Photolysis of Zidovudine in Wastewater Effluent

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Abstract: Pharmaceuticals and personal care products have recently fallen under the category of contaminants of emerging concern. Due to constant release into the environment, and incomplete removal through common water treatment techniques, trace concentrations of these compounds persist in the environment. The goal of this research was to determine the presence of targeted contaminants of emerging concern as well as to observe the natural interactions of these compounds in the presence of sunlight. If these compounds are ultimately found to cause human harm, then legislative action would be required to mitigate direct disposal of pharmaceuticals and personal care products into surface water, as well as to implement additional treatment techniques at wastewater treatment plants. This experiment sought to simulate environmental conditions to evaluate the degree of degradation of zidovudine, a human immunodeficiency virus anti-retroviral drug, in treated wastewater effluent. Wastewater treated via anaerobic baffled reactor technology was collected, filtered, spiked and exposed to sunlight for a total of five hours. Each hour a spiked and unspiked sample was removed to obtain degradation kinetics. Water quality parameters were measured on all samples including pH, temperature, conductivity, dissolved oxygen and chemical oxygen demand. All parameters remained constant over the five hours, which allowed for the isolation of the effects of solar irradiation, alone. Following, the samples were solid phase extracted and analyzed using ultraviolet-visible spectrophotometry, between 200 and 500 nm. Absorbance values at 266 nm were plotted against a standard calibration curve to determine concentrations of zidovudine. The results were inconclusive. The spiked extracted sample was the only one to show a decrease in concentration. Further analyses via high performance liquid chromatography, and liquid chromatography mass spectrometry are necessary to determine zidovudine degradation during exposure. Understanding if and how zidovudine degrades in sunlight, will help to determine its eventual fate in the environment.

1. Introduction

Advances in medicinal technology over recent decades have led to vast improvements in the quality of life and health in many locations across the globe, as well as a striking increase in the use of pharmaceuticals. Due to constant widespread use, pharmaceuticals and personal care products (PPCPs) have been recently categorized as contaminants of emerging concern (CECs). CECs are contaminants that are not currently regulated, but have the potential to cause adverse effects on humans and the environment if proper action is not taken (Prasse et. al 2015). PPCPs are consumed by humans for health, cosmetic uses, or agriculture, and are then introduced to the environment through manufacturing, human excretion, improper disposal and landfill leaching. A majority of PPCPs are removed through conventional water treatment processes, however, trace organic materials are persistent and, as a result, are being released into natural water bodies (Petrovic et al., 2003). In addition, sludge later recycled and used as agricultural fertilizer reintroduces removed contaminants. Concentrations of PPCPs are even found in treated water that is considered, by United States Environmental Protection Agency (USEPA) standards, to be potable (Ebele et al., 2017; USEPA, 2017).

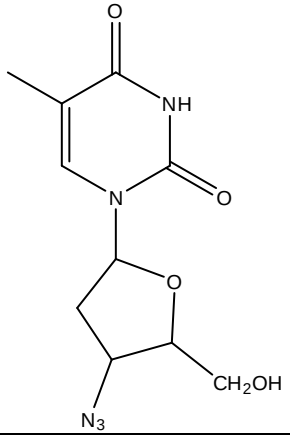
Through biomagnification and crop reuptake, these compounds are continuously cycled through the environment (Ebele et al., 2017). Once present in the aquatic environment, degradation of a compound can occur via bacterial and/or photochemical interactions either simultaneously, or sequentially (Prasse et al., 2015). Compounds that are initially resistant to biodegradation, can degrade from exposure to sunlight, forming hydroxylated derivatives which are then more easily degraded by microbes (Prasse et al., 2015). If the compounds are not completely degraded, their

metabolites remain in the aquatic environment, and can be even more toxic than the original compound (Devrukhakar et al., 2017).

The combination of chemicals present in a particular water body is ultimately dependent on socioeconomic factors and sanitation infrastructure of a specific location. South Africa is a country with unique challenges, housing extremes of both sides of the economic spectrum. The greatest number of people living with human immunodeficiency virus (HIV), according to a 2013 estimate, reside in South Africa (Swanepoel et al., 2013). Because ARVs are not completely removed through conventional wastewater treatment processes, trace concentrations remain in released effluent. Municipal wastewater concentrations of ARVs have been detected above 1 µg/L (Prasse et al., 2015). The environmental fate of these compounds has not been well studied; therefore, the impacts on human and ecosystem health are still not well understood.

Zidovudine, or azidothymidine (AZT), belongs to a group of drugs named nucleoside reverse transcriptase inhibitors (NRTIs). They function by blocking an HIV enzyme, reverse transcriptase, in order to delay the development of acquired immunodeficiency syndrome (AIDS).

Table 1: *Physiochemical properties of AZT in water (Swati et al., 2011, Prasse et al., 2010).*

Drug name and CAS.no	***Chemical structure***	***m/z (g/mol)***	***Solubility in water (mg/mL)***	***pKa***	***Log K_{OW}***
Zidovudine (30516-87-1)		267.242	16.3	9.96	-0.1

AZT is the most effective regimen in the prevention of "mother-to-child HIV-1" transmission, therefore, it is widely used (Devrukhakar et al., 2017). It was chosen for this study because it is known to easily degrade in sunlight (Zhou et al., 2017). Studies have observed that the photoreactivity of AZT is due to the amine moiety which cleaves off leaving behind predominantly thymine (Prasse et al., 2015).

For communities in South Africa with limited access to clean water and sanitation, decentralized treatment is necessary. Anaerobic baffled reactor (ABR) technology is a treatment technique, which operates with low maintenance, requires little energy and reclaims water to be used for

agriculture. It is being studied in South Africa to determine its feasibility in communities that are "off of the grid". Such sites receive real-time influent from nearby communities.

In order to obtain a true representation of the concentrations and interactions of AZT that would exist in the real environment, wastewater treated at a decentralized wastewater treatment system (DEWATS) facility in South Africa, which utilizes ABR technology, was studied. Effluent was collected from the second anaerobic filter (AF2). Following collection, the wastewater was filtered to remove suspended particles that would interfere with light penetration. Photodegradation experiments were carried out in direct sunlight by using wastewater alone, as well as wastewater spiked with AZT. Because AZT is known to be photoactive, the concentration of AZT was expected to decrease over the five-hour degradation period. The effect of sunlight on the degradation was analyzed by means of ultraviolet-visible spectrophotometry (UV/VIS). A portion of each sample was frozen to await further analysis via high performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS).

2. Methods:

Methods were determined to simulate natural environmental conditions and were limited by various factors including the experimental timeline, equipment and materials. Three trials of AZT exposure were conducted, each for a total of five hours. Each trial had five spiked and five unspiked samples, as well as two dark controls (spiked and unspiked). Samples were collected, exposed, tested for water quality, and then analyzed.

In order to ensure the correlation of solar irradiance to the projected degradation of AZT, various precautions were taken to maintain a controlled environment during experimentation. Water quality parameters including temperature, turbidity, conductivity, pH and DO were measured throughout the trials to determine if there were any changes that might affect results. AZT is known to thermally degrade at 190℃ (Shamsipur et al., 2013), so maintaining temperature was important. Turbidity is the cloudiness of a sample and is linked to the amount of suspended particles present. Particles can scatter light, which would inhibit the light from reaching the dissolved AZT. Conductivity is a measure of ions present and is a correlated to of the amount of total dissolved solids present in a sample. Because salts and other inorganic materials generally conduct electrical current, increased conductivity correlates to increased salinity. Aquatic life can only sustain life within a certain salinity ranges, therefore it is an important parameter to determine before releasing effluent into water bodies. The value of pH signifies the acidity or alkalinity of a solution which can incite or inhibit the reaction of compounds present. DO refers to the gaseous oxygen that is dissolved in water. Aquatic life relies on oxygen for respiration, therefore certain concentrations are necessary in order to sustain life. On the contrary, anaerobic bacteria die when a high amount of oxygen is present. All water quality parameters can inflict negative impacts to receiving water bodies, therefore proper monitoring is necessary.

