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Investigation Of The Natural Degradation Of Selected Endocrine Disrupting Compounds In Wastewater

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INVESTIGATION OF THE NATURAL DEGRADATION OF SELECTED ENDOCRINE DISRUPTING COMPOUNDS IN WASTEWATER

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Dedication

To my parents, for everything that they do

INVESTIGATION OF THE NATURAL DEGRADATION OF SELECTED ENDOCRINE DISRUPTING COMPOUNDS IN WASTEWATER

by

TONI CARRICK, B.S.

THESIS

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Abstract

Endocrine disrupting compounds (EDCs) are a class of compounds that are of concern for their potentially adverse effects on fish, wildlife, and humans. They can cause developmental and reproductive anomalies at concentrations in the low ppb to ppt levels. Studies have shown the primary route of exposure of EDCs within the environment is through the discharge of effluent wastewater. Removal efficiencies of wastewater treatment plants have been studied extensively, however, in rural or developing areas, which often lack secondary or tertiary wastewater treatment, little is known about the stability of these compounds and the rate at which they degrade in untreated wastewater. Two of the most commonly found EDCs in wastewater are nonylphenol (NP) (used in personal care products, detergents, and the production of nonylphenol ethoxylate surfactants) and bisphenol A (BPA) (used in the production of polycarbonate plastics, epoxy resins, and other common household items). Therefore, the aim of this research was to investigate the degradation of BPA and NP in untreated wastewater.

It was found that in ultrapure water, abiotic degradation (e.g. hydrolysis and photodegradation) of NP was negligible, regardless of pH throughout a storage period of around 30 days. For BPA, it was found that both photodegradation and hydrolysis occurred. In wastewater, NP, NPEO1, and BPA all underwent biodegradation. It was also determined that a single autoclave cycle of 60 minutes was not enough to kill all of the bacteria present in the wastewater capable of degrading NP and BPA. Filtration of samples did seem to be effective in reducing, although not completely stopping, biodegradation of NP and NPEO1. Further pH adjustment to either an acidic pH (e.g. 3) or basic pH (e.g. 11) helped to further slow degradation in an autoclaved filtered wastewater. For BPA, filtration of wastewater seemed to enhance degradation.

In unfiltered wastewater, biodegradation of NP, NPEO1, and BPA also occurred. In all wastewater samples, BPA followed first order degradation, and both NP and NPEO1 followed second order degradation.

Although most removals were generally incomplete, 100% removal was proven possible at some point for all compounds studied, albeit the rates are very slow. The results presented herein demonstrate the degradation capabilities of NP and BPA in ultrapure and wastewater.

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Chapter 1: Introduction

Endocrine disrupting compounds (EDCs) are a class of compounds which have only recently, within the past two decades, risen to prominence within the scientific community for their potentially adverse effects on fish, wildlife, and humans (Jean-Claude & Amiard-Triquet, 2015; Kabir, Rahman, & Rahman, 2015). In the early 90's, the World Wildlife Federation Wingspread Conference held the first meeting dedicated solely to the discussion of EDCs, at which they were able to conclude that "Many compounds introduced into the environment by human activity are capable of disrupting the endocrine system of animals, including fish, wildlife, and humans. Endocrine disruption can be profound because of the crucial role hormones play in controlling development." (Colborn & Clement, 1992) In 1996 the United States Environmental Protection Agency (EPA) was issued a mandate under the Food Quality Protection and Safe Drinking Water Acts in order to develop validated methods for screening and testing estrogens and other similar substances, as well as evaluate the potential risks of combinations of these compounds (as opposed to risk assessments for these substances acting alone). (Hotchkiss et al., 2008) Due to their widespread use and the increasing number of studies indicating their adverse effects, EDCs have been thrust into the forefront of environmental research.

EDCs can be classified as either naturally occurring, as is the case for phytoestrogens commonly found in soybeans (Murkies, Wilcox, & Davis, 1998), or synthetic chemicals used by humans for industrial processes, such as polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) used as industrial solvents and lubricants; plastic components like bisphenol A (BPA); plasticizers such as various phthalates; pesticides; and surfactants, such as nonylphenol monoethoxylate (NPEO-1), nonylphenol diethoxylate (NPEO-2), and nonylphenol (NP) (Diamanti-Kandarakis et al., 2009). Given their widespread use, EDCs have become ubiquitous within the environment, found in waterways or organisms on all seven continents (Cao, Yu, & Connell, 2010; Focazio et al., 2008; Goutte et al., 2013; Jurado et al., 2012; Kolpin & Meyer,

2002; Manickum & John, 2014; Syakti et al., 2013; Trumble et al., 2012). In the United States alone, the US Geological Survey reported the presence of EDCs in 80% of the 139 streams that they tested across the entire country (Kolpin & Meyer, 2002). Despite this fact, there is still a lack of concrete regulation on the monitoring and removal of these compounds from anthropogenic sources. One common source of EDCs within the environment is through the discharge of these compounds in wastewater treatment plant effluents, where the contaminated water can potentially seep into groundwater. Since these treatment plants are neither designed nor required to monitor or remove these organic compounds (Focazio et al., 2008), they remain persistent within the environment.

Aquatic exposure to levels of EDCs detected in streams has also been shown to cause adverse effects in populations of fish (Kidd et al., 2007) and therefore, EDCs are classified as contaminants of emerging concern. The US EPA defines these emerging pollutants as "new chemicals without regulatory status and with an impact on the environment and human health that is poorly understood" (Priac et al., 2014). Within the classification of EDCs lie two environmentally relevant compounds found to cause adverse effects after their anthropogenic release into the environment: BPA and NP. As mentioned previously, one common source of EDCs within the environment is through the discharge of contaminated wastewater effluent. Based on the literature, the removal efficiency of BPA by wastewater treatment plants can be estimated ranging from 30% to 90% (Tran, Teil, Blanchard, Alliot, & Chevreuil, 2015), whereas the removal of NP is estimated ranging from 9% to 94% (Argese, Marcomini, Bettiol, Perin, & Miana, 1994). These highly variable results indicate incomplete removal of NP and BPA and serve as an explanation as to why they are considered ubiquitous within the environment. BPA and NP are among the most frequently detected compounds in wastewater treatment plant effluents, as well as streams and natural waterways impacted by industrial and wastewater treatment activities. Therefore it is important to look further into the activity of these compounds within the environment.

1.1 BISPHENOL A

Bisphenol A (BPA) is considered a high production volume chemical, with approximately 1.7 billion pounds of it being produced annually in the United States alone (Geens, Goeyens, & Covaci, 2011). It is widely used in the production of polycarbonate plastics as well as epoxy resins, found in common household items such as canned foods, baby bottles, paper, cardboard, reusable food and beverage containers, and microwavable containers. Additionally, it is used in the manufacture of various automotive parts, optical media, medical and healthcare devices, and construction materials, among others. BPA was found in atmospheric samples across six continents (with the exclusion of Africa), suggesting the potential for a great environmental concern in the future, given the compound's ability to accumulate within the environment (Fu & Kawamura, 2010). With humans being exposed to BPA through the aforementioned sources, the compound is continually being released into the environment through effluent wastewater (via human-ingested BPA), landfill leachate, or the natural degradation of polycarbonate plastic. In 2007, Vandenberg et al. concluded that the sources of BPA within the environment that contributed the most to human's exposure was unknown (Vandenberg, Hauser, Marcus, Olea, & Welshons, 2007). Furthermore, they expanded upon this in 2010 by stating further research needs to be done in order to identify all sources of BPA (Vandenberg et al., 2010). Without a clear understanding of where the largest contributors of BPA exposure to humans are, it will remain difficult to estimate the fate of BPA in an effort to reduce the levels within the environment. Given that the presence of BPA within the environment is solely due to anthropogenic activity, this poses a massive threat to both humans and wildlife.

In terms of human exposure, BPA has been detected in drinking water and foods, and as a result, in urine, sweat, breast milk, blood (including follicular, amniotic, and umbilical fluids), and bodily tissues such as the liver, brain, and adipose tissue (Michałowicz, 2014). One of the most common routes of exposure of BPA to humans is through the ingestion of contaminated food and drink, and in 2007 Vandenburg et al. estimated that amount to be from 0.48 to 1.6 g/kg

body weight/day (Vandenberg et al., 2007). In 1988, the EPA estimated the reference dose of BPA to be 50 μg/kg of body weight/day (Vogel, 2009). This estimation remains the standard today regardless of the abundance of new information on the deleterious effects of BPA. One of the most well-known effects of BPA exposure is endocrine disruption. Some studies suggest BPA is only mildly estrogenic (Iso, Watanabe, Iwamoto, Shimamoto, & Furuichi, 2006), with activities towards estrogen receptors being 1,000-10,000 times less than that of 17-β estradiol (the naturally-occurring primary female sex hormone) (Michałowicz, 2014), however studies continue to demonstrate that even in very low concentrations, BPA can still exert physiological effects on receptor-mediated processes. Metabolites of BPA have also been shown to exhibit estrogenic activity, sometimes in greater amounts than BPA itself (Alonso-Magdalena et al., 2012). Meeker et al. demonstrated a relationship between exposure of men to BPA and circulating hormone levels (Meeker, Calafat, & Hauser, 2010). Ziv-Gal et al. were also able to show that BPA was capable of endocrine disruption by decreasing synthesis of estradiol and inhibiting the growth of ovarian follicles by interaction with the aryl hydrocarbon receptor, a protein in humans involved in the regulation of biological responses to aromatic hydrocarbons (Ziv-Gal, Craig, Wang, & Flaws, 2013). Though research is limited, the correlation between exposure to BPA and obesity in humans has also been examined. A cross-sectional evaluation of a study performed from 2003-2004 of the urinary BPA levels of 2,838 children and adolescents between the ages of 6 and 19 revealed that elevated levels of BPA in the urine were significantly associated with obesity (Trasande, 2012). In a similar cross sectional evaluation of the same study, other researchers have also identified a positive correlation between urinary BPA levels and occurrence of diabetes mellitus (Shankar & Teppala, 2011). BPA has also been shown to have damaging effects on other systems within the body, including hepatotoxicity, neurotoxicity, and immunotoxicity, as well as mutagenicity in eukaryotic cells, carcionogenicity, and teratogenicity, *in vitro and in vivo*, all of which were reviewed by Michałowicz (Michałowicz, 2014).

In addition to the effects on humans, BPA has also been detected in urban and rural atmospheric samples, natural waterways and sewage effluents, and dust (Michałowicz, 2014), indicating a need to examine the effects of this anthropogenic compound in wildlife. In wildlife species, the endocrine disrupting effects of BPA have been shown to include alteration of sex determination, alteration of gonadal function, and induction of hepatic vitellogenin production (Crain et al., 2007). BPA has been shown to be systemically toxic to various aquatic organisms in concentrations ranging from 1.1 to 12.8 mg/L, however studies have also demonstrated the harmful effects of BPA on wildlife in more environmentally relevant concentrations (defined as 12 µg/L or lower) (Flint, Markle, Thompson, & Wallace, 2012). In invertebrates, BPA exposure at these low concentrations has shown both developmental effects (in the form of developmental inhibition of the copepod *Tigriopus japonicas*) (Marcial, Hagiwara, & Snell, 2003), and reproductive effects (in the form of superfeminization, which included the growth of additional female sex organs and increased fertility in a freshwater snail, *Marisa cornuarietis*) (J. Oehlmann, Schulte-Oehlmann, Tillmann, & Markert, 2001). In fish, these same growth effects have been seen, but at concentrations above typical environmental levels; zebrafish embryos exposed for only 48 hours to 2280 µg/L of BPA resulted in higher brain levels of cytochrome P450 aromatase, which is responsible for aromatization of androgens into estrogens and can result in feminized brains (Kishida, McLellan, Miranda, & Callard, 2001). In terms of reproductive effects of BPA in fish, studies have shown gonad structural changes in male carp, reduced sperm quality and delayed ovulation in trout (*Salmo trutta f. fario*), and altered sex cell type ratios in the fathead minnow, among others, all of which could potentially affect growth, brain and bone development, cellular division, and cause feminization or masculinization (Jörg Oehlmann et al., 2009). The effects of BPA on amphibians pose a greater threat due to their permeable skin. In one study, BPA concentrations of 2.28 µg/L were shown to inhibit spontaneous metamorphosis in the western clawed frog (*Silurana tropicalis*) after being exposed for nine days (Kashiwagi et al., 2008). Levy et al. also found that in the African clawed frog (*Xenopus laevis*), their larvae underwent complete sex reversal from male to female after being

exposed to 22.8 µg/L BPA for two weeks (Levy, Lutz, Krüger, & Kloas, 2004). BPA has also exhibited negative effects in reptiles. One study exposed crocodile (*Caiman latirostris*) embryos to 1400 µg/L BPA and the result was the development of abnormalities within the testes. Upon increasing the exposure to 140,000 µg/L BPA, a complete sex reversal from male to female was seen in the eggs incubated at the male-determining temperature (Stoker et al., 2003). The majority of the research looking into the effects of BPA exposure to humans has been done by extrapolating the results of tests done on mice and rats, so the effects of BPA on such mammals can be seen in numerous studies throughout the literature. However, there is very little research on the effects of BPA exposure to mammalian wildlife as such effects are difficult to determine in nature. Therefore the assessment of the effects of BPA on mammalian wildlife is based on those laboratory studies of mice and rats used as model organisms, and it has been concluded they are similar to the effects seen in humans, e.g. obesity and reproductive defects, among others (Flint et al., 2012).

The current use and production of BPA is massive and widespread. Given the facts that BPA is found ubiquitously within the environment as well as its deleterious effects to both humans and wildlife alike, the concern for BPA's activity within the environment is great. It is for these reasons that this study will focus on BPA as a targeted EDC.

1.2 NONYLPHENOL

In addition to BPA, one other class of compounds exhibiting endocrine disrupting capabilities is alkylphenols (APs) and their polyethoxylated derivatives (APEOs). More specifically, one of the most commercially important APs is nonylphenol (NP), used in the production of nonylphenol polyethoxylate (NPEO) surfactants. NPEOs have themselves extensive and widespread use in many different applications and consumer products, including personal care products, detergents and cleansing agents (surfactants), paints, adhesives, and stabilizers in plastics (Diehl, Johnson, Xia, West, & Tomanek, 2012). It is estimated that

industrial and municipal wastewater treatment plant effluents are the main routes of NPEO's discharge into the environment (Servos et al., 2003).

NP can be considered, itself, a degradation product, formed by the aerobic degradation of nonylphenol ethoxylates, and subsequent anaerobic degradation of the shorter ethoxylates (Giger, Gabriel, Jonkers, Wettstein, & Kohler, 2009; Shao, Hu, & Yang, 2003), as shown in Figure 1 (Ahel, Giger, & Koch, 1994). Additionally, NPs are expected to be persistent in the groundwater (Servos et al., 2003). Therefore, through the use and discharge of NPEOs, and subsequent degradation, NP can also be considered to occur ubiquitously within the environment (Priac et al., 2014). It has been estimated that the annual production of nonylphenol has reached 154,200 tons in the United States alone, although NP is only fractionally discharged into the environment through industrial sources, while its main source within the environment is through the degradation of NPEOs (Argese et al., 1994).

As with BPA, NP has been proven to function as an endocrine disruptor, as well as being able to interfere with other types of cells and organs present in both humans and animals (Argese et al., 1994). NP also exists as several different isomers, with the potential for each to exhibit its own individual estrogenic capabilities. Some isomers of NP are structurally very similar to 17-β estradiol (shown in Figure 2), and it has therefore been proposed that NP mimics estradiol by binding to the site of the estrogen receptor, thereby blocking the production of natural hormones or inhibiting or stimulating the endocrine system (Lee & Lee, 1996).

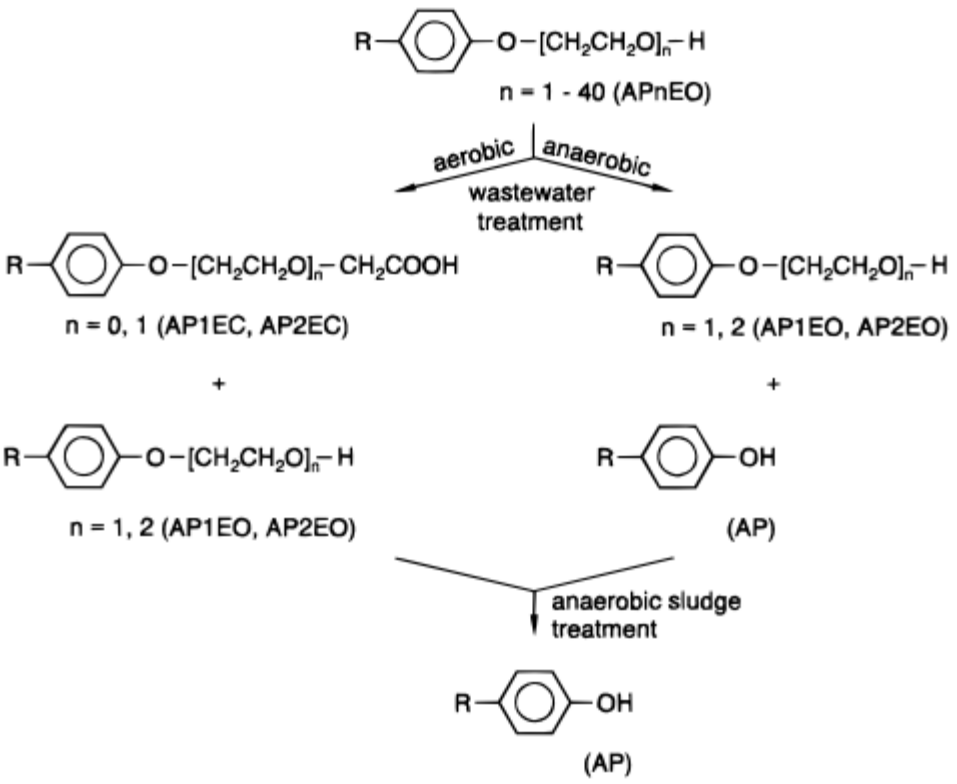

Figure 1: Aerobic and anerobic biotransformation pathways of APEOs (Ahel et al., 1994).

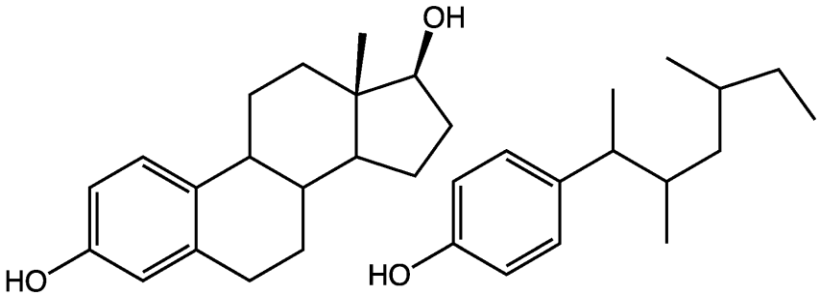

Figure 2: Structural similarities between estradiol (on the left) and nonylphenol (on the right).

