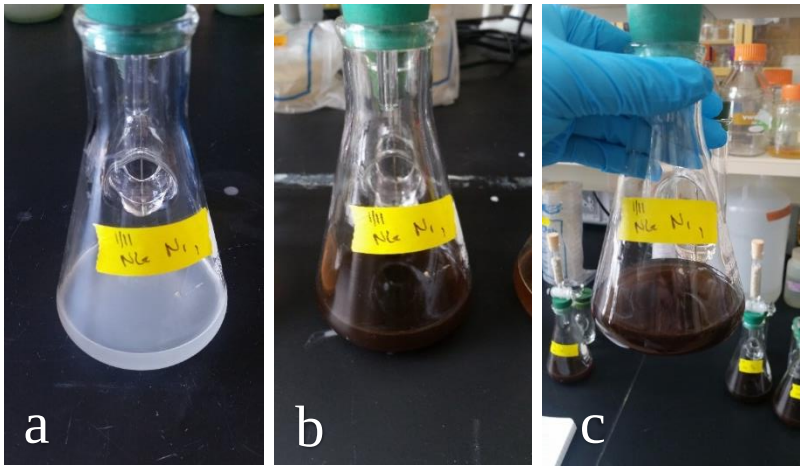

Figure 9. a) $N1_S$ after 0 days, b) 4 days, and c) 10 days of growth on acetaminophen.