2.1 Samples

In order to conduct photolysis experiments, a sample of 2 L was taken from ABR Street 3, anaerobic filter 2 (AF2) and collected by grab sampling. Prior to collection, the metal sampling vessel and borosilicate glass collection flasks were cleaned by collecting effluent and disposing of it in adjacent grass area. The sample was moved to the Newlands-Mashu (NM) laboratory via two 1000 mL Erlenmeyer flasks placed in a plastic tub for secondary containment. The sample was filtered 1-3 days prior to the experiment through a pre-filter of 1.5 µm pore-size followed by a 0.7 µm glass fiber filter to remove turbid material. Each filter was pre-combusted (550ºC for 2 h) to remove impurities. Additional rinsing with 100 mL deionized (DI) water was performed on the third trial as an extra measure to ensure that any fluorescent compounds that might affect the florescence of the sample were removed. Three sub-samples of filtrate were collected in centrifuge tubes and shipped back to the United States for future fluorescence analyses. The filtered samples to be used for the experiment were placed into two 1000 mL glass bottles, labeled and placed in the refrigerator

2.2 Photolysis Experiment:

All three trials were conducted on a sunny day to obtain maximum degradation of AZT. Solar intensity data was determined using a pyranometer which is measured daily on a flat roof building with no obstructions in the Physics Department at the University of KwaZulu-Natal. The trials were conducted in the winter season.

2.2.1 Preparation of stock solution of AZT:

In order to prepare a stock solution, AZT was weighed with a fine balance and placed into a 25 mL volumetric flask. An approximate mass of 0.2 g was used (trial 1 and 2: 0.2020 g, trial 3: 0.201 g). Approximately 15 mL of DI water was subsequently placed into the volumetric flask and mixed until AZT reached its saturation point. DI water was then filled to the mark and the solution was homogenized with a vortex mixer until all the solute dissolved. For trials 1 and 2, the stock solution was made directly before exposure. For trial 3, the stock solution was made the day before, and then wrapped in aluminium foil and kept in a dark, dry cabinet in the laboratory.

2.2.2 Setup:

Prior to experimentation, the quartz tubes and glass bottles were autoclaved at Durban University of Technology (DUT). On the day of experimentation, the refrigerated effluent sample was removed from the fridge. For trial 1 (July 19, 2017) the refrigerated sample was removed at 7:40 am and placed in a 29 °C water bath for one hour. For trial 2 (July 25, 2017) the refrigerated sample was removed at 6:30 am and placed in a 21.6 °C water bath for one hour. For trial 3 (August 7, 2017) the refrigerated samples were removed at 6:30 am and placed in a 25.4 °C water bath for 45 minutes.

To prepare the spiked samples, an aliquot of 8.296 mL of the AZT stock solution was pipetted into a 500 mL volumetric flask. This volume was determined by means of the following equation:

$$C_1V_1 = C_2V_2 \quad (1)$$

where C_1 *(mg/L)* is the concentration *of AZT stock solution (202.0 mg AZT/25 mL = 8080* mg/L), V_1 *(mL)* is the volume *of AZT stock solution to be added to effluent*, C_2 *(mg/L)* is the *desired concentration of AZT in effluent sample* (*133.621* mg/L or 5 × 10^{-4} *mol/L)* and V_2 *(mL)* is the final *volume of spiked sample (500 mL*). The spiked solution was then filled to the 500 mL marked line with effluent and inverted several times to homogenize.

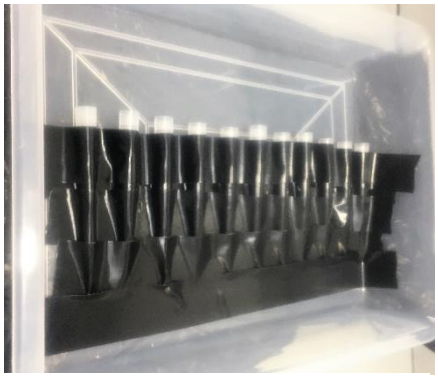


Figure 1. *Container used to hold quartz tubes during exposure*

The unspiked effluent sample was placed into each of five quartz tubes and one 250 mL glass bottle with the aid of a funnel. The quartz tubes were capped with rubber stoppers. The bottle was topped with a Ziploc bag and then capped to provide a seal. The effluent sample spiked with AZT was placed into each of the five remaining quartz tubes and capped with stoppers. After this, an additional 500 mL of effluent was spiked following the same procedure as stated above. This solution was placed into the remaining bottle. The bottle was topped with a Ziploc bag and then capped to provide a seal. The two glass bottles were wrapped in foil and served as dark controls. The quartz tubes and dark controls were then placed in a plastic container *(Figure 2)*. Water was filled approximately half way up the sides to avoid temperature fluctuations within the sample.

Figure 2. *Quartz tubes and dark controls prior to exposure*

Figure 3. *Experimental Setup*

Fifty mL each of the remaining spiked and unspiked samples was placed into a sterile centrifuge tube for extraction. A further 10 mL of each sample was placed into a sterile falcon tube for later fluorescence analysis. Another 10 mL of each sample was placed into a clean falcon tube for COD analysis. The COD samples were preserved with 10 µL of 50% sulfuric acid.

2.2.3 Trials:

For trial 1, the container comprising of the 10 quartz tubes and two dark controls was placed in direct sunlight *(Figure 3)*. The color black is known to absorb all wavelengths of light, so a black plastic bag was placed on the ground below the container.

Each hour, for five hours, two tubes were removed (one spiked, one unspiked). On the final hour, the two dark controls were also removed along with two tubes (unspiked and spiked). Each time, 50 mL of each spiked and unspiked sample was placed into sterile centrifuge tubes for extraction. A volume of 10 mL of each was placed into a sterile centrifuge tube for later fluorescence analysis. A further 10 mL aliquot of each was placed into a clean falcon tube for COD analysis, and preserved. After this, water quality parameters were measured directly in the quartz tubes for the remaining sample *(Figure 4)*. Conductivity and temperature were measured first using a Jenway 4520 benchtop conductivity meter. Next, DO was measured using a WTW Oxi 3401 DO meter. After, pH was measured using a YSI EcoSense® pH meter. Finally, turbidity was measured using a Hach DR-900 Multiparameter Colorimeter.

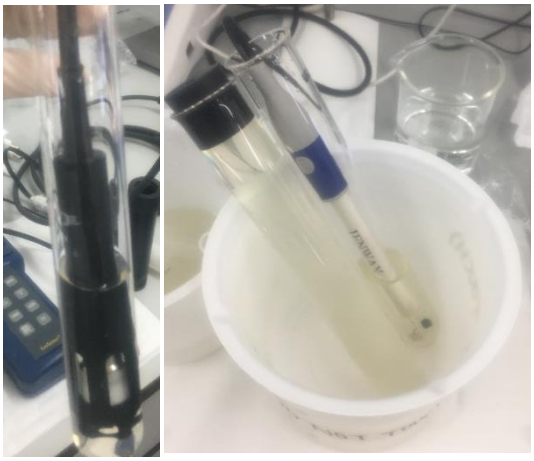


Figure 4. *DO meter (left) and conductivity meter (right) in quartz tubes*

2.3 COD:

Chemical oxygen demand (COD) is a water quality parameter that measures the amount of oxygen needed to chemically oxidize particulate and soluble organic matter. It represents how much oxygen water will consume when discharged. In water treatment, the aim is to reduce the concentration of materials that contribute to COD. If the COD is high, surface waters can become critically depleted of oxygen, which can eventually lead to eutrophication and destroy vital natural ecosystems. COD was measured for the effluent samples to analyze water quality.

