Pharmaceuticals in Wastewater

A Major Qualifying Project proposal submitted to the Faculty of

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Abstract

Pharmaceuticals are known to cause detrimental environmental effects including the increase of antibiotic resistant bacteria and development issues in animals, but research is still limited. This study aims to observe the effect current wastewater treatment methods have on three pharmaceuticals (SMZ, azithromycin, and salicylic acid) by analyzing their concentrations after seven treatment stages. Utilizing SPE and LC-MS, we determined the effluent concentrations of SMZ and azithromycin were 81 ppb and 209 ppb, respectively, with similar concentrations appearing in surface water. This study did not observe a reduction in pharmaceutical concentration with current wastewater treatment methods but noted their potential.

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Capstone Design Statement

The Major Qualifying Project (MQP) at Worcester Polytechnic Institute has several requirements presented by the Accreditation Board of Engineering and Technology (ABET) to help prepare students for practical application "based on the knowledge and skills acquired in earlier course work and incorporating appropriate engineering standards and multiple realistic constraints." These constraints depend on the economic, environmental, social, political, ethical, health and safety, manufacturable, and sustainable factors surrounding the design. Designs are often iterative, involving multiple examinations of data while forming an idea and offering areas of improvement.

Current wastewater treatment practices do not target pharmaceuticals directly, and these contaminants are often missed by general treatment processes. During our investigation of SMZ, azithromycin, and salicylic acid in Upper Blackstone's wastewater, we found that these pharmaceuticals were present throughout the wastewater treatment process. Hence, determining a method of treating pharmaceuticals at Upper Blackstone was prudent. However, the nature of our results added an additional design constraint. UV-Vis spectrometry indicated a significant drop in detected material after aerobic treatment, at wavelengths our pharmaceuticals would be found. With LC-MS, we found all pharmaceuticals present in low concentrations, with little or no reduction from start to finish. Combined, these data indicate that aerobic treatment has the potential to treat pharmaceuticals in wastewater in Upper Blackstone's current system. Because this design is focused on potential treatment in a pre-existing environment, it should impact the current system as little as possible. The design should also offer increased efficiency of overall treatment, in the event that the potential to treat pharmaceuticals is not reached.

To address these constraints and requirements, a second biological reactor and a recycling stream in the aerobic tank were both considered. Initial testing indicated the potential for aerobic tank treatment to effectively treat pharmaceuticals, while research also showed biological reactors had proven effective in treating salicylic acid, making both viable options. After examining potential costs, feasibility, and impact, the recycling stream option was chosen. Wastewater would travel through the aerobic tank and receive initial treatment, before exiting the tank and entering a recycling stream. Air would continuously be blown into the pipe at periodic intervals, adding oxygen necessary for aerobic treatment and thoroughly mixing the water. The wastewater would then re-enter the aerobic tank and undergo treatment a second time before flowing to primary settling. Two different dissolved oxygen values were considered for this design. The first value, 0.6 mg/L, is the average of typical values in Upper Blackstone, and focused on maintaining current levels of oxygen that appease the present bacteria. The second, higher dissolved oxygen of 1.0 mg/L is considered average wastewater treatment plant values and would provide a more complete mixing. While a higher dissolved oxygen value will ultimately reduce the aerobic treatment performed by the bacteria, this difference was found to be minimal. The focus of this design is to increase mixing, as it will allow the bacteria to better feed on pharmaceuticals and other previously untreated contaminants, especially those in low concentrations. A recycling stream has minimal

impact to the current treatment system and requires limited materials, merely piping, pumps to carry the water, and air blowers to add oxygen and mix the wastewater. Recycling streams are known to be safe and non-controversial additions to a system, and can easily be modified or manufactured for multiple locations. It is recommended that Upper Blackstone perform a more in-depth analysis of their aerobic tank's efficiency, bacteria conditions and reactions, and specific reactions to pharmaceuticals to determine optimal dissolved oxygen and mixing levels.

Professional Licensure Statement

Licensure is the act of distributing licenses as well as restricting practices to those who have specific licenses. Licensure is usually run by the government or a specific organization dedicated to the practice. In the United States, engineering licensure falls under the state board jurisdiction, and is represented by the National Council of Examiners for Engineering and Surveying (NCEES). There are two licenses individuals can gain for engineering: Engineering in Training (EIT) and Professional Engineering (PE).

To become a professional engineer, an individual must graduate from an accredited engineering program, then become an EIT by passing the Fundamental Engineering (FE) exam. An EIT gains experience by working under an engineer with a PE license for four years before they are eligible to take the PE exam (Doud, 2021). Becoming an EIT is traditionally a required step to becoming a professional engineer, though 15 states have begun moving away from this in the past decade (National Society of Professional Engineers, 2018). This change is intended to move engineering licensure to a more individually oriented process, where an applicant may take the PE exam as soon as they feel they are prepared. The PE exam itself is dependent on what type of engineering a person is pursuing, though exams are typically 8 to 8.5 hours long with 80 questions (NCEES, 2021). After passing the PE exam, licensed engineers maintain this status by participating in designated courses, webinars, and conferences (School of PE, 2018). The required topics of this continued education and how often they should be completed is determined state by state and can easily be found on state websites. If an engineer wishes to practice in a different state, they will need to obtain a separate PE license for that state (Blake, 2017). This process is much simpler than obtaining an individual's first PE license, as states merely need to recognize that someone has achieved the PE requisites before. At this point, obtaining the new state's PE license is simply a matter of filling out paperwork.

Regardless of engineering type or state, all PE licenses represent the legal privilege an engineer must practice engineering. They have proven they have a comprehensive understanding of the subject and can be trusted to complete their work skillfully. To the engineer themselves, engineering licensure is recognition of their hard work, and the key to open doors that otherwise may be closed. As a professional engineer, they are allowed to prepare, seal, sign, submit, and approve plans and drawings (NSPE What is a PE?, 2021). A PE license is also required for engineers who wish to work in a private practice. A license allows engineers to take higher positions in a company, often with more pay than an unlicensed engineer. All of these aspects involve a high degree of knowledge and competency, which an engineering license stands as proof of (NSPE 2021 Why Get Licensed?). It also reminds engineers of the responsibility they must use this knowledge to ensure public safety.

Licensure allows the engineering industry to preserve its integrity. Without licensure, there would be no official, definitive way of determining whether an individual has the necessary expertise to practice engineering. By setting certain goals to reach before licenses can be obtained, all professional engineers are expected to have these skills and know how to use them effectively.

This helps prevent damage from inexperienced engineers, while also holding professional engineers to a strict ethical code to avoid negligence. In case of a faulty design, having a professional engineer on the design team will corroborate that an accident was beyond the industry's control, helping avoid potential lawsuits (Chinchilla, 2017). These factors ensure a group of individuals provide high quality, efficiently designed products ideal for the client's use. This is the primary meaning of "professional engineer" to the public. Most times, clients or individuals who will frequently use the final product do not have the necessary background to understand how to engineer a product. As a result, they place their trust in an engineer to not only create the product, but to do so in a way that will ensure its smooth and safe functioning. Engineering licensure acts as a way for the public to distinguish which engineers should be hired over others. They know that because the license represents extensive knowledge and skill, the engineer will use these attributes to complete projects. This results in a high-quality finish that was completed safely, efficiently, and within legal guidelines. A PE license shows an engineer can be trusted.

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Executive Summary

Background

Wastewater is defined as used water including human waste, food scraps, oils, soaps, and chemicals. Originally, human waste and wastewater were disposed of directly on land surfaces, but today they are treated in specialized plants to reduce environmental impact and health risks. The major goal of wastewater treatment is to remove as many suspended solids as possible before the effluent is discharged to the environment (Cressler, 2021). Wastewater treatment plants (WWTP) are held to certain standards set by the Environmental Protection Agency (EPA), however pharmaceuticals are not currently regulated by the Safe Water Drinking Act. Thousands of tons of medicines treat both humans and animals each year. These medicines are often improperly disposed of, tossed directly into surface water or down a toilet or sink. Because of the lack of regulations, WWTPs are not currently designed to treat pharmaceuticals, so they are often redistributed into the environment by their effluent streams. Unfortunately, pharmaceuticals have many negative effects on plants and animals, such as development issues and increasing antibiotic-resistant bacteria. This is creating an urgent need to study not only their environmental impacts but also possible mitigation efforts.

This study focuses on three different pharmaceuticals: sulfamethoxazole (SMZ), azithromycin, and salicylic acid. SMZ and azithromycin are widely used antibiotics used to treat infections, while salicylic acid is a typical ingredient in acne treatments. Although research is currently focused on preventing their introduction to the environment, possible treatment methods, such as bioprocesses, show promising results. In this study, we are focused on determining the concentration of SMZ, azithromycin, and salicylic acid in surface water in Worcester, MA, and wastewater from Upper Blackstone Clean Water, one of the largest treatment plants in New England.

Methodology

Although a large portion of pollutants are removed from wastewater during treatment processes, pharmaceuticals are not directly targeted by current methods. This study aims to quantify SMZ, azithromycin, and salicylic acid in surface water samples, as well as investigate the effectiveness of current treatment methods with respect to pharmaceuticals. To accomplish this, we followed the objectives below:

1: Determine current concentration levels of SMZ, salicylic acid, and azithromycin in three different bodies of water in Worcester, MA.

2: Analyze the concentrations of these pharmaceuticals after seven major treatment stages, including the influent and effluent streams of Upper Blackstone WWTP.

3. Recommend strategies to improve the removal of pharmaceuticals in WWTPs.

First, we collected samples from three local bodies of water: Lake Quinsigamond, Green Hill Pond, and Salisbury Pond. We also collected samples from Upper Blackstone to analyze the seven major wastewater treatment methods they utilize, which includes a bioreactor process. We then created standard solutions with the pure compounds at known concentrations; this helped us identify our pharmaceuticals and quantify the samples later on. Once all the samples and standards were gathered, we used UV-Vis to confirm the presence of the selected pharmaceuticals by comparing their chemical properties to the resulting spectra. Then, we performed SPE to isolate and concentrate our samples to prepare for LC-MS. With the pharmaceuticals isolated, they were easier to detect using LC-MS, our main method of identifying and quantifying the samples. LC-MS was the ideal method based on our pharmaceutical's chemical properties, and its sensitivity allows us to detect concentrations in the parts per billion (ppb) range. By creating a serial dilution, we were able to create a calibration curve and compare the MS peak areas of our samples to find their approximate concentrations.

Results

UV-Vis Data Analysis

When UV-Vis samples have a strong signal, the absorbance can be measured, and the Lambert Beer Law is used to analyze the results. Unfortunately, the signals in our samples were too weak to definitively interpret our chosen pharmaceuticals. This is either because their concentrations are too low, or the method did not separate the signals clearly enough. Overall, our graphs showed slight differences and shifts from trial to trial, but the samples contained too many chemicals to identify our antibiotics for certain.

In both the SMZ and salicylic acid spectra, we saw the highest peaks at around 240-250 nm, which were expected as compared to literature. The next comparison we make is between the spectra of the influent and final effluent samples. The final effluent spectrum is smoother than the one of influent. This is expected because the influent is the untreated wastewater and the effluent is fully treated and leaves the WWTP as clean water. Lastly, the UV-Vis spectra between the three bodies of water were compared which can be seen in Results Section 3.1, page 17. Lake Quinsigamond and Salisbury Pond have similar graph shape and therefore may have similar concentrations, while Green Hill Pond has a steeper shape and potentially higher concentration. More details on these graphs can be seen in Appendix B.

Coliform and Bacteria Test

To investigate the presence of *E. Coli* and coliform in wastewater, the tests were performed in three composite samples (influent, primary effluent, final effluent) acquired from Upper Blackstone and in the three bodies of water (Lake Quinsigamond, Green Hill Pond, and Salisbury Pond) as shown in Appendix B. The influent color had shifted completely from yellow to dark purple, the primary effluent color was mainly yellow but with some hints of purple, while the effluent color had remained purely yellow. This color change is consistent with our expectations

as influent is the raw, untreated wastewater which contains different pharmaceuticals, chemicals and effluent is the last stage of the treatment plant which is safer and well-treated for discharge.

As we can see after testing for presence of bacteria, Lake Quinsigamond and Green Hill Pond had remained yellow, while Salisbury Pond had turned slightly purple, indicating a higher level of coliform contamination. The other water quality tests included general hardness, nitrate, nitrite, free chlorine, carbonate, and pH as shown in Appendix E.

LC-MS Analysis

Since our samples contained too many chemicals to identify our pharmaceuticals in the UV-Vis spectrums, we performed SPE to isolate and concentrate our samples. This allowed for easier detection using LC-MS, and we were able to analyze all our samples at once using Single Ion Monitoring (SIM) for each pharmaceutical. Details about the optimization of the LC-MS methods can be seen in Appendix A. We had 51 total samples including 21 standards to create a calibration curve for each pharmaceutical. We then plotted the known standard concentrations against the peak area from the MS report to create a linear trendline. After retrieving the MS reports for the 30 wastewater and surface water samples, we were able to use the trendline equation to approximate their concentrations from their peak areas. A complete list of these samples, their peak areas, and their concentrations can be found in Appendix C, Table 2.

Our goal was to observe the trend in SMZ, azithromycin, and salicylic acid concentrations as the treatment stages progressed. However, our LC-MS data show there is no definitive trend or reduction in any of the three pharmaceuticals as the treatment stages progress. The approximate concentration of SMZ stays between 40 and 100 ppb throughout wastewater treatment. Due to a combination of instrumental errors and unreadable wide peaks, only one concentration was approximated for salicylic acid, and that was about 50 ppb. Figure 23 in Section 3.4 shows the concentration of azithromycin stays consistently between 200 and 300 ppb throughout the wastewater treatment process, much higher than SMZ and salicylic acid concentrations as seen in Figure 19 and 22, even though we expected higher concentrations of SMZ. A more comprehensive report of our LC-MS findings can be seen on page 30.

The LC-MS results for surface water samples taken from Lake Quinsigamond, Green Hill Pond, and Salisbury Pond show similar concentration levels. We were able to plot the concentrations for each pharmaceutical at each body of water, which can be seen on page 30. The missing data for salicylic acid at Green Hill Pond and SMZ at both Green Hill Pond and Salisbury Pond resulted from either instrument error or no sample was tested due to lack of supplies. Azithromycin had the highest concentration at all three locations, with the highest at Salisbury Pond. This is more than double the concentration of salicylic acid at Salisbury Pond and Lake Quinsigamond. Surprisingly, SMZ had the lowest concentration at lake Quinsigamond, despite high concentrations in the environment reported in literature.

Introduction

Antibiotics are one of the most significant discoveries of the last centuries, effectively treating a large array of deadly infections. This greatly increased pharmaceutical consumption in recent years which exposes bacterial communities and ecosystems to a large amount of antibiotics residues. This exposure has led to the increase in antibiotic resistance bacteria (ARB) and poses significant risk to public health and safety. There are thousands of pharmaceuticals and antibiotics that are commonly used today. These pharmaceuticals find their way in the water by excretion of active drugs, being directly discarded into the environment, or by incomplete removal during wastewater treatment. Wastewater treatment removes contaminants from sewage and wastewater then converts it into an effluent stream that returns to the environment. This effluent can be repurposed and thus creating the wastewater cycle. Currently, pharmaceuticals are not regulated in wastewater, so treatment plants are not equipped to target and remove these antibiotics. It is critical to study pharmaceutical treatment methods for the health and safety of the public and to avoid negative effects on the environment.

Our project focused on three pharmaceuticals: sulfamethoxazole (SMZ), azithromycin, and salicylic acid. These antibiotics were chosen because of their everyday use and known presence and effect on the environment. The methods used to detect and analyze the presence of antibiotics were mainly UV-Vis, LC-MS, and general water quality tests. The goal of this study was to better understand environmental effects, pollution sources, and current treatment efficiency. By knowing this, we hope to provide recommendations to improve current wastewater treatment methods in the removal of pharmaceuticals.

Background

1.1 Cycle of Wastewater

Wastewater contains human waste, food scraps, oils, and soaps, and is usually made up of 99.9% water and 0.01% organic matter, microorganisms, or inorganic compounds (Tuser, 2020). Originally, human waste and wastewater were disposed of directly on land surfaces, leading to massive sanitary problems that caused deadly illnesses and diseases. The amount of wastewater increased with the development of water supply systems, shifting the need to create wastewater treatment plants (WWTPs) to protect public health and the environment. Wastewater treatment plays an important role in fisheries, wildlife, quality of life, and other health concerns.

To address these concerns, wastewater treatment plants (WWTPs) treat industrial and residential wastewater to remove pollutants with a combination of treatment processes. The major goal of wastewater treatment is to remove as many suspended solids as possible before the effluent is discharged to the environment (Cressler, 2021). Effluent streams are any wastewater that exits a reservoir, treatment process, or industrial plant that is treated or untreated. Influent, on the other hand, is wastewater that flows into a reservoir, treatment process, or industrial plant (UFL, 2021). In general, the urban water cycle starts with water withdrawal from rivers or lakes that is delivered to the city buildings after purification. After constant use at home or industrial plants, the water is delivered to collection plants and WWTPs. After treatment, the water is returned to its original source, rivers, or lakes. A diagram showing the path of water and wastewater can be seen in Figure 1.

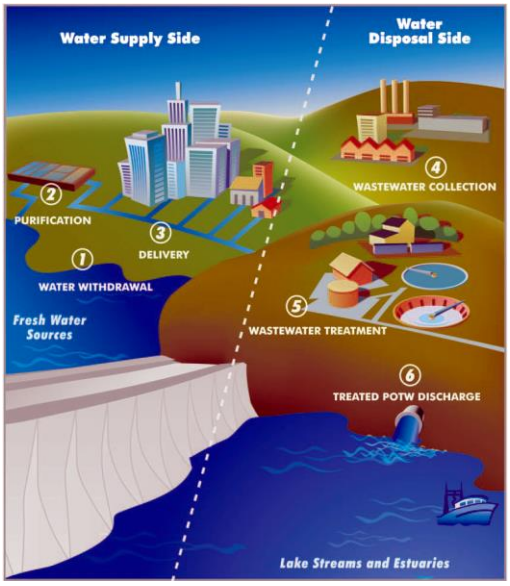


Figure 1. The cycle of wastewater starting from water withdrawals and ending as treated discharge before returning to Step 1.

A wide range of human medicines, including antibiotics, are produced and consumed in the range of thousands of tons per year (Boxall, 2004). While wastewater is processed by WWTPs, pharmaceuticals in the wastewater may not be treated at all. Many WWTPs do not currently target pharmaceuticals, so they end up recirculating in the water cycle. Additionally, some pharmaceuticals, such as veterinary medications, completely avoid WWTPs by entering the environment directly as animal waste.

Pharmaceuticals can enter the environment a multitude of ways: industrial pollution, improper disposal in sewage systems, or animal and human excrement as mentioned above. Excrement has 30% to 90% of the active chemical ingredient in it, and treatment plant removal efficiencies can be as low as 10% (Patel et al, 2019). Water bodies such as lakes are often polluted through human contact, with sweat or sunscreen products washing off skin as people swim. Though they have relatively short lifespans, pharmaceuticals' near-constant presence in effluent streams cause them to be “pseudo-persistent” in the environment.

There are several classes that categorize pharmaceuticals by function. Classes known to impact the environment include antibiotics, antidepressants, pain killers, and hormones. Antidepressants and anti-anxiety medications have led to behavior changes in birds and fish, such as increased food consumption. Additionally, synthetic hormones have caused male fish in Europe to become intersex (Nawrat, 2020). Painkillers and antibiotics commonly used to treat-cattle are linked to acute kidney failure in vultures in Asia (Pharmaceuticals Move throughout the Aquatic Environment). These studies show predators are most affected and the spread of the problem goes far beyond initial perception. Even personal care products negatively impact the environment by inhibiting vital plant growth in high concentrations. Figure 2 shows how quickly pharmaceuticals can spread through soil adsorption, water flow, and animal consumption. The figure depicts various environmental effects of pharmaceuticals, including hormone overstimulation, developmental issues, behavioral changes even death.

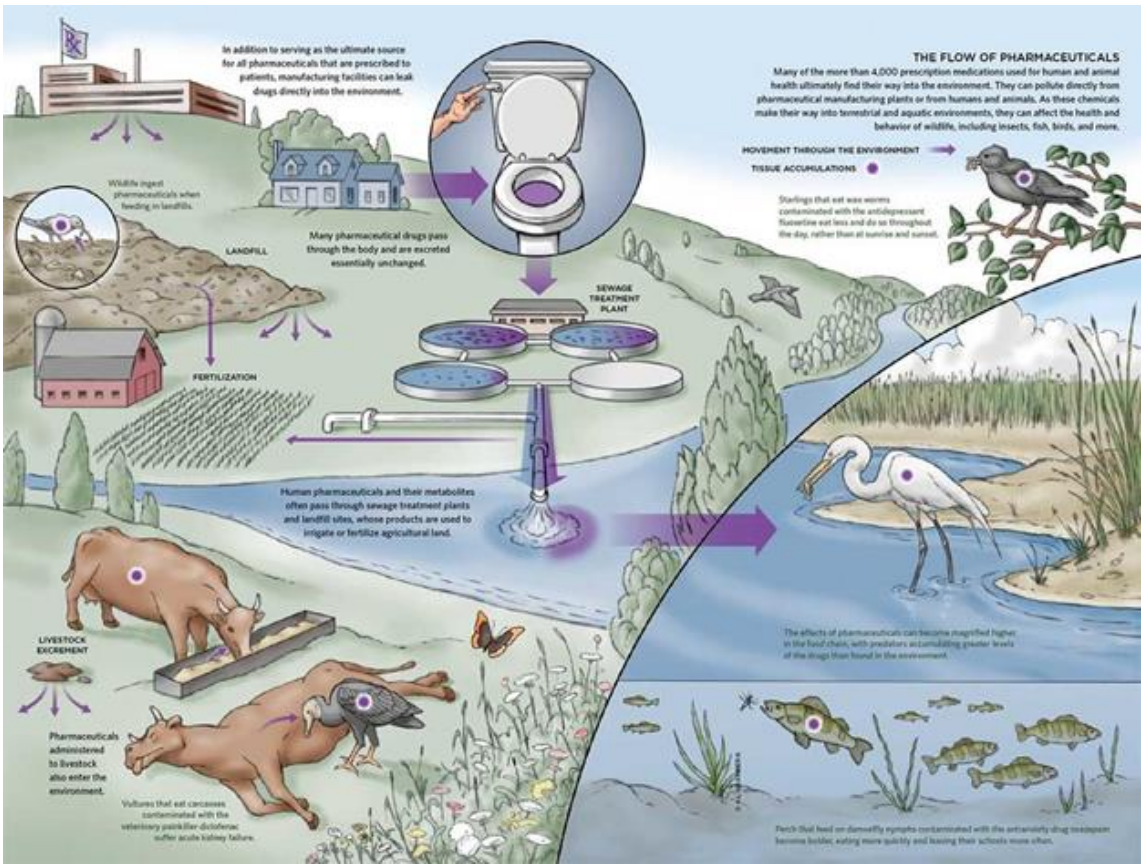


Figure 2. This flow diagram shows the possible spread of pharmaceuticals and the effects they have on flora and fauna.

One of the greatest concerns with the increasing level of pharmaceuticals in wastewater is antibiotic resistant bacteria (ARB). Even low concentrations of antibiotics can lead to antibiotic resistance in any environment. This creates a positive feed loop, where higher doses are necessary to fight infections, which increases the side effects, and ultimately creates stronger bacteria. This is particularly a problem in developing countries who have less access to medication. A recent global review reported that of 713 pharmaceuticals tested in the environment, 631 of those were found above detection limit (Thomas, 2017). Because this is an emerging issue, it is important to not only study the environmental impacts, but also any mitigation strategies that may reduce their effects.