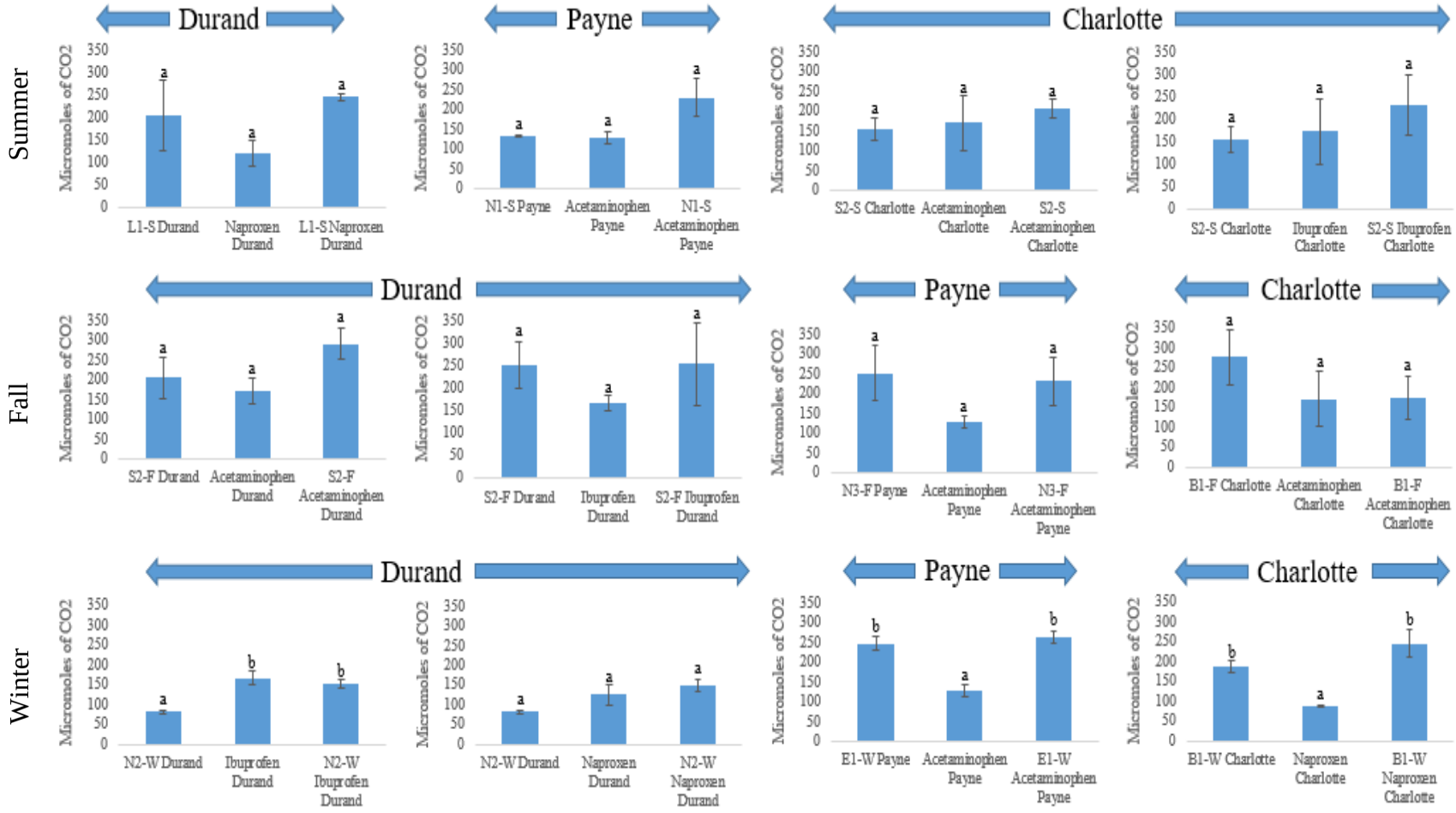

Figure 10. Biodegradation of contaminants by isolates in their natural water sources. The total micromoles of CO2 were determined at the 10-day mark. Standard error bars are representative of each of the three trials tested. A one-way ANOVA comparing the biodegradation of pharmaceuticals in natural water by isolates was performed, followed by a post-hoc Tukey HSD test ($p \leq 0.05$). "b" indicates a significant mean from "a".