2.3.1 Standards:

In order to obtain a standard calibration curve, standard solutions of known concentrations were prepared from pure and dry potassium hydrogen phthalate (KHP). First, a 10,000 mg/L KHP stock solution was made. To achieve this concentration, 0.85 grams of KHP were measured with a mass balance and added to a 100 mL volumetric flask. The flask was then filled to the mark with DI water. To make standards of 100, 300, 600, 900, 1200 and 1500 mg/L, 1, 3, 6, 9, 12 and 15 mL each of stock solution was added to 100 mL volumetric flasks, respectively. A blank was made by using DI water in place of KHP. The standard solutions were digested and analyzed alongside the samples (Figure 5).

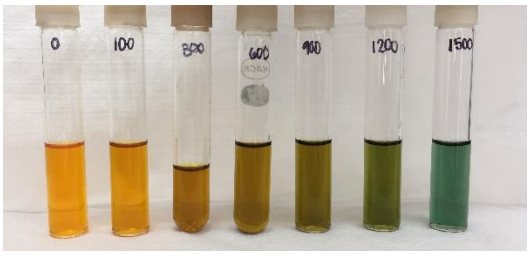


Figure 5. *COD standards from 0 to 1500 mg/L KHP after digestion*

2.3.2 Digestion:

All digestions were carried out inside a laminar flow hood. The digester was set to 148 °C for a time interval of two hours. While preparing solutions, the digester was turned on to allow it to reach temperature. Solutions were prepared adhering to the Spectroquant™ Solution A and B

method. Solutions were made by pipetting 0.3 mL of COD solution A into a cuvette, then 2.3 mL of COD solution B, followed by 3 mL of sample. The cuvettes were capped and homogenized by using a vortex mixer. Once the digester reached 148 °C, the cuvettes were wiped with a paper towel and placed in the digester. The samples were allowed to digest for two hours. After two hours, the lid of the digester was opened, and the samples were allowed to cool for 30 minutes. Thereafter, the samples were placed in a tube rack to await analysis.

2.3.3 Measurement:

Once cool, the samples were analyzed with a benchtop Lasec spectrophotometer. The mode was set to 51 and the wavelength was set to 605 nm. Before placing the cuvettes in the spectrophotometer, they were wiped with KimWipes®. The blank was inserted first to zero the instrument. Following this, each sample cuvette was wiped and placed in the spectrophotometer. The results were given as emission and transmission.

2.3.4 Determining COD values:

A standard calibration curve was obtained from the known concentrations of KHP and the corresponding emission values. From these values, a line of best fit was determined. The equation for the line is in the form:

$$y = mx + b \qquad (2)$$

where y is the emission, x is the concentration of KHP (mg/L), m is the slope, and b is the y-intercept.

To determine the COD, the slope and y-intercept of the line, as well as the measured emission of each sample, was substituted into the equation and the equation solved to find x, where x is now mg/L COD.

2.4 SPE Method:

Solid phase extraction (SPE) is a clean-up method used to extract compounds of interest from solutions, and eliminate contaminants. There are many options for how to extract samples depending on the volume and characteristics of the sample. In SPE the basic steps are condition, load, wash, and elute. The solvents used depend on the solubility characteristics of the analyte. In this extraction Oasis HLB cartridges were used. These cartridges contain polymeric reversed-phase sorbent that is used for a wide range of matrices from acidic to basic. According to the Oasis manufacturer, the proper solvent will elute the compound only, leaving behind matrix interferents such as salts, phospholipids and proteins. Due to the small sample volume, 200 mg cartridges were used. Reversed phase extraction was chosen because our analyte of interest is a non-polar compound.

2.4.1 Setup:

Figure 6. *Vacuum manifold with attached cartridges*

The Supleco Visiprep™ vacuum manifold (Figure 6) was cleaned by rinsing the inside of all portals, as well as the inner tubes, with methanol. The tubes that attached the cartridges to the manifold were not secure and continued to fall. To avoid this during the extraction, each tube was secured to the manifold with Parafilm® *(Figure 7).* The collection tubes were placed inside of the manifold onto a rack. The top of the manifold was placed on securely, and the vacuum was turned on. Each portal was individually cleaned by opening and pouring in a small amount of methanol. After all portals were cleaned, all portals were closed, and the vacuum was left running for 2 minutes.

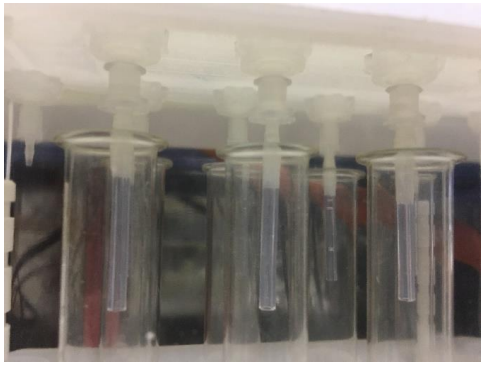


Figure 7. *Manifold tubes wrapped in Parafilm®*

2.4.2 Condition:

The purpose of the conditioning step is to wet the sorbent in order to ensure extraction efficiency and sample recovery. For reversed phase extraction, the sorbent media is first conditioned with a water-miscible organic solvent, like methanol. Following this, the sorbent must be equilibrated with the same aqueous solution as the sample to be loaded.

The labeled cartridges were placed inside the portals. A volume of 4 mL of methanol was pipetted into each cartridge, and allowed to soak until the sorbent bed absorbed it, and turned dark yellow in color. Some of the portals took much longer than others for the sorbent to absorb the methanol. Once all of the cartridges were soaked (approximately 15 minutes), one portal was opened and allowed to drip at a rate of 1 drop/s. Once the proper flow rate was achieved, another portal was opened to match the rate, and so on. The portals were closed just before the liquid reached the sorbent, in order to avoid drying out the sorbent. Once all portals were closed, 3 mL of Millipore water (with a resistance > 18 MΩ) was pipetted, and allowed to drip following the same procedure as for methanol. This step was repeated, to achieve a total of 6 mL (For methanol 1 drop/s was equivalent to 1 mL/min. For water, 1 drop/s was equivalent to 3 mL/min.) The vacuum was then turned off and the filled collection vials were removed, emptied and returned. The manifold lid was placed back on top and the pump was turned on again. The pump was allowed to run for 2 minutes before the loading step.

2.4.3 Loading:

The loading step is necessary to separate the compounds of interest from other contaminants. The compound of interest is retained on the packing and all other contaminants flow through the packing to be discarded. According to the Oasis manufacturer, the loading step is recommended not to exceed 5 mL/min in order to ensure proper retention on the packing.

Each cartridge was filled with corresponding sample. The samples were allowed to drip following the same procedure as previously mentioned. Periodically the vacuum had to be broken in order to remove the filled collection tubes. Each time, the pump was allowed to run for 2 minutes before any of the portals were opened. Once all the samples were loaded, the cartridges were allowed to dry out. For trial 1 samples, the cartridges were dried under nitrogen for 3 minutes. For trial 3 samples, the cartridges were not dried under nitrogen, because it was unavailable. These were allowed to dry under vacuum for 5 minutes.

2.4.3 Wash:

The washing step was omitted from this extraction.