Just as with BPA, humans are primarily exposed to NP by ingesting contaminated foods and drinking water. In addition, use of personal care products or detergents in which these APs are used can lead to human exposure to this EDC. In the US, one study found NP present in greater than 51% of the 394 adult urine samples studied (Calafat et al., 2005), whereas another study reported NP being detected infrequently (in less than 11% of all samples tested, including urine, skin residues, food, beverage, indoor and outdoor air, dust, and soil samples in and around

the homes) in 297 preschool age children (Wilson, Chuang, Morgan, Lordo, & Sheldon, 2007). Another study in China, which tested for the presence of both BPA and NP in typical foodstuffs as part of a total diet study, showed both compounds to be present with ubiquity in food, estimating the average intake to be 520 ng/kg of bodyweight per day, which is still below the tolerable daily intake of 5 µg/kg of bodyweight per day (Niu, Zhang, Duan, Wu, & Shao, 2015). This same study also reported the occurrence of NP in infant formulas and was able to extrapolate the total daily intake of infants, ages 0-1 year, as being 5 to 17 µg/kg of bodyweight per day, which is well above the tolerable daily intake. These findings agree with the trend seen in the United States, in which younger children are exposed to, and have, higher levels of NP within their bodies. Given that children are commonly at a higher risk of experiencing adverse effects from EDCs, especially during typical developmental stages, these trends suggest the need for urgent attention to be paid to the routes of exposure to humans of NP, as well as the activity of the compound within the environment. Widely varying and diverse concentrations of this compound have proven NP's ability to interfere with the homeostatic systems of different types of cells and organs. Argese et al. demonstrated NP's ability to trigger respiratory toxicity in cells, reporting an EC_{50} value of 1.8 mg/L (Argese et al., 1994). Kirk et al. summarized the effects of EDCs, including NP, being able to inhibit the active transport of calcium ions to skeletal muscle cells, reporting IC_{50} values of 820-2420 mg/L (Kirk et al., 2003). Kudo et al. suggested that exposure to NP ay affect neurogenesis in a developing brain after demonstrating the EDC's ability to induce the death of neural stem cells (Kudo et al., 2004). The effective concentration in that study was greater than 660 mg/L. Finally, at concentrations below 0.01 mg/day, one study demonstrated the ability of NP to alter the cell cycle kinetics of the mammary gland by increasing the growth of epithelial cells (Colerangle & Roy, 1996).

In terms of wildlife exposure to NP, the literature also suggests widely varying and diverse effects of NP. In a study by Hoss et al., an exposure between 0 to 350 µg/L of NP to nematodes actually demonstrated an increase in their growth and reproduction (Höss et al., 2002). Although this result supports the recommendation by the USEPA of exposure of various

invertebrates to NP be below 6.6 µg/L in freshwater and 1.7 µg/L in saltwater (Soares, Guieysse, Jefferson, Cartmell, & Lester, 2008), it has been shown that some of the effects of NP exposure cannot be determined within one life cycle. In the case of Japanese rice fish, NP exposure was shown to negatively affect embryo survival as well as the development of sex characteristics (Yokota et al., 2001). However, the first offspring of those fish exposed to NP did not show the same adverse effects in terms of embryo survival. These studies provide further evidence of the variable and diverse effects NP can elicit in both humans and wildlife.

The demonstrated adverse effects of NP on different organisms raise the concern for monitoring the presence of NP in environment. Although there are some countries that are beginning to ban the use and manufacture of NPEOs and NP, the ubiquity of this compound suggests it will remain a presence for years to come. Therefore, NP has been chosen as the second targeted EDC of this study.

1.3 NATURAL DEGRADATION OF EDCS

As previously mentioned, the primary route with which these EDCs enter the environment is through the discharge of wastewater treatment plant effluents, and as such, the removal efficiencies of wastewater treatment plants have already been studied extensively. For BPA, studies have shown there is still generally incomplete removal, ranging from 30-90% depending upon the treatment processes applied (Tran et al., 2015). Before the establishment of centralized wastewater treatment facilities, the naturally occurring processes in lakes and streams were considered enough to treat wastewater. However, with humans' growing demand for natural resources (due to both population and industrial growth) and the discharge of anthropogenic contaminants at an all-time high, those natural processes were no longer an option. Therefore it is important to look into the activity of these contaminants once they've been introduced to an ecosystem. Our research question began with a simple inquiry: can certain EDCs be removed after being discharged into the environment and can they be degraded naturally? It had already been proven biodegradation of BPA by bacteria isolated from

wastewater treatment plants is possible (Eio, Kawai, Tsuchiya, Yamamoto, & Toda, 2014), however very few studies look at the degradation of native EDCs, meaning the degradation of the compounds already present in the natural waters, without the addition of standards. The degradation pathways of BPA in the presence of a bacterial consortium isolated from activated sludge is shown in Figure 3.

Figure 3: Biodegradation pathway of BPA in a bacterial consortium isolated from activated sludge, proposed by Eio et al.. The bacterial degradation pathways are represented by solid arrows, whereas the photodegradation pathways are represented by dashed lines.

The natural degradation of the two selected EDCs has been demonstrated to occur in seawater. In an effort to gain further understanding of the fate and exposure of BPA, and therefore look into hazard evaluation, a 1987 study looked into the degradation of BPA in natural waters around a BPA manufacturing plant (Dorn, Chou, & Gentempo, 1987). The authors were able to conclude that BPA is readily biodegradable, with a half-life of less than 5 days, in natural waters following an acclimation period. In 2003, another study looking into the degradation of both BPA and NP in natural seawater was able to conclude the same thing – that BPA is readily biodegradable after an acclimation period; however they demonstrated a much longer half-life (14.5 days) days (Ying & Kookana, 2003). In the case of NP, they saw a rapid decrease in the compound due to abiotic processes, followed by further biodegradation, with a half-life of only 5. A similar study in 2005 compared the degradation of BPA in both river and seawater (J.-H. Kang & Kondo, 2005) in which the authors were also able to conclude BPA is readily biodegradable under aerobic conditions in river water, with half-lives of 3-4 days, depending on the temperature. A previous study by the same authors revealed BPA was not able to degrade in anaerobic conditions with bacteria isolated from river water (J. H. Kang & Kondo, 2002). Their results for seawater were in agreeance with Ying and Kookana's study in that there is very little BPA degradation for the first several weeks of the storage. In an effort to determine compound stability, Salgueiro-Gonzalez et al. did a short-term stability study and monitored the change in

concentration of BPA and NP in seawater and noted that within eight days, there is very little change in BPA concentration (Salgueiro-González et al., 2012). In the case of NP, there is little degradation within the first five days, followed by a sharp decrease in concentration between day five and day eight. This was attributed to both adsorption to the container walls, as well as degradation. In order to quantify the adsorption potential of NP and BPA in nature, one study looked at the degradation in a soil-water system at a groundwater recharge site (J. Li, Jiang, Liu, & Lv, 2013). It was reported that NP adsorbed very easily onto the soil, whereas BPA was more mobile within the water. In this same study, by looking at their aerobic degradation, they reported half-lives of 2.7 and 3.3 days for NP and BPA respectively. When looking at the degradation with additional EDCs, each of the two half-lives increased, suggesting interactions between natural degradation processes. Another study looking at the photodegradation of NP in natural seawater using simulated sunlight also noted a slowed rate of degradation as compared to the degradation in pure water (Yanxia Li, Duan, Li, & Zhang, 2013). Again, this was attributed to the presence of additional constituents within the natural seawater whose degradation would compete with the NP. Since influent wastewater is generally a very complex mix of organic constituents, the results of their study indicate a need to look at the degradation of EDCs within one of these complex mixtures.

As mentioned previously, the technical mixture of NP exists as several different isomers. Ieda et al. were able to detect a total of 102 different NP isomers in a technical mixture (Ieda et al., 2005). Several of the isomers can be seen below in Figure 4. Additionally, several different isomers of NP have been found in environmental water samples (Kim et al., 2005). Therefore, when examining the degradation of NP, it is important to realize that not all NP isomers will have the same degradation potential. Lu et al. suggested degradability of the NP isomers in an activated sludge bioreactor could be based on the structure and location of the alkyl chain (Lu, Reif, & Gan, 2015). It was noted that isomers with bulky α-substituents were resistant to biodegradation and ended up being enriched in the effluent waters. Therefore attention should be paid to the isomers capable of natural biodegradation within a wastewater treatment plant so that

future research focus can aid in degrading the ones that are not. Lu and Gan also found that there is slight degradation selectivity between the different NP isomers in their oxidation by potassium permanganate, albeit less selectivity than in their biodegradation study (Lu & Gan, 2014b). This reveals a possibility for the improvement of wastewater treatment to remove all recalcitrant NP; however without the knowledge of how this EDC acts within an unadulterated wastewater system, it can still be difficult to accurately assess these options. Additionally, it still provides little information in the risk assessment to humans and wildlife when exposure to untreated natural wastewaters.

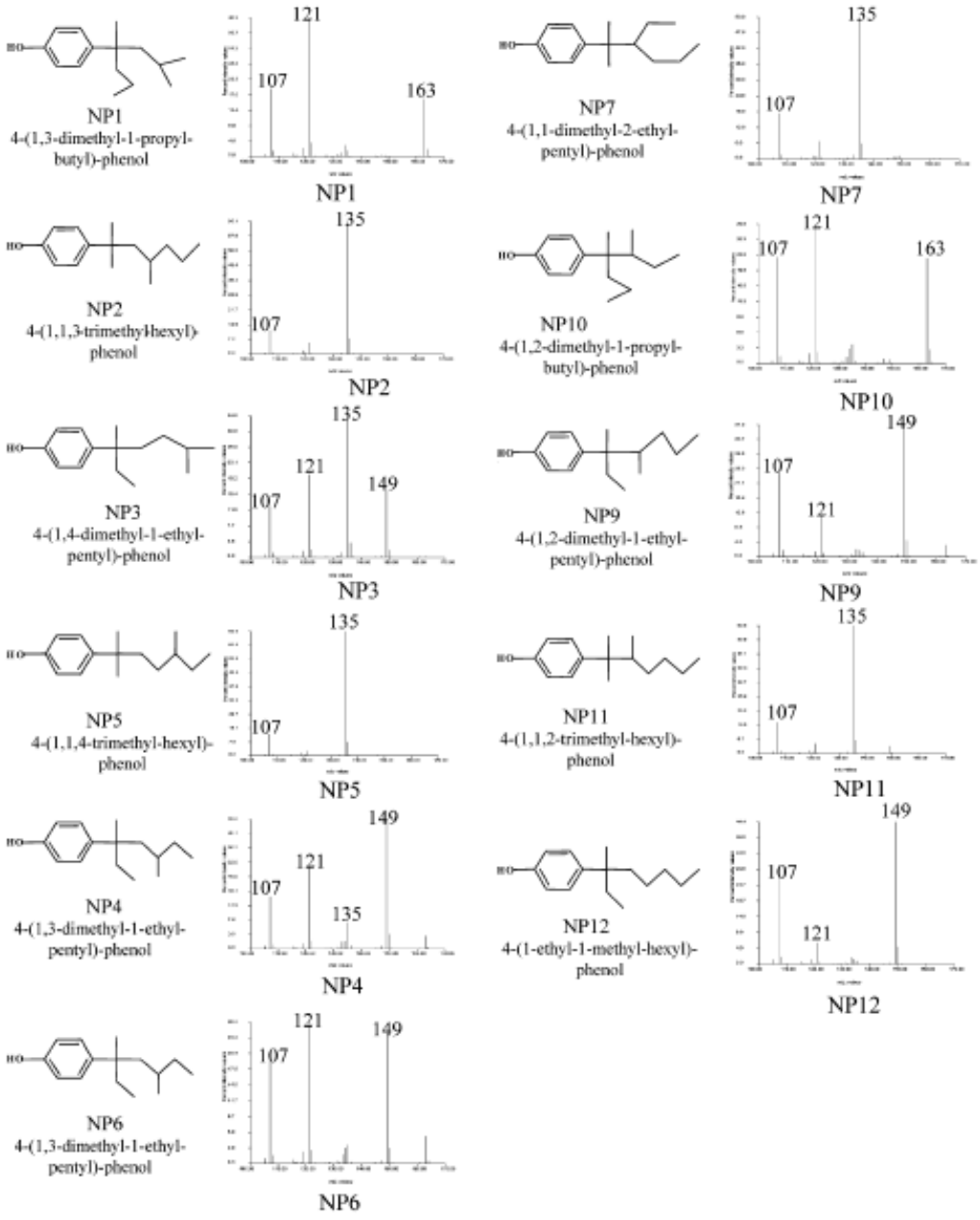

Figure 4: Structure of several different NP isomers, determined by GC-GC/MS and NMR (Ieda et al., 2005).

1.4 RESEARCH QUESTIONS AND OBJECTIVE

Due to their potentially toxic effects, recent studies have focused on determining the concentration of EDCs in various water matrices (Tran et al., 2015) (Soares et al., 2008) (e.g. waste water influent and effluent, drinking water, and natural waterways, such as lakes, rivers, and streams). Additionally, remediation methods (reviewed by Luo et al., 2014), such as

biodegradation, physical removals, and chemical oxidations, are constantly under investigation. An example of three common wastewater treatment set-ups typical of rural areas can be seen in Figure 5. This study showed that these rural wastewater treatment facilities were least effective in removing six EDCs as compared to a centralized facility employing secondary treatment (activated sludge) (Qiang, Dong, Zhu, Qu, & Nie, 2013). However, thus far, limited information is available regarding the stability of the compounds and the rate at which they degrade in untreated wastewater. This becomes especially important in developing countries and rural areas where wastewater treatment facilities do not employ the use of secondary or tertiary treatment processes, including advanced oxidation. These types of facilities rely on natural physical, chemical, and biological processes to treat the influent wastewater. Since one common source of EDCs within the environmental is through the discharge of effluent wastewater still containing relevant concentrations of these compounds, studying their stability without the addition of physical or chemical degradation agents will also help to aid in the determination of risk assessments for these compounds. To our knowledge, no such studies have been carried out on EDC's stability in wastewater. A lack of such information could potentially lead to incorrect determination of EDC levels in the samples and faulty impact assessment. As a secondary benefit, studying the compounds' stability within the unadulterated water could potentially lead to a standard sample preparation method, as well as aid in determining the sample holding times of environmental samples containing NP and BPA. Currently, there are standard methods in the preparation, as well as the holding times, of organic analytes (ASTM Standard D3694, 2011), however they are neither compound specific, nor matrix specific. Therefore, this research represents a vital piece of missing information in the study of EDCs within the environment.

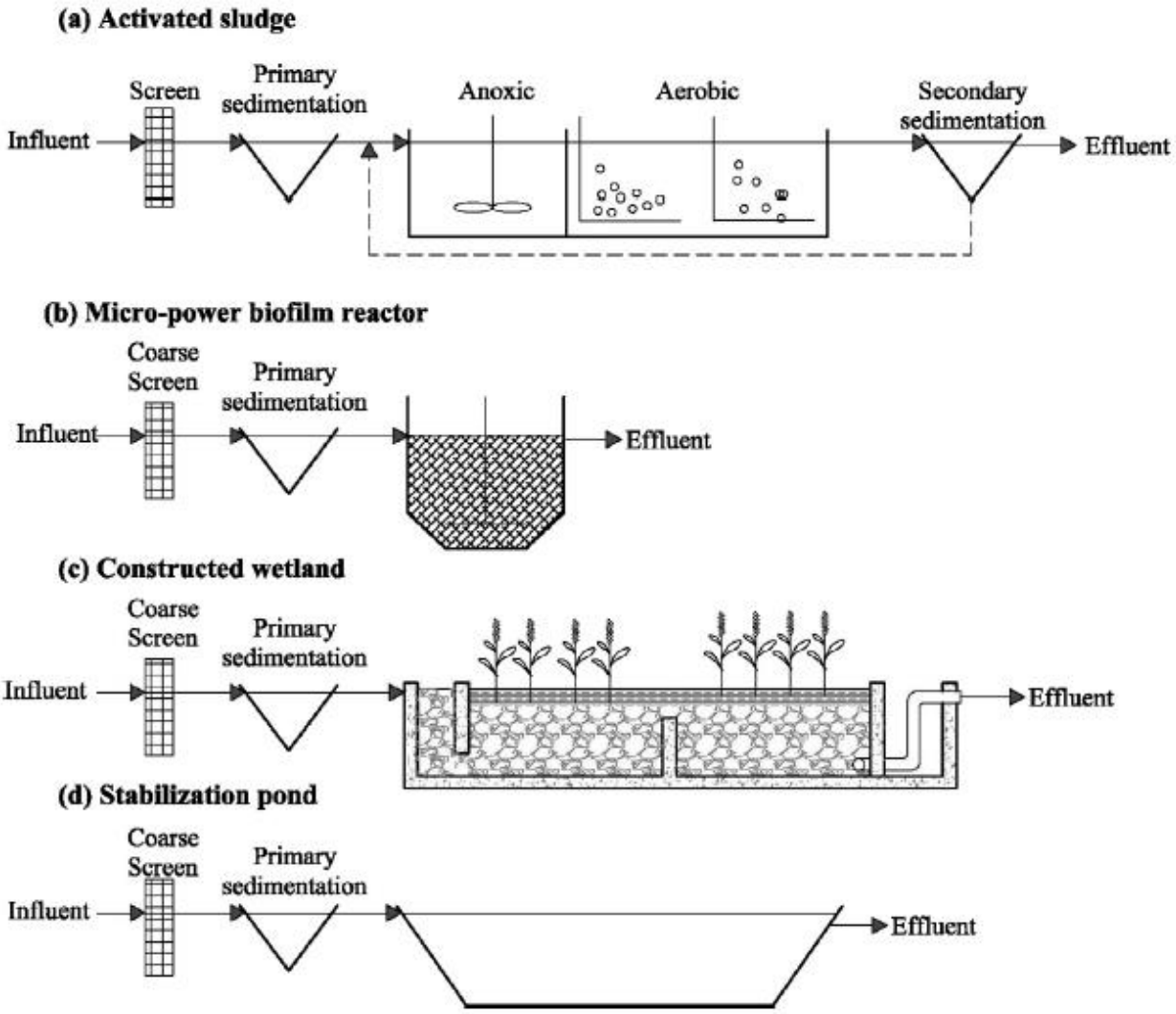

Figure 5: Four wastewater treatment processes from a study in China. Schematic (a) was based on a centralized treatment plant in a densely populated town, whereas schematics (b), (c), and (d) were based on village-scale treatment facilities in rural areas (Qiang et al., 2013).

Objective

The aim of this research was to investigate the degradation of BPA and NP in wastewater without treatment, i.e. without the addition of traditional removal methods. To determine the effects of storage condition on the change in concentration of the compounds, the rates of degradation were determined and compared at five different pHs (3, 5, 7, 9, and 11), as well as under three different storage conditions (4 ºC, room temperature, and room temperature with exposure to ambient light). As an additional benefit of this experimental set up, the degradation

of BPA and NP under typical laboratory storage will also be elucidated, giving rise to more compound-specific sample preservation techniques. The degradation of BPA and NP in a clean water matrix, deionized ultrapure water, was also tested as baseline. To accomplish this goal, stir bar sorptive extraction, coupled to thermal desorption-gas chromatography/mass spectrometry (SBSE-TD-GC/MS) was used, as it has been proven to be effective at detecting concentrations of BPA and NP at trace, environmentally relevant levels.