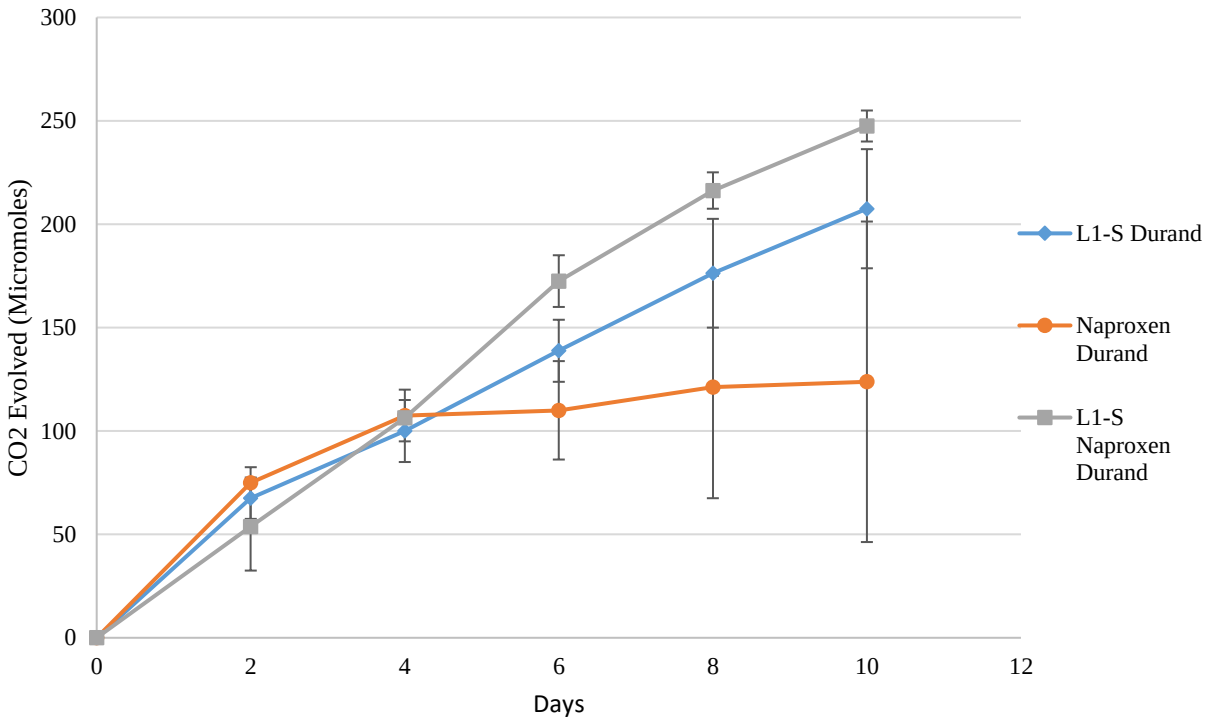

Figure 11. Degradation of naproxen by isolate $L1_S$ in Durand Beach water. Standard error bars are representative of each of the three trials. L1-S Durand represents the isolate in Durand Beach water solely, Naproxen Durand represents naproxen in Durand Beach water solely, and L1-S Naproxen Durand represents the isolate in Durand Beach water with naproxen.