2.4.4 Elute:

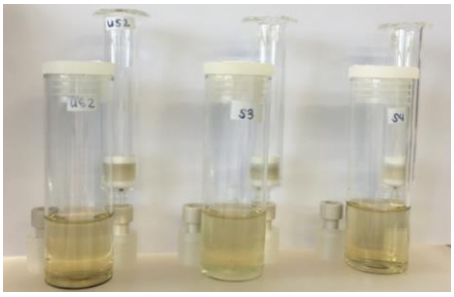


Figure 8. *Extracted samples*

The elution step is necessary to extract the desired compound. The collection tubes were removed and replaced with labeled sample collection vials. The vials were placed under the corresponding cartridge, ensuring that all tubes were positioned inside of the vials. The manifold lid was placed back on top, and the pump was turned on again. The pump was allowed to run for 2 minutes. An aliquot of methanol was added to each cartridge. The portals were opened, and the flow rate was adjusted to 1 drop/s. This step was repeated for a total 10 mL of methanol. The portals were opened completely, one by one, to ensure all the methanol was collected. The collected samples were a light yellow color (Figure 8).

2.4.5 Analysis:

Following extraction, samples were analyzed by means of ultraviolet-visible spectrophotometry (UV/VIS). Dilutions and further details of this analysis will be described in subsequent sections.

2.4.6 Storage:

Each sample vial was capped and wrapped with parafilm®. The samples were then wrapped in foil to avoid interaction with light and placed in a freezer to await further analysis via HPLC and LC-MS.

2.5 UV/VIS Method:

UV/VIS can be used to determine the concentration of compounds that are known to be present in a solution if these compounds are known to absorb wavelengths of light in either the visible or UV

spectrum. A beam of light is passed through the cuvette of different wavelengths, and the intensity of the light absorbed is measured. The sample absorbance is related to concentration by using the Beer-Lambert Law. In comparison to fluorescence spectrophotometry, which deals with transitions from excited to ground state, UV/VIS spectrophotometry measures transitions from ground to excited state. The aim of this experiment was to use UV/VIS spectrophotometry as another analytical technique to observe the photodegradation of AZT.

2.5.1 Dilutions:

The absorbance of the samples was measured before and after extraction. Dilutions were made of both the extracted samples, and the wastewater samples. The dilutions were calculated by using equation **(1)** where C_1 (M) is the concentration of AZT in sample (5×10^{-4} M), V_1 (mL) is the volume of sample to be diluted (400 µL), C_2 (M) is the desired concentration of AZT in the diluted sample (8×10^{-6} M) and V_2 (mL) is the final volume of the diluted sample (25 mL). (Note: This calculation only applies to the unextracted sample due to an error in calculations. After extraction, the volume of sample changed, therefore concentration also changed. Using equation (1), the concentration of AZT in the extracted sample equates to 0.0025 M. Therefore, a 400 µL aliquot of extracted sample creates a diluted sample concentration equal to 4×10^{-5} M.) A 400 µL aliquot was removed from each extracted and unextracted sample, and placed individually into 25 mL volumetric flasks. Each was filled with solvent to the 25 mL line in order to obtain a consistent dilution. The unextracted effluent samples were diluted with DI water, whereas the extracted samples were diluted with methanol. Following, the diluted samples were analyzed using a double-beam PerkinElmer Lambda 35 UV/Vis spectrophotometer.

2.5.2 Analysis:

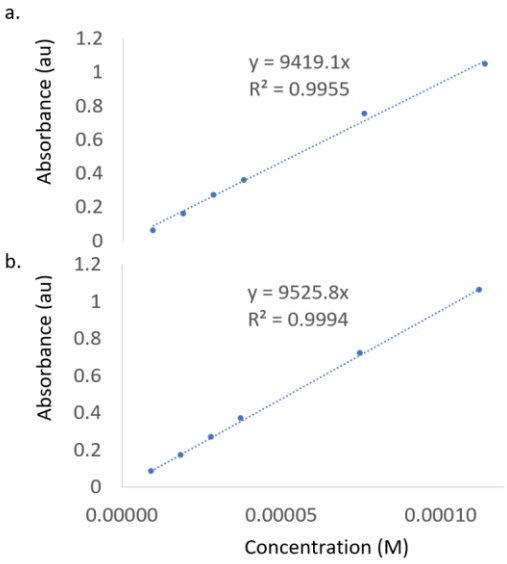


Figure 9. *Calibration curve for AZT in (a) methanol and (b) water, at 266 nm*

Samples were placed into a 10 mm quartz cuvette prior to scanning. For extracted samples the spectrophotometer cuvette was pre-rinsed twice with methanol, and then twice with the diluted sample. For the unextracted effluent samples, the spectrophotometer cuvette was pre-rinsed twice with DI water, and then twice with the diluted sample. The cuvette was filled with diluted sample and then wiped with soft tissue. The samples were scanned under the following conditions: scan speed 480 nm/min, scan wavelength range 200-500 nm, smooth 2 nm, slit width 1.00 nm, lamp change 326 nm, ordinate mode and the number of cycles 1.

According to Beer-Lambert law, there is a linear relationship between the absorbance of a solution and the concentration of the absorber. The absorbance is equal to the product of the wavelength dependent molar

absorption coefficient, the pathlength of the cuvette, and the concentration of the target compound. Beer Lambert's Law does not hold true over all concentrations, however. When the concentration is greater than 0.01 M, the relationship between absorbance and concentration is no longer linear. A linear calibration curve was obtained, for both AZT in water and AZT in methanol, using absorbance data of known concentrations of AZT, at a specific wavelength of 266 nm (Figure 9).

A wavelength of 266 nm was chosen because AZT is known to absorb light maximally at this wavelength. The equations of the calibration lines had the form of equation (2), where y is the absorbance of the sample at 266 nm, x is the concentration of AZT (M), m is the molar absorption coefficient (ε) of AZT at 266 nm multiplied by the pathlength of the cuvette (1 cm) (L mol^{-1}) and b is the y-intercept which in this case is 0. The concentrations of AZT in the samples were calculated and corrected for the dilution in order to obtain the actual concentrations in the irradiated samples. The values obtained are provided in Tables C11 and C12 .

2.6 HPLC and LC-MS

After samples are cleaned by SPE, the samples are analyzed using reversed-phase high performance liquid chromatography (HPLC). This technique allows the separation, identification and quantitation of compounds in a mixture. The mixture to be separated is injected onto a column and is eluted by passing a polar solvent, called a mobile phase, through a non-polar packing, called a stationary phase, that fills the column. The compounds that have a similar polarity to the mobile phase will be attracted to it and move more quickly through the column. The less polar compounds will be retained by the stationary phase and move more slowly through the packing.

2.6.1 Quantification:

After the compounds are separated by the column, they are run through a UV detector for HPLC which measures the absorbance at a wavelength in the ultraviolet or visible spectrum. The output information is recorded as peaks which are displayed in a chromatogram. The resulting chromatogram is displayed graphically as absorbance versus retention time. Known retention times of compounds of interest are compared, and can then determine which peaks correspond to which compound. Following, a calibration curve is created by running through known concentrations of the compound. The peak area displayed in the chromatogram represents the amount of compound that has passed through the detector. The peak area of our compound of interest is then compared to the peak areas of those from the calibration curve, and the concentration is determined. The value in using HPLC analysis is that each compound is separated and the peak of one compound does not interfere with the other. This helps to determine accurate concentrations of compounds. The quantification of compounds for this experiment will be performed at the University of KwaZulu-Natal Westville Campus once necessary equipment is functioning properly.

2.6.2 Analysis:

The analysis of compounds will be done following HPLC.

3. Results:

Key results for this experiment include changes in water quality parameters, solar irradiance values during the trials and UV/VIS data for both extracted and unextracted samples. Through water quality results, the experimental method can then be critiqued. UV/VIS data is the only information used in this study thus far to determine whether or not the hypothesis can be verified. Irradiance data helps to supplement any findings of concentration change.