Chapter 2: Methodology

2.1 MATERIALS

Bisphenol A (BPA) and nonylphenol (NP) technical mixture were purchased from Sigma-Aldrich (MO, USA). Nonylphenol monoethoxylate (NPEO-1) standard solution in methanol was obtained from AccuStandard (CT, USA). Isotopic BPA (ring $^{13}C_{12}$) was supplied by Cambridge Isotope Laboratories, Inc. (MA, USA). ACS grade iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), ACS plus grade hydrochloric acid (HCl), reagent grade sodium hydroxide (NaOH), and 50% w/w aqueous hydrogen peroxide (H_2O_2) were obtained from Fischer Scientific (MA, USA). ACS grade sodium carbonate (Na_2CO_3) was purchased from VWR (PA, USA), and reagent plus grade acetic anhydride was purchased from Sigma-Aldrich (MO, USA). All solvents used were of at least ACS grade, with the specific gradings included below.

2.2 MODEL DEGRADATION EXPERIMENTS

To determine the degradation of the selected EDCs barring all matrix and biological effects, degradation experiments were carried out preliminarily in ultrapure deionized (DI) water from a Millipore Milli-Q Direct Reverse Osmosis system. The Milli-Q water was pH adjusted to pH's of 3, 5, 7, 9, and 11 through the addition of 2M HCl and/or 2M NaOH. The pH-adjusted water was then decanted in 800-mL increments into 1-L amber glass jars fitted with Teflon-lined caps and either individually spiked with BPA and NP standards, or left blank to serve as a control. Enough amber jars of each pH were prepared in order to accommodate storage at both room temperature (approximately 22 ºC) and 4 ºC in order to observe the effect of temperature on the degradation of the EDCs. Additionally, to determine the effects of the EDC's exposure to indoor, ambient light on their degradation, a third set of samples was prepared in the same manner (pH adjustment followed by EDC spiking) with the only exception being that the water was decanted into 1-L clear glass jars and samples were stored at room temperature next to a window that receives natural sunlight on a regular basis. A schematic of the DI water sample set-up can be seen in Figure 6 below for the water with a pH of 3. The schematic displayed below

includes only the set-up for the waters adjusted to a pH of 3, however the set-up was identical for the remaining pHs (5, 7, 9, and 11).

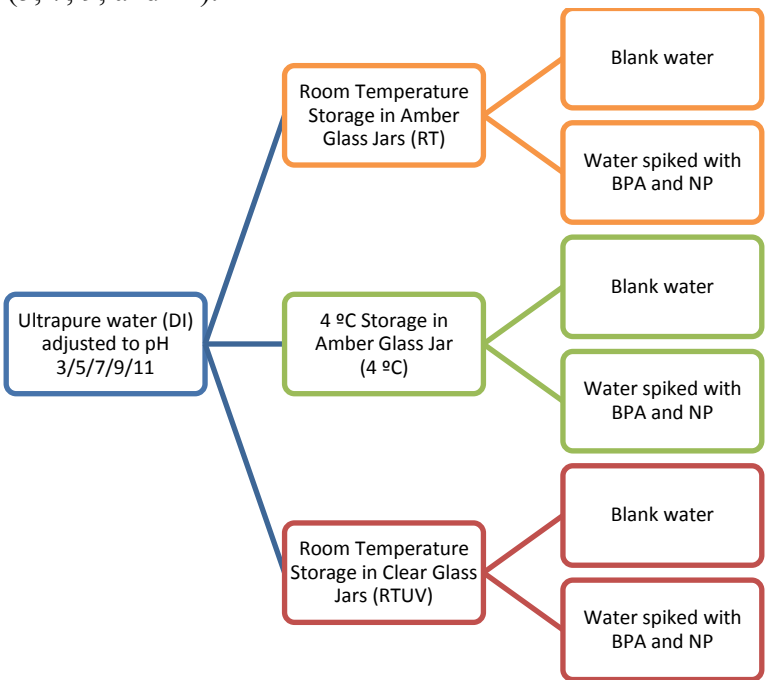

Figure 6: Sampling schematic of the preparation of all DI water samples.

The first extraction of each storage period was performed immediately after pH adjustment of the storage waters and analyte spiking (if applicable), and served as a "zero time point". This was accomplished by removing three 20-mL aliquots of the water from the jar to 20-mL amber glass vials fitted with Teflon-lined septa. After that, extractions were carried out after various periods of time to determine if there was a change in analyte concentration. Initially the samples were extracted every 4 days, however that time period was deemed inadequate, especially in the beginning of the storage period, therefore all subsequent extractions took place after at least 1, 2, 4, 8, 16, and 32 days, with several storage periods lasting beyond one month. Regardless of their exact storage times, a total of at least ten data points were collected for each storage experiment.

2.3 WASTEWATER SAMPLE COLLECTION AND STORAGE SET-UP

In addition to testing the EDC's degradation under a "controlled" environment, i.e. the DI water storage, the degradation of the EDCs naturally occurring (or native) in wastewater was also studied. In order to study the degradation of the EDCs in their natural, untreated state, influent wastewater was chosen as the preferred sample medium. Influent wastewater samples were collected from a local wastewater treatment plant (WWTP) supplied mainly with residential wastewater. The total flow of the wastewater treatment facility can be seen in Figure 7. All samples were collected during the time at which the plant received and treated its highest flow, between 12 and 1 pm.

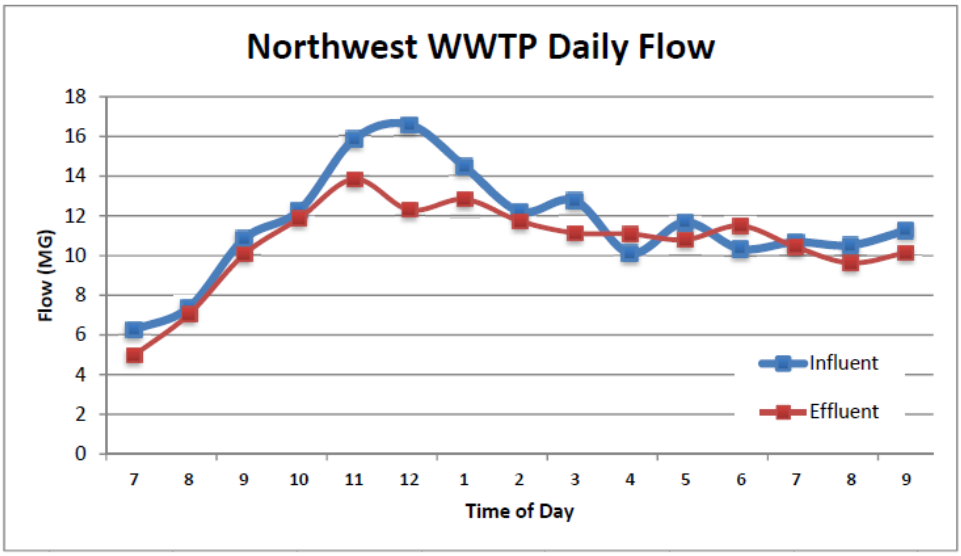

Figure 7: Influent and effluent flow of the selected wastewater treatment facility in El Paso, Texas, displaying millions of gallons of water incoming and outgoing. (Lozano, 2013)

2.3.1 Wastewater Collection

At the WWTP, raw influent wastewater was collected in an area of highly turbulent flow to ensure adequately mixed samples. Initial grab samples of approximately 100 mL were collected at intervals of two minutes, of which 10 mL aliquots were dispensed into each of the pre-washed amber glass jars fitted with Teflon-lined lids to form composite samples. This

process was repeated until several composite samples of approximately 900 mL each were collected. The composite samples were transported back to the laboratory on ice.

2.3.2 Wastewater Filtration

The effects of filtering the wastewater before storage were tested under two different storage experiments. Wastewater samples were filtered before any additional preparation steps, upon immediate arrival at the laboratory, through GF/F Whatman Glass Microfibre filters (70 μm).

2.3.3 Storage Preparation

If the samples were filtered, the following steps were performed immediately after filtration. Otherwise, these steps were carried out immediately upon the wastewater's arrival to the laboratory. To determine the effects of bacterial degradation, half of the 1-L sample jars collected were autoclaved to ensure a sterile environment. After the autoclave process, certain jars of collected wastewater samples were pH adjusted through the addition of 2 M HCl and/or 2 M NaOH to a pH of either 3 or 11. An additional set of sample jars was left unadjusted, i.e. neither acid nor base was added to change the pH, in order to serve as a control sample. A schematic of the wastewater sampling set-up can be seen in Figure 8.

Just as in the DI water storage experiments, the first extraction of each storage period was performed immediately after pH adjustment of the storage waters and analyte spiking (if applicable), and served as a "zero time point". This was accomplished by removing three 20-mL aliquots of the water from the jar to 20-mL amber glass vials fitted with Teflon-lined septa. After that, extractions were carried out after 1, 2, 4, 8, 16, and 32 days to determine if there was a change in analyte concentration. During the storage period, jars were left slightly ajar to allow for oxygen transfer between the jar and the environment, however none of the samples were bubbled with air during storage.

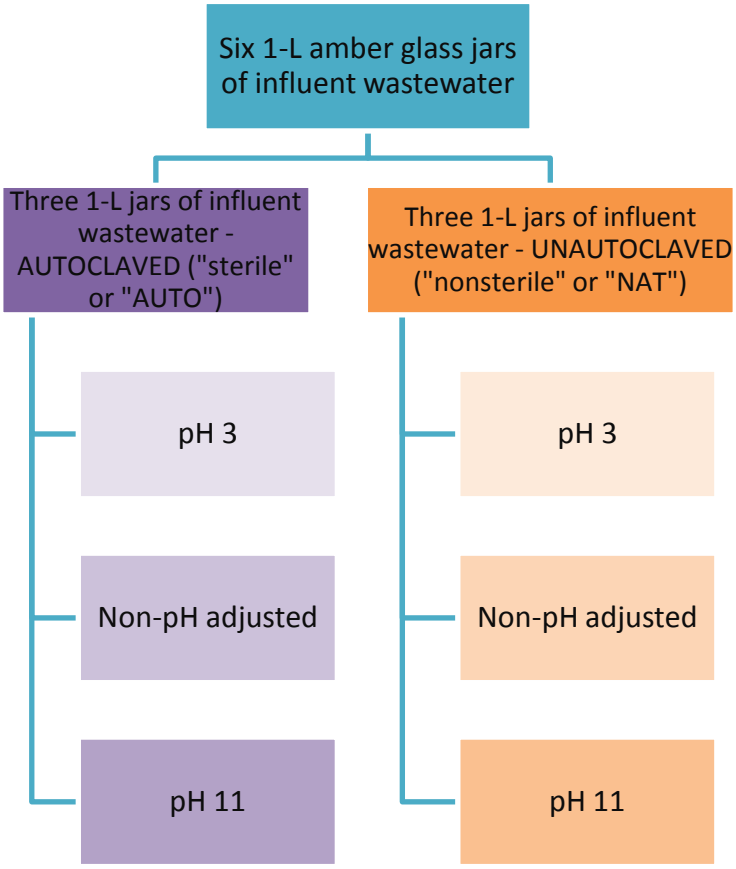

Figure 8: Schematic showing the typical sampling set-up for the wastewater storage experiments.

2.4 FENTON DEGRADATION REACTION

In order to demonstrate that the degradation of both the BPA and NP used in this experiment are in fact feasible, the Fenton degradation reaction was used. The Fenton reagents used for this experiment were based on the reported procedure by Poerschmann, Trommler, & Górecki, 2010 with slight modification. Fenton reactions in DI and wastewater were carried out at room temperature in 250 mL beakers. The oxidation process was first optimized barring all matrix and biological effects of raw wastewater by spiking DI water with environmentally relevant concentrations of BPA and NP, i.e. in low ppb (µg/L) range. For this experiment, stock solutions of BPA and NP were prepared in water to avoid concomitant organic solvent oxidation. 200 mL of water was added to the beaker and pH adjusted by the addition of 2 M HCl to a pH

between 3 and 4 (Pignatello, Oliveros, & MacKay, 2006). After that, 80 μM Fe^{2+}, in the form of a 0.05 M aqueous solution of $FeSO_4 \cdot 7H_2O$, was added to the beaker. A 20-mL sample of water was taken at this point to serve as the "time zero" data point before initiating the Fenton oxidation reaction. Finally, 800 μM H_2O_2 was added to the beaker, commencing the reaction. After 5 minutes, additional Fe^{2+} and H_2O_2 were added to the reaction vessels, to maintain the concentration of 80 μM and 800 μM, respectively. A third addition of Fenton reagents was added again after 30 minutes total reaction time. During the entire reaction time, the solutions were magnetically stirred. 20-mL samples were taken from the reaction vessel after 1, 2, 4, 8, 16, 32, and 64 minutes and the oxidation reaction was immediately quenched by raising the pH of the sample with the addition of the *in situ* derivatization reagents (Na_2CO_3) for stir bar sorptive extraction (SBSE). The samples were then extracted and quantified to determine if there was a change in concentration over time.

2.5 SAMPLE EXTRACTION

All storage samples were extracted using stir bar sorptive extraction according to previously-developed methods (Kawaguchi et al., 2004; Nakamura & Daishima, 2004). To summarize, 20-mL aliquots of the stored water samples were removed from the storage jars to 20-mL amber glass screw top vials, fitted with Teflon-lined septa. In order to accommodate the extraction of BPA and increase its affinity for the polydimethylsiloxane (PDMS) coating of the stir bars, *in situ* derivatization was done through the addition of 200 mg of Na_2CO_3 as a pH adjustment agent (so that the extraction pH was constant at 11.5) and 200 μL of acetic anhydride, which acetylates the –OH groups on BPA. For quality control purposes, an internal standard was added immediately before adding the stir bar. After that, a preconditioned Twister® stir bar 10 mm long containing 1 mm PDMS thickness (Gerstel Inc, MD, USA) was added to each vial, and the solutions were set to stir for two hours at 1000 rpm. After the extraction, the stir bar was removed with forceps, rinsed with ultrapure DI water, and dried with a lint-free tissue before being placed into a thermal desorption tube. Stir bars were then thermally desorbed on a Thermal

Desorption Unit (TDU) to release analytes from the PDMS coating, before subsequent injection into a GC/MS.

2.6 INSTRUMENTAL ANALYSIS – TDU-GC/MS

In order to release the target analytes from the Twisters®, the stir bars were desorbed in a TDU under splitless mode. The desorption process was programmed as follows: an initial temperature of 45 ºC with a ramp of 60 ºC/min to a final temperature of 280 ºC and held for 7 minutes. The transfer line temperature was held at 300 ºC. After desorption from the stir bars, the analytes were cryo-focused in a baffled glass liner in a cryo-injection system (CIS4) under liquid nitrogen at -40 ºC. The CIS4 was programmed as follows: an initial temperature of -40 ºC was ramped at a rate of 12 ºC/second to 300 ºC, after which it was held for 10 minutes. Separations of the target analytes was accomplished using an HP 6890/5973 GC/MS (Agilent, CA, USA) fitted with an HP-5MS capillary column (0.25 mm × 30 m × 0.25 um, Agilent, CA, USA). The GC oven was programmed for an initial temperature of 60 ºC, followed by a 15 ºC/min ramp to a final temperature of 300 ºC, and held for 5 minutes. Ultra-high purity helium was used as the carrier gas, with a constant flow of 1.2 mL/minute. In order to be able to analyze all possible degradation products with unknown molecular masses, the mass spectrometer was operated with electron impact ionization of 70 eV and in scan mode for 40 to 500 m/z. Eight-point calibration curves were extracted with concentrations ranging from 0.1 to 20 µg/L for NP and NPEO1, and 0.05 to 10 µg/L for BPA. All coefficients of determination (R^2) produced from the linear response to the calibration curves were at or above 0.99 for all EDCs.

2.7 QUALITY CONTROL

To ensure the quality of the data presented herein, quality control measures were taken throughout the entirety of the research. During initial storage set-up of the DI and wastewater, method blanks were established by preparing identical storage waters. In the case of the DI storage experiment, these method blanks were prepared from the same pH-adjusted DI water, the main difference being that the final storage water was not spiked with the analytes of interest.

For the wastewater samples, a field blank was obtained by exposing a DI water storage sample to the same environment from which the wastewater samples were taken. Both of these blanks were stored and extracted alongside the spiked DI water and the wastewater to evaluate the extent of method contamination of the target EDCs.

To ensure the efficiency of the SBSE, all samples were extracted in triplicate. Continuous calibration standards were also run alongside the storage samples to verify that the initial calibration remained analytically viable. Isotopic BPA ($^{13}C_{12}$) was also used as an internal standard during all sample extractions to monitor the performance of the GC/MS.

2.8 STATISTICAL ANALYSIS

MSD ChemStation Data Analysis Application software, 2003, was used to perform instrumental data analysis. Given that standard NP and NPEO1 are available as technical mixtures, 13 isomers from the NP standard and 12 isomers from the NPEO1 standard were able to be separated by the GC/MS. Therefore, for the quantification of these compounds in natural wastewater, the same isomers were used and all concentrations are reported as a sum of those isomers. The m/z values for the targeted analytes can be seen in Table 1. To calculate concentrations, ratios of the analyte peak area to the internal standard peak area were used in order to account for normal instrumental sensitivity changes. Minitab® version 16 was used for all statistical evaluations. To determine significant changes in concentration over time, the Analysis of Covariance (ANCOVA), that included the various pH's and storage mediums (sterile vs. natural wastewater, and room temperature vs. 4 ºC vs. room temperature exposed to ambient light) as factors, and time (in days) as a continuous covariate. All two-way and three-way interactions between the factors and time were included in the models. The terms were evaluated at the 0.05 significance level. Concentration changes over time were plotted as the log (base 10) of concentration so that the Gaussian assumption needed for validity of the F-test statistics used in the Analysis of Covariance was fulfilled. To determine if rates of degradation were

statistically significantly different than zero, linear regression analysis was done on lines formed by plotting log concentration change over time (days).

Table 1: m/z of Targeted Analytes

Compound	m/z	Compound	m/z
$^{13}C_{12}$ BPA	225, 240	NPEO1-1	179, 135
BPA	213, 228	NPEO1-2	179, 135
NPa	135	NPEO1-3	179, 135
NPb	135	NPEO1-4	193, 179, 235
NP1	121, 163	NPEO1-5	179, 193, 135
NP2	135, 177	NPEO1-6	193, 179, 163
NP3	135, 149	NPEO1-7	179, 135, 193
NP4	135	NPEO1-8	193, 179, 165
NP5	135, 177, 163	NPEO1-9	179, 207, 135
NP6	149	NPEO1-10	193
NP7	177	NPEO1-11	179, 135
NP8	163	NPEO1-12	193
NP9	135, 177		
NP10	149, 191, 107		

Chapter 3: Results and Discussion

3.1 Degradation in DI Water Experiments

3.1.1 Degradation with Exposure to Ambient Light

In order to determine the effects of waters containing the NP and BPA being exposed to ambient light during storage treatments, a comparison of the degradation of BPA and NP in ultrapure water was conducted by storing the water concurrently in clear and amber glass jars. Perfunctory tests to determine the difference in light that the amber glass jars could block were performed and the results are shown in Figure 9 and Figure 10. First, the amount of solar radiation capable of passing through the laboratory windows was determined by measuring the amount of solar radiation inside the glass window in the spot the samples would be stored. Although they are not equal (as shown in Figure 9), it is evident a certain degree of solar radiation is present indoors, indicating the capability of UV, visible, and near infrared light to pass through the glass windows. While the intensities are lower inside the laboratory, it was therefore assumed that samples stored in the laboratory could be exposed to solar radiation.