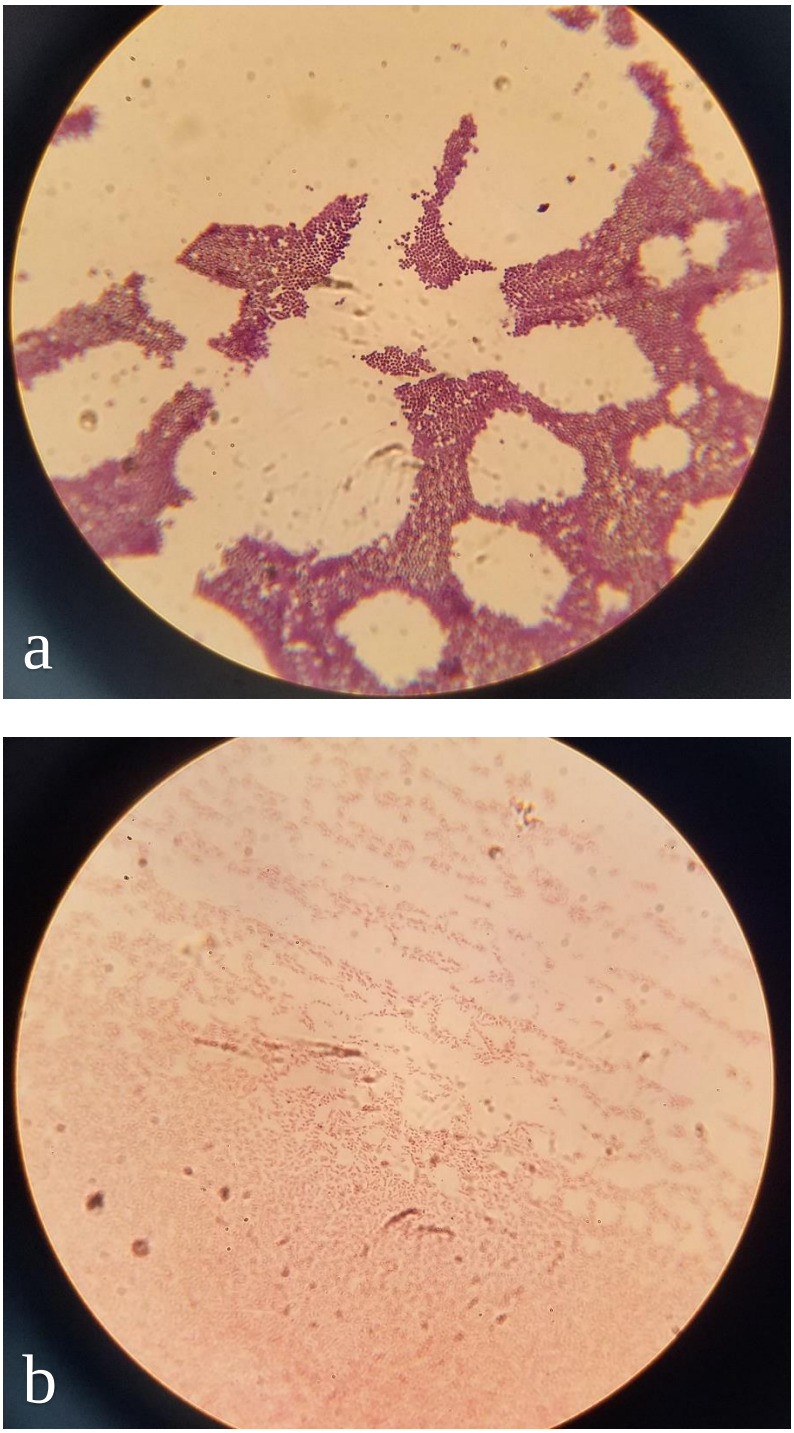

Figure 12. Gram stains of two different isolates; a) $N1_S$ and b) $B1_F$.

Appendix

Supplemental Tables

Table S-1a. Monthly precipitation rates for the Rochester region (data from (Weather Underground, 2016a)). Precipitation was measured from the Greater Rochester International Airport.

Region	**Monthly Precipitation**			
	May 2015	**September 2015**	**March 2016**	**August 2016**
Rochester	4.2 cm	4.0 cm	0.97 cm (snow depth: 2.54 cm)	2.8 cm

Table S-1b. Monthly precipitation rates for the Syracuse region (data from Weather Underground, 2016b)). Precipitation was measured from the Syracuse Hancock International Airport.

Region	**Monthly Precipitation**			
	June 2015	**September 2015**	**February 2016**	**August 2016**
Syracuse	6.7 cm	4.8 cm	5.0 cm (snow depth: 30.5 cm)	2.8 cm

Table S-2. Environmental measurements of the surface water samples taken on August 15, 2016.

Surface Water	Temperature (°C)	pH	Phosphate (mg/L)	Nitrate (mg/L)
Onondaga	27	8.61	0.24	2.2
Charlotte	26	8.61	0.14	2.2
Payne	25	8.25	0.13	1.32
Durand	25	8.32	0.07	0.44

Table S-3a. Average CFU/mL of colonies from each sampling site growing on the selected pharmaceuticals at week 4, day 7. The average was determined from the summer, fall, and winter enrichments. A one-way ANOVA and a post-hoc Tukey HSD test were performed on the data ($p \leq 0.05$). "b" indicates a significant mean from "a", * = Indicates use of Fisher LSD test. The results of the ANOVA test are listed in Table S-3b.

Sample Plates	Average cfu/mL
Acetaminophen	
Durand Beach^{b}	1.3×10^{8}
Payne Beach^{a}	4.7×10^{6}
Charlotte Beach^{ab}	2.1×10^{7}
Onondaga Lake^{a}	7.8×10^{6}
***Ibuprofen**	
Durand Beach^{b}	6.1×10^{7}
Payne Beach^{a}	1.3×10^{6}
Charlotte Beach^{ab}	2.0×10^{7}
Onondaga Lake^{a}	4.4×10^{6}
Naproxen	
Durand Beach^{a}	5.2×10^{7}
Payne Beach^{a}	5.2×10^{7}
Charlotte Beach^{a}	4.9×10^{6}
Onondaga Lake^{a}	1.9×10^{6}
17β-Estradiol	
Durand Beach^{a}	1.1×10^{8}
Payne Beach^{a}	2.5×10^{7}
Charlotte Beach^{a}	2.3×10^{7}
Onondaga Lake^{a}	3.5×10^{6}

Table S-3b. One-way ANOVA comparing the CFU/mL of colonies growing on the selected pharmaceuticals versus the sampling sites ($p \leq 0.05$). Week 4, day 7 data (without averaging) was considered for all calculations. Significant isolates are highlighted in gray.