3.1 Effluent Quality

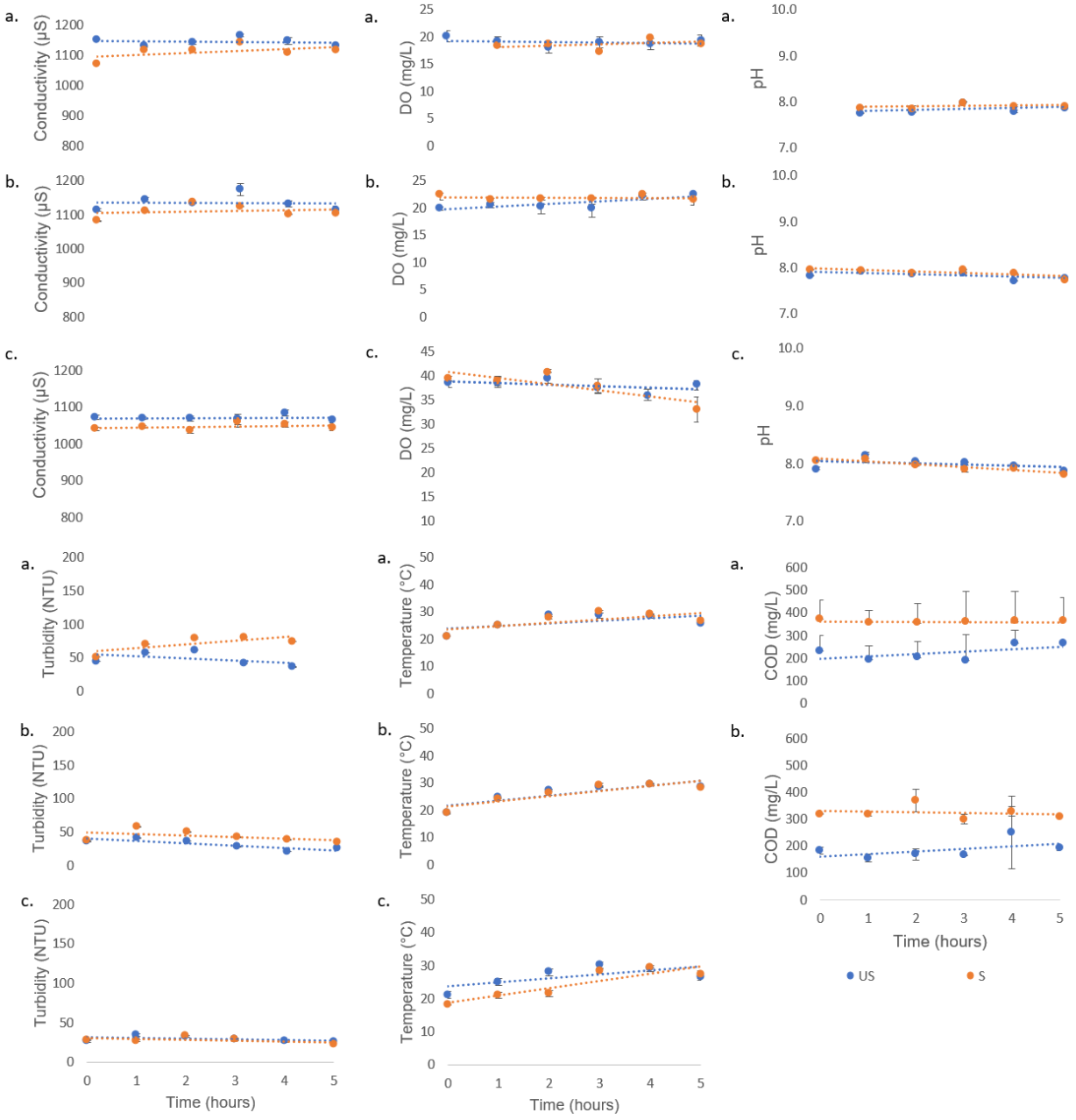


Figure 10. *Water Quality data including conductivity, DO, pH, turbidity, temperature and COD for (a) Trial 1, (b) Trial 2 and (c) Trial 3*

Water quality parameters including conductivity, turbidity, DO, pH and COD were monitored over the 5-hour solar exposure period for the effluent and AZT-spiked effluent samples. These parameters remained fairly constant, with only minor changes over the five-hour exposure *(Figure 10)*. The data obtained can be found in Tables A1-A11, B1-B11, and C1-C10 for the three trials respectively. COD values were not obtained for trial 3. The temperature increased by less than 10 °C for each trial and there was not a distinct difference between the spiked and unspiked samples. Trial 3 had the greatest increase in temperature. The pH was very similar for both the spiked and unspiked samples maintaining a pH value around 7.9. Conductivity was slightly greater in the unspiked samples than the spiked samples. In all trials, COD was higher in the spiked samples than the unspiked ones.

3.2 AZT concentrations from UV/VIS analyses

UV/VIS analysis was only determined for trial 3 samples due to time constraints, therefore only data for trial 3 will be presented here. UV/VIS data was given in terms of absorbance versus wavelength (Figure 11). Using the absorbance values at 266 nm for each sample and the calibration curve, the concentrations of AZT were found.

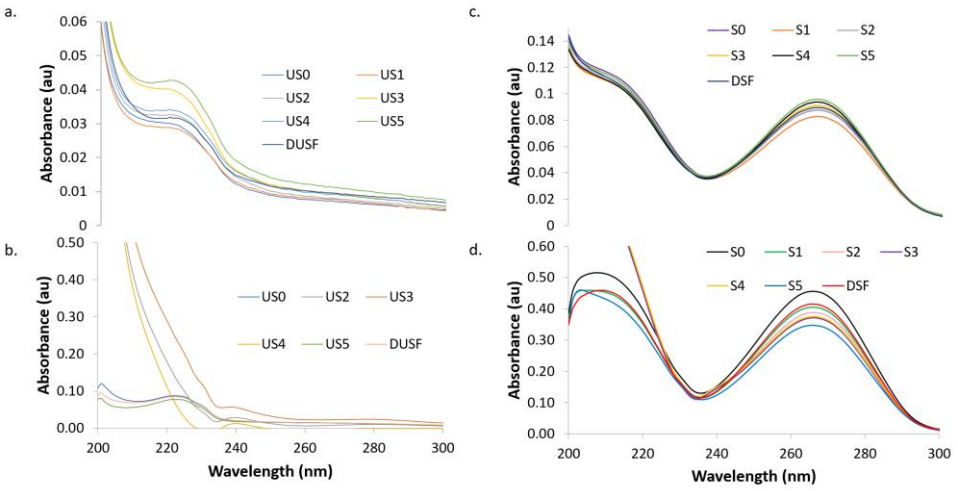


Figure 11: *Absorbance vs Wavelength data from UV/VIS for (a) unspiked samples before extraction, (b) unspiked samples after extraction, (c) spiked samples before extraction and (d) spiked samples after extraction, for Trial 3.*

Concentrations of AZT in the wastewater samples prior to extraction remained fairly constant, with standard deviations to the degree of 10^{-7}, for both spiked and unspiked samples. Concentrations of the extracted samples were shown to remain fairly constant, with a minimal increase of 2×10^{7}M over the five-hour time period *(Figure 12, Tables C11 & C12)*. The spiked samples showed higher concentrations of AZT than the unspiked samples, as expected.