1.2 Selected Pharmaceuticals

In the wide range of drugs, sulfamethoxazole, salicylic acid, and azithromycin have been known to have significant effects on the environment. These three pharmaceuticals are commonly used in medicine and personal care products, promoting microbial resistance that creates microbes with the potential to upend fragile systems.

Sulfamethoxazole (SMZ) is in the sulfonamide class of antibiotics that is widely effective against bacteria, including UTIs, pneumonia, and ear infections. SMZ is commonly paired with trimethoprim (TMP) to create the antibacterial products Bactrim, Bactrim DS (double strength), and Septra. It is among the top ten most common generic antibiotics and has been found in environments both near and far from hospitals and manufacturing plants, making it a prime pharmaceutical to analyze (drugs.com).

A study in a developing country found bacteria with a high resistance to TMP– SMZ (Gangcuangco, 2005). This is problematic since this antibiotic drug has been around since the 1930s and is usually the first line of treatment for infections (Huovinen, 2001). This study also showed an association between failed treatments for acute urinary tract infections (UTI) and previous UTI episodes, meaning past use of antimicrobial drugs could lead to treatment failures in the future (Gangcuangco, 2005).

There are many emerging studies attempting to treat SMZ and other antibiotics in wastewater, with varying degrees of success. Knowing this is a growing problem with no solution in sight, there should be a focus on preventing antibiotics from entering the environment as well as finding successful treatment. Recently, the Center of Disease Control issued a list of serious and concerning antibiotic resistant bacteria (ARB) afflicting the US healthcare system in hopes of doing just that, stopping the problem at the source (Ventola, 2015).

Salicylic acid is a monohydroxy benzoic acid plant hormone that plays a major role in metabolism, growth, and stress response (Hayat, 2009). It is used as a topical treatment for acne and skin conditions which cause scaling and overgrowth and is commonly found in over-the-counter skin care products such as creams or pads. The U.S. National Library of Medicine discourages pouring salicylic acid down the drain or toilet and recommends dropping the product off at a drug take-back program location (Medlineplus, 2021). However, many might not realize this common skin care product is a pharmaceutical drug that requires proper disposal to prevent negative environmental effects.

In low concentrations, exogenous salicylic acid can even be beneficial to plant health, increasing seed germination, fruit yield, and photosynthetic rate. Additionally, plants may benefit from salicylic acid in stressful situations as it naturally controls hormonal stress responses. For example, a study focusing on tobacco and cucumbers noted that salicylic acid reduced or completely eliminated oxidative stress conditions from said plants being exposed to the herbicide paraquat (Hayat, 2009). However, these effects are not consistent with all plant species; only certain strains of Rhizobia bacteria reacted to additional salicylic acid, whereas the common bean and the Asiatic dayflower did not react at all. Concentrations higher than 10^{-5} M start to have an inhibitory effect, reducing growth and increasing stress.

Salicylic acid has not been closely monitored in the past, and only recently became classified as a contaminant of emerging concerns (CEC) (Lopez-Serna, 2019). A 2019 study evaluated the effectiveness of treating several pharmaceuticals, including salicylic acid, with agal bacterial photobioreactors. Two different configurations were considered: an anoxic-aerobic photobioreactor; and an anaerobic-anoxic-aerobic photobioreactor. These types of processes have

only recently been considered for treating CECs and utilize the "solar-driven conversion of carbon and nutrients from wastewater into algal-bacterial biomass" (Lopez-Serna, 2019). The first configuration had removal efficiencies between 63% and 83%, while the second varied between 34% and 97%. This may be due to the extremely high concentration of salicylic acid, roughly 10,756 ng/L, as this experiment focused on whether the configurations would remove salicylic acid rather than estimating from realistic wastewater values (Lopez-Serna, 2019). Theoretically, these photobioreactors could remove 93% to 98% of salicylic acid under average environmental conditions (Lopez-Serna, 2019). Despite this wide range of efficiency, the removal efficiency dropped after three to four days of HRT, indicating that biodegradation plays a key role in salicylic acid treatment.

Azithromycin is an acid stable, orally administered antimicrobial drug in the macrolide class used to treat different bacterial infections, especially in the lower and upper respiratory tracts (Peters, 2012). Recently, this antibiotic has been studied in combination with hydroxychloroquine and other medications to treat COVID-19. It is recommended to dispose of it through medicine take-back programs rather than flushing it down the toilet (Medlineplus, 2020).

Researchers in a YEAR study collected 72 samples from two Mid-Atlantic and two Midwest treatment plants and measured antibiotic concentrations using liquid chromatography (LC). Azithromycin had the highest concentration of all antibiotics in influent and effluent samples from both regions (Kulkarni, 2017). Other findings showed low-level antibiotic concentrations exist in reclaimed water used for irrigation. Even low concentrations of antibiotics can result in antibiotic resistance when combined with nutrients and bacteria (Kulkarni, 2017). The researchers concluded that the pharmaceuticals had entered the Mid-Atlantic wastewater treatment plant through domestic and hospital wastewater, whereas they entered the Midwest through domestic and agriculturally influenced stormwater.

Another study in Croatia investigated the effects of antibiotic-containing wastewater on bacteria, algae, and animals by analyzing river water directly downstream of pharmaceutical plants (Lehman, 2018). Azithromycin can persist in the natural environment for a long time and was one of the antibiotics detected in the river water. The study showed that fish embryos grown in this wastewater experienced development issues and they died within 24 hours of development. Additionally, the high levels of antibiotics also inhibited all algae growth. In general, discharge effluents containing pharmaceuticals, including azithromycin, alter physicochemical characteristics of receiving river sediments, which can contribute to macrolide-resistant genes (Milakovic, 2019).

1.3 Current Regulations

In the United States, the Environmental Protection Agency (EPA) is the primary governing body for effluent limitations in wastewater treatment plants (WWTP). As an extension of the Clean Water Act, the EPA publishes an annual review where these standards are evaluated and discussed for industrial categories (EPA,2018). The EPA also reviews previous Effluent Limitation Guidelines (ELGs) and publishes the Effluent Guidelines Program Plan every two years. These

regulations center around current wastewater treatment technologies and are updated as needed to "restore and maintain the chemical, physical, and biological integrity of the nation's waters" (Flanders, 2021). ELGs control the discharge standards from all different kinds of industries. These nationwide regulations aim to control the largest pollutants from each industry depending on toxicity, frequency, and location (Flanders, 2021). The EPA has prioritized 126 known pollutants based on their toxicity level; however, everyday pharmaceuticals do not fall on this list (Flanders, 2021). Because knowledge of their effect on the environment is limited, pharmaceuticals are slipping through the cracks of WWTP and their effects are increasing in impact.

In 1976, the United States implemented the Resource Conservation and Recovery Act, a set of regulations meant to control hazardous wastes from cradle to grave. Frequently used medicines such as epinephrine, warfarin, and chemotherapeutic drugs are subjected to these guidelines, but WWTPs are ill-equipped to treat these chemicals. Regulations on pharmaceuticals outside of the Resource Conservation and Recovery Act are lacking worldwide; What little information is presented, comes in the form of recommended guidelines for medical centers as opposed to concrete regulations. Currently, Australia is the only country whose drinking water regulations specifically address pharmaceuticals.

Pharmaceutical treatment has become a research focus in science in recent years but overcoming years of environmental impacts is not an easy feat. While research and regulations try to catch up, actions are being taken to stop the problem at the source. Prescription Drug Take Back programs currently provide the best way to dispose of unused or expired medicine safely. The Drug Enforcement Administration (DEA) and Environmental Protection Agency (EPA) have begun regulatory proceedings on the behalf of public health and negative environment effects (Barlas, 2009). The DEA periodically hosts National Prescription Drugs Take Back events where temporary drug collection sites are set up in communities nationwide. There are also permanent collection sites that can include retail, hospital, or clinic pharmacies.

1.4 Current Treatments

Typical prescription doses have such low concentrations, at least compared to the rest of the effluent, that their impacts were historically considered negligible. As a result, current knowledge of environmental effects and effective treatment methods is growing but still underdeveloped. Because of the wide range of pharmaceutical classes and functions, chemical properties vary too much for current treatment methods or to design broad treatment methods. Despite this, some effective treatment methods for prominent pharmaceutical pollutants have been discovered through research. For example, salicylic acid has been known to degrade in bioreactors containing *C. sorokiniana*. However, bioreactors are considered a sophisticated method for WWTP and an expensive addition even if they do show promise. Fortunately, Upper Blackstone Clean Water treatment plant utilizes such treatment processes and is located nearby in Millbury, MA, allowing the opportunity to investigate the effectiveness of bioreactors in treating pharmaceuticals.

As one of the largest treatment plants in New England, Upper Blackstone treats water for over 250,000 individuals in the Greater Worcester area (UpperBlackstone, 2016). This WWTP has a multi-step treatment process, beginning with bar screens and aerated grit chambers that remove large objects and dense solids that could potentially damage more sensitive machines. Wastewater is then moved into a primary clarifier to allow organic material and suspended solids to settle, while floating matter such as grease is skimmed from the top. Through this process, roughly half of present suspended solids and one third of the organic matter is removed (UpperBlackstone, 2016). The scum and settled sludge are moved to a holding tank that will mix the material before moving it into a press to remove excess water. The dried sludge enters a scrubber, which exits to a thermal oxidizer that decomposes gases before releasing them into the environment. Some sludge does not enter the oxidizer, instead being moved to a landfill as sterile ash. Meanwhile, the wastewater moves to a biological nutrient removal system. A bioreactor and final settling tanks to remove finer solids, dissolved metals, and organic material (UpperBlackstone, 2016). The final stop before being released into the Blackstone River is the disinfection tank. Wastewater is cleansed with sodium hypochlorite, then de-chlorinated with sodium bisulfate before finally leaving as the effluent stream. A more detailed flow diagram of the wastewater treatment process can be seen in Figure 3.

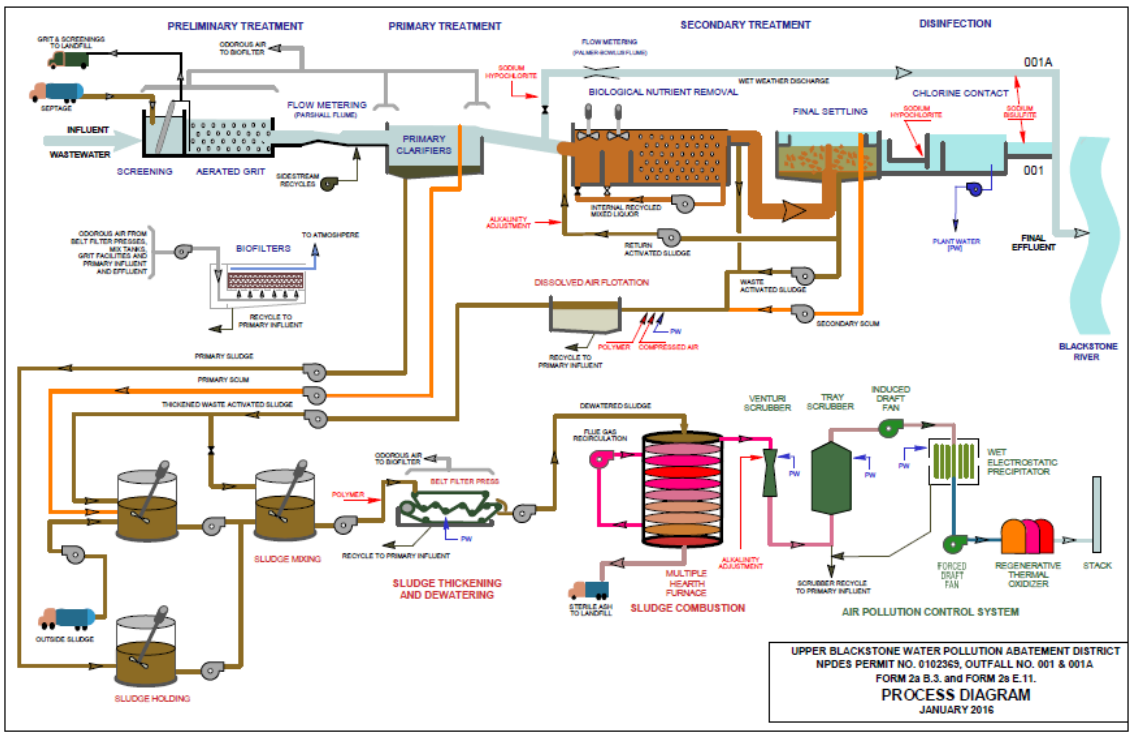


Figure 3. A visual representation of Upper Blackstone's treatment process, from Upper Blackstone's website.

The purpose of this study is to observe what effect each of these steps in Upper Blackstone's wastewater treatment process has on our selected pharmaceuticals. To achieve this, the first step is developing detection methods to identify and quantify the concentration levels of each pharmaceutical. Liquid chromatography and mass spectrometry (LC-MS) is currently the most popular detection and quantification method due to its ability to detect concentrations in the parts per billion (ppb) range. After determining the concentrations after each process, we hope to make recommendations to further improve the removal of pharmaceuticals in wastewater and analyze the concentrations of pharmaceuticals that may have leached into local bodies of water.

Methodology

The majority of micropollutants are removed from the influent stream via a variety of wastewater treatment plant processes. However, pharmaceuticals are not targeted by current treatment methods due to their low concentrations and relatively unknown impact on the environment and human health. The purpose of this study is to analyze the concentrations of select pharmaceuticals in the local Worcester county area in Massachusetts and to observe the effect that current wastewater treatment processes have on pharmaceuticals and water quality. To achieve this, the following objectives were pursued:

1: Determine current concentration levels of SMZ, salicylic acid, and azithromycin in three different bodies of water in Worcester, MA,
2: Analyze concentration of pharmaceuticals in influent and effluent streams of Upper Blackstone, as well as after each major treatment stage.
3. Recommend strategies to improve the removal of pharmaceuticals in WWTPs.

By performing solid phase extraction (SPE), we were able to isolate and analyze the pharmaceuticals. Through UV-Vis and LC-MS, the concentrations of SMZ, salicylic acid, and azithromycin were determined, as well as the overall effectiveness of Upper Blackstone's wastewater treatment process.

2.1 Sampling Scheme

Samples were collected from Upper Blackstone Clean Water treatment plant and three water bodies in Worcester, MA: Lake Quinsigamond, Green Hill Pond, and Salisbury Pond. By analyzing surface water samples, we were able to determine what concentration of pharmaceuticals were leaching into the environment and if they affected water quality. From the wastewater samples, we were able to determine if certain treatment steps were able to remove pharmaceuticals. Although the treatment process does not target pharmaceuticals directly, it is possible there may be a decrease in the concentration of pharmaceuticals during these processes as they target other pollutants.

After discussing Upper Blackstone's treatment process with several employees, we chose seven major treatment steps that potentially remove pharmaceuticals from the influent and provided glass collection bottles for each of those steps. Three composite samples were taken over a 24-hour period for the influent, primary effluent, and final effluent streams. Four grab samples were collected at some point during the 24-hour period after the anaerobic, anoxic, aerobic sections of the bioreactor, and after the final settling. Both the grab and composite samples were retrieved one day after collection. Because the composite samples consist of wastewater collected over a longer period of time, these samples were only compared to each other. Similarly, grab samples were only compared to each other. This is because the different kinds of samples, grab and

composite, were collected over different amounts of time and likely have different chemical profiles.

After settling has removed suspended particles from the influent stream, the wastewater travels to the biological nutrient removal bioreactors containing the following three sections: the anaerobic section, which converts organic pollutants into biogas in an oxygen-free environment (Veolia Water, 2021); the anoxic section that removes nitrogen from wastewater; and the aerobic section, where oxygen breaks down organic contaminants and other pollutants like nitrogen and phosphorus. After the bioreactor process, the wastewater comes out as the primary effluent. Final settling is the last process separating pollutants in the wastewater, before leaving as the final effluent.

In order to better understand environmental levels of SMZ, salicylic acid, and azithromycin, we collected samples from Lake Quinsigamond, Green Hill Pond, and Salisbury Pond. As an aggregate for runoff and common recreational spot, Lake Quinsigamond makes for a representative body that has a direct impact on the surrounding community. Green Hill and Salisbury Ponds are close by and likely have different chemical profiles we were also curious to explore. All samples were collected in one-liter plastic containers and rinsed three times with the sample water before a final sample was collected. The samples were stored in a cool dark area to prevent potential degradation of the pharmaceuticals. Once all of the raw samples were collected, they were prepared for analysis.

2.2 Sample and Standard Preparation

To calibrate the equipment, we created stock standards for each of our pharmaceuticals following the procedures in Appendix A. We purchased one gram of each pharmaceutical from Sigma-Aldrich. All other chemicals were available to us in the laboratory. Since the chosen pharmaceuticals are poorly soluble in water, they were dissolved in methanol to create standards for UV-Vis. For LC-MS, we created a serial dilution by dissolving the powdered pharmaceuticals in a few drops of methanol and diluting to different concentrations with deionized water. Procedures are available in Appendix A.

Because these pharmaceuticals are present in such low quantities, it was necessary to concentrate them for detection during UV-Vis sample analysis. To accomplish this, we left the sample containers open in a fume hood over a 24-hour period at room temperature to allow excess water to evaporate. By slowly evaporating our samples at room temperature, excess water will be removed without damaging the sample, subsequently providing a better analysis. This also prevented the pharmaceuticals from possibly suffering heat degradation .

To further isolate our chosen pharmaceuticals within the samples for LC-MS, we performed solid phase extraction (SPE). SPE is a common extraction method that separates compounds dissolved or suspended in the liquid mixture according to their physical and chemical properties. It works to isolate target analytes from a complex sample and remove sample components that may block instrument columns. It also significantly improves detection sensitivity by increasing analyte concentration, which is critical to our objective of achieving accurate levels

and signals of our antibiotics in surface water and wastewater samples. Since wastewater often contains very low chemical concentrations, as low as micrograms per liter, they may be undetectable by LC-MS without increased sensitivity. Thus, by performing SPE we hope to yield more successful results. According to literature, SPE has specifically proven effective when separating pharmaceuticals, which is the focus of this research. This method is quicker, less expensive, and more efficient at separation than liquid phase extractions.

To prepare for the solid phase extraction, we used optimized procedures specific to each antibiotic as shown in Appendix A. The materials used for extraction were acetone, methanol, sulfuric acid (H_2SO_4), ethylenediaminetetraacetic acid (EDTA), acetonitrile (ACN), hydrochloric acid (HCl), triethanolamine and calcium chloride ($CaCl_2$).Three mL SPE cartridges were used for all three antibiotics. For azithromycin, cartridges were preconditioned with acetone, methanol, and water. After the samples were acidified by adding H_2SO_4, they were passed through the cartridges and eluted with methanol in accordance to the procedures in Appendix A. For SMZ, the cartridges were preconditioned using the same methods as for azithromycin, but with adding EDTA to improve recovery efficiency. Note that Na_2EDTA was our first option as it is more soluble in water than regular EDTA, however this disodium salt did not ship in time for this procedure. For salicylic acid, after the SPE cartridge was prepared with ACN and HCl, the sample was prepared with triethanolamine, EDTA and $CaCl_2$ and then loaded on the cartridge. All samples were eluted with methanol, more detailed procedures can be found in Appendix A.

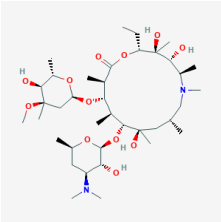
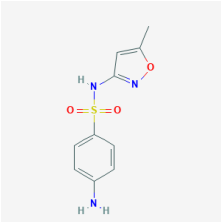
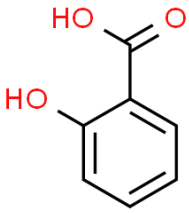
2.3 Analysis Methods

For our second objective, we chose the following analytical methods: Liquid Chromatography-Mass Spectrometry (LC-MS) and UV-Vis Spectroscopy. LC-MS has become increasingly popular for analyzing pharmaceuticals in recent years, as it is capable of performing very sensitive analyses. Chromatography can separate substances fairly easily due to its dependence on a compound's mobility in a certain solvent, making it ideal for analyzing complex samples. MS is a major analysis technique that relies on the molecular masses and abundance of different compounds. By analyzing its results, hypothetical chemical structures including these compounds can be formed. The results from liquid chromatography creates a clean sample that can be easily analyzed by a mass spectrometer, and clearly demonstrates if one of our pharmaceuticals is present. Then, by creating a calibration curve from the serial dilution in Appendix A, we were able to quantify the peaks produced by LC-MS. However, if the concentrations of our pharmaceuticals are too low, or there are too many contaminants, it may be difficult to determine which values belong to our pharmaceuticals. Because of this, we have also included UV-Vis analysis.

UV-Vis is a quick, simple analysis method readily available in most laboratories. It measures a chemical's ability to absorb different wavelengths of light. Because different chemical bonds and functional groups absorb at characteristic wavelength values, they can be used to determine present functional groups and their relationships to one another. This provides an easy way to determine whether our pharmaceuticals are present or not. Because this method analyzes

all chemicals in a sample, it will also tell us how well the water is being treated overall. With UV-Vis in mind, we found the structure, functional groups, volatility, and wavelengths for all three antibiotics to separate the analytes based on these characteristics. The table below was used to determine the above characteristics and were used when interpreting our graphs.

Table 1. This table shows the physical and chemical characteristics that belong to each of the chosen pharmaceuticals, azithromycin, sulfamethoxazole, and salicylic acid.

Antibiotic	**Structure**	**Functional Groups**	**Volatility**	**Wavelength**
Azithromycin		Ether (C-O-C), Alcohol (C-O-H), amine (N-C,N-H), ester (-COOC-), acetal (-O-C-O-)	No data	412 nm, if colored
SMZ		Amide (RC(=O)NR′R″)	Nonvolatile	200-300 nm
Salicylic Acid		Hydroxyl (-OH), carboxylic (-COOH), benzene ring	Steam volatile	230-300 nm

2.3.1 Ultraviolet-Visible (UV-Vis) Spectrophotometer

Ultra-violet (UV-Vis) spectroscopy is an effective tool for qualitative analysis and quantitative detection of contaminants in water samples including antibiotics. To use the UV-Vis, we blanked the instrument with water, then analyzed the three standard pharmaceuticals dissolved in methanol. The resulting graphs showed where to expect a signal. Then, we analyzed all other samples from WWTP and the environment. The range we chose was 200-400nm, due to expected pharmaceutical values and equipment limitations. We used quartz cuvettes instead of test tubes because they can absorb light more easily.

2.3.2 Liquid Chromatography - Mass Spectrometry (LC-MS)

Liquid chromatography is a widely used separation technique often used in tandem with mass spectrometry. The solubilized compounds, called the mobile phase, pass through a column packed with a stationary, or solid, phase. This method separates compounds based on their affinity to the mobile or stationary phase. The sample then passes through a mass spectrometer for analysis. This is a good option for larger compounds that are not volatile, such as SMZ. Optimization of the preparatory methods is required for successful LC-MS, with further details in Appendix A.