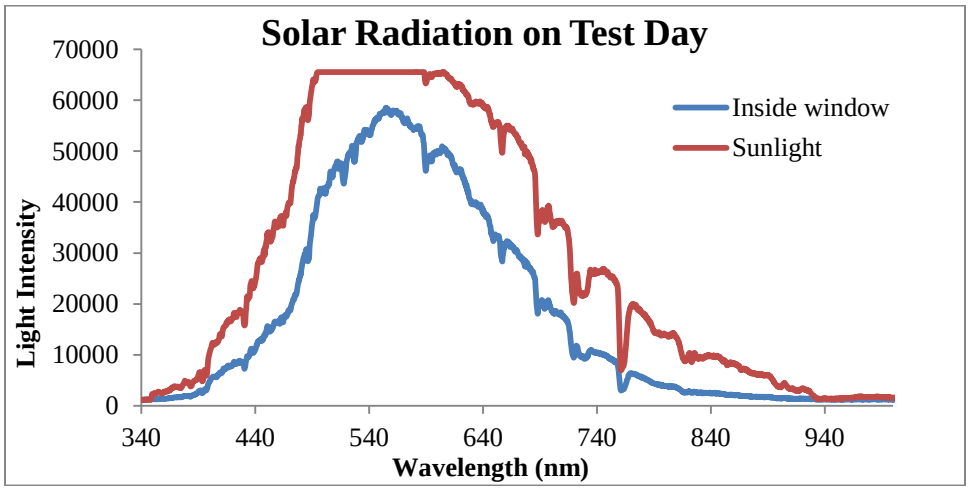

Figure 9: Graph indicating the intensity of sunlight inside the laboratory window where the RT samples were stored, compared to outside in direct sunlight. This shows that the samples stored in the lab will be exposed solar radiation, albeit to a lesser degree than if they were outside.

BPA absorbs light in the UV range (Rosenfeldt & Linden, 2004), therefore it was also necessary to determine if the glass storage jars housing the samples would block light in this range (340-400 nm, as capable by the instrument). The light intensity was measured inside the bottles and it was determined that the amber glass bottles do in fact allow the transmission of light as compared to the amber glass bottles. This is shown in Figure 10.

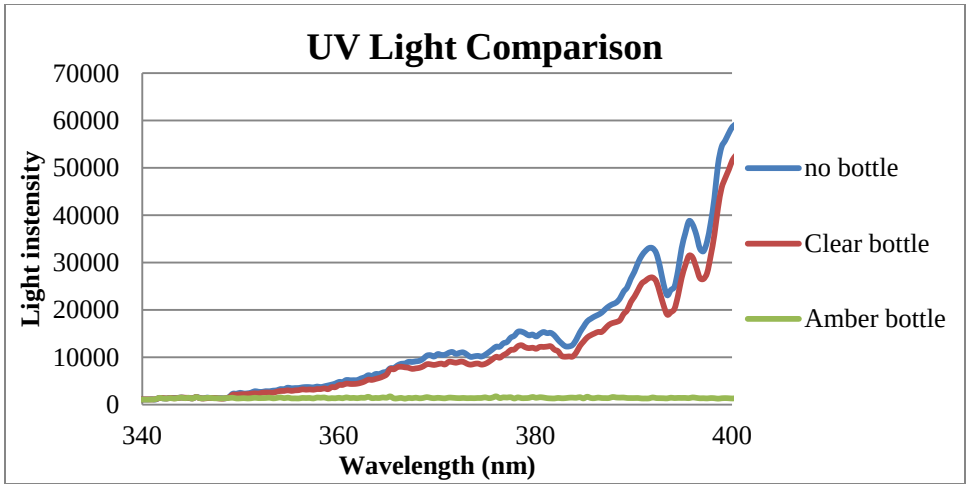

Figure 10: Graph indicating the intensity of ambient light inside the laboratory. Bottle measurements were taken inside the empty bottles to determine the degree to which the amber bottle will block ambient light.

Given the difference in the amount of light passing through the glass, waters were stored in amber glass and clear glass jars to serve as a comparison. For this experiment, ultrapure water spiked with both BPA and NP was pH adjusted to a pH of 3, 5, 7, 9, or 11 through the addition of HCl and/or NaOH and held at room temperature. Samples were initially extracted every three days up until day 12, after which longer periods of time elapsed between extractions, for a total storage time of 91 days.

Throughout this storage experiment, the pH of the waters was measured intermittently to ensure the samples were maintaining the proper pH, and it was subsequently noted that within a week's time, the waters stored at pH 5 and 9 were not able to maintain the intended pH, as shown in Figure 11. Immediately following the addition of the acid/base on day 0, the full range

of correct pH was seen. After 35 days, the pHs in the middle of the range (5, 7, and 9) were nearly equal and close to neutral, ranging from 6-7, and continued to maintain this pH throughout the entirety of the storage period. This was seen in both the blank and spiked waters. As stated previously, this observation was discovered within the first week of storage, though it is not shown. Since ultrapure deionized water was used, the water lacks any buffering capacity and was therefore unable to maintain the pH of 5 and 9. Since the concentration of the acid/base in pH's 3 and 11 is much higher, the solution was able to self-buffer and maintain itself at the adjusted pHs. Buffers were not added to the waters as it was our goal to investigate the degradation of the EDCs without nonessential supplementary chemicals, and therefore the following results represent analysis on only those samples stored under pH 3, 7, and 11. Additionally, for all subsequent storage experiments, only pH 3, 7, and 11 were used.

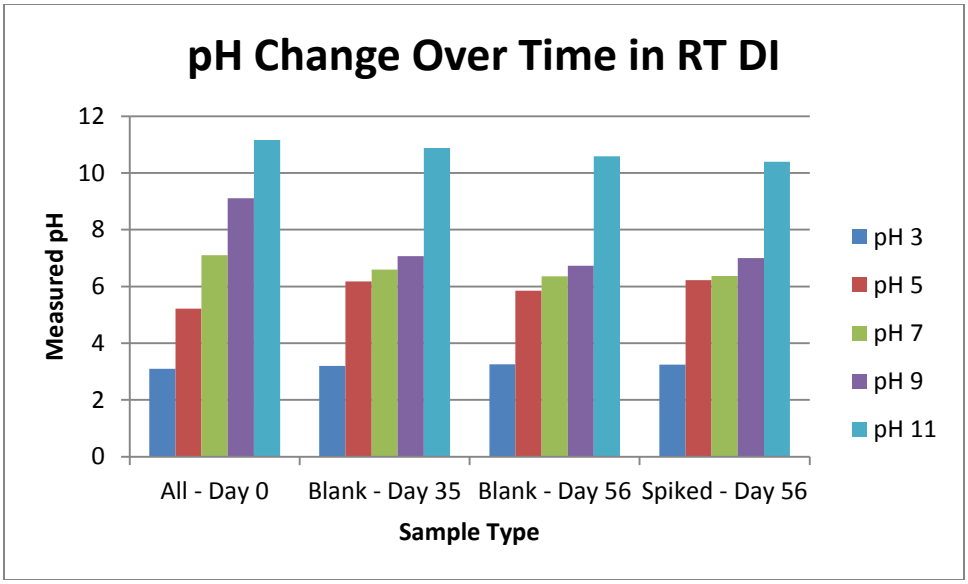

Figure 11: pH change in waters stored at room temperature.

NP

The concentration change of NP stored in ultrapure water at room temperature can be seen in Figure 12 and Figure 14. Error bars are not displayed on the graph in order to more clearly visualize the magnitude of the responses, and instead the standard error between

replicates (n=3) is summarized in Table 18 in the appendix. It is worth noting that during this particular storage experiment, the compound mirex was used as an internal standard. Given the structural differences between mirex (see Figure 57 in appendix) and both NP and BPA, it was noted that the response of mirex was highly variable. Since analyte concentrations were calculated as ratios of the instrument response of the analyte to the internal standard, the results for this experiment were also highly variable. Nonetheless, it could generally be concluded that for the samples stored in the amber glass jars (referred to only as "room temperature," or "RT"), as well as the clear glass jars (referred to as "room temperature with exposure to UV light, or "RTUV"), there seemed to be a loss of NP of approximately 40-70% within the first twelve days. The change in log concentration over time during the first 36 days of storage can be seen in Figure 12. There is a statistically significant change in concentration as well as a significant difference between the two storage conditions (see Table 2), however neither the pH nor the storage condition will change the slope of the concentration change over time, so the concentration is changing at the same rate. As mentioned previously, for all statistical analyses concentration changes over time were plotted as the log (base 10) of concentration so that the Gaussian assumption needed for validity of the F-test statistics used in the ANCOVA was fulfilled. An example of such a plot is shown in Figure 13. A significant change in concentration over time was determined by looking at the slopes of the lines formed when plotting log concentration as a function of time. The slopes represent the change in log concentration over the change in time (days). Therefore, for all of the following analyses, if a slope is found to be statistically different than a zero slope line, that slope will represent the rate of degradation with units of log[ppb] day^{-1}. Since only the waters stored in the amber glass jars' slopes were statistically different than zero, the change in log concentration of NP over time is expected to decrease at a rate of 0.00906, 0.00751, and 0.00581 log[ppb] day^{-1} for pH 3, 7, and 11, respectively. The pH 3 showing the largest rate of decrease in the log concentration of NP can be explained due to the fact that NP's solubility is pH dependent (EPA, 2005). At more acidic pHs, NP is less soluble in water, and the results obtained for the rate of decrease of NP agree with this.

When looking at the waters stored in the clear glass jars, the only rate of change in the log concentration of NP that was statistically different from zero was that of pH 3, which again could be the result of the decrease in solubility in water at acidic pHs. The significance in the adsorption difference between the clear and the amber glass samples could be due to glass manufacture, as well as weathering of the bottles. Although both sets of the bottles were made from type III soda-lime glass, they came from different manufacturers. Additionally, the amber glass jars had been used before, therefore having been subjected to possible weathering of the glass from cleaning or previous solutions stored within, whereas the clear glass jars were brand new.

Table 2: ANCOVA results for the concentration change of NP stored in ultrapure water at room temperature in either amber or clear glass jars ("Storage" condition), at three pHs (3, 7, and 11) over a period of 36 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	0.00156	0.04846	0.02423	1.53	0.222
Day	1	0.67029	0.48609	0.48609	30.60	0.000
Storage	1	0.10922	0.07151	0.07151	4.50	0.036
pH*Day	2	0.06612	0.08829	0.04415	2.78	0.067
pH*Storage	2	0.00801	0.02255	0.01127	0.71	0.494
Storage*Day	1	0.00676	0.00663	0.00663	0.42	0.520
pH*Storage*Day	2	0.05501	0.05501	0.02751	1.73	0.182
Error	107	1.69991	1.69991	0.01589		
Total	118	2.61688				

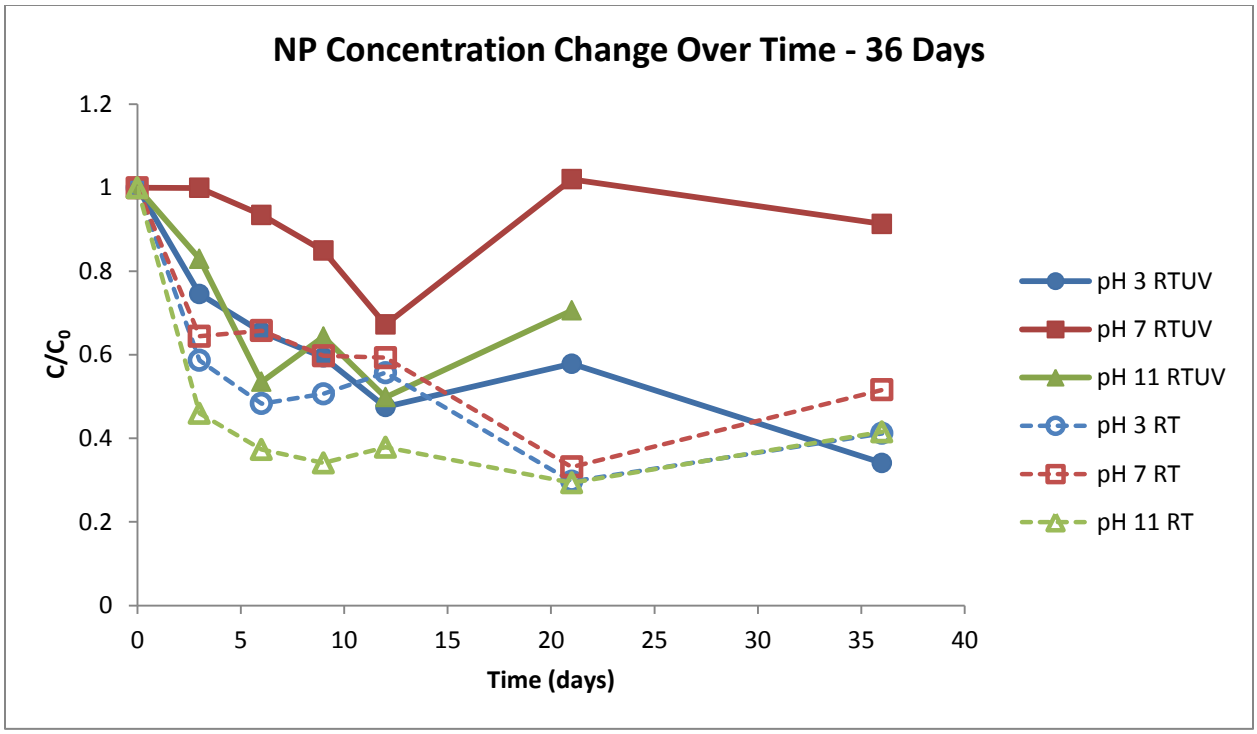

Figure 12: Concentration change of NP in ultrapure water, stored for 36 days at room temperature in amber glass jars (RT), and room temperature in clear glass jars (RTUV), each at three different pH's (3, 7, and 11).

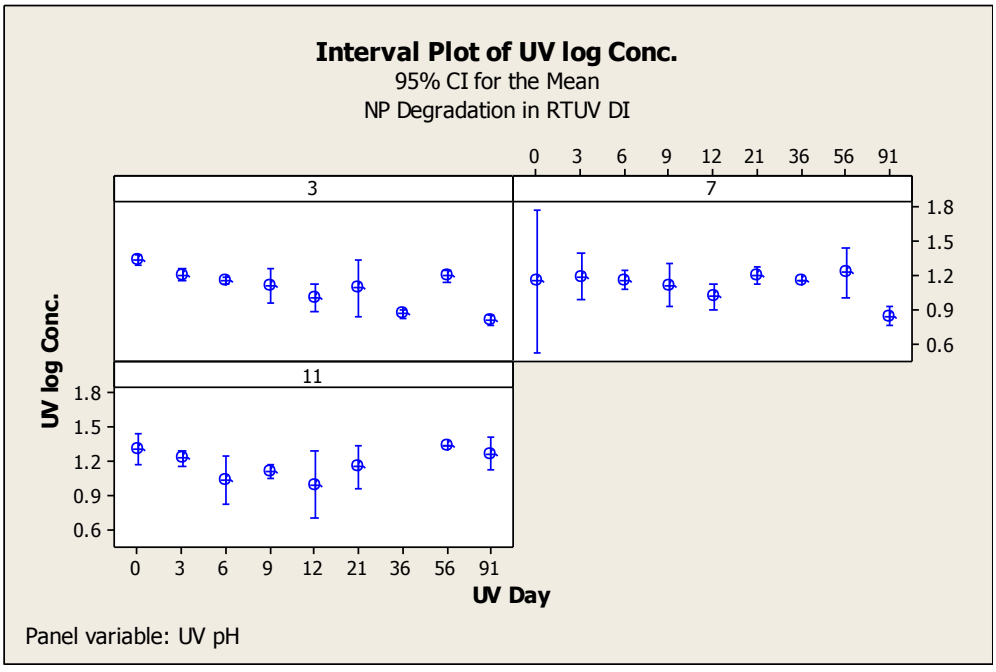

Figure 13: Change in log concentration of NP over time (days) for ultrapure water stored in clear glass jars (RTUV DI).

Samples were also kept for longer periods of time to continually monitor concentration changes (Figure 14) and statistically speaking, there is no significant change in concentration throughout the entire 91 day storage (see Table 3) at a 95% confidence level. Therefore, the initial loss of NP within the first 36 days is believed to be due to adsorption of NP onto the glass walls of the storage containers. Given NP's low solubility in water (~5 mg/L at 25 ºC, as reported by (Brix, Hvidt, & Carlsen, 2001)) and high $\log K_{OW}$. $K_{OW,}$ also known as the octanol-water partition coefficient, is the ratio of a compound's concentration in n-octanol, i.e. a hydrophobic solvent, to its concentration in water. $\text{Log}K_{OW}$ is generally used as a relative indicator of the tendency of an organic compound to adsorb to an organic layer, such as soil. The higher the $\log K_{OW}$ value is, the more hydrophobic the compound is. NP has an average $\log K_{OW}$ value of 4.48, as reported by Lu & Gan, 2014a, and therefore there is an increased tendency for the compound to adhere to glass surfaces during either sample storage or extraction. To remedy this phenomenon, methanol is commonly added in amounts ranging from 0.1-5% to samples stored in glass containers (Benijts, Lambert, & De Leenheer, 2004).

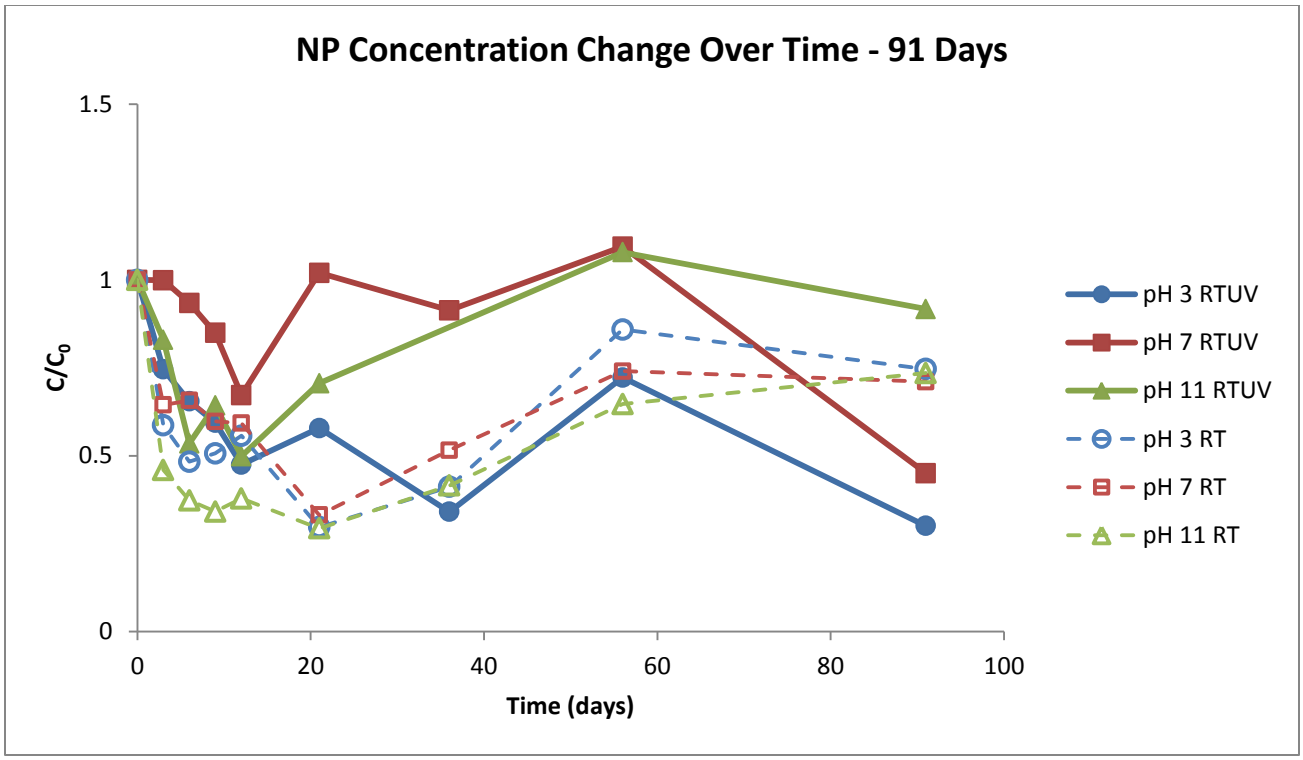

Figure 14: Concentration change of NP in ultrapure water, stored for 91 days at room temperature in amber glass jars (RT), and room temperature in clear glass jars (RTUV), each at three different pH's (3, 7, and 11).