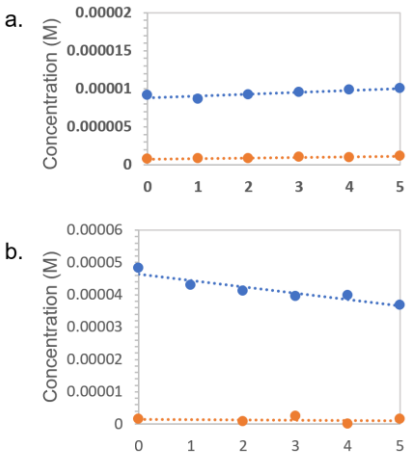


Figure 12. *AZT concentrations for both spiked and unspiked samples* ***(a)*** *before and* ***(b)*** *after extraction for Trial 3*

The values for the concentration of AZT of the dark samples and exposed sample are very similar *(Figure 13)*. For the unextracted samples, the concentrations of the dark control were slightly greater than the exposed sample. For the extracted samples, the concentrations were slightly greater in the exposed samples than the dark controls. The greatest change in concentration was in the spiked unextracted sample with a difference of 4.3×10^6 M. All other samples showed a difference around 2×10^7 M.

Solar irradiance varied for each trial (Table D-2), with the greatest irradiance measured in Trial 2 of 173,512.6 W/m^2. Comparing irradiance values with AZT concentration of spiked extracted sample from trial 3, shows that as irradiance increases, the concentration of AZT decreases (Figure 14). For trial 3, the concentration drops rapidly over the first hour, then slowly decreases over the next three hours, and then for the last hour there is more intense drop. Only this sample was compared with irradiance data because it was the only sample to show a decrease in concentration over the exposure period.

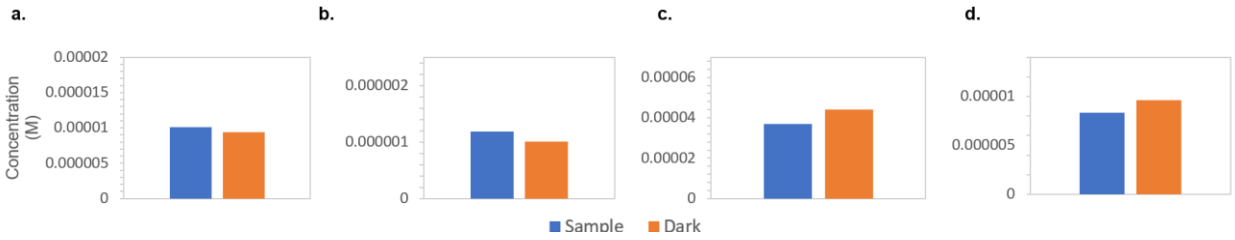


Figure 13. *Differences between exposed samples and dark controls after 5 hours for (a) unspiked samples before extraction, (b) spiked samples before extraction, (c) unspiked samples after extraction and (d) spiked samples after extraction, for Trial 3*

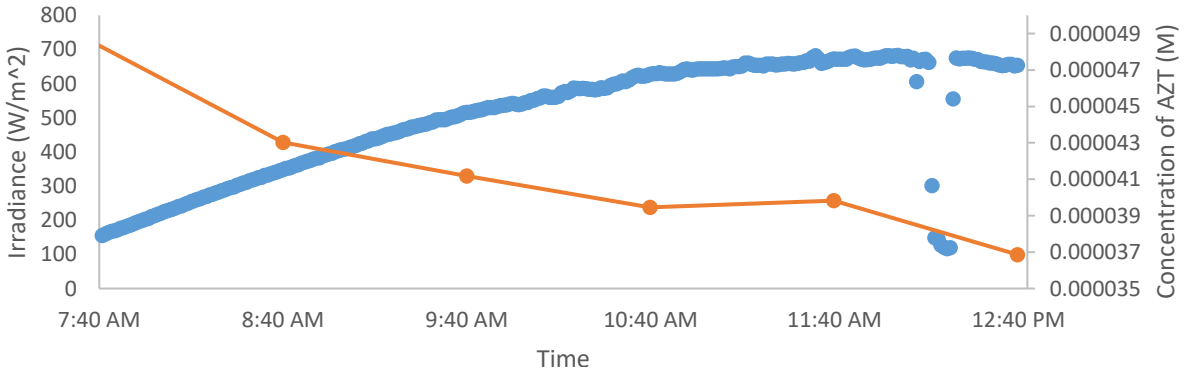


Figure 14. *Irradiance vs. Concentration over the five-hour exposure for spiked extracted samples, for Trial 3*

4.Discussion:

Results from the water quality analyses showed constant values over the five-hour exposure. This is a positive result, which will be discussed in further detail in the following sections. Degradation of AZT was determined by comparing initial and final concentrations, as well as comparing final exposed and dark control concentrations.

4.1 Effluent Quality:

Overall, there were minor changes in the water quality parameters over the five-hour irradiation period. Keeping the temperature constant was important to isolate the effects of sunlight alone. Minor changes in the temperature show that the water bath was effective. The measured temperatures of samples were well below 190℃, the temperature at which AZT is known to thermally degrade. This eliminates the potential for any degradation to be caused by heat. Determining DO values in important in measuring the usability of an effluent source for experimentation. Anaerobic bacteria required for treatment can die if DO levels are too high. If the DO values are too high, it may imply that the anaerobic treatment in not operating properly. DO values collected were extremely high for water, signifying that these values were not accurate. This was because the meter was not calibrated for the elevation and temperature prior to use. This step was not known to be necessary prior to use. Before measuring these parameters, common values expected for ABR treated wastewater effluent should be known. Though the numerical values are inaccurate, the change between the values is useful. Conductivity also remained constant over the five hours. Conductivity values were greater in the unspiked samples for all three trials. This is because AZT does not break down into ions, therefore a decrease in conductivity can be expected. Minor fluctuations were also found in pH data over the five-hour exposure. Maintaining the pH was not important for isolating the effects of solar irradiation because AZT is not known to degrade with changes in pH (Prasse et al., 2015). The value of pH is important in inferring potential compounds or bacteria that would be present and interact under acidic, basic or neutral conditions. Determining the initial value of pH is also important when assessing the likelihood of an effluent to be discharged into the environment. Should the effluent pH be recorded at extreme acidic or basic conditions, it is unlikely that the effluent would be regularly discharged due to harmful effects on aquatic life. The treatment technique would therefore probably be functioning inefficiently and should not be used for the experiment. Natural waters have a pH range between 6-8. The initial pH values recorded were nearly neutral, indicating that the effluent can be used. Small variations between the water quality parameters suggest that sunlight would not change these parameters in a natural setting over a short time period. This is an important finding when trying to understand how contaminants interact in the environment.

4.2 AZT concentration change

It is expected that the wastewater would contain various contaminants that interfere with the UV/VIS analysis of the sample. Thymine is a common photoproduct of AZT which, like AZT, also absorbs light at 266 nm. Absorbance is additive, meaning that if two present compounds

absorb at the same wavelength, data cannot isolate the concentration of AZT alone. This is an area of limitation when using UV/VIS spectrophotometry. HPLC is a better method because this is not a problem. Absorbance data shows that the concentrations of AZT in the wastewater samples, prior to extraction, did not follow the same trend as the extracted samples. This is due to interferences from the sample matrix. There was a small difference in the spiked, extracted sample between the dark control and final exposed sample. The samples prior to extraction showed a slight increase in concentration between the final exposed sample and dark control. This is attributed either to error, or to other interfering compounds still present in the sample. Concentrations resulting from UV/VIS are inconclusive to whether or not AZT concentrations were decreased over the five hours, due to limitations mentioned above. The difference in concentrations between exposed and dark control is very small, and therefore the it cannot undoubtedly be said that the degradation was due to solar irradiation. Further analyses are necessary to isolate the effects due to sunlight.

4.3 Errors:

Potential sources of error occurred predominately in the extraction process. The air bubbles that formed made it very difficult to maintain a constant flow rate between all cartridges. Also, the volume of methanol and water per mL was not measured until after extraction. Therefore, the flow rate was different for methanol than it was for water. For dilutions, the calculation for the volume of the extracted sample was incorrect. The change in concentration of AZT in the sample was not accounted for after the extraction. Because the volume of sample was not the same in the effluent sample as the extracted sample, a difference volume should have been used for the dilutions. This only affects the comparison in concentration values between the extracted and unextracted samples. For UV/VIS spectrophotometry, the cuvette used had scratches on the sides, which could have interfered with the absorbance data.