2.3.3 Additional Water Quality Tests

The physio-chemical characteristics of our water samples are important to analyze not only for the presence of pharmaceuticals, but also for water quality. About 1.8 billion people worldwide use unsafe water, with an additional 1.2 billion using water from sources with significant sanitary risks (Sila, 2018). We tested for water quality characteristics such as: general hardness (mg/l), nitrate (mg/l), nitrite (mg/l), free chlorine (mg/l), carbonate (mg/l) and pH, salinity, and coliform. The first five tests were completed using "SJ Wave" water test strips, pH tests with a pH meter, salinity tests through an electric conductor, and coliform using an "Aquavial" *E. Coli* kit. All tests were conducted with the original samples. For water quality characteristics, we immersed the strip fortwo seconds in the sample, removed it and then held it horizontally for 30 seconds. We compared the results against the given color chart shown in Figure 4.

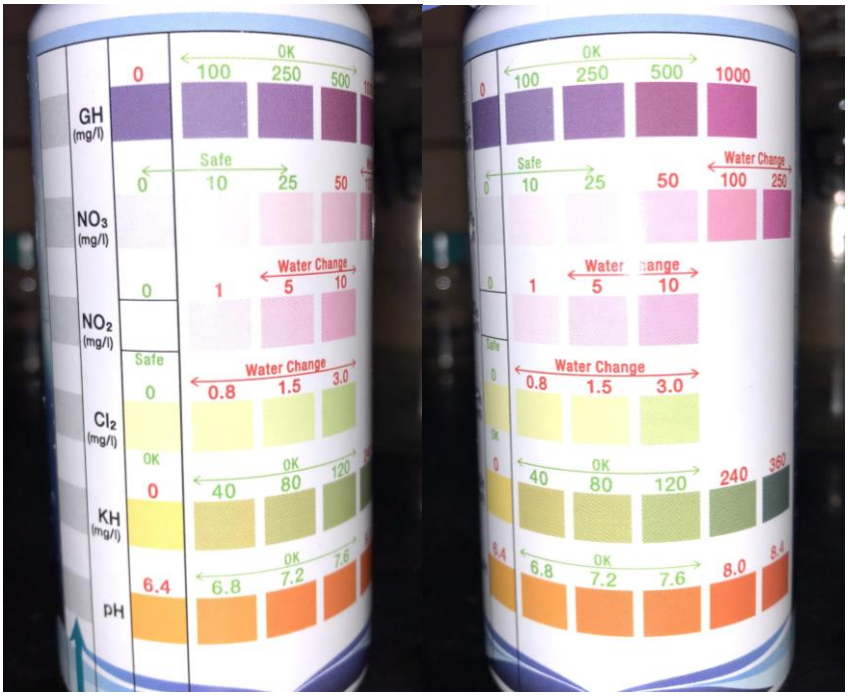


Figure 4. The water quality parameters starting from the top General Hardness, Nitrate, Nitrite, free Chlorine, Carbonate, and pH. This shows the acceptable values for each characteristic.

General Hardness

Hardness is a measurement of the dissolved minerals water contains. Soft water contains a low mineral content, while hard water has a high mineral content. This characteristic is important, as it is a critical factor in biological processes such as fish egg development. The strip for this property ranged from 0-1000 mg/l, where 100-500 mg/l was the range considered safe.

Nitrate/Nitrite

Microorganisms in soil, water and sewage convert nitrate to nitrite, which can be a significant health concern. For example, this changes hemoglobin in the blood to methemoglobin and reduces the amount of oxygen in the blood (California Health Services, 2000). It is ideal to have a nitrate level below 50 ppm, while nitrite level should be maintained at 0 ppm. To test for nitrate/nitrite, we used test strips that changed color depending on the amount of nitrate present. The nitrate value ranged from 0-250 mg/l, where 0-25 mg/l is considered safe. The nitrite value ranged from 0-10 mg/l, with 0-4 mg/l being the safe zone.

Free Chlorine

Free chlorine is a halogen chemical element that damages aquatic life-forms when concentrated. The presence of free chlorine in water indicates that enough chlorine to inactivate bacteria and some viruses was initially added to the water, and that the water is protected from recontamination during storage. A free chlorine level of 0.5 mg/L of free chlorine is enough residual to maintain the quality of water through the distribution network, but most likely will not be able to maintain the quality of the water when stored at home in a bucket or jerry can for 24 hours (CDC, 2020). The test strip we used ranged from 0-3 mg/l with 0-0.7 mg/l being the safe zone.

Carbonate

The carbonate hardness scale is based on the concentrations of carbonate and bicarbonate and reflects the water's buffering capacity. It is an important property, as water with high buffering capacity resists changes in pH, creating very stable water conditions. We used the test strip which ranged from 0-360 mg/l, with 40-240 being the safe zone.

pH

One of the most common water quality tests performed is a pH test, which indicates the sample's acidity. Since pH can be affected by chemicals in water, it serves as an indicator if water is changing chemically, and a very low or high pH may be from chemical or heavy metal pollution (Butler, 2019). To measure the pH, we used the same water quality test strips that once submerged in the sample it read its pH value.

Coliform

The coliform bacteria test is used to indicate the suitability of consumption for drinking water. Coliform bacteria are organisms that are present in the environment and that may cause gastrointestinal illnesses if present (Minnesota Department of Health, 2021). To test for it, water is added to a tube of broth and then incubated for 48 hours at 35-37 ^{o}C. The coliforms will be present if the broth changes from a yellow to red color.

2.4 Additional Analytical Methods for Future Studies

The following analytical methods have been used to identify and quantify other pharmaceuticals in literature. Gas chromatography (GC) and Fourier-transform infrared spectroscopy (FTIR) are two additional methods that may be helpful depending on the chemical properties of other pharmaceuticals. Given the nonvolatility of our selected pharmaceuticals, we opted for LC-MS and did not pursue GC to great lengths. Similarly, because wastewater samples may include a plethora of other chemicals, FTIR may be too sensitive for surface water or wastewater samples.

2.4.1 Gas Chromatography (GC)

Gas chromatography is an analytical separation technique used to analyze volatile substances in the gas phase (Thet, 2020). We introduced our sample into GC by injecting it through a syringe. After injection, the chemical components of our sample were first vaporized and since they were low concentration samples, the vapor cloud was transferred into the analytical column by carrier gas. The sample components were separated by their different interactions with the stationary phase, that is why it was important to be aware of the volatility and functional groups of the analytes as mentioned in Section 2.3 Table 1.

2.4.2 Fourier-transform infrared spectroscopy (FTIR)

Fourier-Transform Infrared Spectroscopy is the preferred, and most popular, form of infrared spectroscopy. Different functional groups absorb infrared light at varying wavelengths, with each type of chemical bond resulting in a unique wavelength value. An FTIR machine sends infrared light through a water sample, then forms a graph based on how the light is either absorbed by or passes through the sample. Because both different functional groups and different types of bonds (such as single, double, or triple) affect the graphs' peak width and length, an FTIR graph can be used to easily identify chemicals present in the sample.

Results and Discussion

3.1 UV-Vis Data Analysis

When UV-Vis samples have a strong signal, the absorbance of that signal could be measured, and the Lambert Beer Law used to analyze the results. This law relies on a beam of monochromatic parallel light that radiates the surface of the tested medium, and is the basis of quantitative analysis using UV-Vis. The mathematical expression is as follows:

$$A = log(1/T) = K * a * L$$

A= absorbance, T= ratio of the intensity of outgoing light to incident light, K= molar absorption coefficient, a= concentration of the absorbing substance, L=thickness of the absorbing layer (cm).

Unfortunately, the signals in our samples were too weak to definitively interpret as one of our chosen antibiotics. This is most likely because the concentrations of said antibiotics are very low, or the method did not separate the signals clearly. Overall, our graphs showed differences and shifts in graphs from sample to sample. The UV-Vis spectra of the standards clearly signal the presence of antibiotics as shown in Figure 5 and 6. For SMZ spectra, we see that it absorbs light between 210-310 nm, while salicylic acid absorbs between 210-330 nm. These spectra have many little peaks because the samples were dissolved in methanol, while the instrument was auto blanked using water. The antibiotics were dissolved in methanol because they were poorly soluble in water. For future studies, the graphs can be obtained more clearly using only one drop of methanol to dissolve and then diluted with water. In both the SMZ and salicylic acid spectra, we can see their highest peaks at around 240-250 nm, which were expected as compared to literature.

The azithromycin spectra were not similar to the previous two as shown on Figure 7. From literature values, azithromycin signal was expected at 200 nm. Since, the limit of the instrument was limited from 200-700 nm, no data could be collected before 200nm. However, we can see that around 200 nm, the spectrum starts to rise to a peak, which can be a signal of azithromycin.

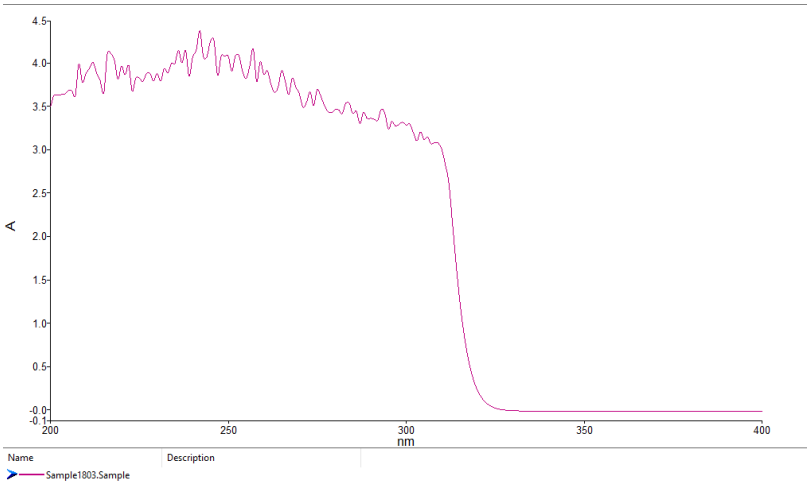


Figure 5. The UV-Vis Spectra of SMZ

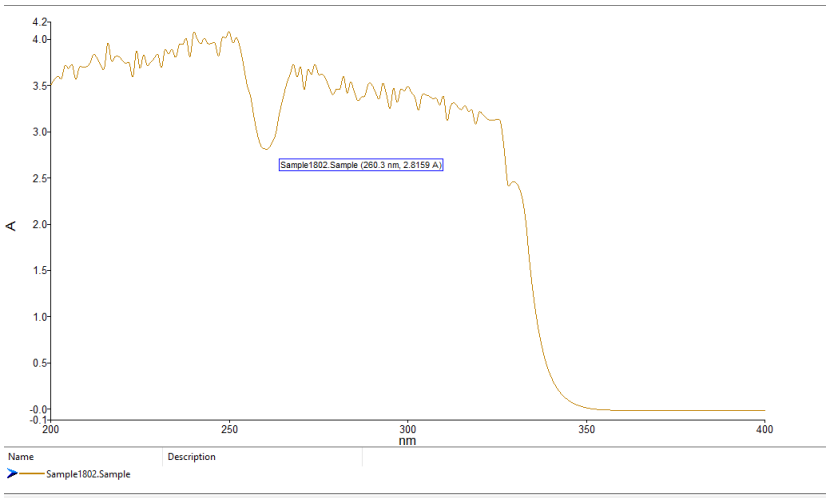


Figure 6. The UV-Vis Spectra of Salicylic Acid

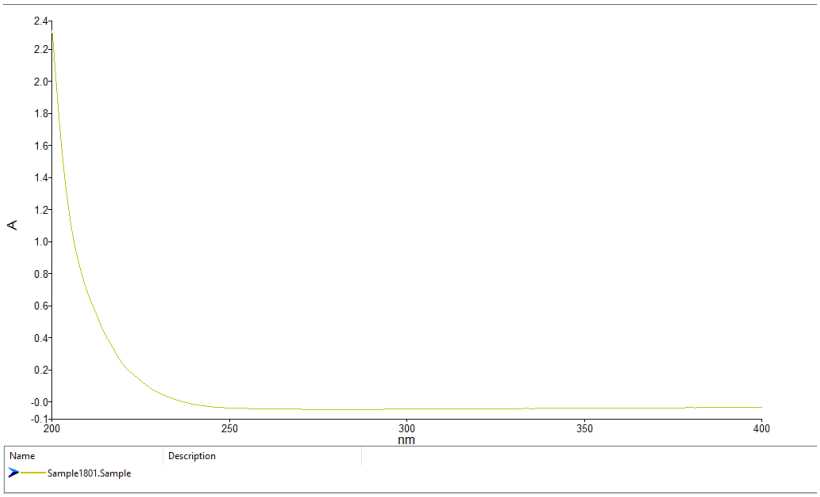


Figure 7. UV-Vis Spectra of Azithromycin

The next comparison we make, is between spectra of Influent and Final Effluent, as shown in Figure 8 and 9. As we can see, there is a difference in the shape of both spectrums. The Final Effluent spectra is smoother than the one of Influent, and this is expected as water has been treated between stages. The two graphs do not give us enough data to calculate or signal our antibiotics, but it gives us enough information to see which of the stages contains more pharmaceuticals or other elements. The Influent spectrum is steeper and has more curves than the Final Effluent. This makes sense because influent is the raw, untreated wastewater, while effluent is the last water treatment stage where the cleanliness of water is expected. The curves on the Influent graph and the flatness of Effluent show that the water treatment is effective on removing water pharmaceuticals and other waste elements. The other graphs of stages between Influent and Final Effluent are shown in Appendix B, and they also represent a waste elimination trend and flatness from one stage to another further down the wastewater treatment.

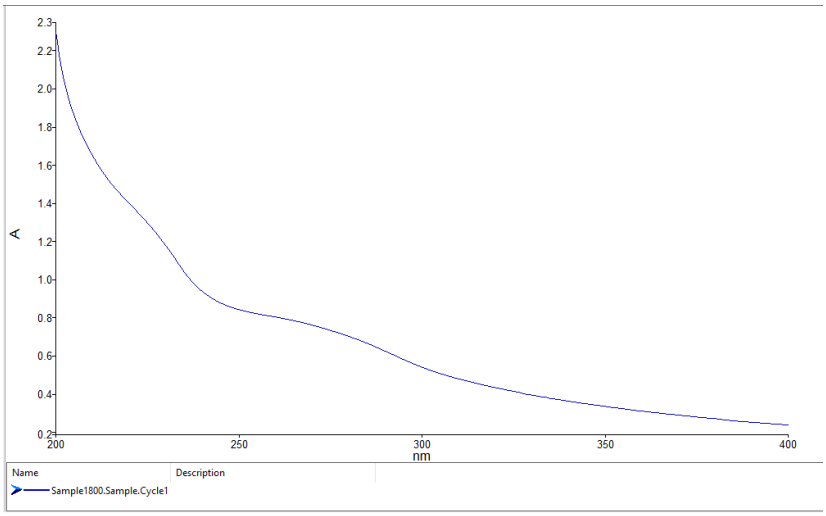


Figure 8. The UV-Vis Spectra of Influent

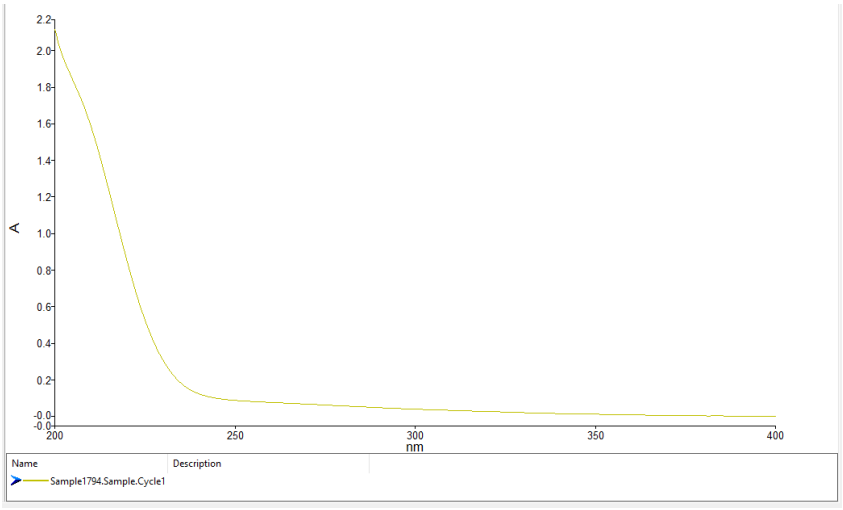


Figure 9. The UV-Vis Spectra of Final Effluent

Lastly, the UV-Vis Spectra between the three lakes were compared as shown in Figure 10, 11, and 12. Lake Quinsigamond (LQ) and Salisbury Pond (SP) have similar graph shape, while Green Hill Pond (GH) has a steeper shape. This can be interpreted as pharmaceuticals present in LQ and SP are in similar concentrations. However, it is expected that SP will be more polluted than the other two because it's suffocation from years of sedimentation build-up below its surface. More specific data could not be obtained from UV-Vis.

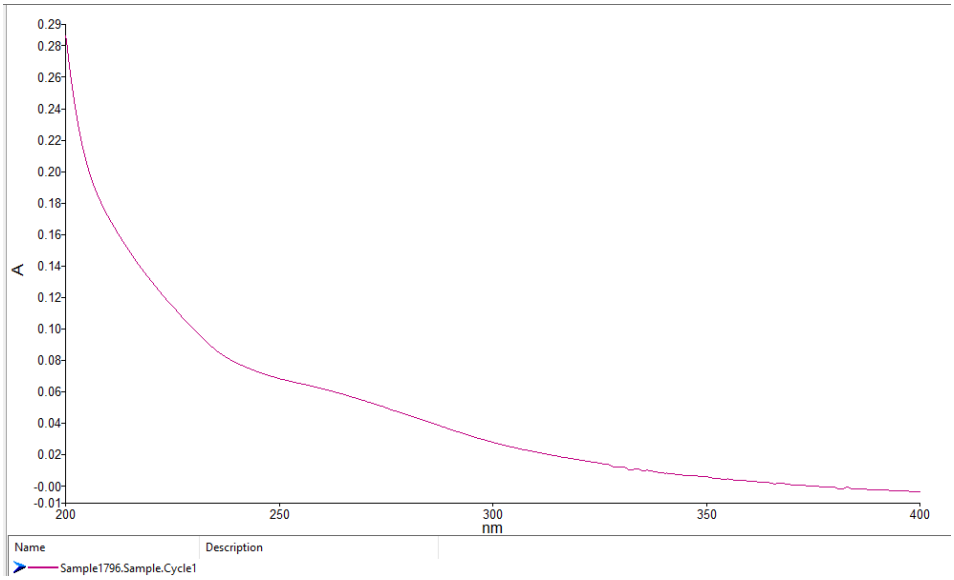


Figure 10. UV-Vis Spectra of Green Hill Pond

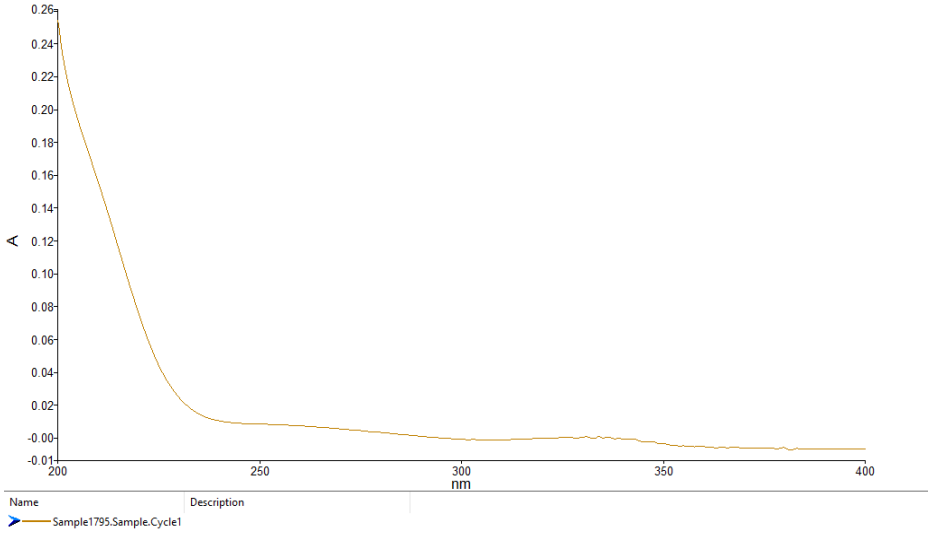


Figure 11. UV-Vis Spectra of Lake Quinsigamond

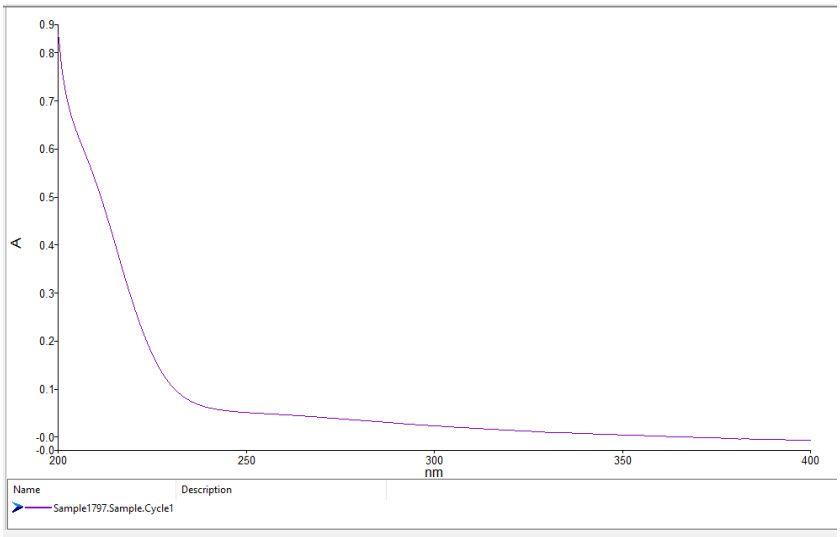


Figure 12. UV-Vis Spectra of Salisbury Pond

3.2 Coliform and Bacteria Test

To investigate the presence of *E. Coli* and coliform in wastewater, the tests were performed in three composite samples (influent, primary effluent, final effluent) and in the three bodies of water (Lake Quinsigamond, Green Hill Pond, and Salisbury Pond) as shown in Appendix E. Once the water sample was transferred to the test tube, it turned yellow, and after 48 hours of incubation at room temperature for certain samples the color had shifted from yellow to purple. Figure 13 shows the bacteria tests for the three water composites from WWTP. The Influent color had shifted completely from yellow to dark purple, the primary effluent color was mainly yellow but with some hints of purple, while the effluent color had remained purely yellow. This color change is consistent with our expectations as Influent is the raw, untreated wastewater which contains different pharmaceuticals, chemicals and effluent is the last stage of the treatment plant which is safer and well-treated for discharge. Primary effluent is the stage between which justifies the color it has as it is not completely treated. This result also implies that the wastewater treatment methods currently in place are effective.

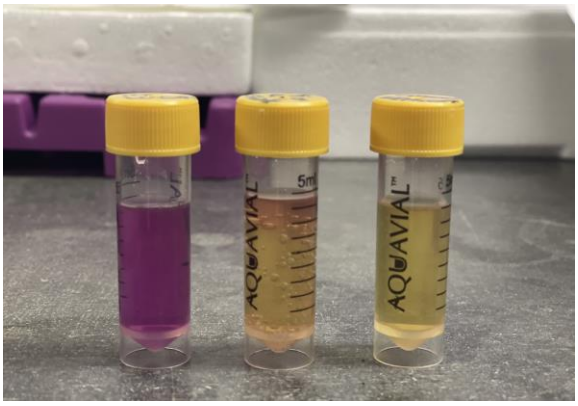


Figure 13. From left to right: influent, primary effluent, and final effluent coliform testing

The next comparison was between the three lakes as shown in Figure 14. As we can see after testing for the presence of bacteria, Lake Quinsigamond and Green Hill Pond had remained yellow, while Salisbury Pond had turned slightly purple, indicating a greater level of coliform contamination. This test was important because it quickly detects water quality issues before they become serious health risks. Even though this test is most commonly used on drinking water, seeing the level of bacteria within the environment can raise awareness about necessary steps to improve its quality. The test kit was able to detect *E. Coli* and Coliform bacteria concentrations as little as 1 CFU/ml.