Table 3: ANCOVA results for the concentration change of NP stored in ultrapure water at room temperature in either amber or clear glass jars ("Storage" condition), at three pHs (3, 7, and 11) over a period of 91 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	1	0.00646	0.00488	0.00488	0.23	0.635
pH	2	0.04086	0.02817	0.01408	0.65	0.522
Storage	1	0.31902	0.00830	0.00830	0.38	0.536
pH*Day	2	0.22491	0.23131	0.11565	5.36	0.006
Storage*Day	1	0.23235	0.21671	0.21671	10.04	0.002
pH*Storage	2	0.07726	0.00004	0.00002	0.00	0.999
pH*Storage*Day	2	0.09859	0.09859	0.04930	2.28	0.106
Error	140	3.02178	3.02178	0.02158		
Total	151	4.02122				

Since over the 91-day storage period the concentration of NP was found to not change significantly regardless of the storage condition, it was concluded that there is no effect from storing waters with NP exposed to ambient light. This is in accordance with the literature as in the environment, the photolysis of NP is expected to be negligible (EU, 2002).

BPA

The change in concentration of BPA stored in ultrapure water at room temperature in amber glass jars (RT) and clear glass jars (RTUV) can be seen in Figure 15 and Figure 16. The error bars, representing standard error between replicates (n=3), were omitted from the graph to more clearly visualize the magnitude of the responses, and the values can be seen in

Table 19 in the appendix.

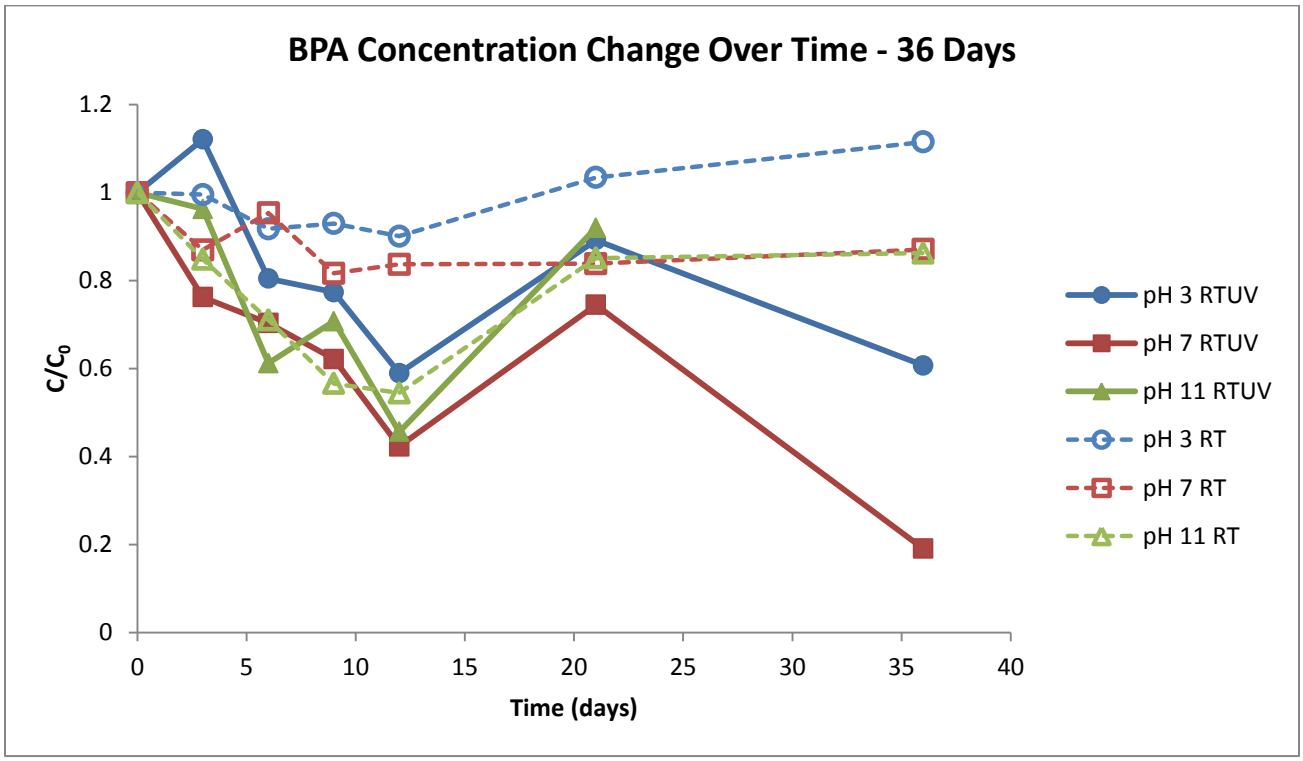

Figure 15: Concentration change of BPA in ultrapure water, stored for 36 days at room temperature in amber glass jars (RT) and clear glass jars (RTUV), each at three different pH's (3, 7, and 11).

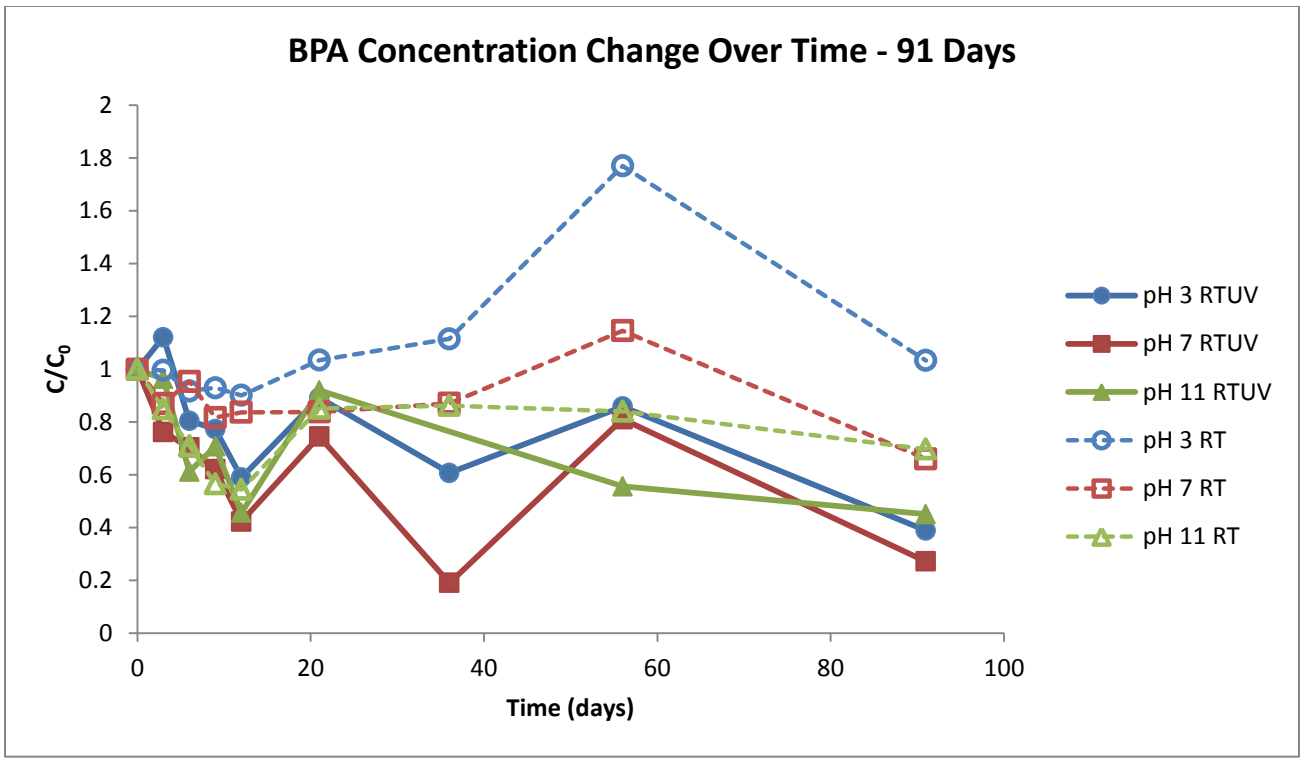

Figure 16: Concentration change of BPA in ultrapure water, stored for 91 days at room temperature in amber glass jars (RT) and clear glass jars (RTUV), each at three different pH's (3, 7, and 11).

Examining the concentration change for the first 36 days (Figure 15) there is a statistically significant change in log concentration over time, and there is an interaction between storage condition and change in log concentration over time (Table 4). This means that the change in log concentration over time will be statistically different between the clear glass storage and the amber glass storage. Additionally, analysis of the first 36 days of storage shows an additional interaction between the pH and log concentration change over time, indicating that the log concentrations are also changing at different rates between the three pHs. However, when looking at the individual ANCOVA analyses of each storage (appendix,

Table 22 and

Table 23), only the clear glass jar storage shows a significant change in concentration. For the samples stored in the clear glass jars, the only change in log concentration of BPA that was statistically significantly different from zero was that of pH 7, which was found to decrease at a rate of 0.01321 log[ppb] day^{-1}. It has previously been reported that the rate of photodegradation of BPA will increase with an increase in pH over 9, and that the rate of degradation would increase with increasing concentrations of BPA (Wang, Feng, Peixia, & Deng, 2007). However, the aforementioned studies used concentrations much higher than the environmentally-relevant concentrations used in this study, therefore showing that perhaps an interaction exists between both the starting concentration of BPA as well as the pH, in terms of BPA degradation.

Table 4: ANCOVA results for the concentration change of BPA stored in ultrapure water at room temperature in either amber or clear glass jars ("Storage" condition), at three pHs (3, 7, and 11) over a period of 36 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	0.01008	0.05170	0.02585	2.29	0.106
Day	1	0.06370	0.12283	0.12283	10.88	0.001
Storage	1	0.04925	0.02528	0.02528	2.24	0.138
pH*Day	2	0.05229	0.07618	0.03809	3.37	0.038
pH*Storage	2	0.01598	0.00391	0.00196	0.17	0.841
Storage*Day	1	0.18853	0.15510	0.15510	13.74	0.000
pH*Storage*Day	2	0.03030	0.03030	0.01515	1.34	0.266
Error	105	1.18563	1.18563	0.01129		
Total	116	1.59576				

Throughout the 91 day storage, there is a statistically significant change in BPA concentration over time as well as an interaction between the storage condition and the change in concentration (see Table 5). By running separate ANCOVA analyses on the individual storage conditions, we can see that the change in concentration over time is significant for the samples

stored in the clear glass jars, but not significant in the amber glass jar storage (appendix, Table 20 and Table 21). This indicates that photodegradation of BPA can occur in ultrapure water when exposed to ambient light (Da Silva, Reis Teodoro, De Cássia Franco Afonso, Aquino, & Augusti, 2014). The loss in concentration for the RTUV storage ranges between 55-61% (Table 6), and the differences in log concentration over time at different pHs were not significant, indicating the degradation will change at the same rate over time, regardless of pH. Though they are not statistically different, the log concentrations of BPA in ultrapure water stored at room temperature in clear glass jars at pH 3, 7, and 11 are expected to decrease by 0.00248 log[ppb] day^{-1}. Additionally, the photodegradation of BPA in ultrapure water seems to follow first order degradation laws, as can be seen in Figure 17. Although the correlations of determination are relatively low, the slopes of each line are negative, characteristic of a first order reaction. However, the solubility and log Kow values for BPA are also relatively low and high, respectively (Groshart, Okkerman, & Pijnenburg, 2001). Although not to the same degree as NP, adsorption of BPA to the glass walls can also be expected. If methanol was added to the water to reduce glass adsorption, it can be postulated that the erratic behavior of the concentration (i.e. sharp increases and decreases after day 0, rather than a steady decline in concentration) would be reduced, and thus the correlation of determination would increase. However, based on these reaction rates, BPA is expected to have a half-life of around 90 days in ultrapure water exposed to ambient light.

Table 5: ANCOVA results for the concentration change of BPA stored in ultrapure water at room temperature in either amber or clear glass jars ("Storage" condition), at three pHs (3, 7, and 11) over a period of 91 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	1	0.30602	0.34270	0.34270	29.59	0.000
pH	2	0.03910	0.03334	0.01667	1.44	0.241
Storage	1	0.23556	0.00303	0.00303	0.26	0.610
pH*Day	2	0.06054	0.06101	0.03051	2.63	0.075
Storage*Day	1	0.42912	0.41971	0.41971	36.23	0.000
pH*Storage	2	0.01175	0.00047	0.00024	0.02	0.980
pH*Storage*Day	2	0.01938	0.01938	0.00969	0.84	0.435

Error	136	1.57530	1.57530	0.0115		
Total	147	2.67676				

Table 6: Change in BPA concentration throughout 91 days of storage in ultrapure water at room temperature in clear glass jars. Concentrations are reported as ppb.

	[BPA] on Day0	[BPA] on Day 91	% Loss
pH 3 RTUV	8.969	3.669	61.091
pH 7 RTUV	9.753	3.040	72.801
pH 11 RTUV	10.302	4.737	54.902

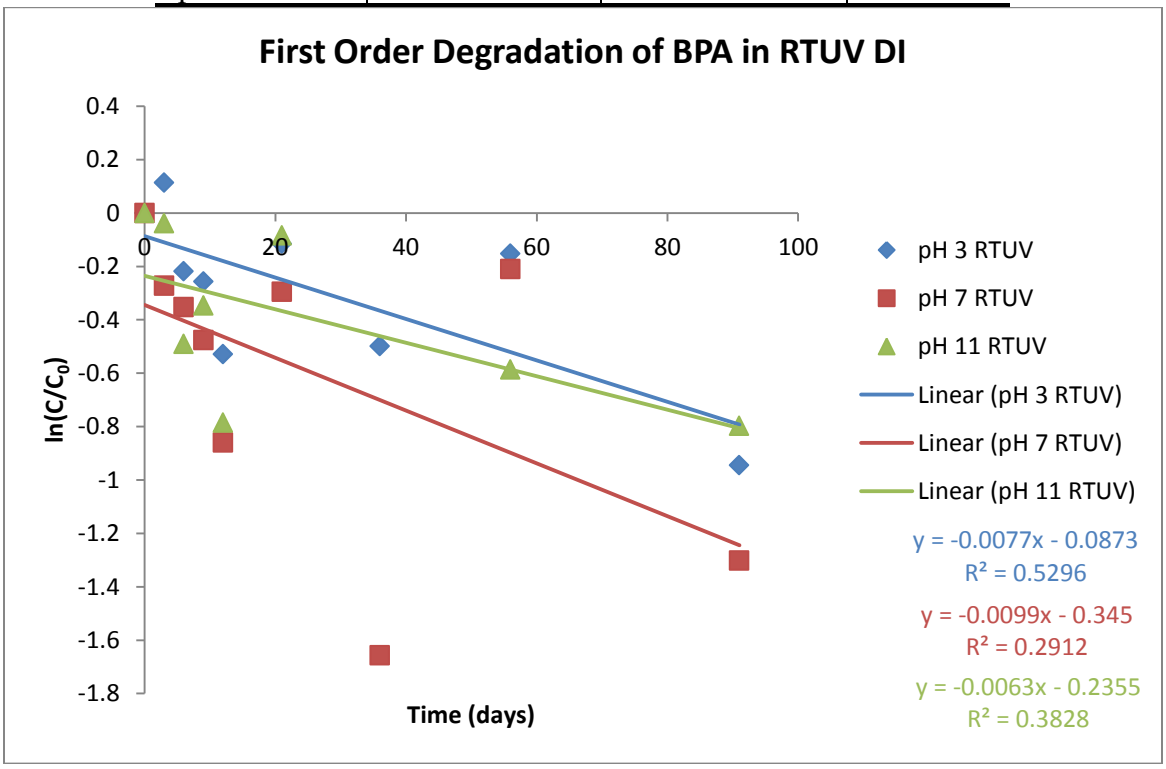

Figure 17: First order degradation of BPA in ultrapure water stored in clear glass jars at room temperature for 91 days.

Given that a decrease in concentration is seen in only the samples stored in the clear glass jars, with exposure to ambient light, it can therefore be inferred that BPA is capable of photodegradation, albeit very slow. This is in agreeance with the study done by Da Silva et al., in which they were able to prove BPA capable of photolytic degradation through direct irradiation by UV-C (200-280 nm) (Da Silva et al., 2014). Given that this experiment demonstrated the

ability of BPA to photodegrade, the following analyses were done in amber glass storage jars only.

3.1.2 Degradation at Varying Storage Temperatures

In order to determine the effects of waters containing the target analytes stored at differing temperatures, a comparison of the degradation of BPA and NP in ultrapure water was conducted by storing the water concurrently in amber glass jars at room temperature as well as at 4 ºC. For this experiment, ultrapure water spiked with both BPA and NP was pH adjusted to the same three pHs and held at either room temperature or 4 ºC. To gain a better understanding of the concentration changes, samples were initially extracted daily for three days, after which they were extracted at days 6, 10, 18, 26, and 48. Because holding times of water samples containing organic analytes is generally no longer than 28 days (ASTM Standard D3694, 2011), a longer storage period beyond 48 days was not conducted. For this and all remaining experiments, $^{13}C_{12}$ BPA was used as an internal standard to better improve response consistency.

NP

The concentration change of NP in ultrapure water at room temperature and 4 ºC can be seen in Figure 18. In the case of the room temperature storage, the concentration of NP remains relatively stable, until day 27 where it can be seen that all three pH's exhibited a loss of approximately 50%. Given that at the following extraction at day 48 the concentration has risen back to around 100% of the original concentration, it can be assumed that that loss was due to adsorption of NP onto the glass walls of the storage containers. Just as mentioned previously, because of NP's low solubility and high $\log K_{OW}$, there is an increased tendency for the compound to adhere to the glass surface. In the case of the refrigerated samples, a similar trend can be seen where there is a gradual decrease with a sudden increase in concentration across all pH's. It is assumed that it is for the same reasons as stated previously. Based on these graphs, it can be seen that there are very little differences between the storage temperatures, as well as the pH's, indicating that in ultrapure water, NP is relatively stable. It can therefore be postulated that

NP will not undergo abiotic degradation such as hydrolysis, which is in accordance with previous studies (EU, 2002).