5. Conclusion:

With the current data, the degradation of AZT cannot yet be attributed to solar degradation. Data showed that water quality parameters including pH, DO, turbidity and conductivity were unchanged. Further analyses are necessary to determine the degradation of AZT without interferences from other compounds. Samples of both spiked and unspiked wastewater will be further analyzed via fluorescence spectrometry, HPLC and LC-MS that should provide more conclusive evidence than could be obtained by UV/VIS spectrophotometry.

6. Recommendations

Future analysis of samples using HPLC and LC-MS is necessary in order to assess the degradation of AZT without the interference of other compounds. Any analyses should be run on all trials in order to compare trends. If the experiment was to be repeated, a longer exposure time would be recommended. It is recommended that the trials be carried out additionally on ultra-pure water and AZT alone, for comparison. All meters used should be properly calibrated to obtain accurate results of all water quality parameters. For improvements to the SPE, it is recommended to extract a similar sample prior, in order to solidify the methods used.

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Appendices:

Appendix A: Trial 1

Table A-1: Conductivity of AZT Photolysis **T1, Unspiked**

Conductivity (µS)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	1151	1131	1140	1164	1135	1132	1174
Replicate 2	-	1130	1136	1170	1155	1128	1171
Replicate 3	-	1127	1147	1161	1149	1132	1176
Average	**1151**	**1129**	**1141**	**1165**	**1146**	**1131**	**1174**

Table A-2: Conductivity of AZT Photolysis **T1, Spiked**

Conductivity (µS)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	1070	1120	1115	1136	1101	1121	1148
Replicate 2	-	1116	1118	1150	1113	1119	1138
Replicate 3	-	1112	1118	1139	1106	1110	1148
Average	**1070**	**1116**	**1117**	**1142**	**1107**	**1117**	**1145**

Table A-3: Dissolved Oxygen of AZT Photolysis **T1, Unspiked**

DO (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	20.1	18.9	17.7	18.9	18.4	19.1	17.5
Replicate 2	-	19	18.1	19.1	18.7	19.4	18.5
Replicate 3	-	19.3	18.1	19.0	19.1	19.6	18.7
Average	**20.1**	**19.1**	**18.0**	**19.0**	**18.7**	**19.4**	**18.2**

Table A-4: Dissolved Oxygen of AZT Photolysis **T1, Spiked**

DO (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	18.4	18.1	16.5	19.5	17.7	19.6	18
Replicate 2	-	18.8	17.1	19.8	18.9	19.8	18.3
Replicate 3	-	19.3	18	19.9	19.2	19.8	18.4
Average	**18.4**	**18.7**	**17.2**	**19.7**	**18.6**	**19.8**	**18.2**

Table A-5: pH of AZT Photolysis **T1, Unspiked**

pH	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	-	7.77	7.79	7.99	7.76	7.86	7.54
Replicate 2	-	7.76	7.78	7.99	7.81	7.89	7.54
Replicate 3	-	7.76	7.78	8.01	7.81	7.89	7.56
Average	**-**	**7.76**	**7.78**	**8.00**	**7.79**	**7.88**	**7.55**

Table A-6: pH of AZT Photolysis **T1, Spiked**

pH	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	-	7.77	7.79	7.99	7.76	7.86	7.54
Replicate 2	-	7.76	7.78	7.99	7.81	7.89	7.54
Replicate 3	-	7.76	7.78	8.01	7.81	7.89	7.56
Average	-	**7.76**	**7.78**	**8.00**	**7.79**	**7.88**	**7.55**

Table A-7: Turbidity of AZT Photolysis **T1, Unspiked**

turbidity (NTU)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	44	57	61	42	36	-	34
Replicate 2	-	57	61	42	35	-	34
Replicate 3	-	57	1	41	36	-	34
Average	**44**	**57**	**61**	**42**	**36**	**-**	**34**

Table A-8: Turbidity of AZT Photolysis **T1, spiked**

turbidity (NTU)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	51	70	80	80	74	-	39
Replicate 2			78	80	74	-	38
Replicate 3			79	80	74	-	39
Average	**51**	**70**	**79**	**80**	**74**	**-**	**39**

Table A-9: Temperature of AZT Photolysis **T1, unspiked**

temp (˚C)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	21.1	25	28.5	29.7	29.2	26	26.5
Replicate 2	-	-	29.1	28.9	28.2	25.9	26.3
Replicate 3	-	-	28.7	27.7	28.1	25.6	26.3
Average	**21.1**	**25.0**	**28.8**	**28.8**	**28.5**	**25.8**	26.4

Table A-10: Temperature of AZT Photolysis **T1, spiked**

temp (˚C)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	21.1	25	28.1	30.8	29.5	26.6	27
Replicate 2	-	25.1	28.4	29.8	29	26.6	26.8
Replicate 3	-	25.1	27.5	29.8	28.9	26.5	26.7
Average	**21.1**	**25.1**	**28.0**	**30.1**	**29.1**	**26.6**	26.8

Table A-11: COD of AZT Photolysis **T1, unspiked and spiked**

COD (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5
Unspiked	232	194	204	190	267	266
Spiked	372	357	356	361	364	365

Appendix B: Trial 2

Table B-1: Conductivity of AZT Photolysis **T2, Unspiked**

Conductivity (µS)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	1115	1146	1135	1193	1140	1113	1120
Replicate 2	1118	1137	1135	1166	1131	1102	1077
Replicate 3	1111	1149	1133	1162	1123	1127	1090
Average	**1115**	**1144**	**1134**	**1174**	**1131**	**1114**	**1096**

Table B-2: Conductivity of AZT Photolysis **T2, Spiked**

Conductivity (µS)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	1092	1130	1143	1113	1108	1120	1121
Replicate 2	1090	1104	1125	1127	1114	1115	1116
Replicate 3	1066	1103	1145	1131	1084	1078	1113
Average	**1083**	**1112**	**1138**	**1124**	**1102**	**1104**	**1117**

Table B-3: Dissolved Oxygen of AZT Photolysis **T2, Unspiked**

DO (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	20	20.1	19	18.1	22.1	22.3	22.3
Replicate 2	19.6	20.4	20.2	20.5	22.2	22.4	22.5
Replicate 3	20.2	21.1	21.5	21.3	22.2	22.7	22.3
Average	**19.9**	**20.5**	**20.2**	**20.0**	**22.2**	**22.5**	**22.4**

Table B-4: Dissolved Oxygen of AZT Photolysis **T2, Spiked**

DO (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	22.1	21.4	21.4	21.5	22.3	20.4	23
Replicate 2	22.5	21.6	21.7	21.7	22.7	21.9	23.3
Replicate 3	22.6	21.7	21.9	22	22.6	22.4	23.2
Average	**22.4**	**21.6**	**21.7**	**21.7**	**22.5**	**21.6**	**23.2**

Table B-5: pH of AZT Photolysis **T2, Unspiked**

pH	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	7.85	7.94	7.87	7.9	7.72	7.78	7.74
Replicate 2	7.84	7.93	7.89	7.91	7.72	7.79	7.76
Replicate 3	7.84	7.94	7.9	7.91	7.74	7.79	7.75
Average	**7.84**	**7.94**	**7.89**	**7.91**	**7.73**	**7.79**	**7.75**

Table B-6: pH of AZT Photolysis **T2, Spiked**

pH	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	7.96	7.96	7.9	7.97	7.89	7.75	7.75
Replicate 2	7.97	7.96	7.91	7.97	7.9	7.75	7.75
Replicate 3	7.97	7.96	7.91	7.98	7.9	7.76	7.76
Average	**7.97**	**7.96**	**7.91**	**7.97**	**7.90**	**7.75**	**7.75**