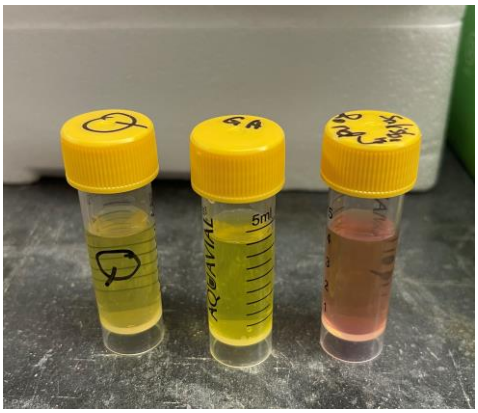


Figure 14. From left to right: Lake Quinsigamond, Green Hill Pond, and Salisbury Pond Coliform Testing

3.3 General Hardness, Nitrate, Nitrite, free Chlorine, Carbonate, and pH

The other water quality tests included parameters such as general hardness, nitrate, nitrite, free chlorine, carbonate, and pH. All images of the test strips can be found in Appendix E. The color changes were compared to the instructions given. To check if the tests worked accurately, we first submerged them in our three antibiotic standards which were dissolved in methanol and water as shown in Figure 15. Azithromycin is an acid-stable antibiotic, salicylic acid has two acidic groups and SMZ interferes with folic acid synthesis. This means that our antibiotics have an acidic profile, resulting in a pH lower than seven. This was confirmed with our test strips, where the pH square turned completely yellow indicating that the standard solution had a pH even lower than 6.4. This test confirmed that the quality test strips worked accurately.

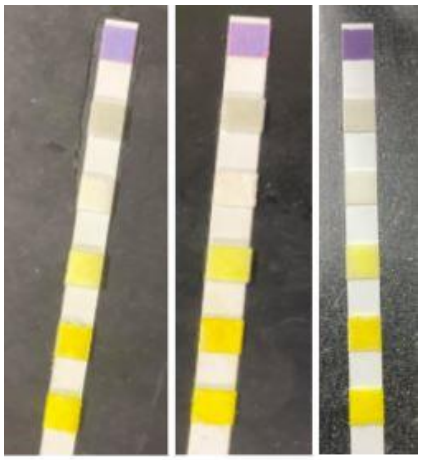


Figure 15. Standards test strips (from left to right: Azithromycin, Salicylic Acid, SMZ)

Next, test strips were used in all of our water samples as shown in Figure 16 and 17. There was consistency in the color changes and all of them within limits of each quality, however with slight changes. The general hardness turned purple in all of them indicating a general hardness value between 0-250 mg/l which is normal for a water sample. However, the aerobic, anoxic, and anaerobic samples have a slightly lighter purple color that means higher general hardness values. Different species require different water hardness, with 0-17.1 mg/l (soft), 17.1-60 mg/l (slightly hard), 60-120 mg/l (moderately hard), 120-180 mg/l (hard) and 180&over (very hard) (2). In our test strips which were commonly used for aquarium water testing, 100-500 mg/l was considered safe for fish species. The second quality was nitrate, which was considered safe from 0-25 mg/l with test strip color from white to light pink. We can see that from influent (white) to final effluent (slight pink) the level of nitrates has increased. High nitrate in the effluent is very normal for biological processes using aerobic processes, due to oxidation of nitrogen by nitrification process, which is present in our WWTP. This is similar to what happens with nitrite levels, the third test strip square that changes between processes from white to light pink. Compared to the test strips instructions, if the water was used for aquariums or for fish species, it would not be completely safe, and it would require water change.

The other water qualities tested were free chlorine and carbonate values. Free chlorine values varied between 0.8-3.0 mg/l and carbonate values between 40-240 mg/l. These ranges for free chlorine require water change for fishes, while the carbonate value above 120 mg/l isn't considered completely safe. However, during wastewater treatment chlorine is most widely used as a disinfectant because it destroys target organisms by oxidizing cellular material. That's why the results of the test strips for chlorine values are consistent with what was expected. The same analysis can be made for carbonate values which are related with alkalinity that is often used as an indicator of biological activity. These results make sense because lack of carbonate alkalinity stops nitrification which is an important step of WWTP. Lastly, pH was measured using the test strips. According to instructions, pH for all of the stages ranged between 6.8-7.6 which is considered safe.

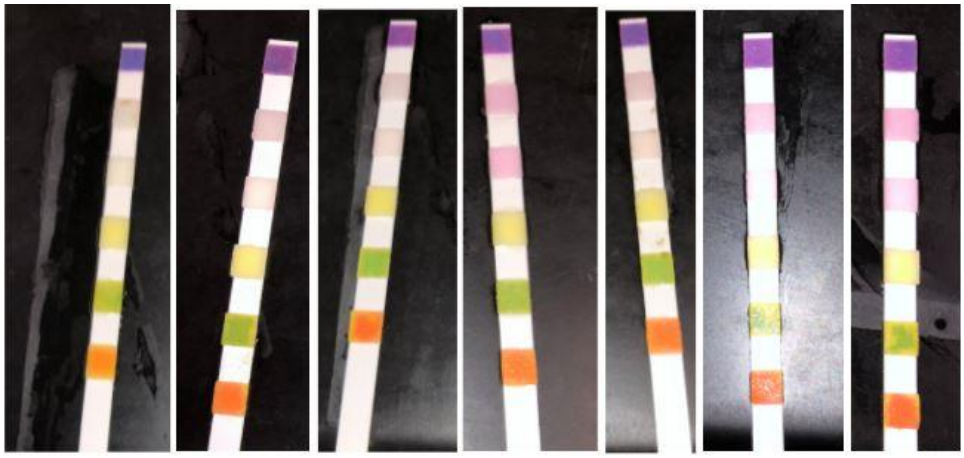


Figure 16. Water quality test strips (from left to right: Influent, Aerobic, Anoxic, Anaerobic Primary Effluent, Final Settling, Final Effluent)

The same analysis was performed for the three bodies of water: Salisbury Pond, Lake Quinsigamond and Green Hill Pond. The color changes on the test strips were similar to each other, but slightly different from the WWTP samples. The main difference is on nitrate/nitrite values, which for the lakes it is close to 0 mg/l. This is expected as these samples have not been treated yet and have not gone through the nitrification steps. Other qualities remain consistent between the lakes with close to safe regions.

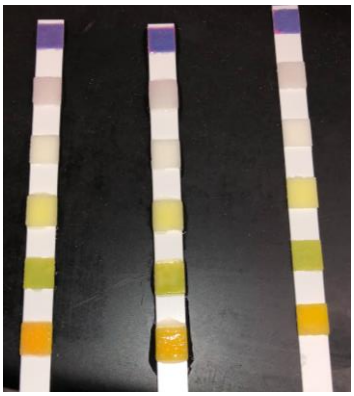


Figure 17. Water quality test strips (from left to right: Salisbury Pond, Lake Quinsigamond, Green Hill Pond)

3.4 LC-MS Analysis

After performing SPE, we had about 30 samples to test using LC-MS: seven wastewater treatment stages plus three surface water for each of the three selected pharmaceuticals. A complete list of these samples can be found in Appendix C, Table 2. We tested several standards to optimize the methods for LC-MS, and details of this optimization can be seen in Appendix A. To detect the pharmaceuticals, we used the scanning mode to identify the MS peaks using their chemical properties. Once we were able to identify the peaks in our standards, we were able to switch to the selected ion monitoring (SIM) mode to focus on each pharmaceutical. Since we

performed three different SPE procedures to elute the pharmaceuticals separately, this reduced the workload on the LC-MS and we could run all 51 standards and samples at once. We had 51 samples total, including 21 standards and 30 samples of unknown concentration.

In order to find the concentrations of our samples, we needed to make a calibration curve. By creating standards of known concentrations, we were able to compare these concentrations to the peak area in the MS reports. Once we have these data, we can use the trendline equation to work backwards to find the unknown concentration of our samples. For example, Figure 18 shows the calibration curve for SMZ. The linear relationship is represented by the equation below:

$$y = 246.91x \qquad \text{(Trendline equation for SMZ)}$$

We assumed the y-intercept to be zero to avoid receiving negative concentrations in our samples. This problem would be avoided altogether if we had run a blank sample on the LC-MS that went through our previous methods. Without this information, we are assuming all of the peaks present in the MS reports indicate our pharmaceuticals and trace contaminants from SPE.

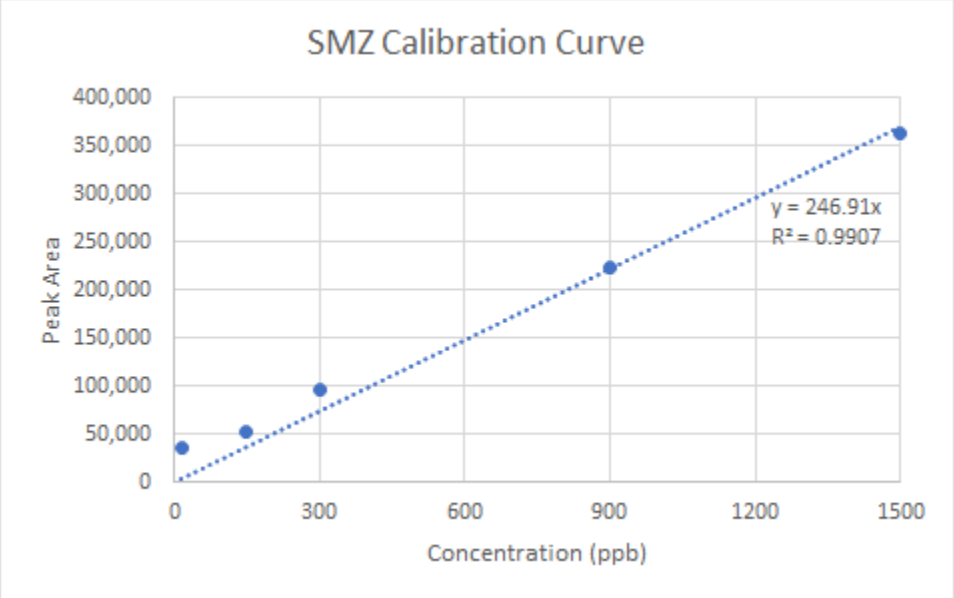


Figure 18. The linear calibration curve for SMZ shows concentration in parts per billion against peak area. We created standards of SMZ at various concentrations (see Appendix A) and analyzed them using LC-MS to find peak areas. By adding a trendline with y-intercept set to zero, we were able to retrieve the trendline equation that we then used to find the unknown concentration in our samples.

The R-squared value of 0.9907 in Figure 18 shows the high correlation between peak area and concentration in ppb. Using the equation above, the concentration of SMZ was found for each stage of Upper Blackstone's treatment process shown in Figure 19.

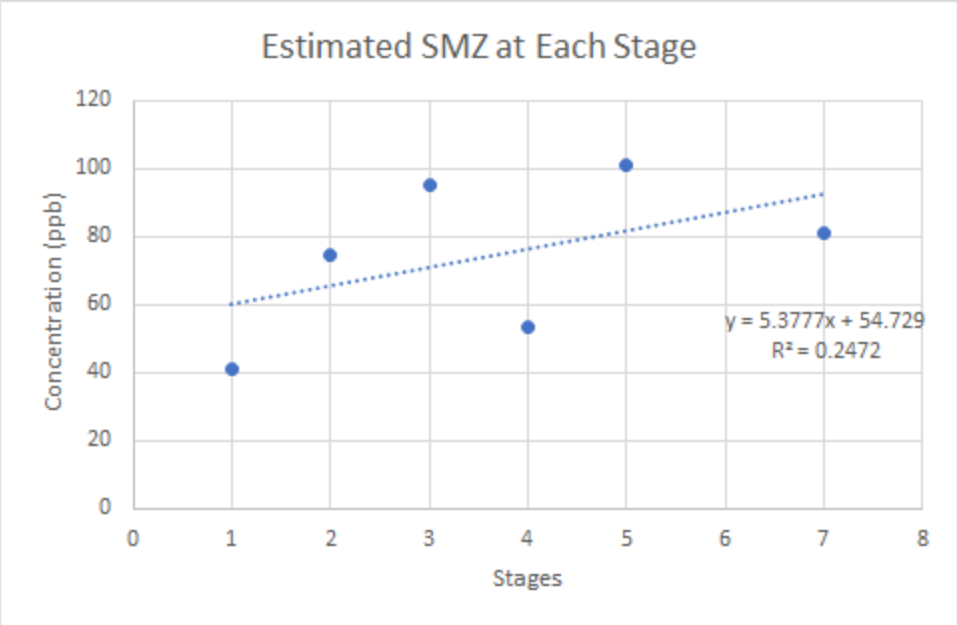


Figure 19. We estimated the concentration of SMZ by using the trendline equation in Figure 18 to calculate the concentration of our wastewater treatment samples. Stage one through seven on the x-axis represent the stages Influent (1), Primary Effluent (2), Anaerobic (3), Anoxic (4), Aerobic (5), Final Settling (6), and Final Effluent (7) of Upper Blackstone's treatment process.

Although we hoped to see a reduction in SMZ as treatment stages progressed, we see no significant trend in these results, as seen with the low R-squared value. Rather, the scatter represents the variance in samples and procedures, and is expected at such low concentrations. The concentration of SMZ stays between 40 and 100 ppb throughout the treatment processes. The data point at stage 6, the final settling sample, was not tested by LC-MS to save supplies for other samples.

The same procedure was followed for creating a calibration curve for both salicylic acid and azithromycin, which can be seen in Figure 20 and Figure 21, respectively. The linear trendline for salicylic acid had an R-squared value of 0.9697, which is high enough to estimate reliable concentrations for our unknown samples using the equation below:

$$y = 207.12x$$ (Trendline equation for salicylic acid)

The equation for azithromycin had a lower R-squared value of 0.9075, which makes for a less reliable concentration estimation for our samples. This could have occurred because as seen in Figure 21, the concentrations for the 15 ppb, 75 ppb, 150 ppb, and 300 ppb standards all resulted in very similar peak areas and threw off the trendline for azithromycin. Keeping this in mind, the following trendline equation was given:

$$y = 207.12x$$ (Trendline equation for salicylic acid)

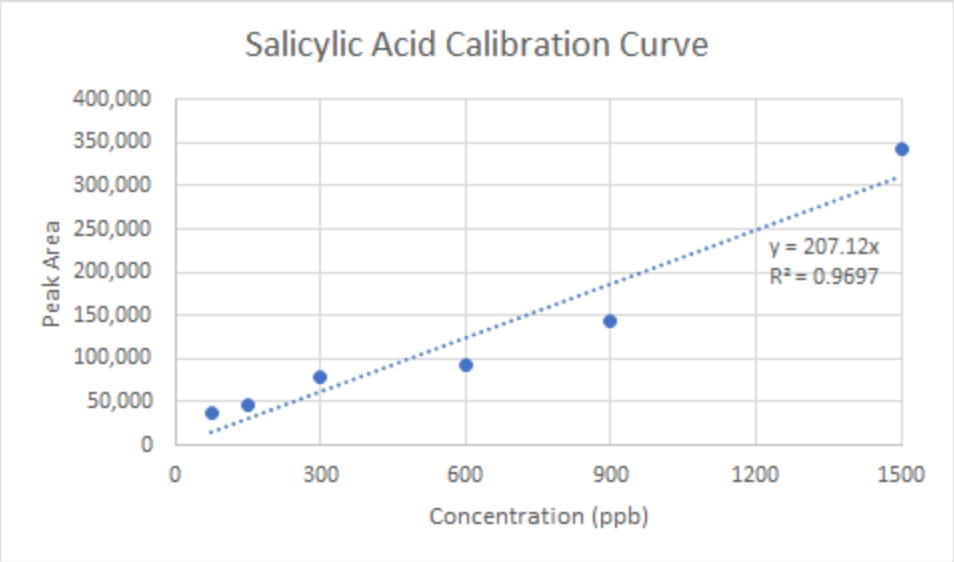


Figure 20. The calibration curve for salicylic acid shows concentration in parts per billion against peak area. By creating standards using procedures in Appendix A and analyzing them using LC-MS, we were able to create this linear curve to find the unknown concentrations of our samples for salicylic acid from the peak areas measured by LC-MS.

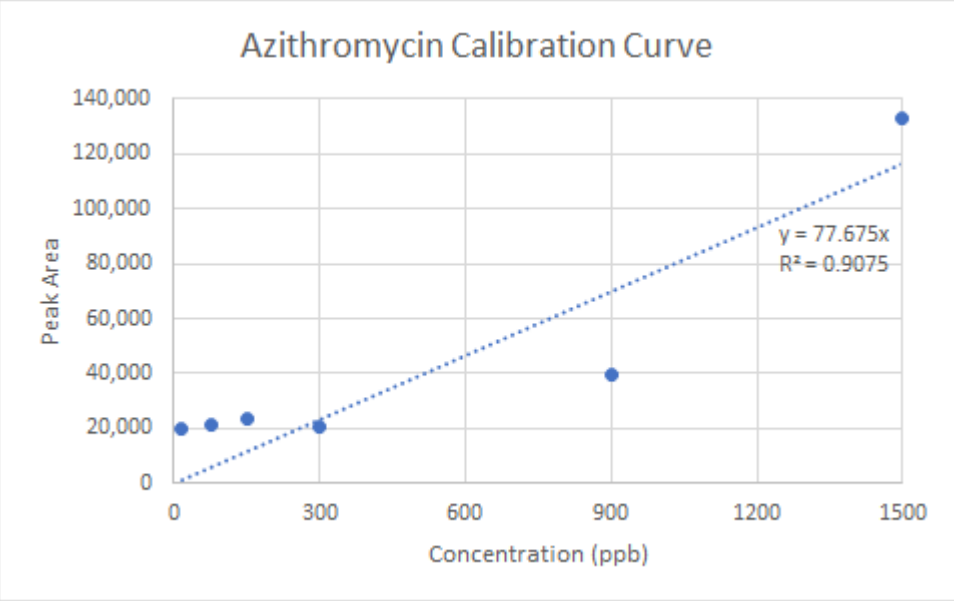


Figure 21. The calibration curve for azithromycin shows concentration in parts per billion against peak area. By analyzing standard concentrations of azithromycin using LC-MS, we were able to create this linear calibration curve to find the unknown concentrations of our samples from the peak areas.

There were five MS reports that were unable to be produced due to an error with the instrument. Details of these errors were noted in Table 2 in Appendix C. The other problem we encountered, in addition to no MS reporting, was not seeing peaks for salicylic acid. We think this occurred because the monoisotopic mass of salicylic acid was much lower than the other two

pharmaceuticals. Smaller chemicals are more common in the environment, and therefore salicylic acid has a higher chance of blending in with other contaminants or chemicals. As seen in Figure 22, only one sample had a peak area we could observe, and that was for stage 3, the anaerobic section of the bioreactor. With these limited data, we cannot say whether there was a decrease in the concentration as treatment processes continued, but we can report that the approximate concentration of salicylic acid in Upper Blackstone's wastewater is about 50 ppb.

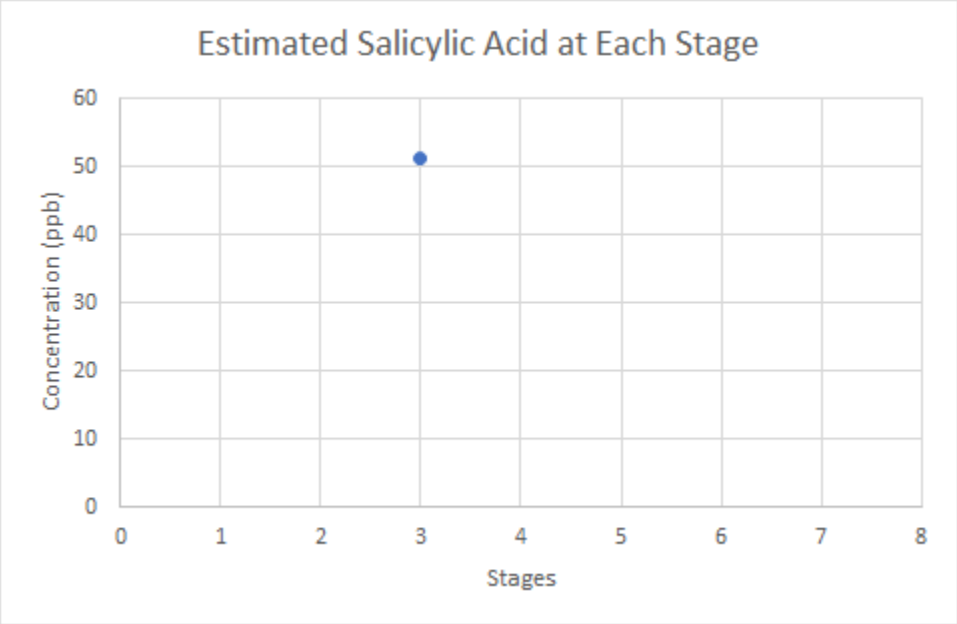


Figure 22. Due to a combination of instrument error and possible contamination, two MS reports were not produced, and the remaining reports showed no sign of salicylic acid peak, therefore the area was not found. The one successful MS report showed an approximate concentration of 50 ppb salicylic acid at the anaerobic stage.

Figure 23 yielded a more complete set of results for the estimated concentration of azithromycin. Although the trendline shows a negative slope, the scatter and R-squared value show there is no reliable trend at each stage. However, the data show the concentration of azithromycin stays pretty consistently between 200 and 300 ppb throughout the wastewater treatment process. This is much higher than SMZ and salicylic acid concentrations in these wastewater samples, even though we expected higher concentrations of SMZ.

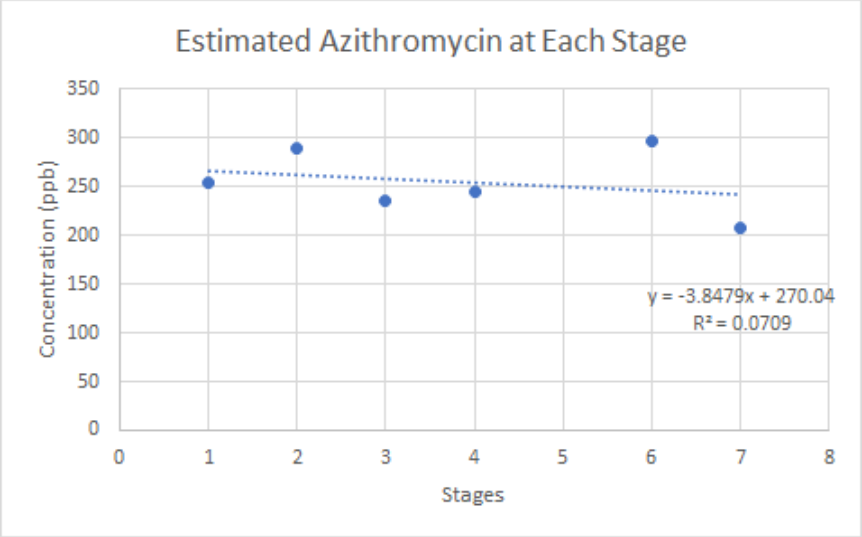


Figure 23. By comparing the estimated concentration of azithromycin at each stage, this scatter shows no reliable reduction of the pharmaceutical by Upper Blackstone's treatment processes.