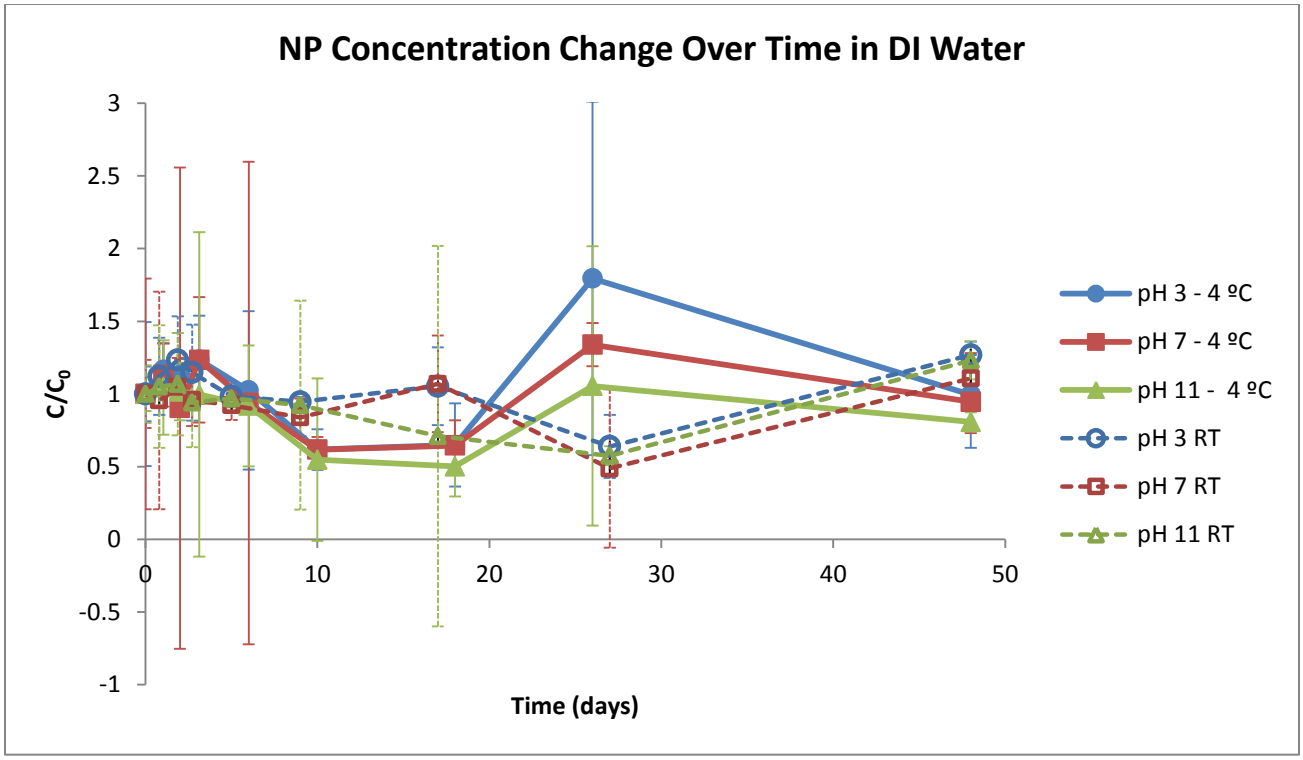

Figure 18: Concentration change of NP in ultrapure water, stored in amber glass jars for 48 days at room temperature (RT) and 4 ºC, each at three different pH's (3, 7, and 11). Error bars represent standard error between replicates (n=3).

To further investigate the significance of the concentration changes of NP, the ANCOVA was performed and these results can be seen in the appendix in Table 24. Based on these results, it can be concluded that in ultrapure water, there is no evidence that the concentration of NP will significantly change over time, nor that the pH or the storage temperature will affect the concentration of NP over time, further confirming our assumption.

BPA

The concentration change of BPA in ultrapure water at room temperature and 4 ºC can be seen in Figure 19. Just as with NP, there are very little observable differences between the

storage temperatures, as well as the storage pH's on the concentration of BPA. The results of the ANCOVA analysis for BPA stored in ultrapure water can be seen in the appendix,

Table 25. Based on these results, it can be concluded that in ultrapure water, there is no evidence that the concentration of BPA will significantly change over time, nor that the pH or the storage temperature will affect the concentration of BPA over time.

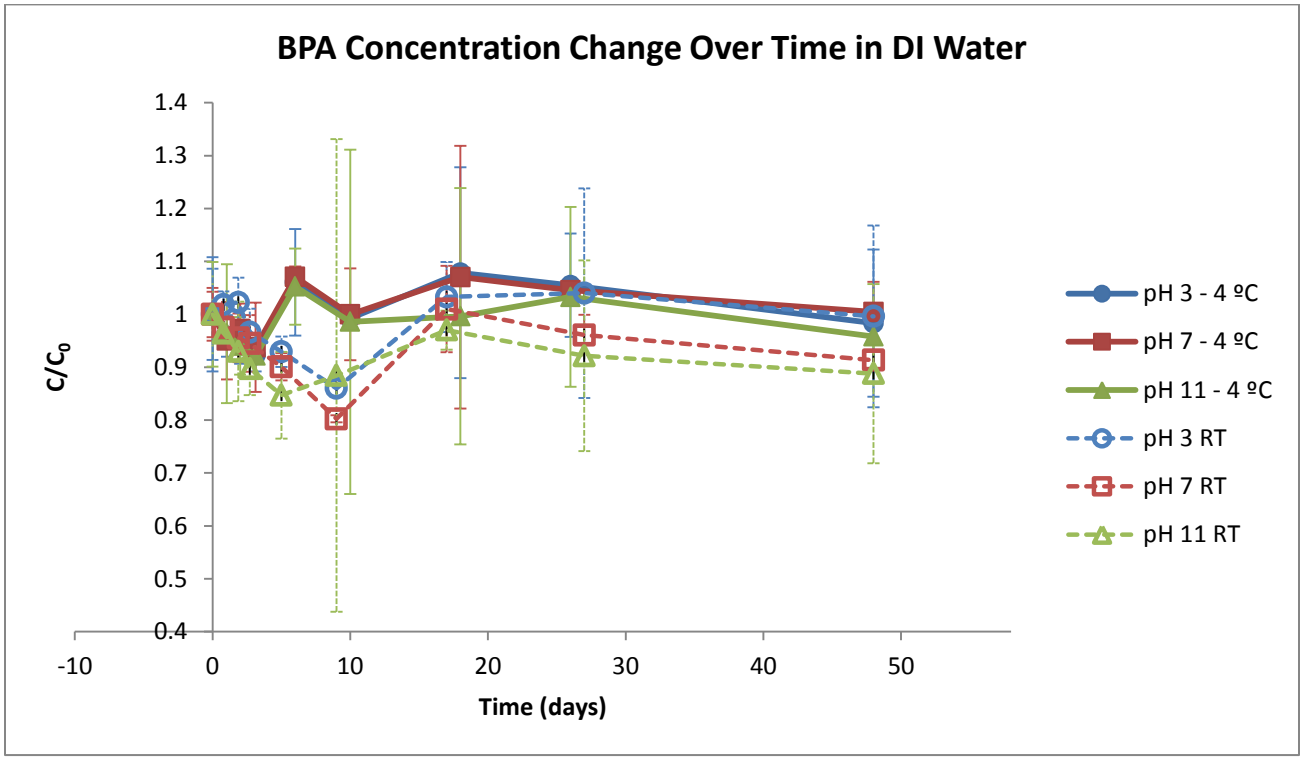

Figure 19: Concentration change of BPA in ultrapure water, stored in amber glass jars for 48 days at room temperature (RT) and 4 ºC, each at three different pH's (3, 7, and 11). Error bars represent standard error between replicates (n=3).

Based on the results of the previous two experiments, it can be concluded that room temperature water in amber glass storage bottles will contribute to the least to chemical

degradation of the targeted EDCs. Since there were no major differences between the room temperature and 4 ºC storage, room temperature was chosen as the best storage condition to use for the remaining experiments. Therefore, all remaining data presented is based on waters stored at room temperature in amber glass bottles. The three tested pHs will remain the same.

3.1.3 Degradation at Room Temperature under Three Different pHs

To confirm the results of the previous two studies by including the random effect of replicate experiments, two subsequent storage experiments were performed using room temperature water stored in amber glass bottles at three different pHs. For this experiment, ultrapure water spiked with both BPA and NP was pH adjusted to a pH of 3, 7, and 11 through the addition of HCl and/or NaOH and held at room temperature. Samples were initially extracted daily for four days, after which they were extracted at days 8, 16, and 32. This storage experiment was performed twice and the ANCOVA analyses included the random effects of experimental replicates.

NP

The change in concentration of NP stored at room temperature can be seen in Figure 20. Both experimental replicates are displayed and are differentiated by the abbreviations RT 1, meaning the first experimental replicate, and RT 2, or the second experimental replicate. According to the ANOVA results, there is a significant change in log concentration during this storage period, but the pH of the storage water does not statistically effect concentration, nor does it change the slope (appendix Table 26). Furthermore, the addition of the random effect of replicate shows statistical significance, indicating that some of the variation in the log concentration of NP over time can be explained by variations between experimental replicates.

During the storage period, the first replicate's sample jars were kept intact for further analysis after the 32-day storage period. Recall that during the previous experiments, comparing the degradation exposed to light and the degradation under different temperatures, we saw a decrease in concentration during the first 30-40 days but no statistical change in concentration in

time periods longer than that. For this reason, the first replicate's samples were extracted once again after a total of 196 days storage. Including this data in the ANCOVA analysis of replicate 1's change in concentration, the result changes and we see that there is no statistically significant change in the concentration of NP (appendix Table 27). Therefore, we can further conclude that NP did not undergo significant degradation in ultrapure water stored at room temperature, regardless of the storage pH.

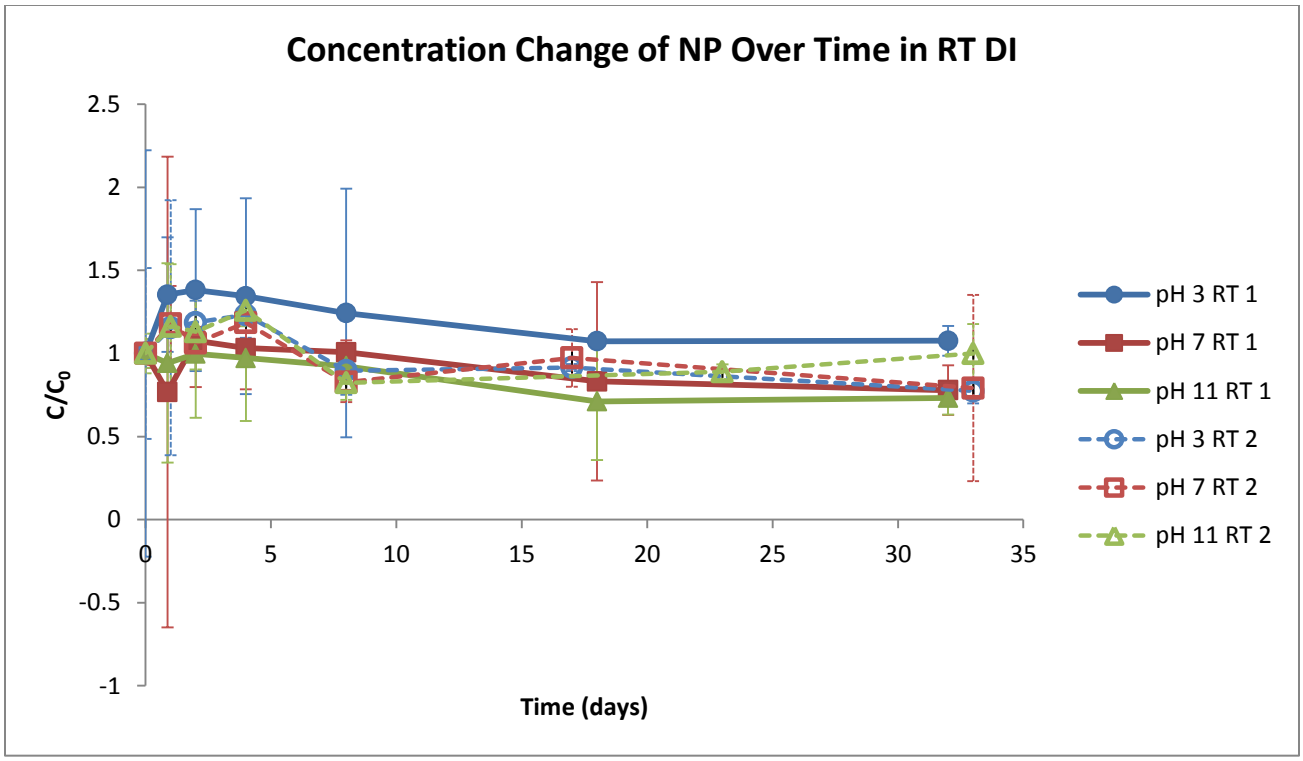

Figure 20: Concentration change of NP in ultrapure water, stored in amber glass jars for 32 days at room temperature (RT) and at three different pH's (3, 7, and 11). Error bars represent standard error between sample replicates (n=3).

BPA

The change in concentration of BPA stored at room temperature can be seen in Figure 21. According to the ANOVA results, there is a significant change in the concentration of BPA during this storage period, and contrary to NP, the pH of the storage water has a significant effect on the concentration, but does not change the slope (appendix

Table 28). Furthermore, the addition of the random effect of replicate shows statistical significance, indicating that some of the variation in the log concentration of BPA over time can be explained by variations between experimental replicates.

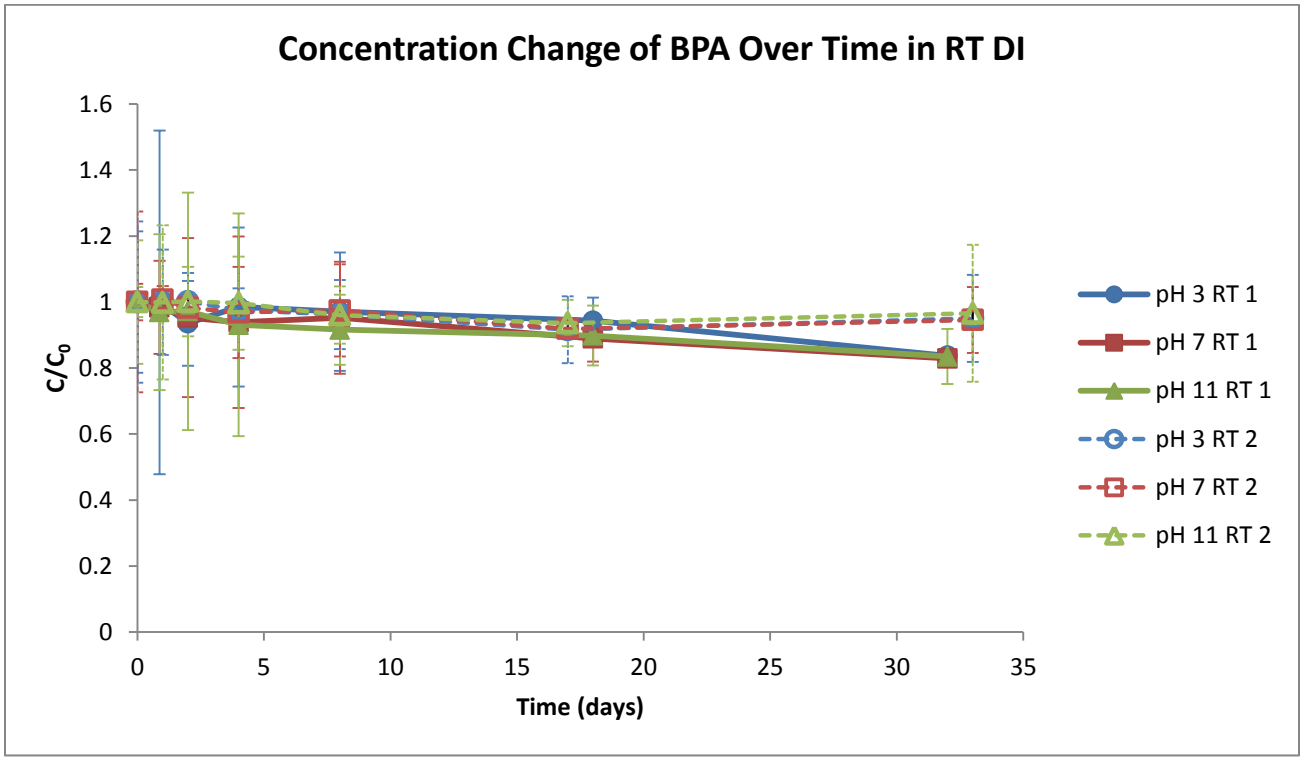

Figure 21: Concentration change of BPA in ultrapure water, stored in amber glass jars for 32 days at room temperature (RT) and at three different pH's (3, 7, and 11). Error bars represent standard error between sample replicates (n=3).

Looking at replicate 1's extraction data for 196 days and including it in an ANCOVA analysis of replicate 1's change in concentration, the results remain the same and we see that there is a statistically significant change in the concentration of BPA, and that pH will not change the slope in time (appendix Table 29), meaning the concentration will change at the same rate across all pHs.

The loss in concentration of BPA ranges between 3-31% (Table 7). It was observed that that within the first 33 days of storage, degradation was much faster than that at 196 days. For the first 33 days of storage, the log concentration of BPA decreased by 0.00143 log[ppb] day^{-1}. However, by looking at the change in concentration throughout the entire 196 day storage of replicate 1, the log concentration decreased by 0.00063 log[ppb] day^{-1}. Given that the rate of degradation throughout 196 days of storage was less than half that of the 33 days of storage, it could be inferred that there was some degree of adsorption of BPA to the glass storage containers and that the degradation was concentration dependent. In NP, we've seen erratic concentration changes that we attributed to adsorption. The results of the present experiment indicate that the degradation of BPA seems to follow first order kinetics, with relatively good correlations of determination, shown in Figure 22. Even when looking at only the first 33 days, the correlations of determination are also relatively high, with values of 0.9131 for pH 3, 0.9334 for pH 7, and 0.9736 for pH 11. When stored at room temperature in amber glass jars, the half-life of BPA was calculated to be 475 days.

Table 7: Change in BPA concentration throughout 32 and 196 days of storage in ultrapure water at room temperature in amber glass jars. Concentrations are reported as ppb.