Table B-7: Turbidity of AZT Photolysis **T2, unspiked**

turbidity (NTU)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	36	42	36	29	21	26	21
Replicate 2	37	41	36	28	21	26	22
Replicate 3	36	42	36	29	21	25	22
Average	**36**	**42**	**36**	**29**	**21**	**26**	**22**

Table B-8: Turbidity of AZT Photolysis **T2, spiked**

turbidity (NTU)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	38	59	51	43	38	34	22
Replicate 2	36	58	50	42	39	34	23
Replicate 3	36	58	49	41	38	35	22
Average	**37**	**58**	**50**	**42**	**38**	**34**	**22**

Table B-9: Temperature of AZT Photolysis **T2, unspiked**

temp (˚C)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	19.1	25	27.4	28.9	29.9	28.7	28.1
Replicate 2	19	24.7	27.5	28.6	29.3	28.6	28.1
Replicate 3	19	24.3	26.9	28.6	29.1	28.3	28.0
Average	**19.0**	**24.7**	**27.3**	**28.7**	**29.4**	**28.5**	**28.1**

Table B-10: Temperature of AZT Photolysis **T2, spiked**

temp (˚C)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	18.9	24.8	27.4	29.9	29.7	28.7	27.7
Replicate 2	18.9	24.0	26.4	29.4	29.5	28.3	27.1
Replicate 3	19.0	23.9	25.4	28.8	29.0	28.2	26.9
Average	**18.9**	**24.2**	**26.4**	**29.4**	**29.4**	**28.4**	**27.2**

Table B-11: COD of AZT Photolysis **T2, unspiked and spiked**

COD (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Unspiked	232	194	204	190	267	266	154
Spiked	372	357	356	361	364	365	305

Appendix C: Trial 3

Table C-1: Conductivity of AZT Photolysis **T3, Unspiked**

Conductivity (µS)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	1078	1073	1062	1051	1092	1072	1073
Replicate 2	1071	1068	1069	1075	1080	1057	1083
Replicate 3	1068	1066	1076	1074	1078	1067	1061
Average	**1078**	**1069**	**1069**	**1067**	**1083**	**1065**	**1174**

Table C-2: Conductivity of AZT Photolysis **T3, Spiked**

Conductivity (µS)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	1041	1049	1035	1061	1052	1048	1064
Replicate 2	1041	1047	1039	1056	1051	1041	1052
Replicate 3	1041	1044	1036	1061	1055	1044	1045
Average	**1041**	**1047**	**1037**	**1059**	**1053**	**1044**	**1055**

Table C-3: Dissolved Oxygen of AZT Photolysis **T3, Unspiked**

DO (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	38.3	37.3	37.3	35.4	34.4	37.1	34.2
Replicate 2	38.8	38.6	40	38.1	36.3	38.5	34.9
Replicate 3	38.7	39.7	41	39	37.1	38.8	35.2
Average	**38.3**	**38.5**	**39.4**	**37.5**	**35.9**	**38.1**	**34.8**

Table C-4: Dissolved Oxygen of AZT Photolysis **T3, Spiked**

DO (mg/L)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	39.4	37.9	40.7	19.5	36.2	30.3	34.9
Replicate 2	39	39	40.7	19.8	38.6	33.4	35.2
Replicate 3	40	40	40.9	19.9	38.9	35.5	35.7
Average	**39**	**39**	**40.8**	**19.7**	**37.9**	**33.1**	**35.3**

Table C-5: pH of AZT Photolysis **T3, Unspiked**

pH	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	7.9	8.19	8.04	8.03	7.97	7.87	7.54
Replicate 2	7.93	8.08	8.05	8.03	7.98	7.88	7.54
Replicate 3	7.93	8.17	8.05	8.04	7.98	7.88	7.56
Average	**7.92**	**8.15**	**8.05**	**8.03**	**7.98**	**7.88**	**7.55**

Table C-6: pH of AZT Photolysis **T3, Spiked**

pH	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate1	8.07	8.04	7.96	7.85	7.9	7.81	-
Replicate 2	8.05	8.18	8	7.94	7.95	7.83	-
Replicate 3	8.06	8.06	8	7.96	7.94	7.84	-
Average	**8.06**	**8.09**	**8**	**7.92**	**7.93**	**7.83**	-

Table C-7: Turbidity of AZT Photolysis **T3, unspiked**

turbidity (NTU)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	27	33	33	28	26	26	23
Replicate 2	27	35	31	28	26	25	23
Replicate 3	26	34	33	29	27	27	24
Average	**27**	**34**	**32**	**28**	**26**	**26**	**34**

Table C-8: Turbidity of AZT Photolysis **T3, spiked**

turbidity (NTU)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	27	27	33	29	-	22	22
Replicate 2	28	27	32	28	-	22	21
Replicate 3	28	27	33	29	-	22	23
Average	**28**	**27**	**33**	**29**	**-**	**22**	**22**

Table C-9: Temperature of AZT Photolysis **T3, unspiked**

temp (˚C)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	18.1	20.4	22.1	29.1	29.3	28.6	26.1
Replicate 2	18.2	20.5	20.5	28.2	29.3	26.6	26.7
Replicate 3	18.2	22.1	22.1	27.7	29.3	26.6	26.4
Average	**18.2**	**21.0**	**21.6**	**28.3**	**29.3**	**27.3**	**26.4**

Table C-10: Temperature of AZT Photolysis **T3, spiked**

temp (˚C)	t=0	t=1	t=2	t=3	t=4	t=5	DARK
Replicate 1	21.1	25	28.1	30.8	29.5	26.6	26.1
Replicate 2	-	25.1	28.4	29.8	29	26.6	26.7
Replicate 3	-	25.1	27.5	29.8	28.9	26.5	26.7
Average	**21.1**	**25.1**	**28.0**	**30.1**	**29.1**	**26.6**	**26.5**

Table C-11: Concentrations of AZT in samples from UV/VIS before extraction **T3, spiked and unspiked samples**

Concentration (M)	*t=0*	*t=1*	*t=2*	*t=3*	*t=4*	*t=5*	*DARK*
Unspiked	7.56E-07	8.19E-07	8.5E-07	1.01E-06	9.66E-07	1.19E-06	1.01E-06
Spiked	9.19E-06	8.68E-06	9.21E-06	9.56E-06	9.85E-06	1.01E-05	9.41E-06

Table C-12: Concentrations of AZT in samples from UV/VIS after extraction **T3, spiked and unspiked samples**

Concentration (M)	*t=0*	*t=1*	*t=2*	*t=3*	*t=4*	*t=5*	*DARK*
Unspiked							
Spiked	4.83E-05	4.30E-05	4.12E-05	3.95E-05	3.98E-05	3.69E-05	4.41E-05

Appendix D:

Table D-1: Irradiance for each hour on trial days

Irradiance per hour (W/m^2)	**hr 0-1**	**hr 1-2**	**hr 2-3**	**hr 3-4**	**hr 4-5**
Trial 1	33006.12	36133.35	35527.43	31185.64	24264.93
Trial 2	31163.27	36739.63	38433.02	36483.21	30693.47
Trial 3	15052.23	26055.15	34036.3	38986.17	36443.46

Table D-2: Total irradiance for exposure time on trial days

Total irradiance (W/m^2)	**Time = 1 hour**	**Time = 2 hours**	**Time = 3 hours**	**Time = 4 hours**	**Time = 5 hours**
Trial 1	33006.12	69139.47	104666.9	135852.5	160117.5
Trial 2	31163.27	67902.9	106335.9	142819.1	173512.6
Trial 3	15052.23	41107.38	75143.68	114129.8	150573.3