The LC-MS results for surface water samples taken from Lake Quinsigamond, Green Hill Pond, and Salisbury Pond show similar levels. Only one surface water sample was tested for SMZ, Lake Quinsigamond, and about 63 ppb was detected. In Figure 24, we can see the concentration is slightly higher for salicylic acid, at 74 ppb. The salicylic acid concentration jumps for Salisbury Pond to around 134 ppb. Azithromycin has the highest concentrations by far for all three bodies of water at 276 ppb, 219 ppb, and 282 ppb for Lake Quinsigamond, Green Hill Pond, and Salisbury Pond, respectively. This shows azithromycin levels should be monitored in all Worcester bodies of water, and Salisbury Pond, specifically, should be monitored for pharmaceutical contamination in general.

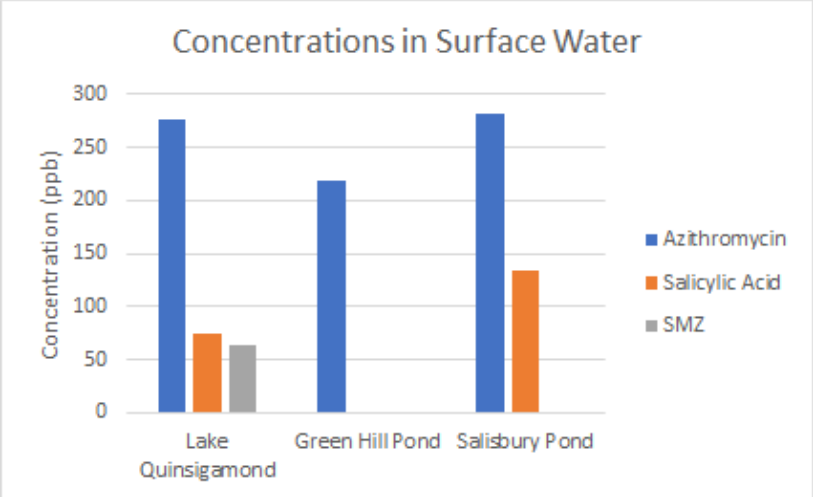


Figure 24. MS data show the concentration of SMZ, salicylic acid, and azithromycin in Lake Quinsigamond, Green Hill Pond, and Salisbury Pond. Due to complications, SMZ samples for Green Hill Pond could not be tested, in addition to both SMZ and salicylic acid samples for Salisbury Pond.

For our final LC-MS sample, we ran two tests on the same 1500 ppb standards for each of the pharmaceuticals. This was to show reproducibility and consistency in data. As seen in Figure 25, only a small amount of each sample may have evaporated by the end of the 19-hour sequence. Preparing the samples in LC-MS compatible vials was performed the same day as the run because the samples were eluted in methanol, a highly volatile chemical. Figure 25 shows the most evaporation occurred for SMZ. This was expected since SMZ was the first pharmaceutical we tested, and once the cap of the vial was breached by the LC-MS it became much less airtight, leading to some evaporation.

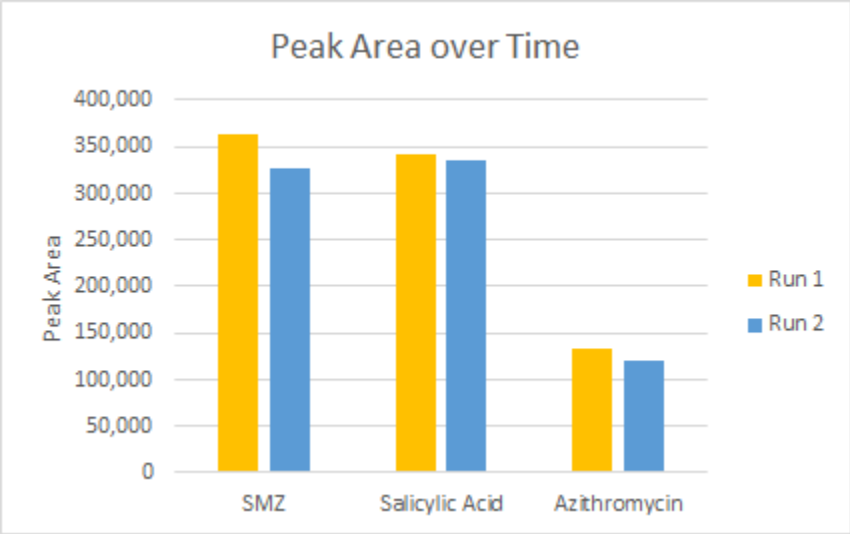


Figure 25. Run 1 and Run 2 of the same 1500 ppb standards for each pharmaceutical. Run 1 was tested first, and the second run happened at the end of all the LC-MS runs. This was to observe reproducibility with this method, and to see if evaporation occurred. This figure shows only a small amount of each sample evaporation by the end of the total run, 19 hours later.

Conclusions and Recommendations

Overall, our study confirmed the rising concern of pharmaceuticals in wastewater and the necessity of further research on possible treatments. We saw a large decrease in UV-Vis detection and bacteria between the influent and final effluent samples, indicating the overall effectiveness of Upper Blackstone's wastewater treatment process. However, our LC-MS data are unable to prove whether the WWTP's current system is capable of effectively treating SMZ, azithromycin, salicylic acid, or other pharmaceuticals. There was difficulty detecting salicylic acid in the wastewater samples due to possible contamination and instrument error, while SMZ and azithromycin concentrations remained relatively consistent throughout treatment. This proves current wastewater treatment methods alone are not enough to treat pharmaceuticals. If pharmaceuticals were to become more regulated, modifications or additional treatment methods may be required to directly treat pharmaceuticals. SMZ, azithromycin, and salicylic acid were found in such low concentrations it becomes uncertain whether a treatment plant is even capable of reducing these contaminants.

For future studies, we recommend LC-MS, FTIR, and GC analysis depending on the characteristics and chemical properties of the chosen pharmaceuticals. As for our methods, we recommend using as little methanol as possible to dissolve the pharmaceuticals for UV-Vis, this may improve identification in spectra. For LC-MS, a blank sample containing only water should undergo the same procedures as unknown samples. This blank will help identify pharmaceuticals in the MS reports by giving a baseline and showing possible contamination due to SPE or in general.

We recommend WWTPs repeat our experiments to a more in-depth level and consider our aerobic recycling stream design if a bioreactor is already in place. From the data gathered, we have designed one possible minimally intrusive modification to Upper Blackstone's current treatment system, which is detailed below.

Design of an Aerobic Recycling Stream for Pharmaceutical Treatment

To increase Upper Blackstone's efficiency in treating pharmaceuticals, particularly SMZ, azithromycin, and salicylic acid, a design was developed for an aerobic tank recycling stream. Results from our LC-MS analysis clearly indicated the presence of all three pharmaceuticals in both our wastewater and environmental samples, making it prudent to consider methods of treating said contaminants. Both a second biological reactor and recycling stream were considered. Biological reactors have proven effective in definitively treating salicylic acid, but have not been researched for SMZ or azithromycin, are very expensive, and would require temporarily closing Upper Blackstone while the tank was implemented into the treatment system. Adding bacteria known for treating salicylic acid to the pre-existing aerobic tank was

considered, but similar problems arose, and it is unknown how these bacteria would react with the current bacteria or treatment of other contaminants. LC-MS results show that little treatment was successfully completed with the current system, but it is unknown if this is because of inadequate methods or because of extremely low concentrations. Thus, to aid us in narrowing our design, we looked at the results from our UV-Vis spectroscopy. This showed a clear, significant decrease in contaminants around 300 nm, the range our respective pharmaceuticals are visible in. Because of this, the aerobic tank was chosen to focus on.

Aerobic tanks require air to function, specifically oxygen. It provides the bacteria necessary fuel, while also mixing the bacteria, wastewater, and contaminants. The more mixed treatment is, the more bacteria will have access to contaminants that can be consumed and removed from the water. Thus, it was important to design a recycling stream around oxygen values within the water. The average dissolved oxygen in the aerobic tank was 0.6 mg/L, or 103 kg/day, while the biological oxygen demand was 22,077 kg/day for the entire aerobic tank. This means that an average of 6,043 kg oxygen is consumed each time the aerobic tank completes a single run time. The volume of the tank can only absorb 189.1 kg of oxygen at a time, so this is the amount that would be added into the recycling stream. The recycling stream itself is 359.8 ft long, requiring 10 pumps to fully transport the wastewater to the beginning of the aerobic tank. The air blowers used at Upper Blackstone are capable of 17,900 acfm, so only one is needed to reach the dissolved oxygen limit. To ensure even mixing throughout the pipe, 5 air blowers are placed every 72.0 ft along the recycling stream. The remaining 5,853.9 kg of necessary dissolved oxygen is added in the aerobic tank with pre-existing air blowers during the treatment process. After the wastewater has been treated a second time, it exits the aerobic tank and travels to final settling. A second set of calculations was completed with a dissolved oxygen level of 1.0 mg/L. This is the lower average dissolved oxygen level for wastewater treatment plants. While overall oxygen consumed is reduced to 6025 kg/run time, this value still allows adequate treatment, and the increased air flow provides a more thorough mixture. The wastewater in the recycling stream has already been treated by the aerobic tank before, so additional mixing may allow for contaminants that were missed due to low concentrations-such as pharmaceuticals- to be treated a second time through.

Both dissolved oxygen levels have benefits- 0.6 mg/L ensures that the system remains at levels known to function well within the aerobic tank, while 1.0 mg/L increases mixing and may provide a more thorough pharmaceutical treatment. It is recommended that Upper Blackstone conducts a more in-depth analysis of this design to ensure optimal treatment efficiency and system assimilation. This recycling stream is fairly simple in design, allowing for modifications as necessary, and reproducibility for other treatment plants that could benefit. It is a safe, inexpensive method of increasing treatment efficiency with minimal impact on the environment or surrounding community, for preemptive pharmaceutical treatment or simply increasing efficiency. Detailed calculations for this design with both dissolved oxygen values can be found in Appendix D.

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Appendix A: Lab Procedures

Creating 300 ppm Stock Standards:

1. Measure out about 0.03g of the pharmaceutical
2. Dissolve in as little methanol as possible
3. Add 100 mL of DI water to create a concentration of approximately 300 ppm or 300 μg/mL and store in sealable 5-dram vial

Creating Serial Dilution:

15 ppb:

1. Add 1 drop of 300 ppm stock standard to a 200 mL beaker
2. Add 100 mL of DI water
3. Transfer 1 mL of that solution to a 5-dram vial
4. Add 9 mL of DI water to create a 15 ppb or 0.015 μg/mL lower limit standard

75 ppb:

5. Add 5 drop of 300 ppm stock standard to a 200 mL beaker
6. Add 100 mL of DI water
7. Transfer 1 mL of that solution to a 5-dram vial
8. Add 9 mL of DI water to create a 75 ppb or 0.075 μg/mL lower limit standard

150 ppb:

1. Add 1 mL of 300 ppm stock solution to a 200 mL beaker
2. Add 99 mL of DI water
3. Transfer 1 mL of that solution to a 5-dram vial
4. Add 19 mL DI water to create a 150 ppb or 0.15 μg/mL standard

300 ppb:

1. Add 1 mL of 300 ppm stock solution to a 200 mL beaker
2. Add 99 mL of DI water
3. Transfer 1 mL of that solution to a 5-dram vial
4. Add 9 mL DI water to create a 300 ppb or 0.3 μg/mL standard

600 ppb:

1. Add 1 mL of 300 ppm stock solution to a 200 mL beaker
2. Add 99 mL of DI water
3. Transfer 2 mL of that solution to a 5-dram vial
4. Add 8 mL DI water to create a 600 ppb or 0.6 μg/mL standard

900 ppb:

5. Add 1 mL of 300 ppm stock solution to a 200 mL beaker
6. Add 99 mL of DI water
7. Transfer 3 mL of that solution to a 5-dram vial
8. Add 7 mL DI water to create a 900 ppb or 0.9 μg/mL standard

1500 ppb:

1. Add 1 mL of 300 ppm stock solution to a 200 mL beaker

2. Add 99 mL of DI water
3. Transfer 5 mL of that solution to a 5-dram vial
4. Add 5 mL DI water to create a 1500 ppb or 1.5 µg/mL upper limit standard

Macrolide Antimicrobials (Azithromycin) Extraction:

1. SPE cartridges were preconditioned with the following:
    a. 2 mL of acetone
    b. 2 mL of methanol
    c. 2 mL of water (pH 6.0)
2. The effluent samples (2 mL) were passed through the cartridges at a rate of approximately 1 drop/second.
3. After passage of the samples, each cartridge was eluted with 2 mL of methanol.

Sulfonamide Antimicrobials Extraction:

1. The previous SPE extraction procedure was adapted where this time the chelating agent, ethylenediamine tetraacetate (EDTA), was added to samples to improve recovery efficiency.
2. The SPE cartridges were preconditioned with the following:
    a. 2 mL of acetone
    b. 2 mL of methanol
    c. 2 mL of 50 mM EDTA
3. The effluent samples (2 mL) were acidified to pH 3.0 with 3.0 M H_2SO_4
4. Then followed by the addition of EDTA (0.1 g)
5. Samples were then passed through the SPE cartridges at a rate of approximately 1 drop/second
6. Just as before, after passage of the samples, each cartridge was eluted with 2 mL of methanol
7. The eluates were collected in a sealable 5-dram vials

Salicylic Acid Extraction:

1. SPE cartridges were preconditioned with the following:
    a. 1 mL ACN
    b. 2 mL 50 mmol/L HCl
2. The effluent samples were prepared by adding the following:
    a. 1 mL of a 50 mmol/L triethanolamine,
    b. 0.2 mmol/L EDTA,
    c. 0.4 mmol/L $CaCl_2$ to the sample, then adjusting the pH to 7.5 with HCl.
3. Each cartridge was eluted with 2 mL of methanol

LC-MS: Agilent Technologies 5130 Quadrupole Methods:

- Pharmaceuticals were separated with an Epic C18 MSO 2.3 µ 150 Å 5 cm × 2.1 mm column
- Water with 0.1 % formic acid (A) 95 % acetonitrile with 5 % water and 0.1 % formic acid (B)
- The column was maintained at 30 °C at a flow rate of 0.3 ml/min
- Injection volume 2 µL with 10 sec needle wash and m/z 80-900

LC-MS Optimized Methods:

We used the scanning mode of LC-MS to observe the MS peaks for our samples. The first method used m/z 180-1200, which was too high and excluded salicylic acid. Since salicylic acid is a relatively small compound it may be harder to identify the MS peak. Once we were able to separate and identify the peaks in our samples, we were able to switch to the selected ion monitoring (SIM) mode to focus on each pharmaceutical. Since we extracted each pharmaceutical individually, we were able to use SIM to target the specific pharmaceutical in each sample. This reduced the workload on the instruction and improved MS results. We had 51 samples in total, shown in Table 2 of Appendix C, 21 standards and 30 samples. We chose to create standards ranging from 15 ppb to 1500 ppb to create the calibration curve.

Monoisotopic Masses:

- SMZ - 254 g/mol
- Azithromycin - 749 g/mol
- Salicylic Acid - 138 g/mol

Appendix B: UV-Vis Spectra

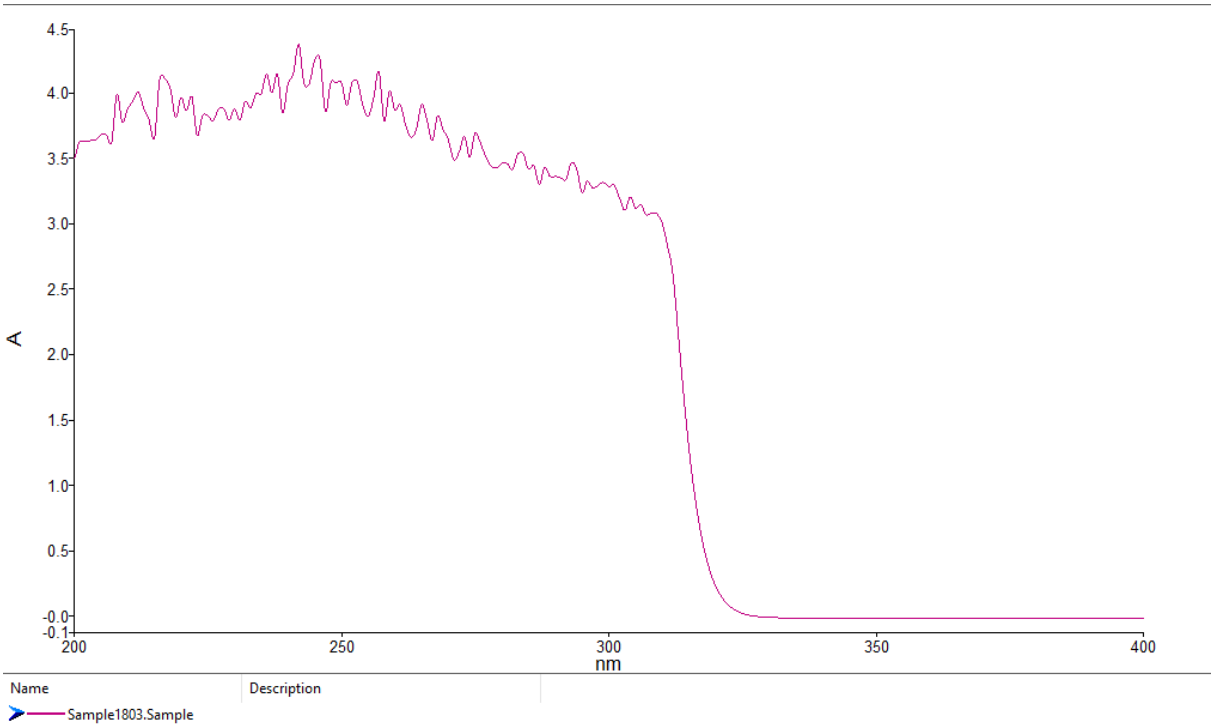


Figure 5. UV-Vis Spectra of SMZ Standard

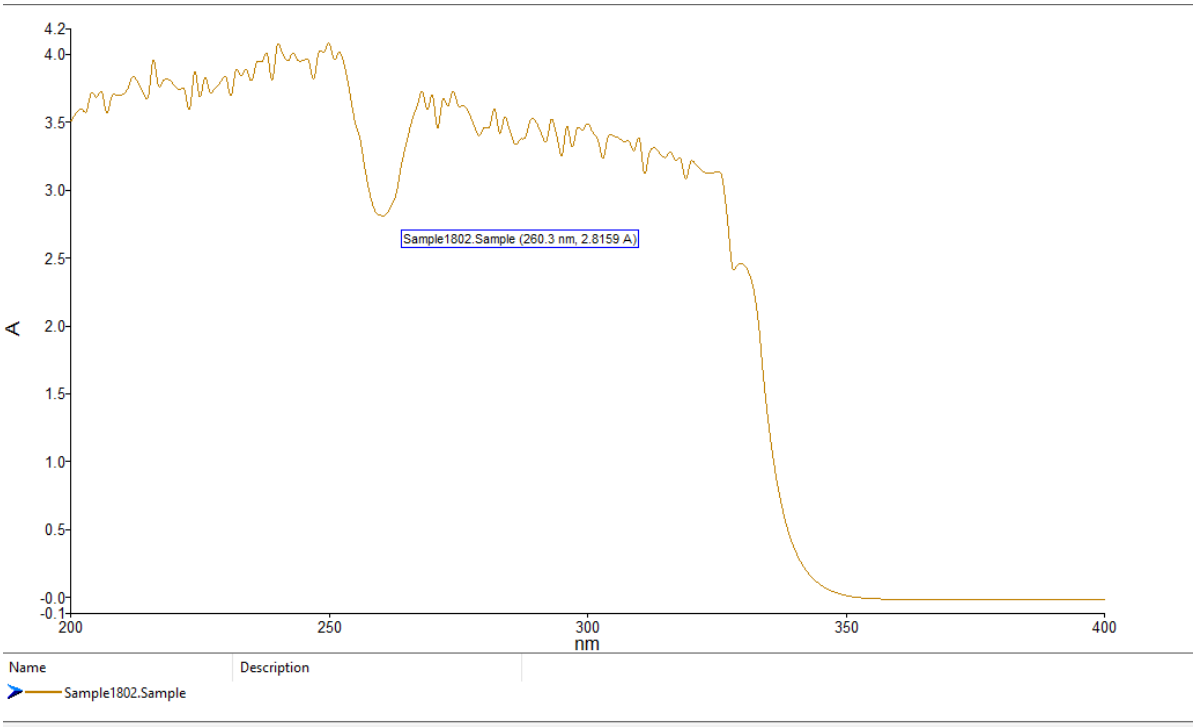


Figure 6. UV-Vis Spectra of Salicylic Acid Standard

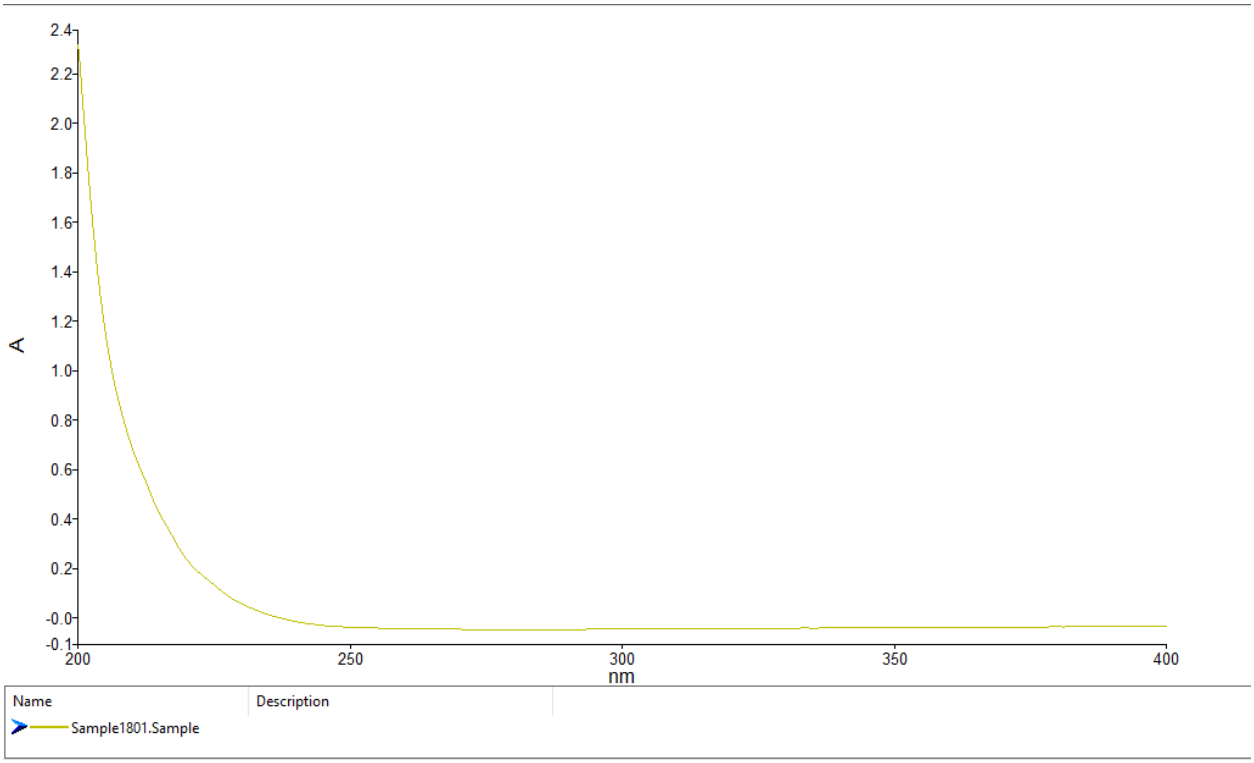


Figure 7. UV-Vis Spectra of Azithromycin Standard

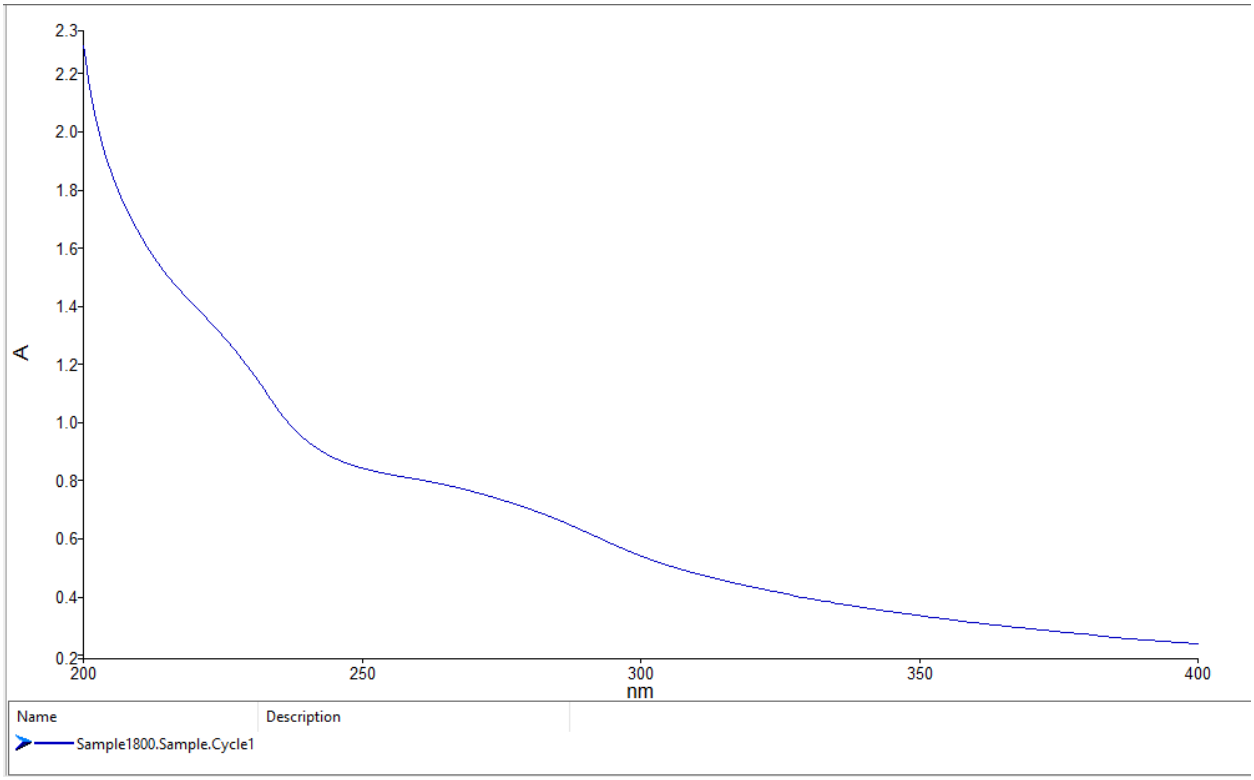


Figure 8. UV-Vis Spectra of Influent Sample

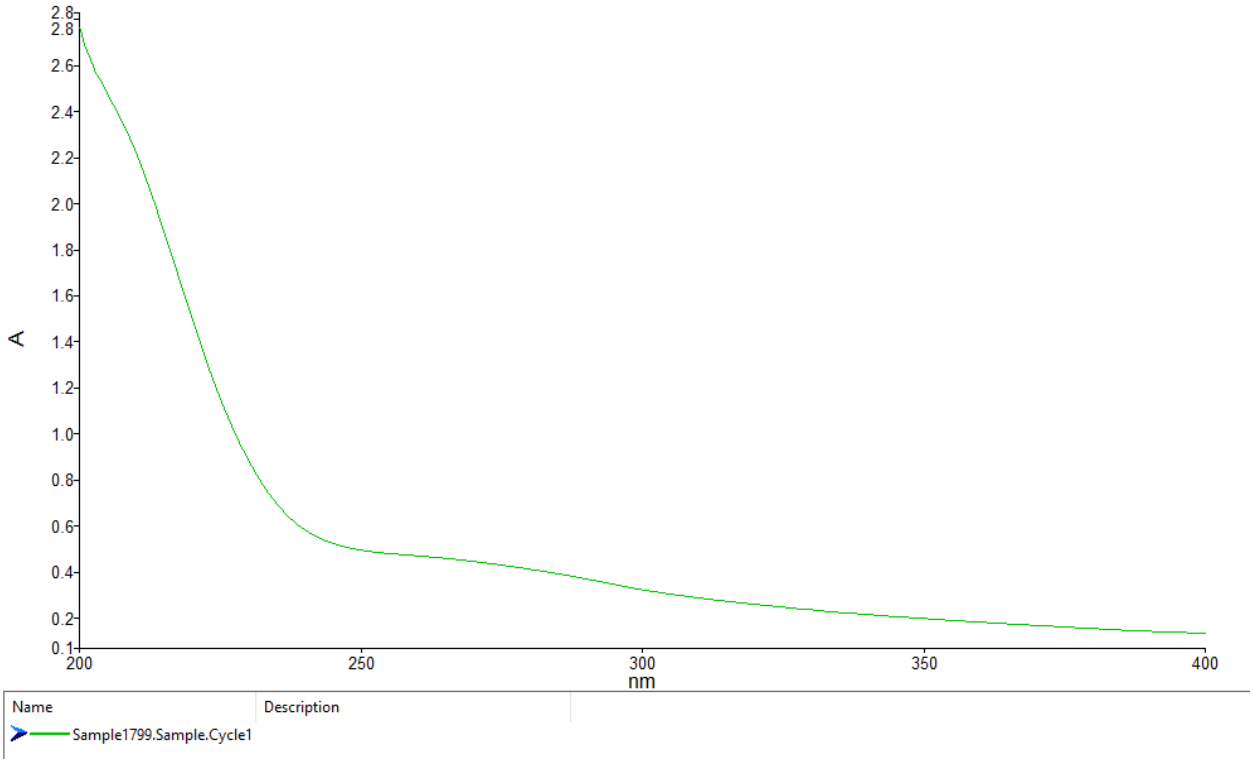


Figure 26. UV-Vis Spectra of Primary Effluent Sample

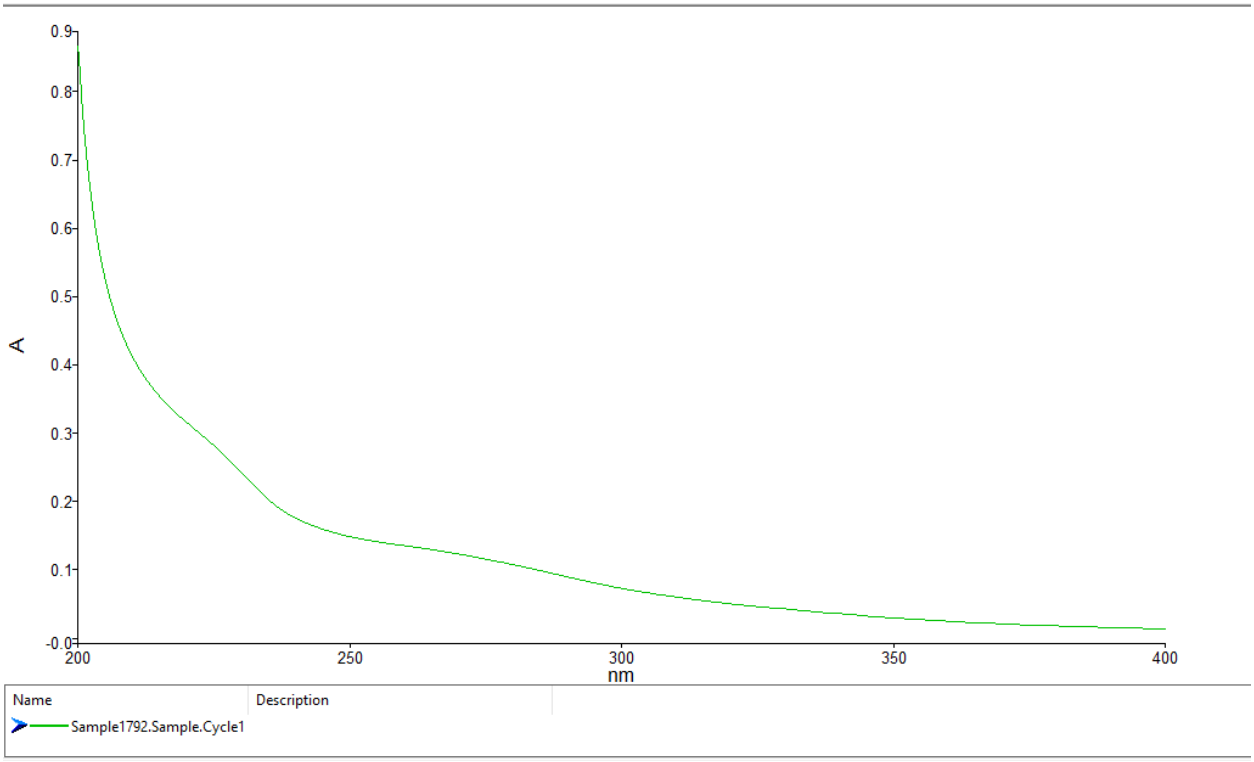


Figure 27. UV-Vis Spectra of Anaerobic Sample

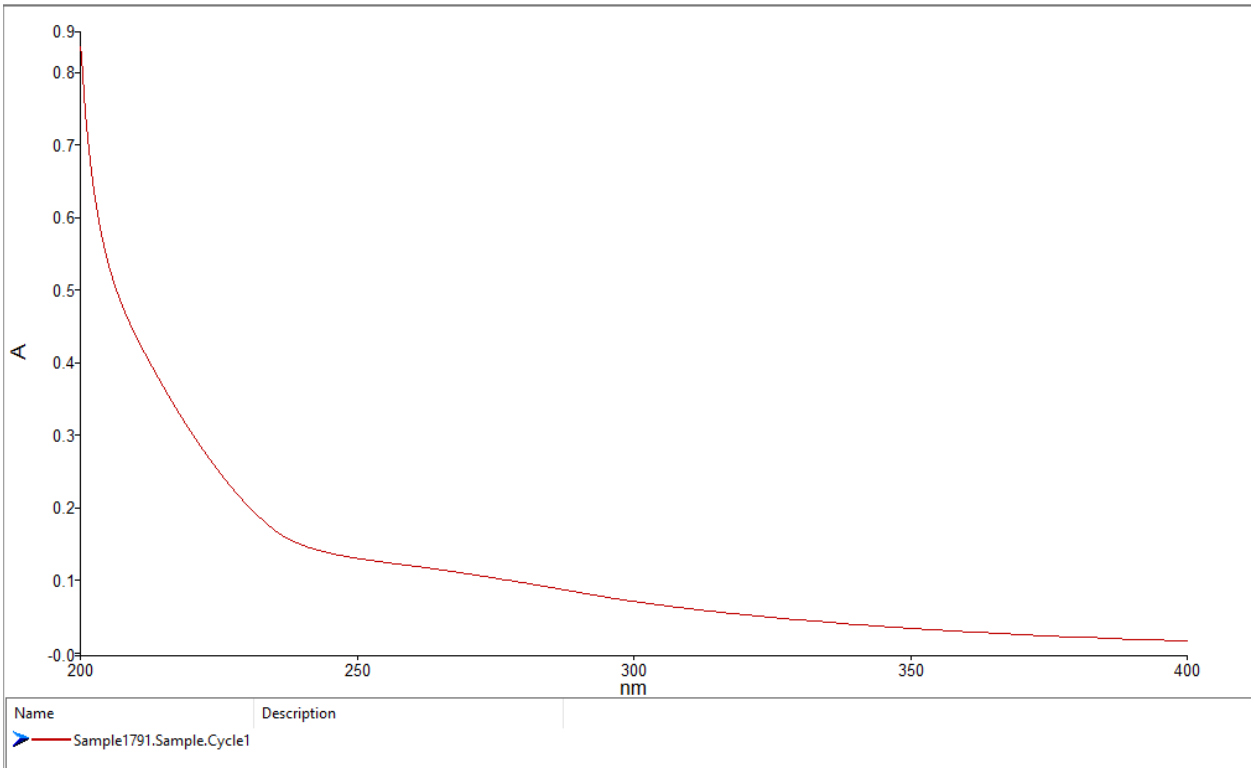


Figure 28. UV-Vis Spectra of Anoxic Sample

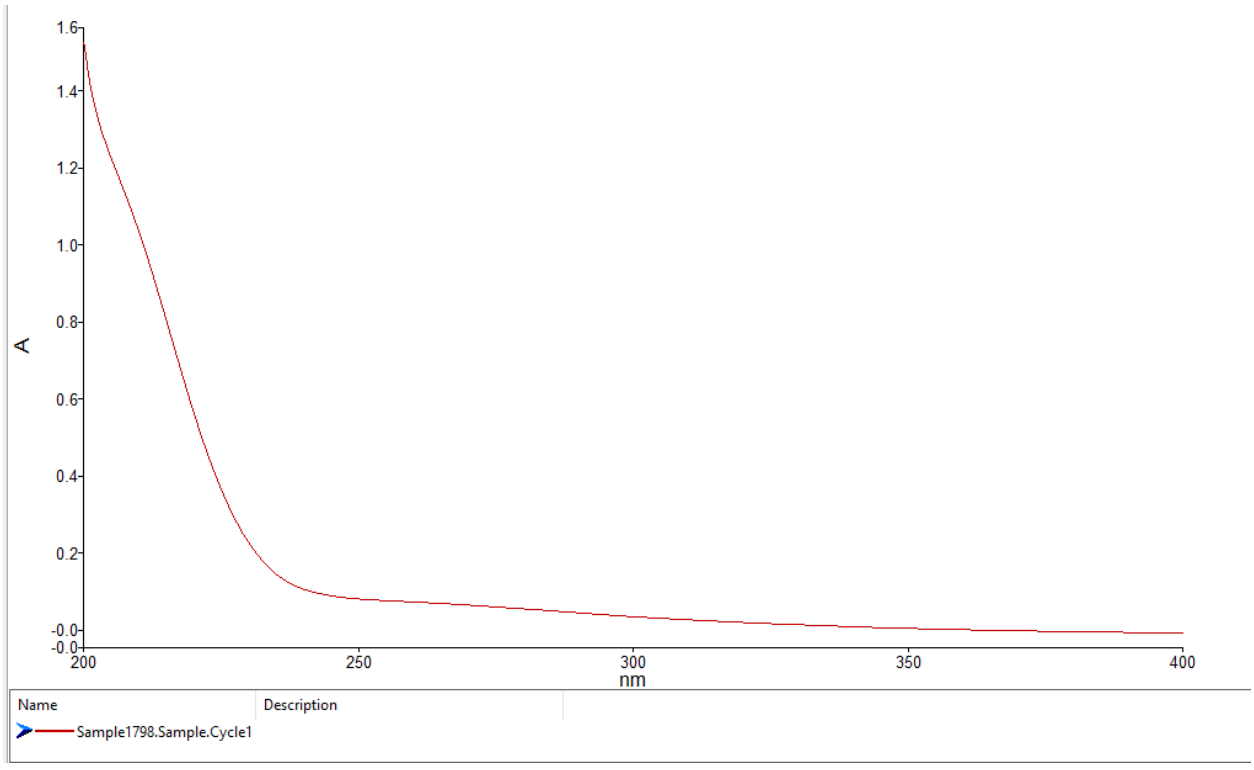


Figure 29. UV-Vis Spectra of Aerobic Sample

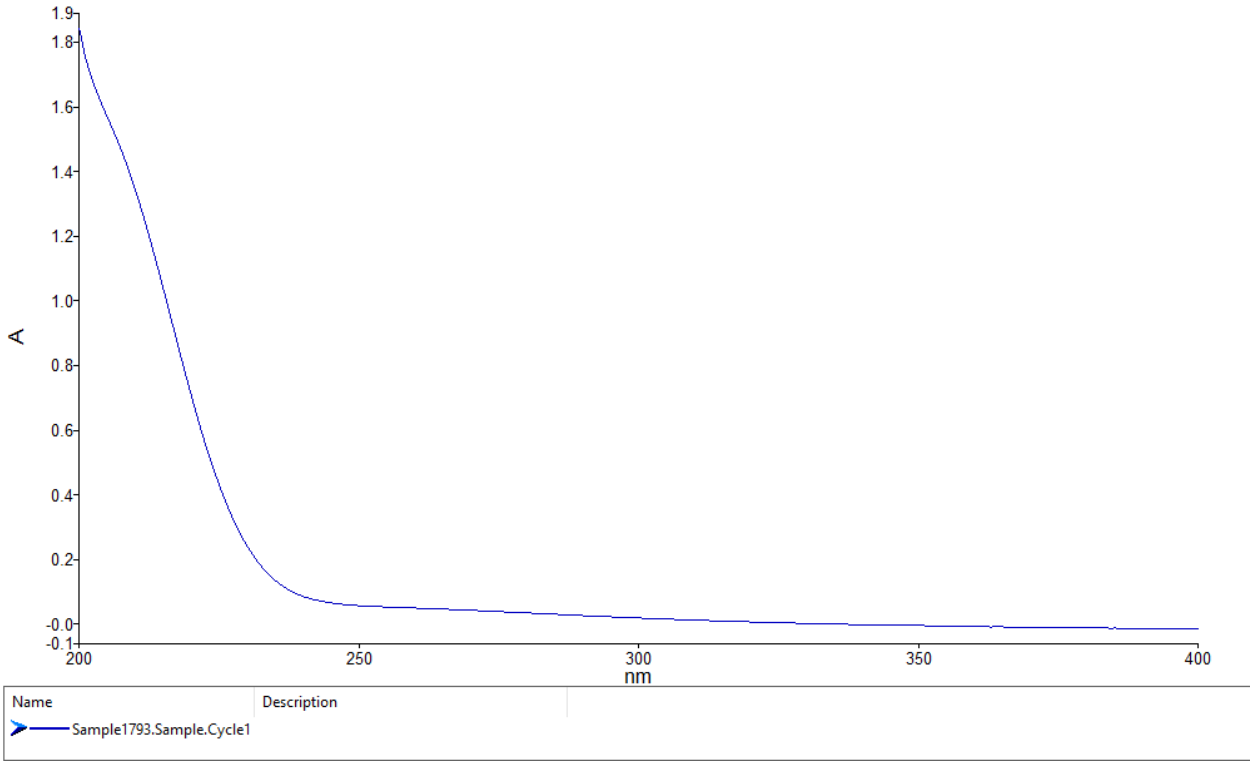


Figure 30. UV-Vis Spectra of Final Settling Sample

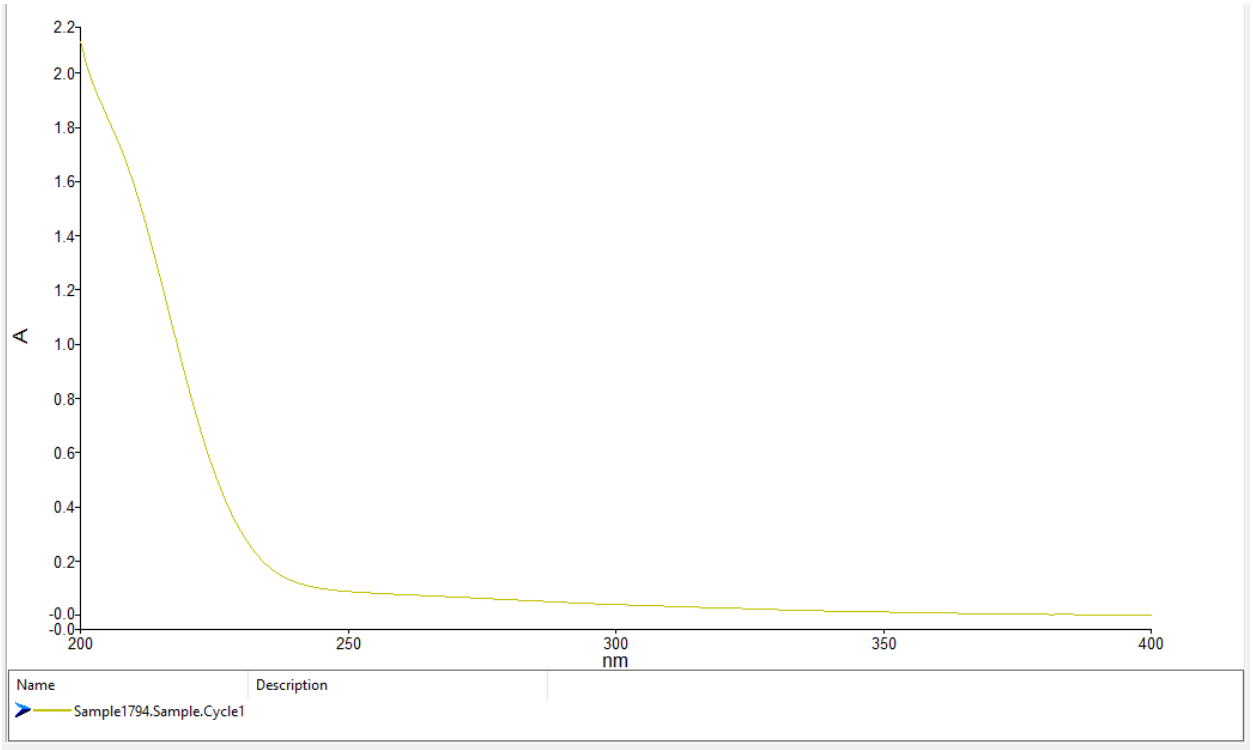


Figure 9. UV-Vis Spectra of Final Effluent Sample

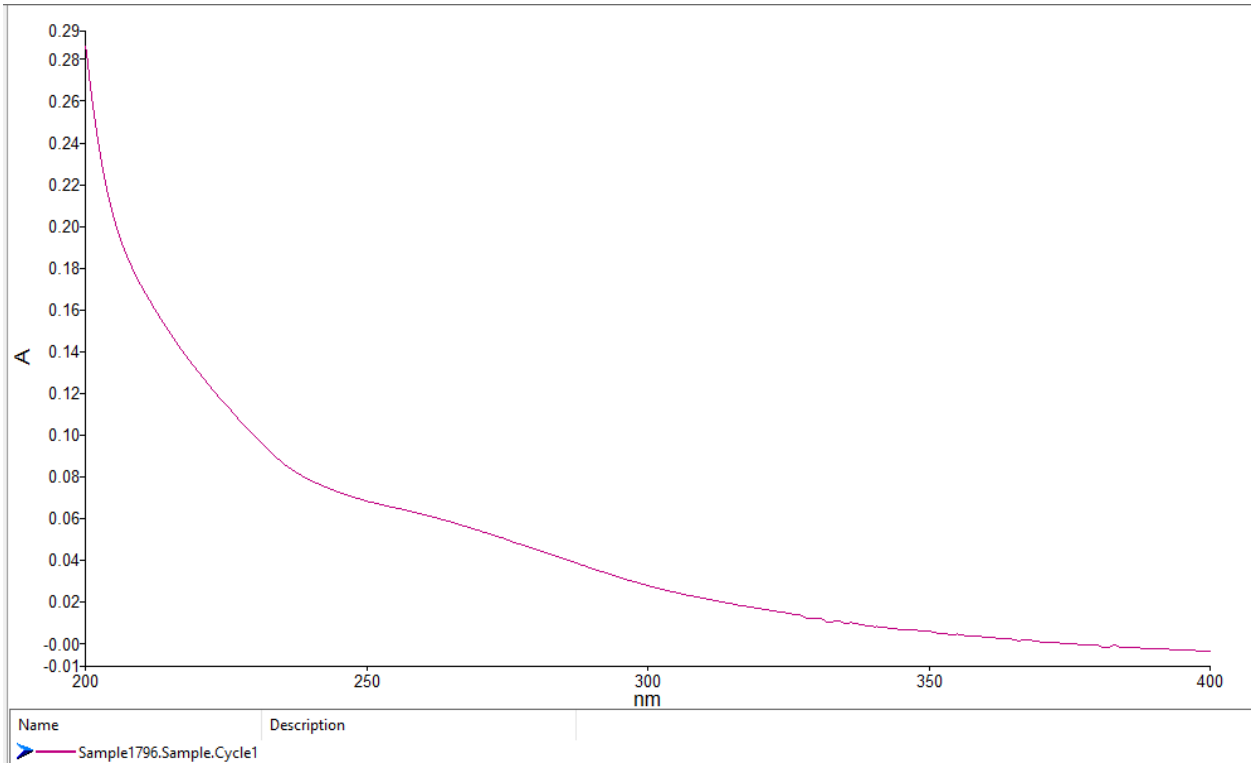


Figure 10. UV-Vis Spectra of Green Hill Pond Sample

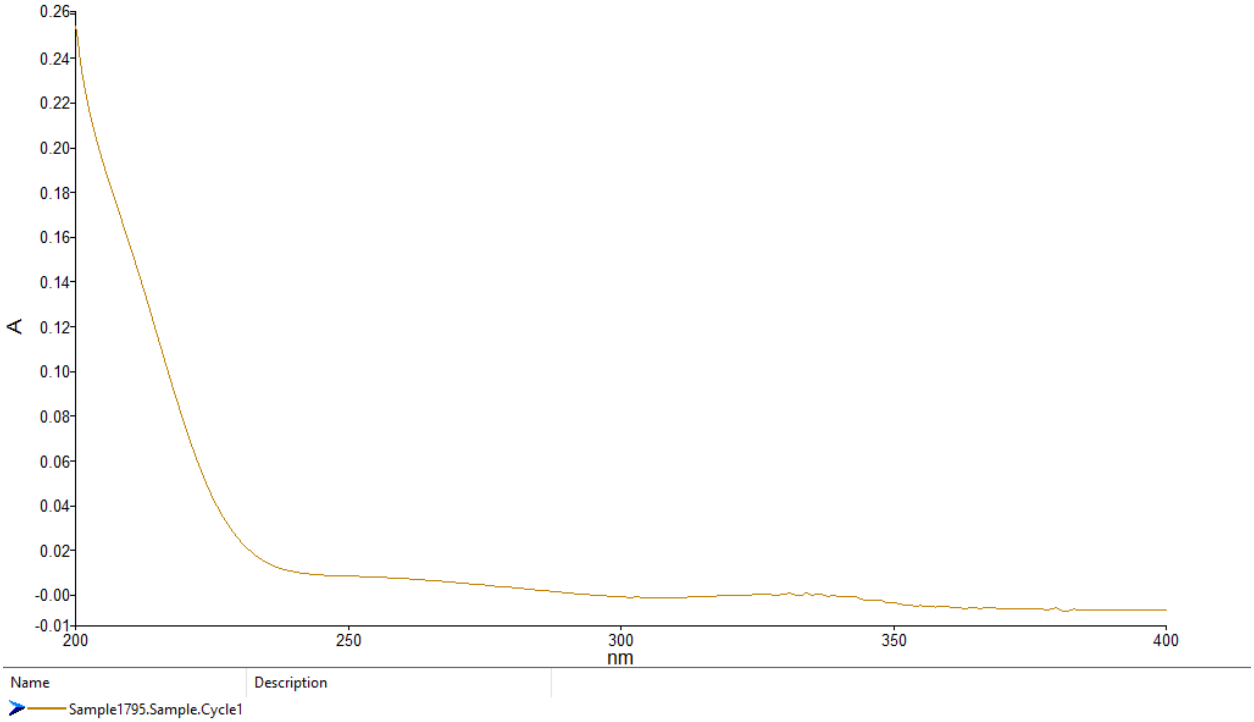


Figure 11. UV-Vis Spectra of Lake Quinsigamond Sample

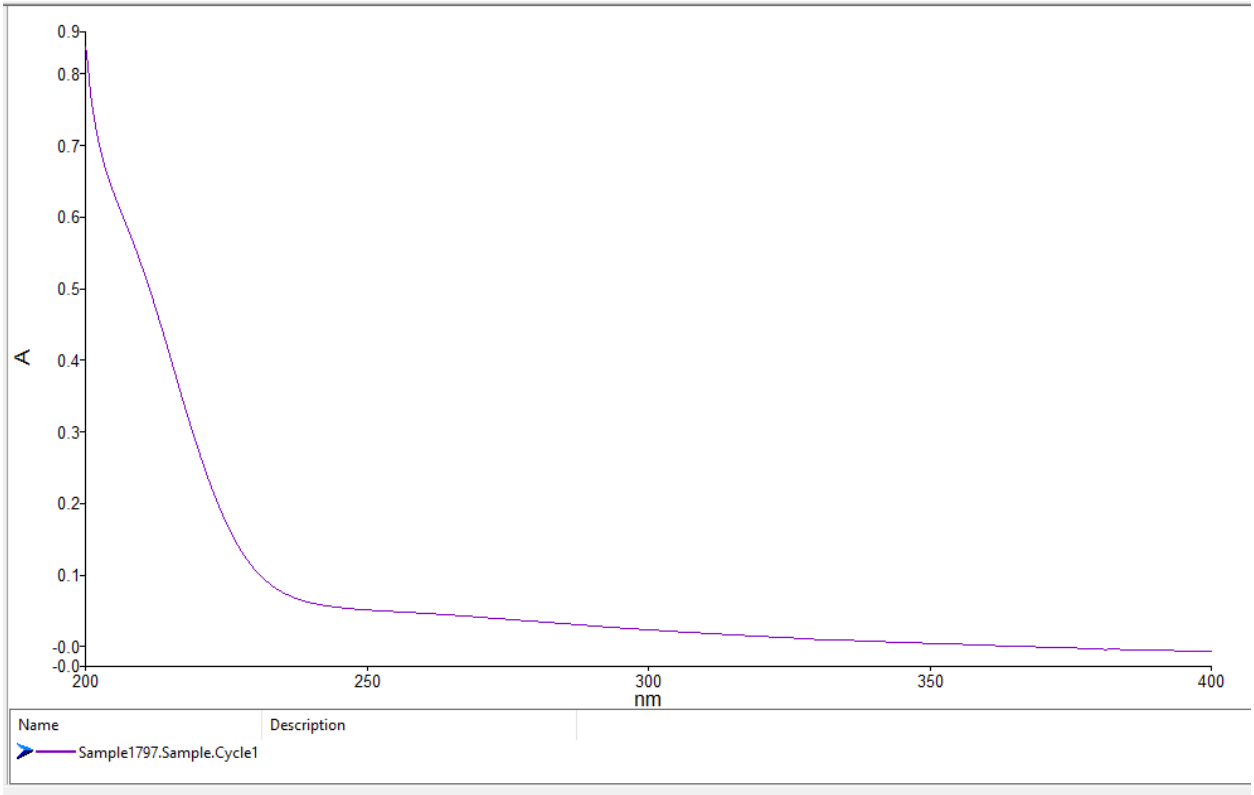


Figure 12. UV-Vis Spectra of Salisbury Pond Sample

Appendix C: LC-MS Data

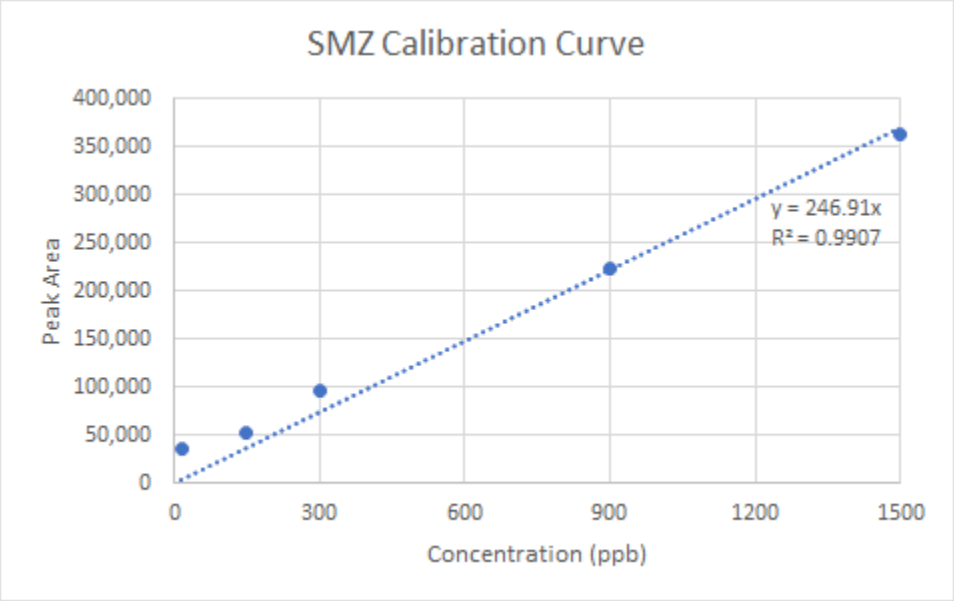


Figure 18. The linear calibration curve for SMZ shows concentration in parts per billion against peak area. We created standards of SMZ at various concentrations (see Appendix A) and analyzed them using LC-MS to find peak areas. By adding a trendline with y-intercept set to zero, we were able to retrieve the trendline equation that we then used to find the unknown concentration in our samples.

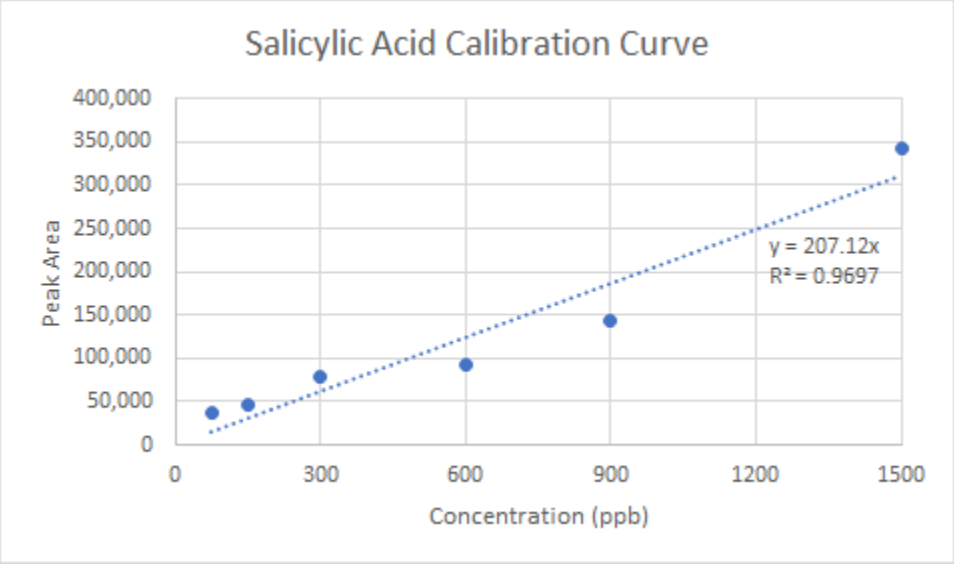


Figure 20. The calibration curve for salicylic acid shows concentration in parts per billion against peak area. By creating standards using procedures in Appendix A and analyzing them using LC-MS, we were able to create this linear curve to find the unknown concentrations of our samples for salicylic acid from the peak areas measured by LC-MS.