	[BPA] on Day 0	**[BPA] on Day 32**	**[BPA] on Day 196**	**% Loss at Day 32**	**% Loss at Day 196**
pH 3 RT 1	12.158	10.177	9.431	16.296	22.430
pH 7 RT 1	11.253	9.329	8.156	17.097	27.525
pH 11 RT 1	10.184	8.503	6.994	16.506	31.318
pH 3 RT 2	12.928	12.283		4.984	
pH 7 RT 2	12.902	12.202		5.426	
pH 11 RT 2	12.755	12.319		3.416	

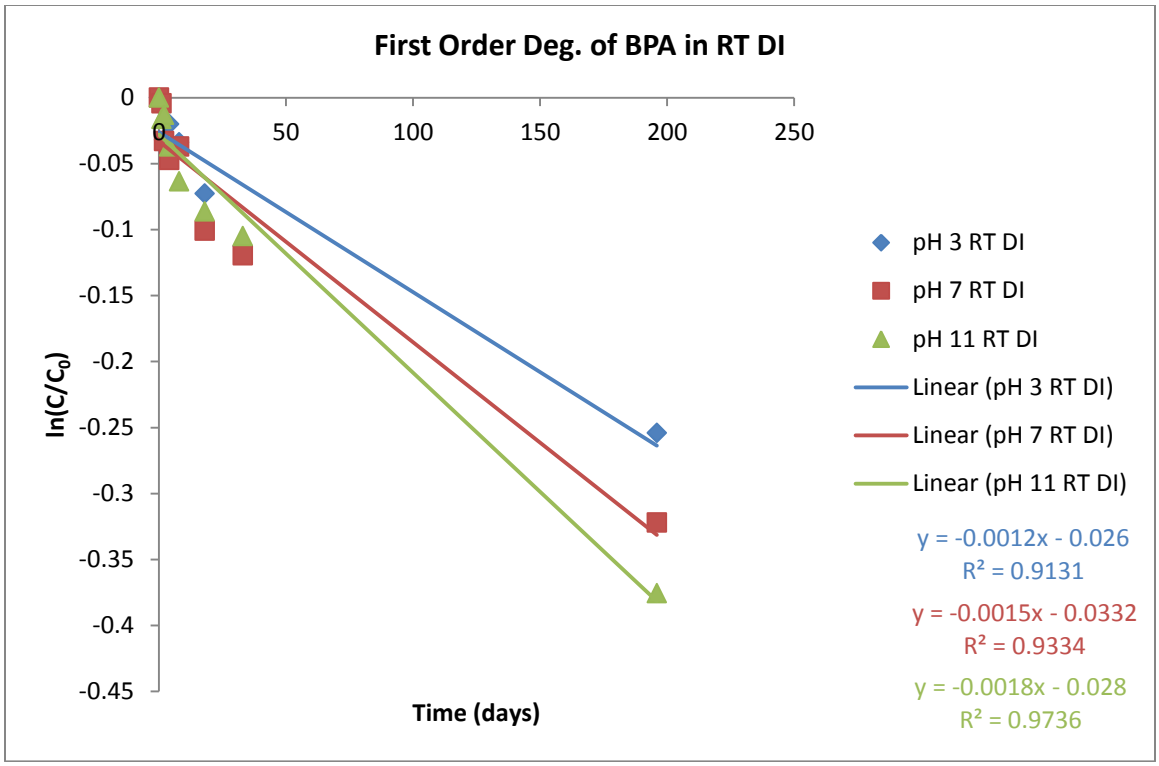

Figure 22: First order degradation of BPA in RT DI water. These results are presented as the average of the two experiment replicates.

3.2 WASTEWATER DEGRADATION EXPERIMENTS

3.2.1 Degradation in Sterile and Non-sterile Filtered Wastewater

In order to determine the effect of biodegradation on NP and BPA, a comparison experiment was done using filtered nonsterile and autoclaved influent wastewater. Influent wastewater was collected in six 1-L amber glass bottles, after which they were filtered through glass fiber filters. Three of the 1-L jars of wastewater were then sterilized in an autoclave, while the other three were kept untreated (referred to hereafter as "nonsterile"). Both sterile and nonsterile waters were pH adjusted to a pH of 3 or 11 through the addition of HCl and/or NaOH, and one set of jars was left unadjusted (referred as "pH 7" hereafter). All samples were held concurrently at room temperature. Samples were extracted at days 0, 1, 2, 4, 8, 16, 33, and 110. The concentrations of native NP and BPA were determined by SBSE followed by TD-GC-MS.

NP

The concentration change of NP in filtered sterile and nonsterile wastewater is shown in Figure 23, and zoomed in in Figure 24. There was a similar trend to what has been observed in our previous experiments, where we noted an increase in the concentration of NP. While adsorption to the glass walls is certainly still a possibility, the fact that the NP levels for some of the treatments on Day 17 were above the time zero concentration point indicates that a new factor must also be considered. In wastewater, there is a vast range of other organic compounds present within the water, such as nonylphenol ethoxylates (NPEOs). It is known that NPEOs degrade into NP, and therefore such an increase in concentration described above could be due to the degradation of these NPEOs. Therefore, for all of the wastewater experiments, the concentration change of nonylphenol monoethoxylate (NPEO1) was also studied. These results are presented in the next topic.

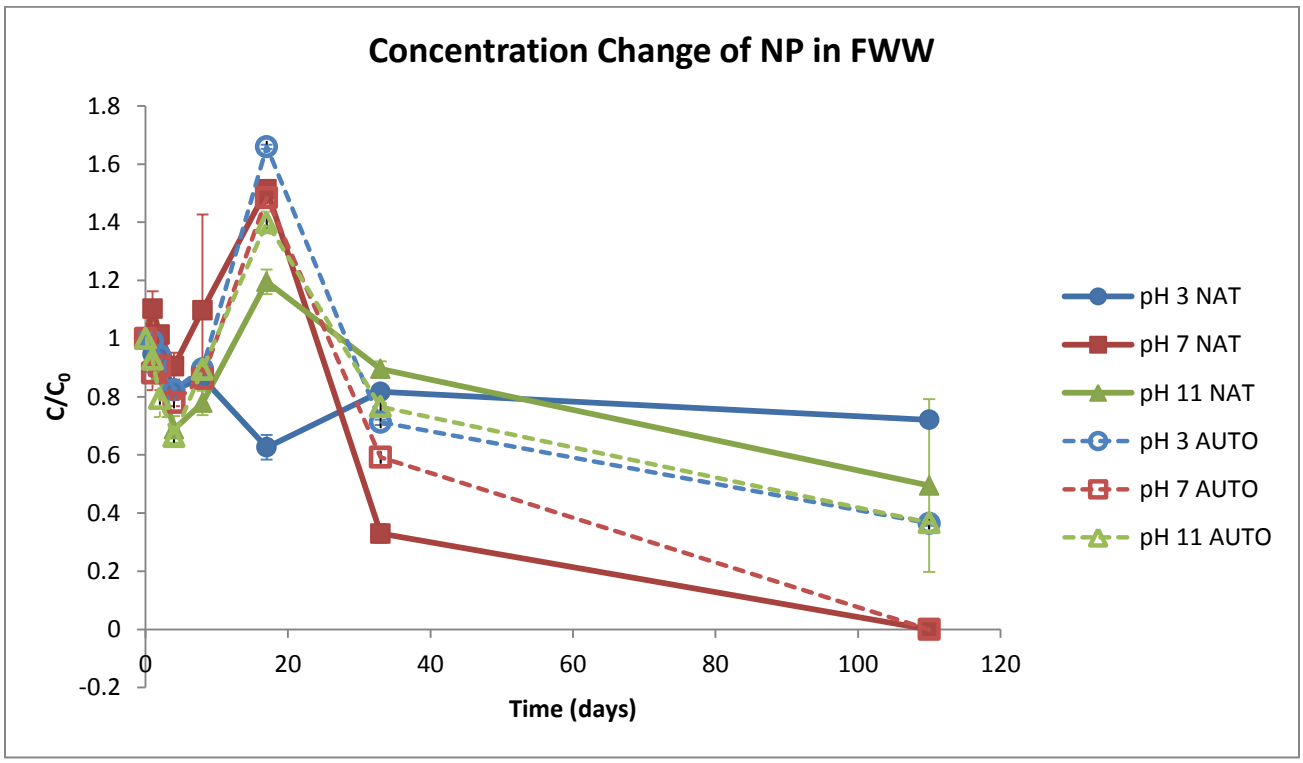

Figure 23: Concentration change of NP stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature, and under three different pHs, for 110 days. Error bars represent standard error between sample replicates (n=3).

By looking at the ANCOVA results, the change in NP's log concentration in sterile or nonsterile filtered wastewater was statistically significant (see appendix Table 30). Although the effect of pH alone on the degradation of NP in sterile wastewater was not significant, there is an interaction between pH and time, indicating the different pHs will cause a change in the degree of degradation over time. The same can be said for the three-way interaction between pH, medium, and time.

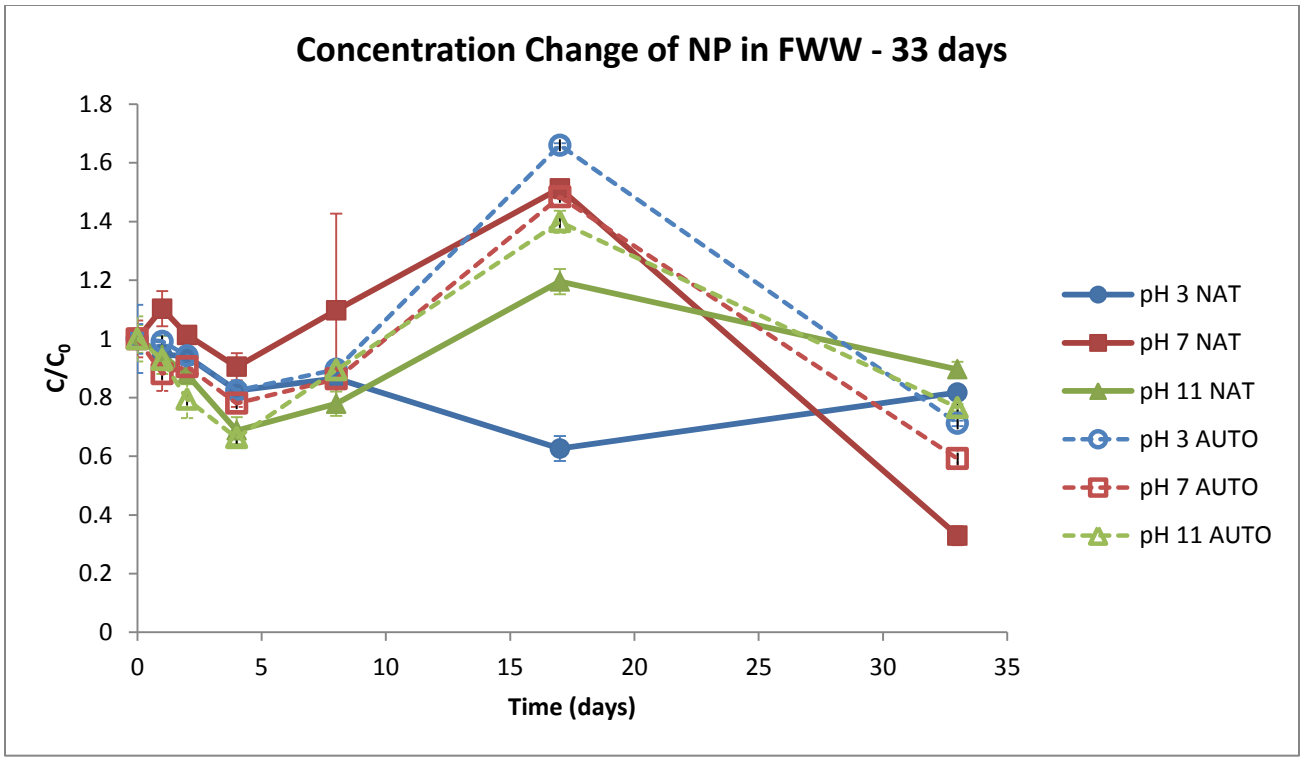

Figure 24: Concentration change of NP stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature, and under three different pHs, for 33 days. Error bars represent standard error between sample replicates (n=3).

Looking individually at each storage medium, we can see that throughout the 110 days of the storage period, the effect of pH and time on the degradation of NP are significant, as well as the interaction between pH and time (see appendix Table 31) for the autoclaved (sterile) wastewater. These results indicate a statistically significant change in concentration across all three pHs, and that the pH will affect the rate in change in concentration. During this time period,

the log concentration of NP over time decreased by 0.00396, 0.05327, and 0.00345 log[ppb] day^{-1} for pH 3, 7, and 11, respectively. However, upon further analysis, it is revealed that the change in log concentration at pH 3 and pH 11 is not statistically significantly different than zero, indicating there is no significant degradation in those pHs ($p=0.057$ for pH 3 and $p=0.098$ for pH 11). Since we have shown NP is not likely to undergo abiotic degradation, the degradation in pH 7 indicates the autoclave process is not enough to kill all bacteria, but that the additional step of pH adjustment to make the storage media acidic or basic (e.g. 3 and 11) could eliminate the potential for biodegradation of NP. When looking only at days 0-33, no factor is significant for the autoclaved wastewater, indicating that there is no statistically significant change in the log concentration of NP during this time period (appendix Table 32). These results were similar to that of the ultrapure water storage experiments. The presence of a significant change in concentration after 33 days of storage could be the result of the regrowth of bacteria capable of degrading NP. Another factor to consider would be the degradation of NPEOs. During the first 33 days of storage, the degradation of NPEOs into NP could cause the NP concentration to increase, thus canceling out the degradation of NP, statistically speaking. When looking at the analysis of the nonsterilized wastewater, we observed a significant change in NP concentration over time, as well as an interaction between the pH and time (appendix Table 33). During the storage period of 110 days, the log concentration of NP over time decreased by 0.00093, 0.05722, and 0.00260 log[ppb] days^{-1} for pH 3, 7, and 11, respectively. Again, only the change over time in log concentration of NP stored at pH 7 is statistically significantly different than zero. This provides further evidence of the idea that the pH adjustment to 3 or 11 is enough to deactivate the bacteria capable of degrading NP. At 110 days, the degradation rate of NP is similar in the non-sterilized wastewater (a reduction of 0.05722 log[ppb] days^{-1}) as it is to the autoclaved wastewater (a reduction of 0.05327 log[ppb] days^{-1}). In contrast to the autoclaved wastewater, when we examined the degradation of NP in non-sterilized, i.e. natural wastewater (NAT), during the first 33 days of storage, we observed a statistically significant change in concentration (appendix Table 34). pH also causes an effect in the change in concentration, and

the interaction between pH and time is also significant, indicating the concentration change will be different between the three pHs. The log concentration of NP decreased by 0.0032 and 0.0129 log[ppb] $days^{-1}$ for pH 3 and 7, respectively, and increased by 0.0013 log[ppb] $days^{-1}$ for pH 11 for the 33 day storage period. However, just as before, only the change in concentration at pH 7 is different from zero, indicating only the samples stored at pH 7 underwent significant degradation.

Attempt was made to investigate the kinetics of NP degradation in wastewater. The first order degradation curves for NP in nonsterile wastewater can be seen in Figure 25, and second order degradation curves are displayed in Figure 26. Although the individual correlations of determination are relatively low, we can clearly see the degradation of NP in the nonsterile pH 7 wastewater follows a sharper decrease than that of the pH 3 or pH 11 waters. Looking at the degradation in pH 7, it appears that NP better followed a second order degradation. Assuming the degradation of NP is also affected by the degradation of the NPEOs, the reaction would follow the form $A + B \rightarrow P$, and therefore the half-life cannot be determined. The values for the losses in native NP concentration are shown in Table 8. It should be noted that the autoclave process might have caused volatization of NP. The concentrations of NP after autoclaving at time zero were lower than that in natural wastewater.

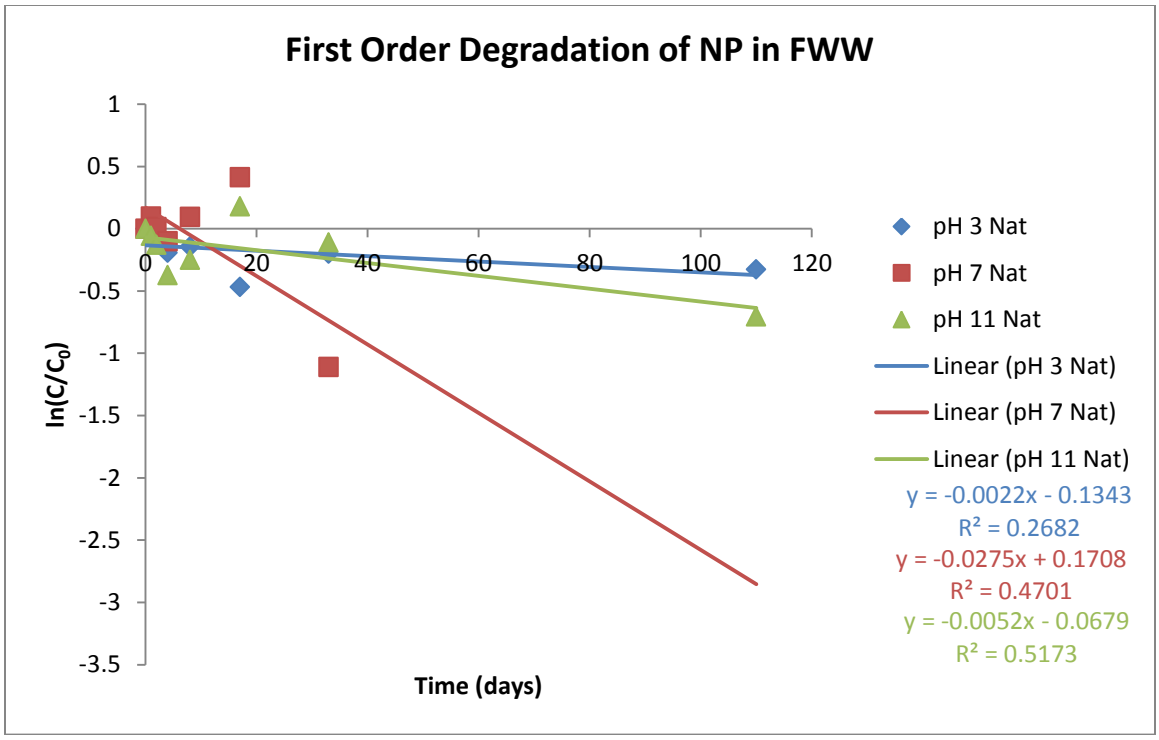

Figure 25: First order degradation of NP in nonsterilized filtered wastewater at room temperature, stored in amber glass jars under three different pHs.

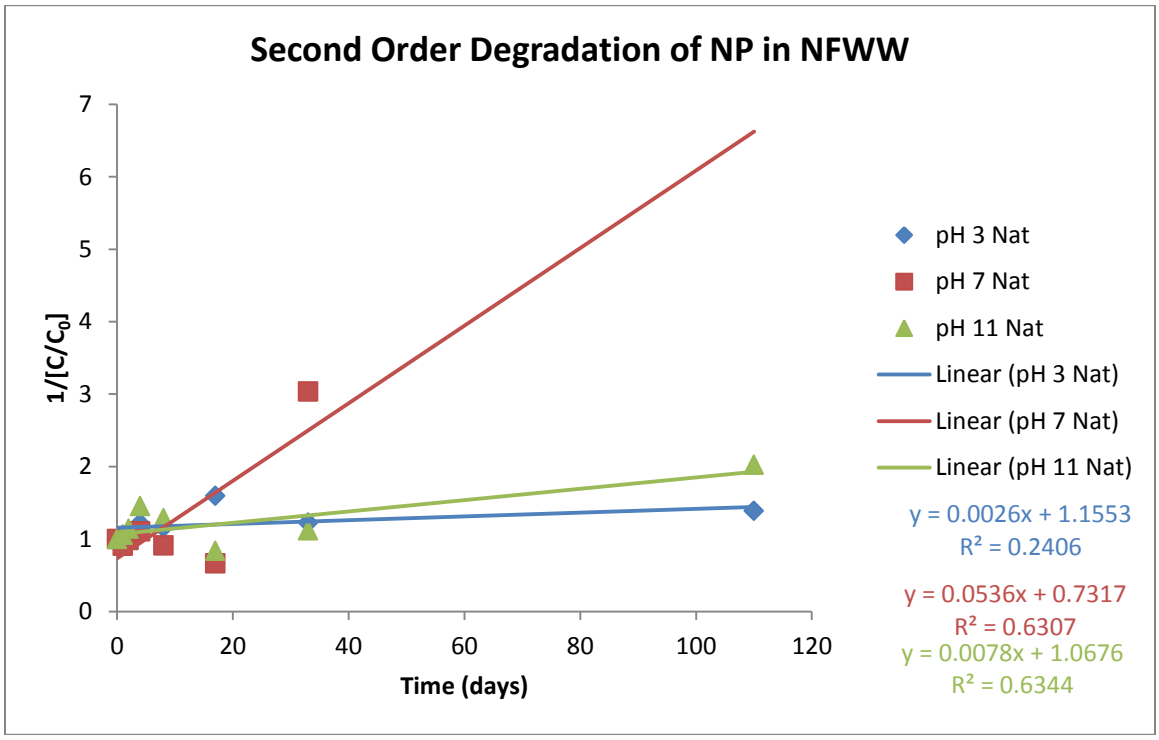

Figure 26: Second order degradation of NP in nonsterilized filtered wastewater at room temperature, stored in amber glass jars under three different pHs.