CFU/mL of Colonies Versus Sampling Site			
Pharmaceutical	**P-value**	**F-value**	**Total df**
Acetaminophen	0.021	5.80	11
Ibuprofen	0.046	4.22	11
Naproxen	0.426	1.04	11
17β-Estradiol	0.316	1.63	11

Table S-4a. Average diversity of colonies from each sampling site growing on the selected pharmaceuticals at week 4, day 7. The average diversity was determined from the summer, fall, and winter enrichments. A one-way ANOVA and a post-hoc Tukey test were performed on the data ($p \leq 0.05$). "a" indicates no significant difference in the mean. The results of the ANOVA test are listed in Table S-4b.

Sample Plates	Number of Different Colonies
Acetaminophen	
Durand Beach[a]	4.3
Payne Beach[a]	4.7
Charlotte Beach[a]	4.7
Onondaga Lake[a]	5.3
Ibuprofen	
Durand Beach[a]	4.7
Payne Beach[a]	5.3
Charlotte Beach[a]	4.7
Onondaga Lake[a]	5.3
Naproxen	
Durand Beach[a]	4.3
Payne Beach[a]	5.3
Charlotte Beach[a]	4.7
Onondaga Lake[a]	6.0
17β-Estradiol	
Durand Beach[a]	5.0
Payne Beach[a]	4.5
Charlotte Beach[a]	5.5
Onondaga Lake[a]	4.5

Table S-4b. One-way ANOVA comparing the diversity of colonies growing on the selected pharmaceuticals versus the sampling sites ($p \leq 0.05$). Week 4, day 7 data (without averaging) was considered for all calculations.

Diversity of Colonies Versus Sampling Site			
Pharmaceutical	**P-value**	**F-value**	**Total df**
Acetaminophen	0.760	0.40	11
Ibuprofen	0.750	0.41	11
Naproxen	0.168	2.19	11
17β-Estradiol	0.689	0.52	11

Supplemental Figures

Figure S-1. 500 mL graduated water dipper; a) 60-ft long dipper, b) 500 mL cup.