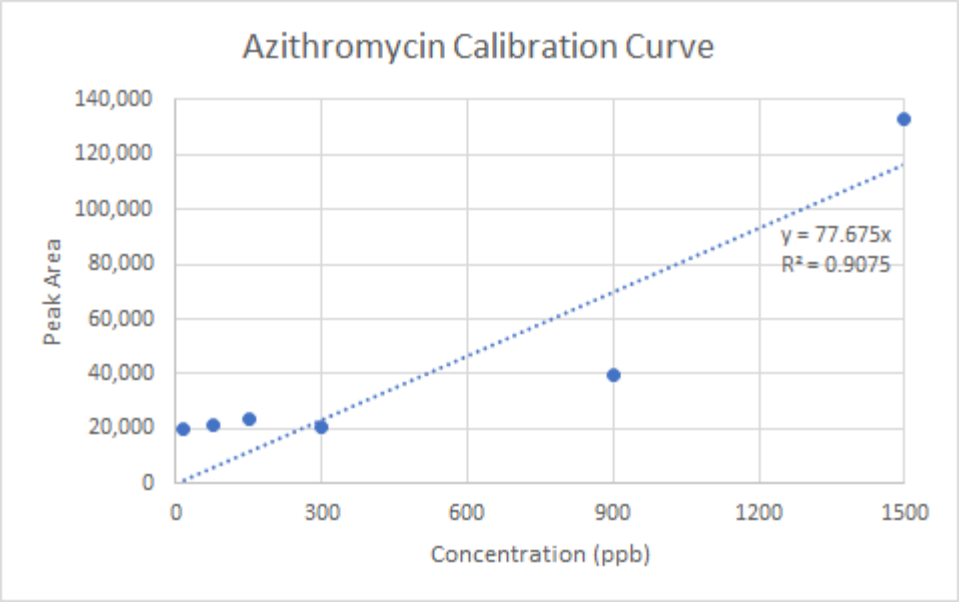


Figure 21. The calibration curve for azithromycin shows concentration in parts per billion against peak area. By analyzing standard concentrations of azithromycin using LC-MS, we were able to create this linear calibration curve to find the unknown concentrations of our samples from the peak areas.

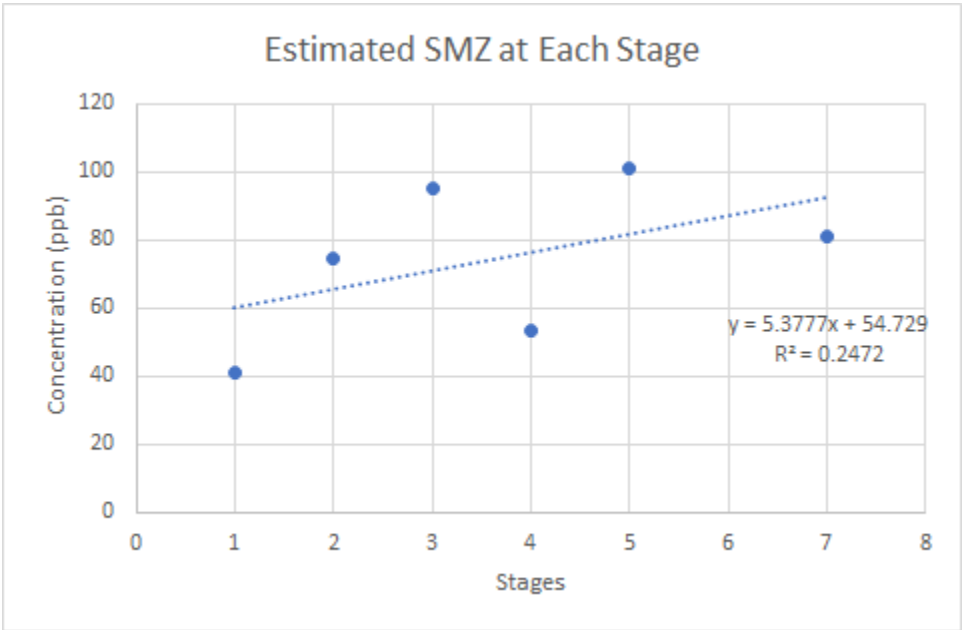


Figure 19. We estimated the concentration of SMZ by using the trendline equation in Figure 18 to calculate the concentration of our wastewater treatment samples. Stage one through seven on the x-axis represent the stages Influent (1), Primary Effluent (2), Anaerobic (3), Anoxic (4), Aerobic (5), Final Settling (6), and Final Effluent (7) of Upper Blackstone's treatment process. As the time goes on, we see no significant trend in the reduction of SMZ, as seen with the low R-squared value. The data point at stage 6, the final settling sample, was not tested by LC-MS.

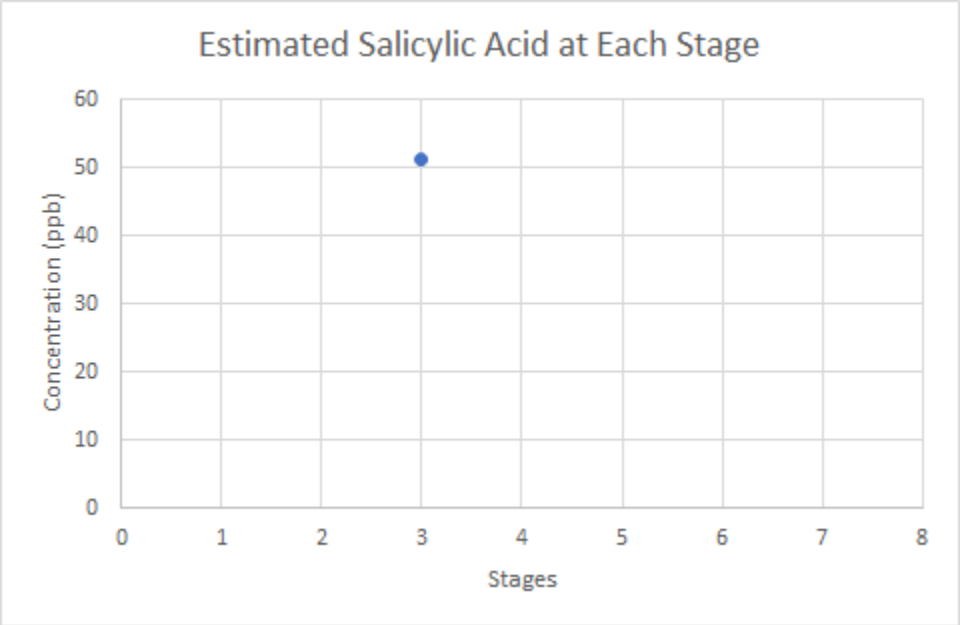


Figure 22. Due to a combination of instrument error and possible contamination, two MS reports were not produced, and the remaining reports showed no sign of salicylic acid peak, therefore the area was not found. The one successful MS report showed an approximate concentration of 50 ppb salicylic acid at the anaerobic stage. With these limited data, we cannot say whether there was a decrease in the concentration as treatment processes continued.

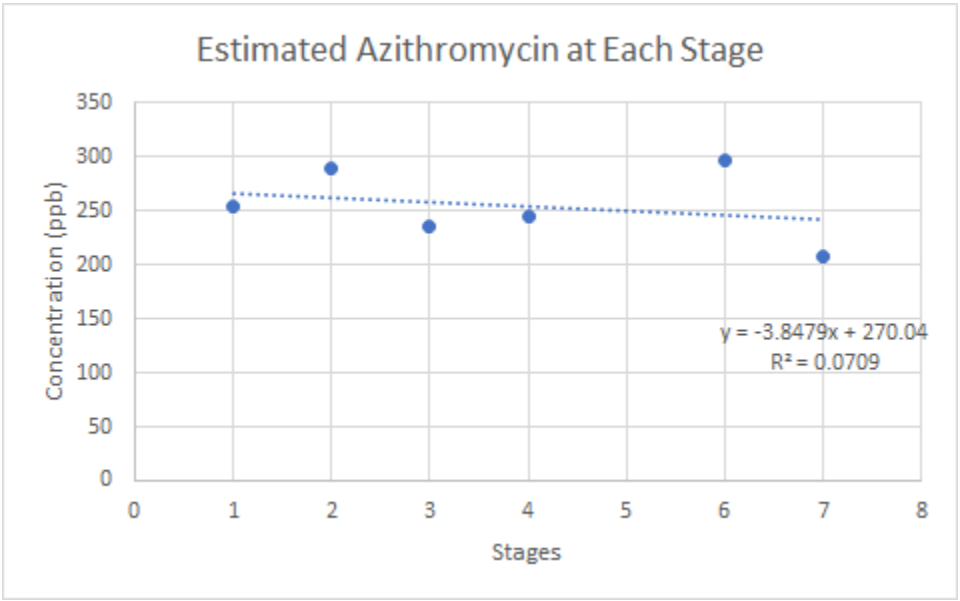


Figure 23. By comparing the estimated concentration of azithromycin at each stage, this scatter shows no reliable reduction of the pharmaceutical by Upper Blackstone's treatment processes.

Table 2. This shows our tabulated data from LC-MS reports for each of our samples. We ran 51 samples total, 21 standards to create calibration curves and 30 unknown samples either from wastewater treatment methods or surface water in Worcester, MA.

Pharmaceutical	Sample Name	Time (Minutes)	Peak Area	Standard Concentration (ppb)	Estimated Concentration (ppb)
SMZ	15 ppb Standard 1	6.393	36,740.3	15	149
	15 ppb Standard 2	No MS		15	
	150 ppb Standard	6.337	51,686.5	150	209
	300 ppb Standard	6.323	95,444.9	300	387
	600 ppb Standard	Removed			
	900 ppb Standard	6.329	223,248.4	900	904
	1500 ppb Standard 1	6.329	363,687.1	1500	1473
	1500 ppb Standard 2	No MS		1500	
	Lake Quinsigamond	6.339	15,481.1		63
	Green Hill Pond	No Sample			
	Salisbury Pond	No Sample			
	Influent	6.33	10,142.1		41
	Primary Effluent	6.334	18,410.9		75
	Anaerobic	6.335	23,526.0		95
	Anoxic	6.337	13,250.8		54
	Aerobic	6.325	24,945.0		101
	Final Settling	No Sample			
	Final Effluent	6.333	20,015.9		81
Salicylic Acid	75 ppb Standard	6.599	36,566.7	75	177
	150 ppb Standard	6.582	47,624.6	150	230
	300 ppb Standard	6.584	78,884.6	300	381
	600 ppb Standard	6.58	92,936.8	600	449
	900 ppb Standard	6.586	144,504.7	900	698
	1500 ppb Standard	6.585	342,301.9	1500	1653
	Lake Quinsigamond	6.583	15,314.7		74
	Green Hill Pond	No MS			
	Salisbury Pond	6.584	27,658.7		134
	Influent	No Peak			
	Primary Effluent	No Peak			
	Anaerobic	6.581	10,631.2		51
	Anoxic	No Peak			
	Aerobic	No Peak			
	Final Settling	No Peak			
	Final Effluent	No MS			
Azithromycin	15 ppb Standard	6.43	20,107.8	15	259
	75 ppb Standard	6.422	21,550.8	75	277
	150 ppb Standard	6.424	23,635.8	150	304
	300 ppb Standard	6.425	20,901.6	300	269
	600 ppb Standard	No MS		600	
	900 ppb Standard	6.424	39,483.4	900	508
	1500 ppb Standard	6.409	133,073.4	1500	1713
	Lake Quinsigamond	6.425	21,425.5		276
	Green Hill Pond	6.43	17,004.8		219
	Salisbury Pond	6.421	21,906.8		282
	Influent	6.427	19,817.8		255
	Primary Effluent	6.425	22,469.2		289
	Anaerobic	6.425	18,313.2		236
	Anoxic	6.426	19,053.8		245
	Aerobic	No Sample			
	Final Settling	6.421	23,118.7		298
	Final Effluent	6.445	16,205.9		209

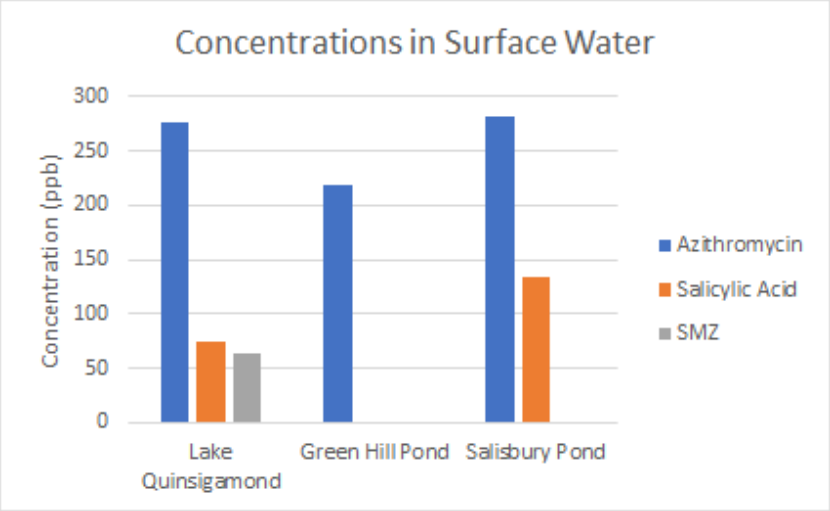


Figure 24. MS data show the concentration of SMZ, salicylic acid, and azithromycin in Lake Quinsigamond, Green Hill Pond, and Salisbury Pond. Due to complications, SMZ samples for Green Hill Pond could not be tested, in addition to both SMZ and salicylic acid samples for Salisbury Pond. Our MS data show the concentration of SMZ in the Lake Quinsigamond sample is about 60 ppb. We did not have supplies to test for Green Hill Pond and Salisbury Pond, so no samples were tested using LC-MS. Due to an instrumental error with the Green Hill Pond sample, no MS report was produced. However, this shows the concentration of salicylic acid in Worcester surface water is around 74 for Lake Quinsigamond and 134 for Salisbury Pond. The surface water concentration of azithromycin was higher than both SMZ and salicylic acid, at about 219 to 282 for these bodies of water.

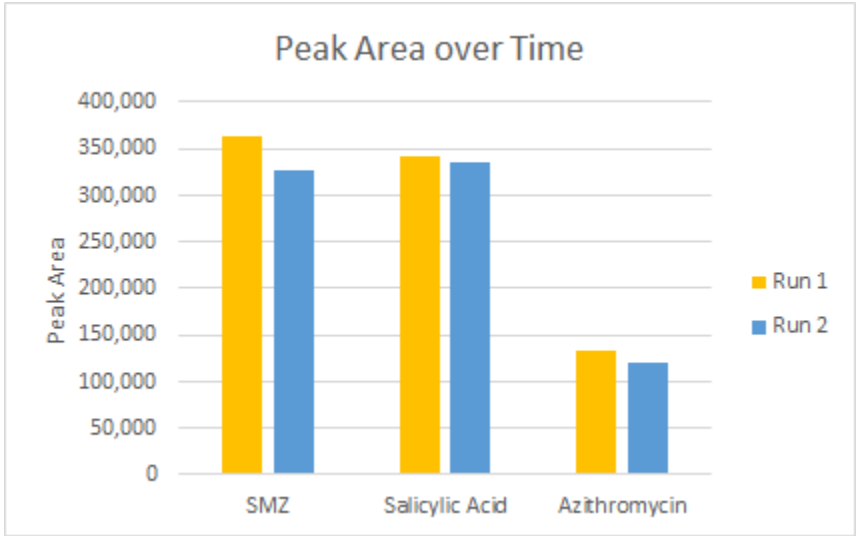
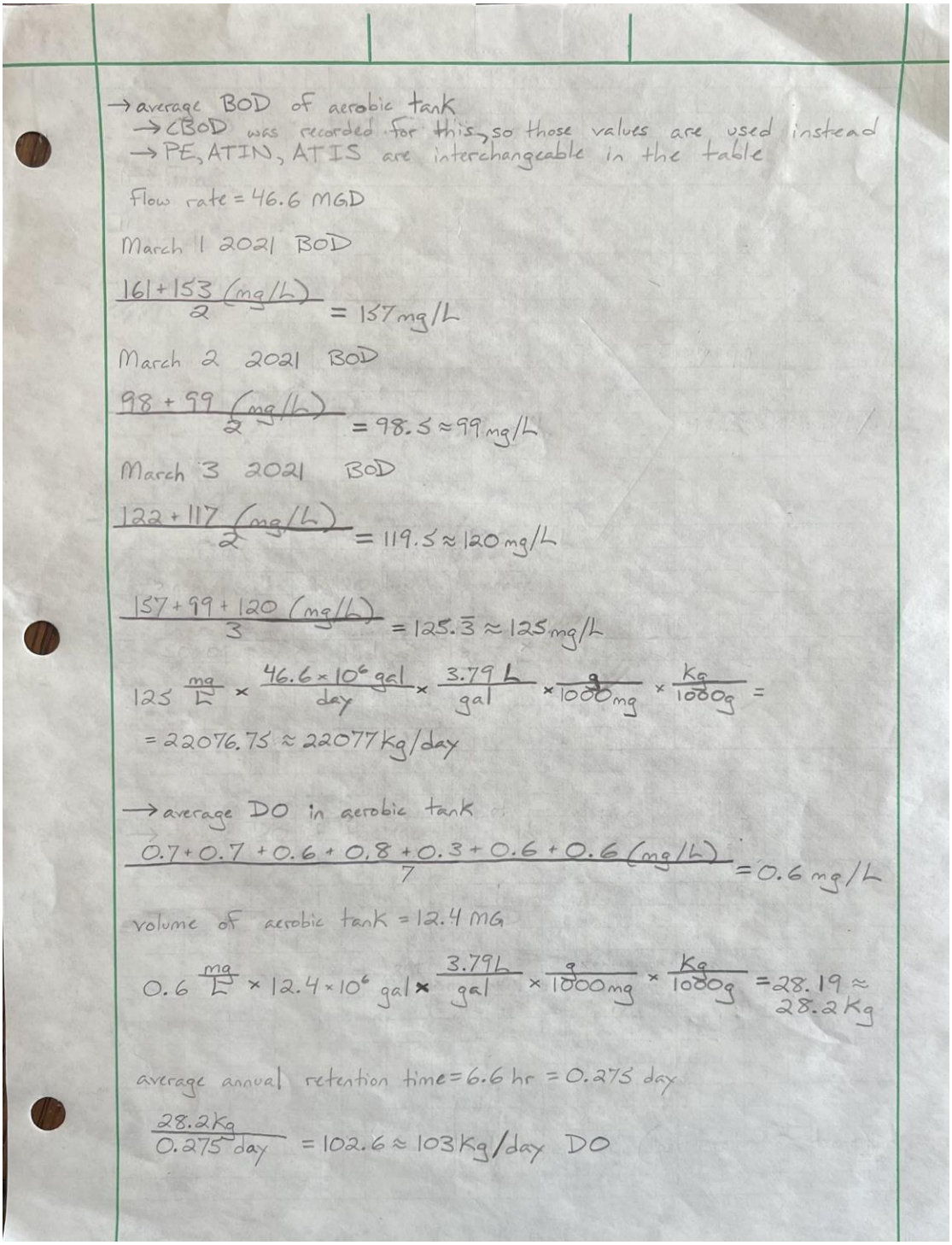


Figure 25. We ran two tests on the 1500 ppb standards for each of the pharmaceuticals, one at the beginning of the run and once at the end. This was to see what kind of reproducibility there is with this method, and to see if evaporation occurred. This figure shows only a small amount of each sample may have evaporated by the end of the run, 19 hours later.

Appendix D: Aerobic Recycling Stream Design Calculations



→ average BOD of aerobic tank
→ cBOD was recorded for this, so those values are used instead
→ PE, ATIN, ATIS are interchangeable in the table

Flow rate = 46.6 MGD

March 1 2021 BOD

$\frac{161+153\ (mg/L)}{2} = 157\ mg/L$

March 2 2021 BOD

$\frac{98+99\ (mg/L)}{2} = 98.5 \approx 99\ mg/L$

March 3 2021 BOD

$\frac{122+117\ (mg/L)}{2} = 119.5 \approx 120\ mg/L$

$\frac{157+99+120\ (mg/L)}{3} = 125.\bar{3} \approx 125\ mg/L$

$125\ \frac{mg}{L} \times \frac{46.6\times10^6\ gal}{day} \times \frac{3.79\ L}{gal} \times \frac{g}{1000\ mg} \times \frac{kg}{1000\ g} =$

$= 22076.75 \approx 22077\ kg/day$

→ average DO in aerobic tank

$\frac{0.7+0.7+0.6+0.8+0.3+0.6+0.6\ (mg/L)}{7} = 0.6\ mg/L$

volume of aerobic tank = 12.4 MG

$0.6\ \frac{mg}{L} \times 12.4\times10^6\ gal \times \frac{3.79\ L}{gal} \times \frac{g}{1000\ mg} \times \frac{kg}{1000\ g} = 28.19 \approx 28.2\ kg$

average annual retention time = 6.6 hr = 0.275 day

$\frac{28.2\ kg}{0.275\ day} = 102.6 \approx 103\ kg/day$ DO

Figure 31. This is the first page of design calculations, showing average BOD and dissolved oxygen.

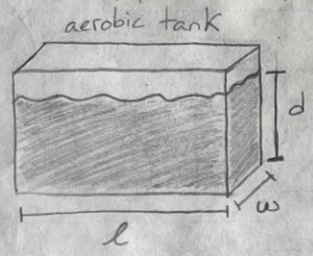
→ oxygen consumed during aerobic treatment; DO = 0.6 mg/L

22077 − 103 = 21974 (Kg/day)

average annual retention time = 6.6 hrs

$$21974 \frac{Kg}{day} \times \frac{day}{24 hrs} \times \frac{6.6 hrs}{run\ time} = 6042.85 \approx 6043 Kg/run\ time$$

→ length of pipes necessary for recycling stream



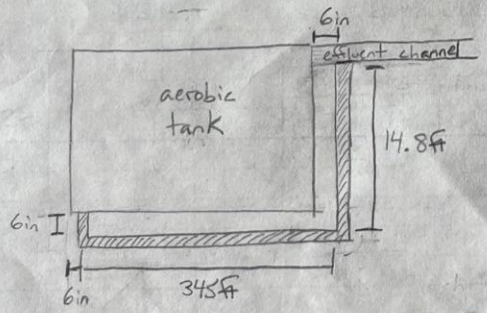
$l = 345 ft$
$w = 84 ft$
$d = 14.3 ft$
effluent pipe height = d

→ pipe diameters throughout the plant: 24 − 72 inches

→ amount of water that needs to flow through recycling stream at a given time: 12.4 MG (approximately ¼ of total flow)

→ channel size before bio reactor: 36 inches

→ reasonable pipe diameter: 24 inches



* not drawn to scale

pipe d = 24in
pipe corner r = 24in

→ leave 6inches between pipe and tank

359.8ft of pipe total

→ pumps needed for recycling stream
→ use the pump models used for return activated sludge

vertical non clog centrifugal, 60hp, 4900gal/min @ 36ft

$$\frac{359.8 ft}{36 ft} = 9.99 \approx 10 \text{ pumps needed}$$

Figure 32. This is the second page of design calculations. Oxygen consumed, pipe length necessary, and number of pumps required are shown.

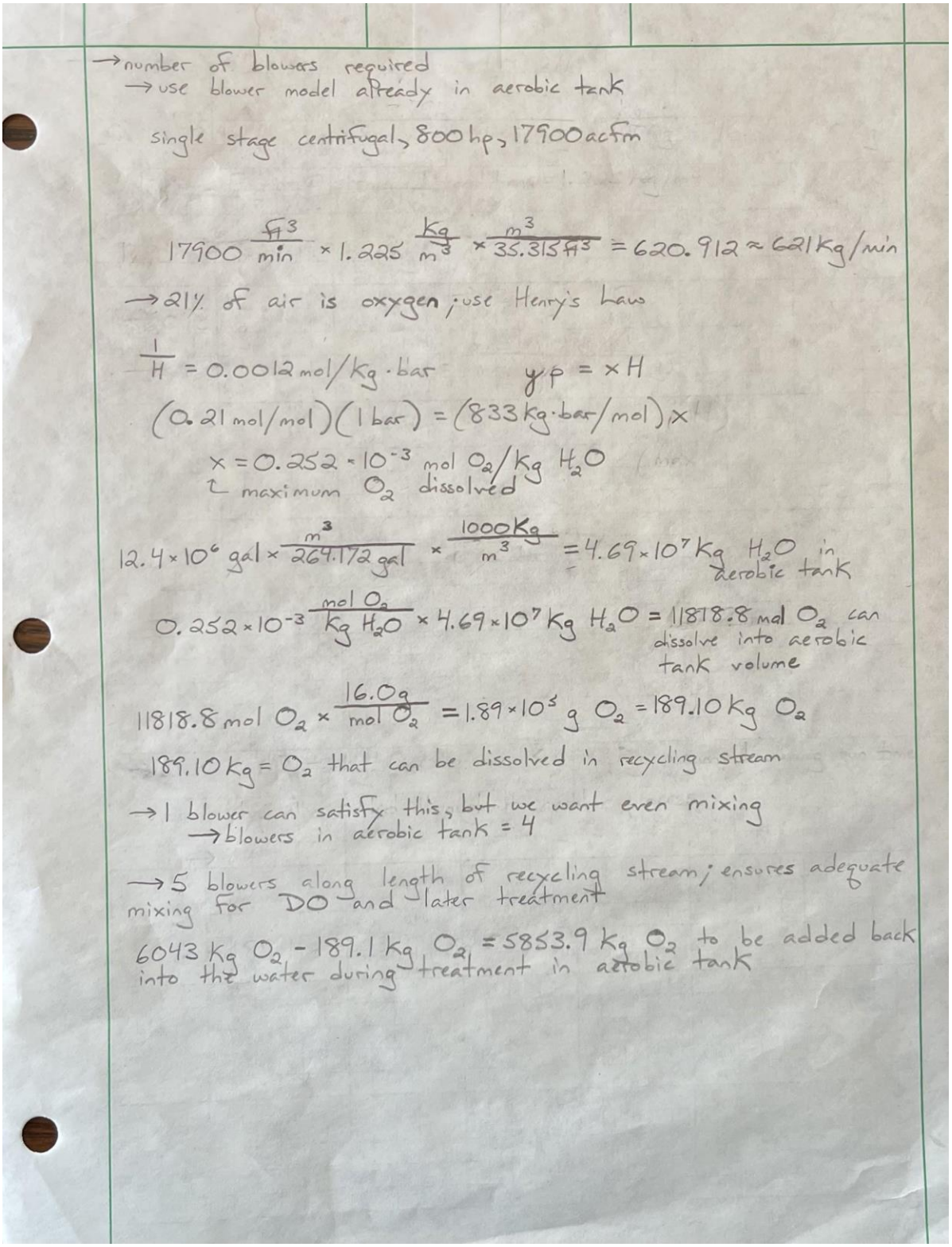


Figure 33. This is the third page of design calculations, highlighting the amount of oxygen added during the recycling stream versus the aerobic tank, as well as the number of air blowers.

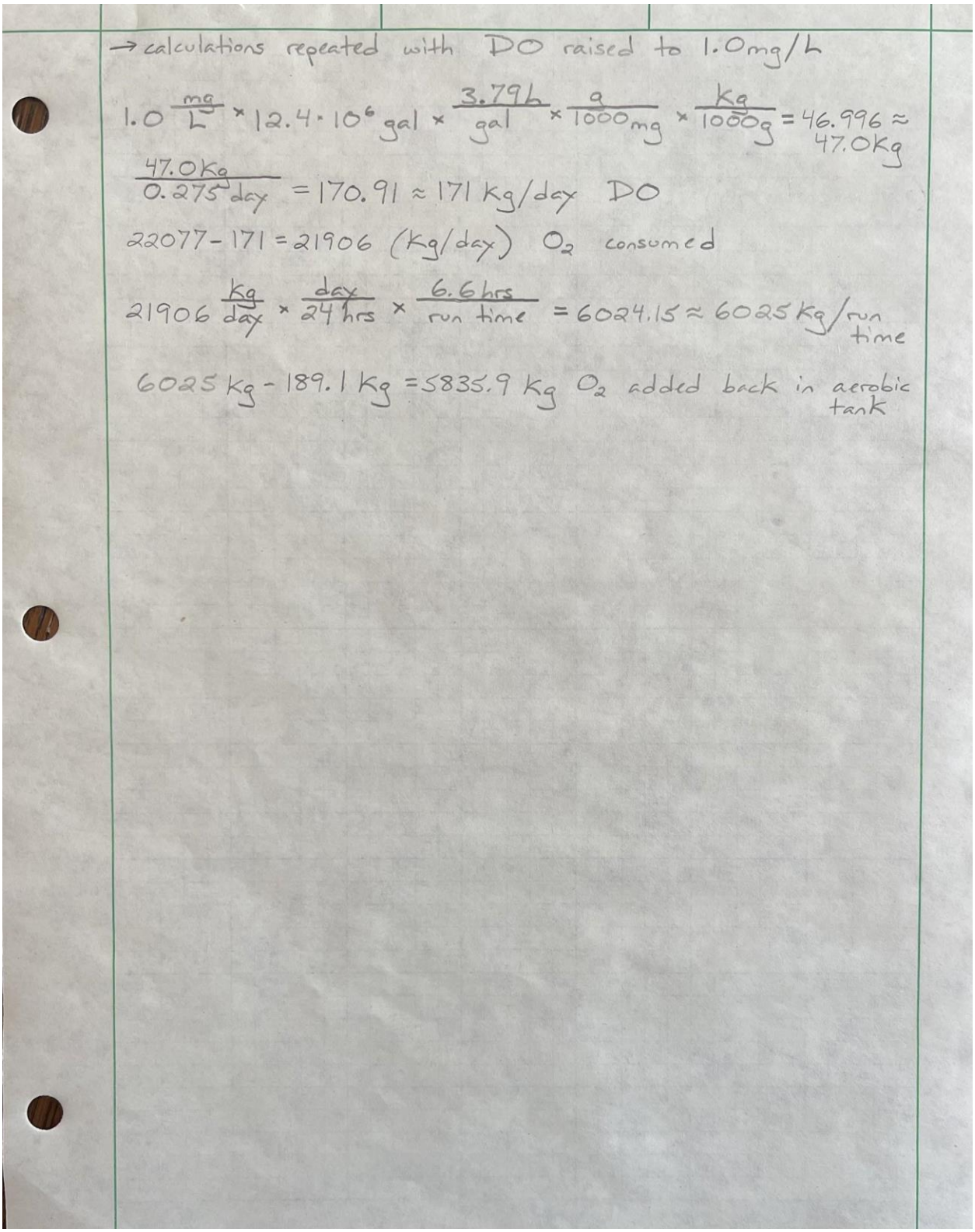


Figure 34. This is the fourth and final page of design calculations, focusing on repeating the above calculations with a dissolved oxygen value of 1.0 mg/L.

Appendix E: Water Quality Test Strips

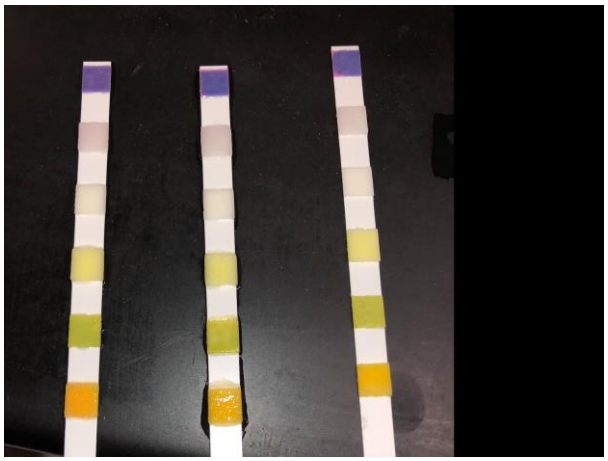


Figure 35. Salisbury Pond, Lake Quinsigamond, Green Hill Pond water test strips

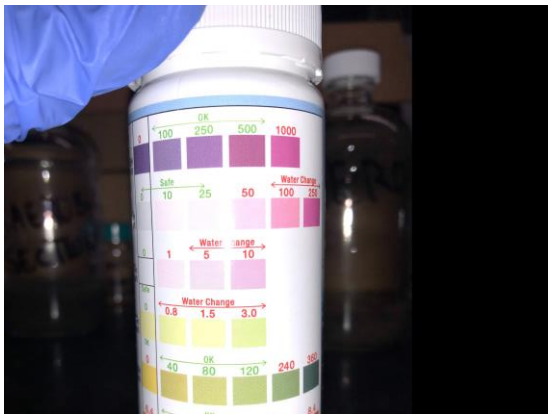


Figure 4. Water quality strips concentration range values

Figure 36. Influent and Primary Effluent test strips

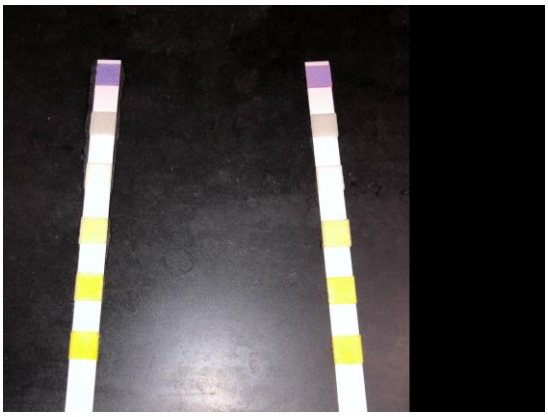


Figure 37. Azithromycin and Salicylic Acid water test strips

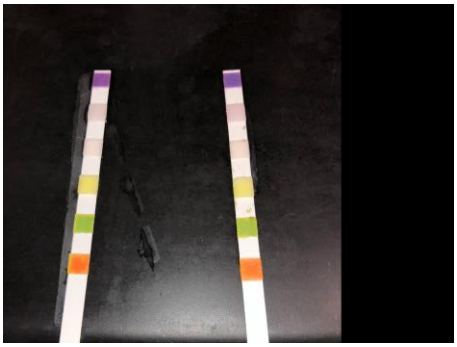


Figure 38. Anaerobic and Anoxic water test strips

Figure 39. Aerobic, Final Settling, Final Effluent water test strips