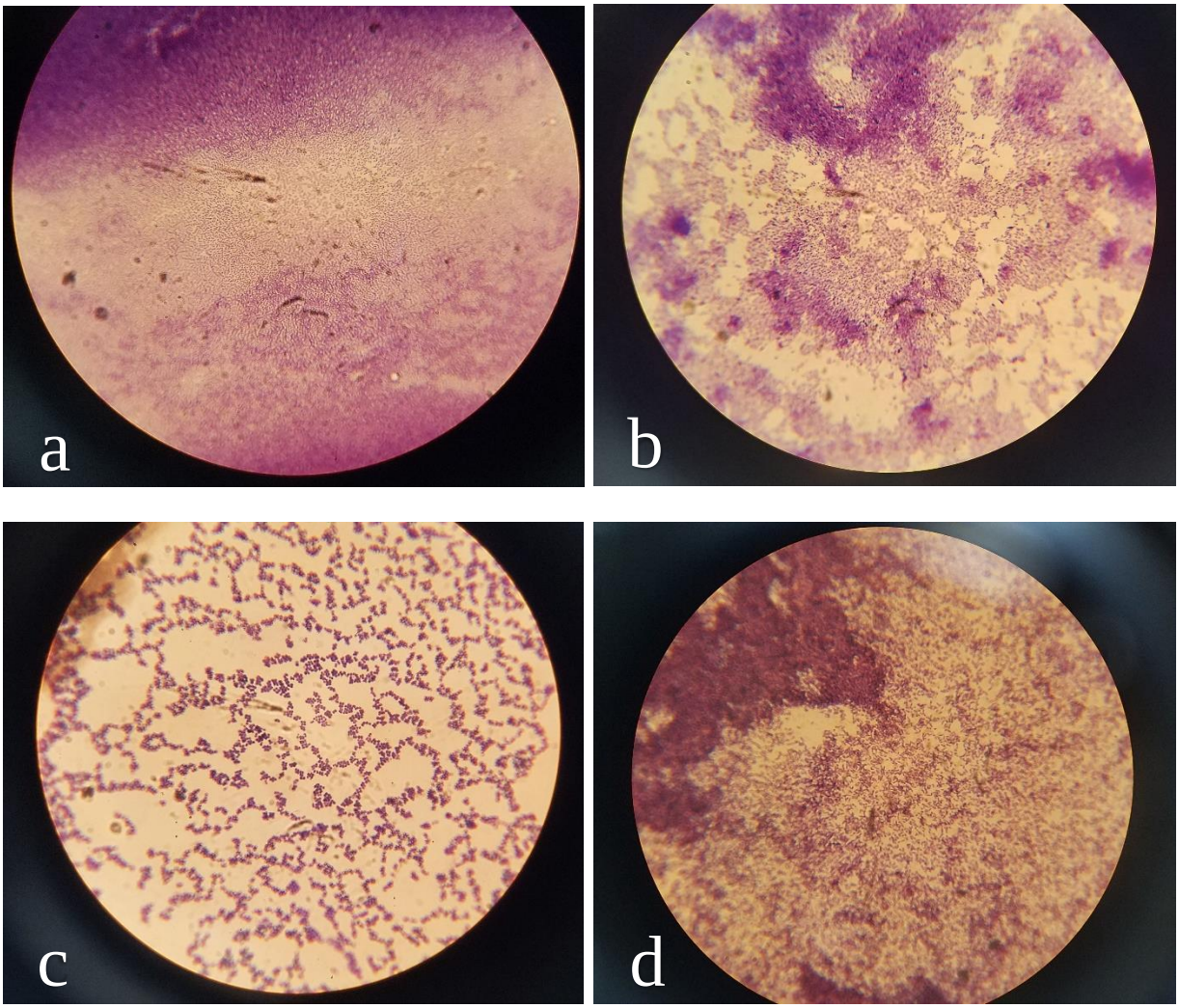

Figure S-2a. Gram stains of a) $J1_S$, b) $L1_S$, c) $L2_S$, and d) $R2_S$.

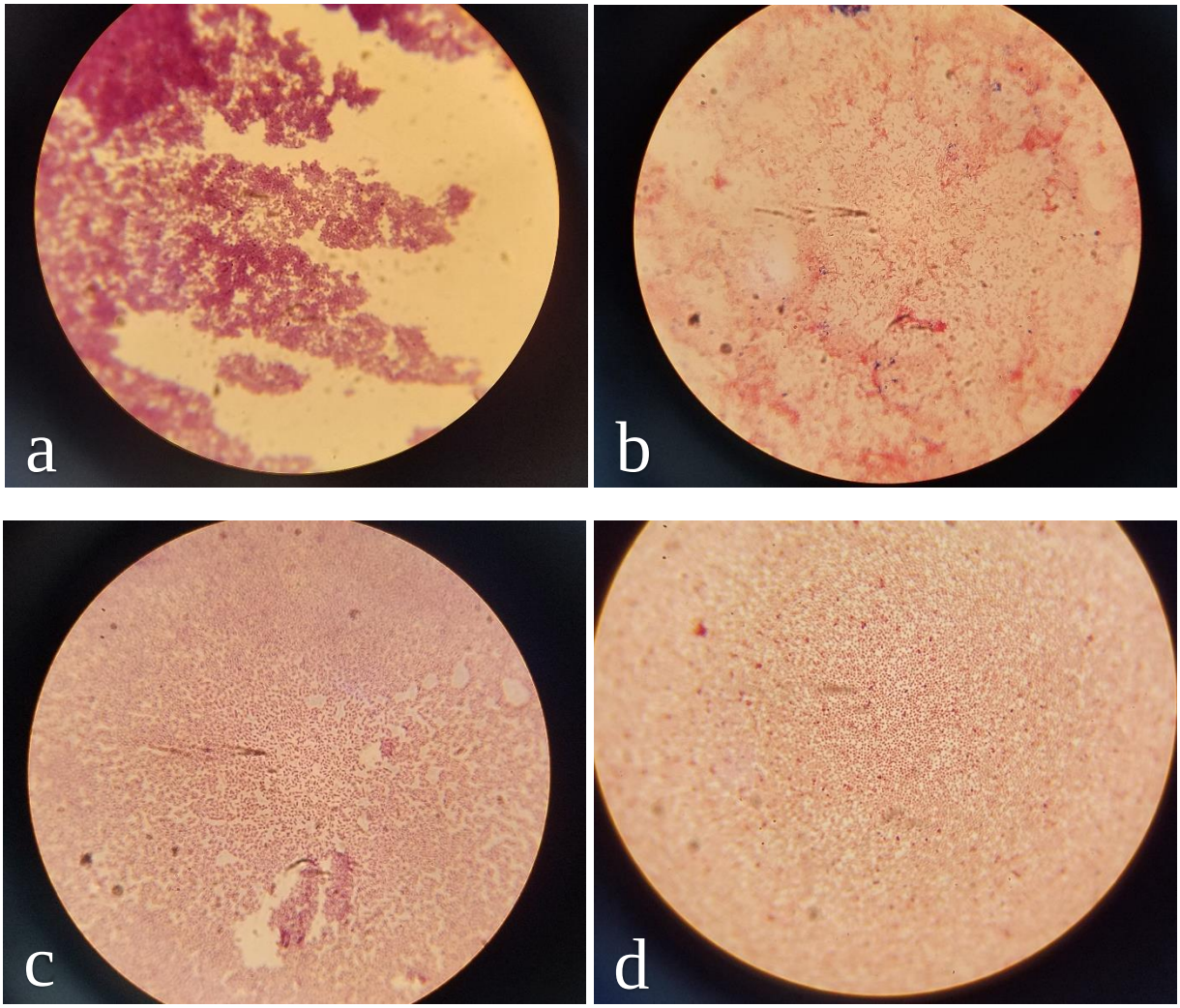

Figure S-2b. Gram stains of a) $L1_F$, b) $M2_F$, c) $O2_F$, and d) $S2_F$.

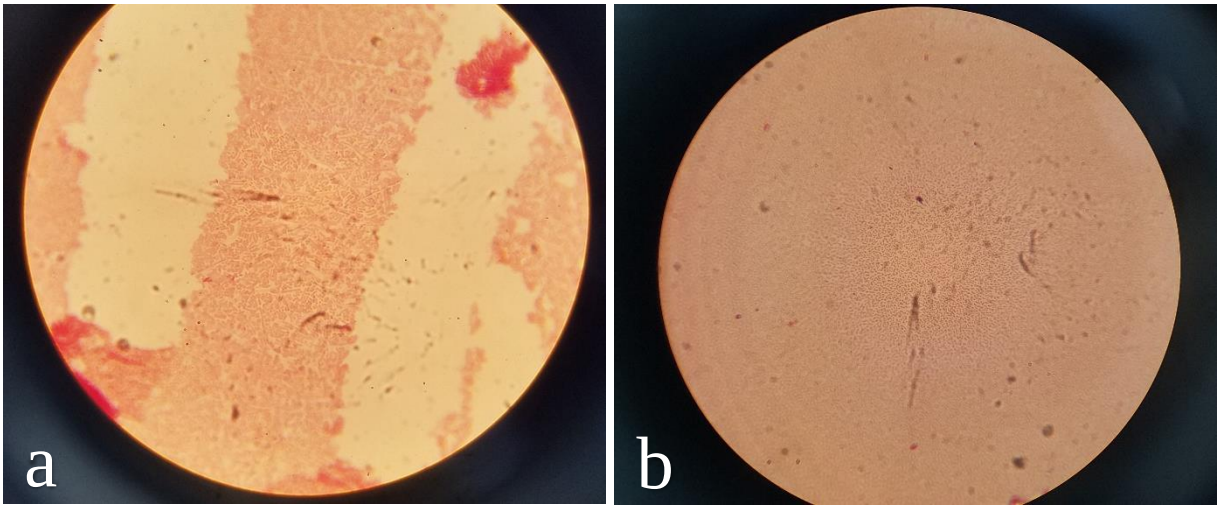

Figure S-2c. Gram stains of a) $H2_W$ and b) $N2_W$.