Table 8: Change in NP concentration throughout 33 and 110 days of storage in filtered wastewater at room temperature in amber glass jars. Concentrations are reported as ppb.

	[NP] on Day 0	[NP] on Day 33	[NP] on Day 110	% Loss at Day 33	% Loss at Day 110
pH 3 AUTO	0.777	0.553	0.284	28.765	63.501
pH 7 AUTO	0.796	0.471	0	40.824	100
pH 11 AUTO	0.865	0.662	0.317	23.429	63.322
pH 3 NAT	1.756	1.435	1.266	18.294	27.922
pH 7 NAT	1.807	0.596	0	67.039	100
pH 11 NAT	2.064	1.849	1.021	10.419	50.540

Generally all NP concentrations are reported as a sum of the isomers found in the technical standard. Since it has been documented that certain NP isomers are more readily biodegradable as well as exhibiting differing degrees of estrogenicity (Lu & Gan, 2014a, 2014b; Lu et al., 2015), the degradation of each individual isomer was examined as well. As shown in Figure 27 and Figure 28, no significant differences in degradation of NP was observed among the isomers in either sterile or non-sterile wastewater. Note that although the concentration of NP at 110 days was reported as 0, the instrument is still able to identify and separate isomers; however the overall sum of the isomers is below the calibration level. This result was the same across all pHs (data not shown).

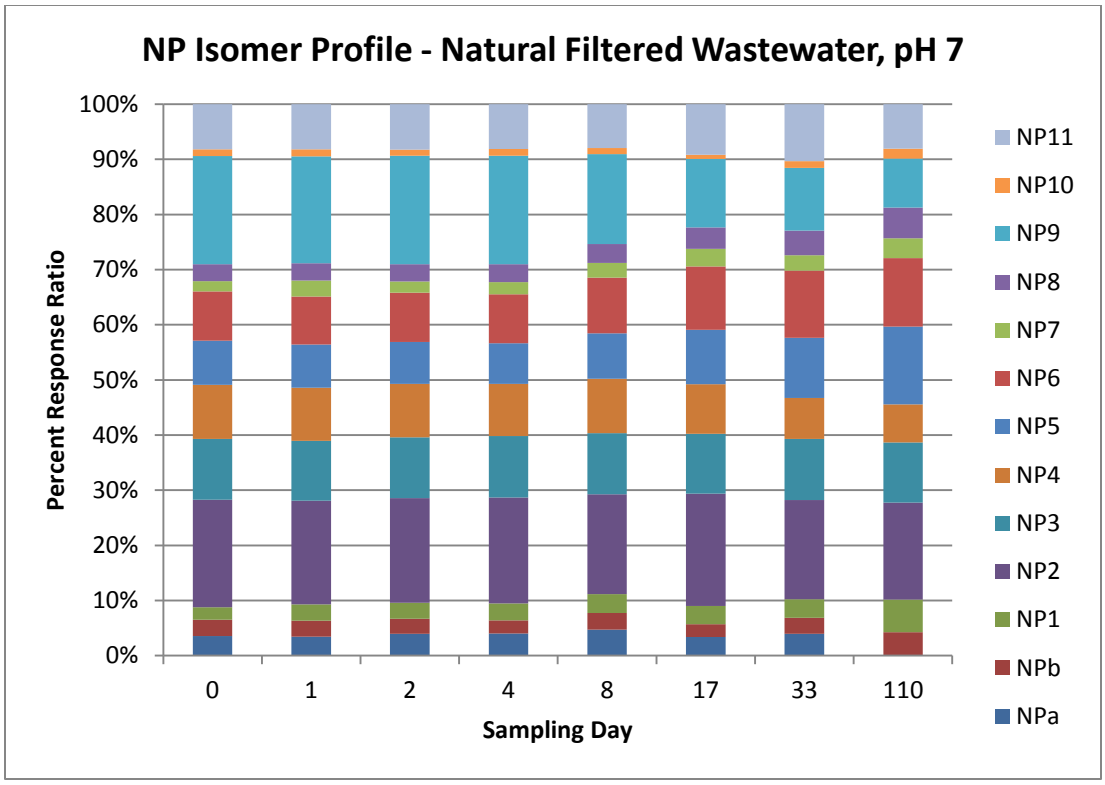

Figure 27: Response ratios of NP isomers in nonsterilized filtered wastewater at pH 7. Ratios are reported as the instrument response of the NP isomer to the instrument response of the internal standard.

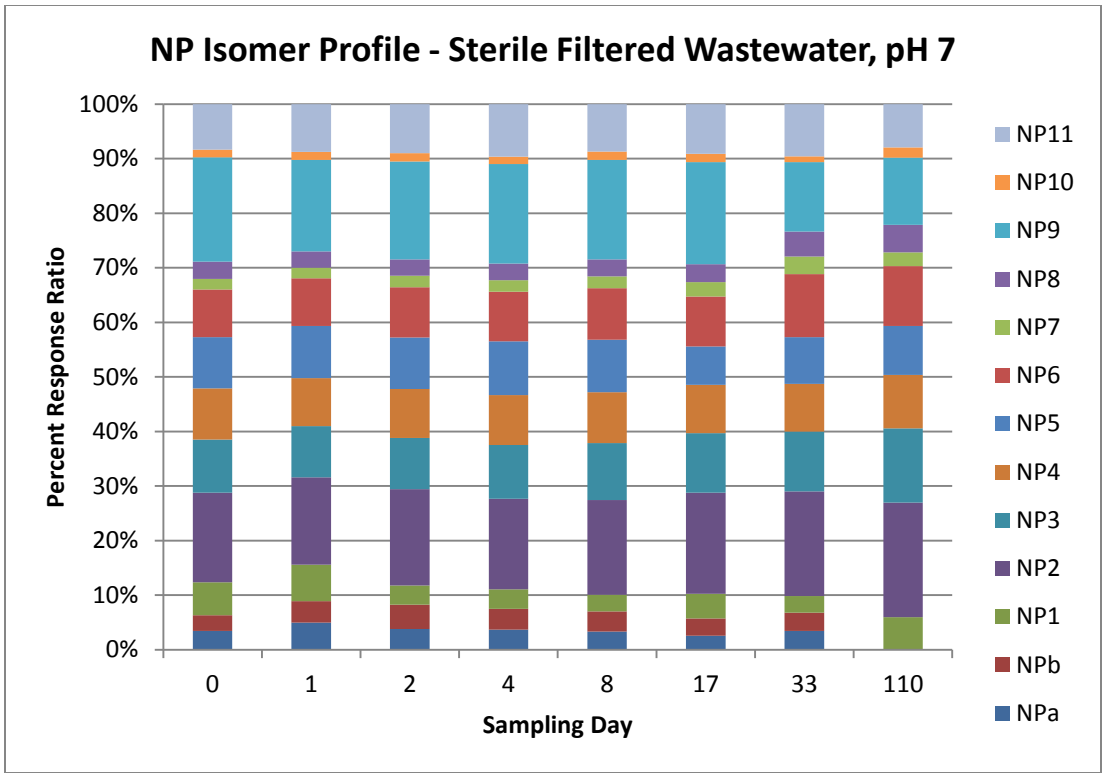

Figure 28: Response ratios of NP isomers in sterilized (autoclaved) filtered wastewater at pH 7. Ratios are reported as the instrument response of the NP isomer to the instrument response of the internal standard.

NPEO1

As previously mentioned, we suspected that the increase in NP's concentration in this degradation experiment could be due to the metabolism of its parent compounds, such as NPEOs (which was shown in Figure 1). Analysis of the concentration change of nonylphenol monoethoxylate (NPEO1) was therefore conducted. The change in concentration over the 110 day storage period can be seen in Figure 29 and Figure 30, which focused on the first 33 days. Statistically speaking, all factors, except pH, were statistically significant in the degradation of NPEO1 (appendix

Table 35), including all two- and three-way interactions, over the storage period of 110 days. This means that there is a significant change in concentration across the three pHs as well

as the two storage conditions. Additionally, this data shows that while pH doesn't seem to effect the NPEO1 concentration individually, the slopes in time are significantly different across the three different pHs (see appendix

Table 35, "pH" and "pH*Time").

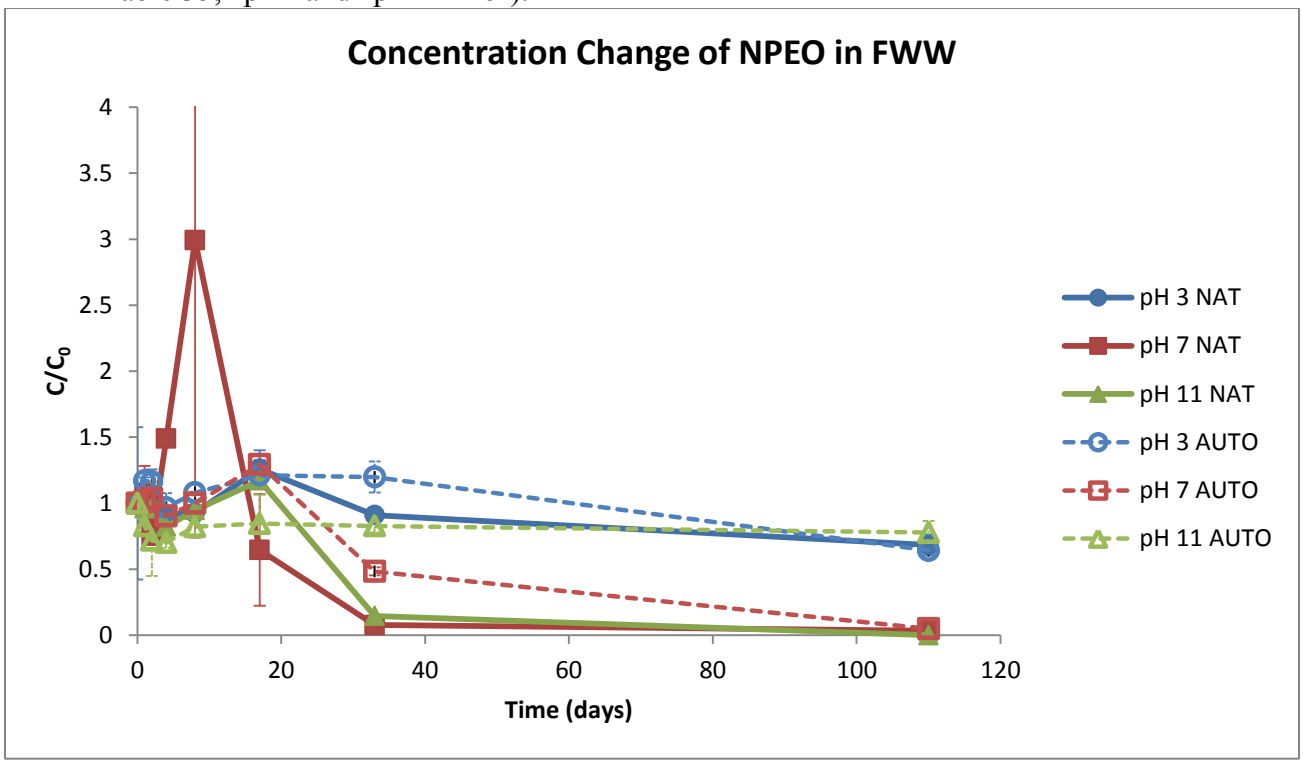

Figure 29: Concentration change of NPEO1 stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature, and under three different pHs, for 110 days. Error bars represent standard error between sample replicates (n=3). To prevent excessive skewing, pH 7 NAT, day 8's error bar is cut off; the true value is ±2.253.

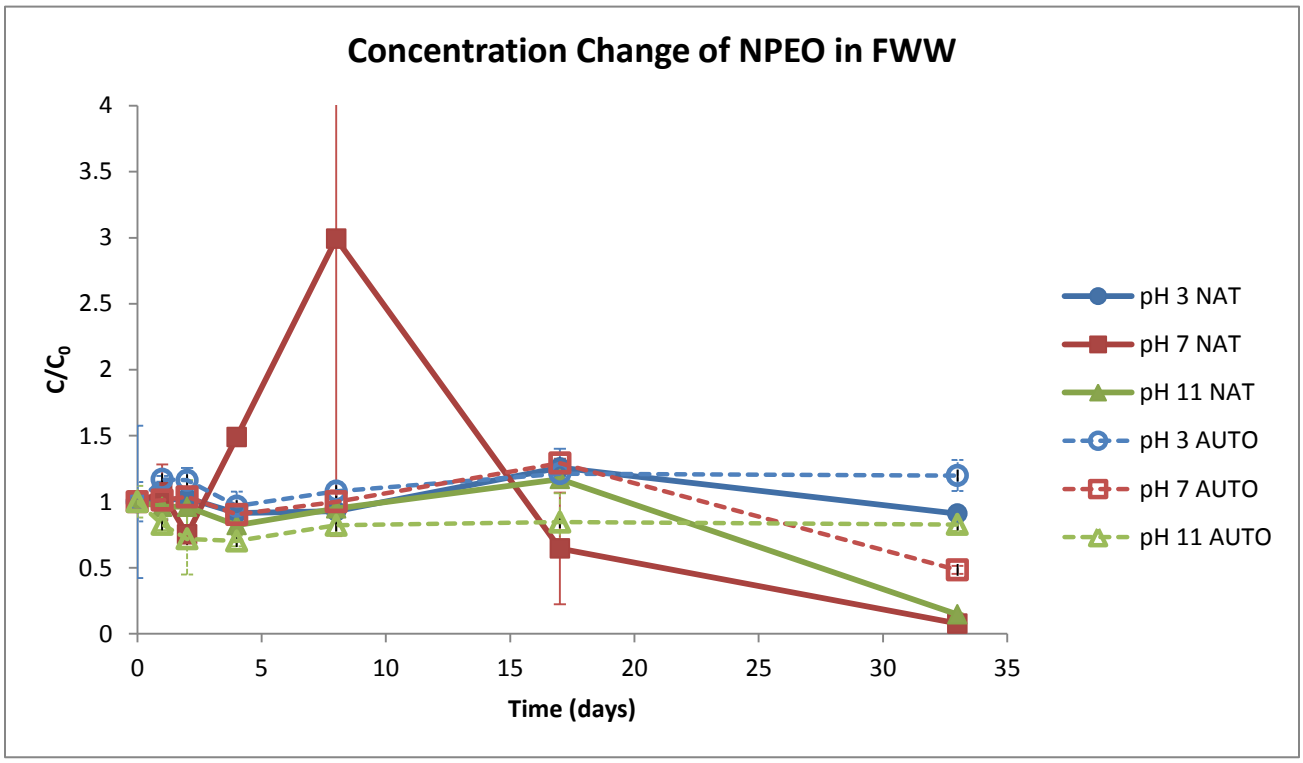

Figure 30: Concentration change of NPEO1 stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature, and under three different pHs, for 33 days. Error bars represent standard error between sample replicates (n=3). To prevent excessive skewing, pH 7 NAT, day 8's error bar is cut off; the true value is ±2.253.

When looking individually at the storage conditions, the change in concentration is significant for both. Additionally, the pH factor, as well as the interaction between the pH and the change in concentration is also significant for the autoclaved wastewater as well as the natural wastewater (data not shown). The rates of change of the log concentration of NPEO1 can be seen in Table 9. When we look closer at the autoclaved wastewater only, for the first 33 days of storage, only the change in log concentration of NPEO1 stored at pH 7 is statistically different from zero, indicating only the water stored at pH 7 will experience significant degradation within the first 33 days. As a result, there was no significant degradation observed for NP at 33 days of storage, as previously shown (Figure 23 and Figure 24). Since we see degradation of NPEO1 in the pH 7 waters, this could cause an increase in NP during this time period, and since we didn't

see degradation of NP, we can conclude that perhaps concurrent degradation of NP is occurring at a pH of 7. It could also explain the low correlations of determination in NP first order or second order degradation plot. When looking at the complete 110 day storage, the degradation of NPEO1 at pH 3 becomes statistically significant, which could indicate an acclimation period of the bacteria to the pH adjustment. For the nonsterile waters, degradation is seen in pH 7 and pH 11 for both storage periods (0-33 days and 0-110 days). At both 33 and 110 days, we can see that the degradation of NPEO1 in pH 7, regardless of whether it was sterilized or not, is higher than the other two pHs. This shows that the additions of high enough concentrations of acid or base are capable of slowing down the degradation of NPEO1 to a certain degree. Percentages lost for each water storage is summarized in Table 10. The first-order degradation curves of NPEO1 in autoclaved and natural filtered wastewater are shown in Figure 31 and Figure 32, respectively. In the autoclaved wastewater it is abundantly clear there is a large difference between the degradation in pH 7 versus pH 3 and pH 11, further demonstrating our observation that the pH adjustment will slow down degradation. For the natural wastewater, it is likely that the degradation of NPEO1 under all three pHs followed a first order reaction rate. The sharp increase seen in NPEO1 concentration at pH 7 could be explained by assuming the higher order NPEOs are readily biodegradable at a pH of 7, thus degrading into NPEO1 and causing an increase. As such, it would seem that at pH 7 in natural wastewater, NPEO1 follows more of second order degradation, as shown in Figure 33 and this holds true for all pH adjusted wastewater. It should be noted that the filtration step did not affect the degradation of NPEO1.

Table 9: Rates of change in the log concentration of NPEO1 over time, stored in sterilized (AUTO) and nonsterilized (NAT) filtered wastewater at room temperature and three different pHs. Those rates shown in red are statistically different from zero.

	Rate of change between 0-33 days	**Rate of change between 0-110 days**
pH 3 AUTO	+0.00177	-0.00198
pH 7 AUTO	-0.00761	-0.01217
pH 11 AUTO	+0.00057	-8.023×10^{-5}
pH 3 NAT	$+4.424 \times 10^{-5}$	-0.00148
pH 7 NAT	-0.03343	-0.01515
pH 11 NAT	-0.02149	-0.06077

Table 10: Change in NPEO1 concentration throughout 33 and 110 days of storage in filtered wastewater at room temperature in amber glass jars. Concentrations are reported as ppb.

	[NPEO1] on Day 0.	**[NPEO1] on Day 33.**	**[NPEO1] on Day 110**	**% Loss at Day 33**	**% Loss at Day 110**
pH 3 AUTO	2.17	2.598	1.393	-19.932	35.701
pH 7 AUTO	2.035	0.985	0.103	51.590	94.956
pH 11 AUTO	2.454	2.033	1.911	17.185	22.146
pH 3 NAT	4.069	3.704	2.783	8.962	31.593
pH 7 NAT	4.216	0.327	0.146	92.245	96.477
pH 11 NAT	4.292	0.631	0	85.297	100

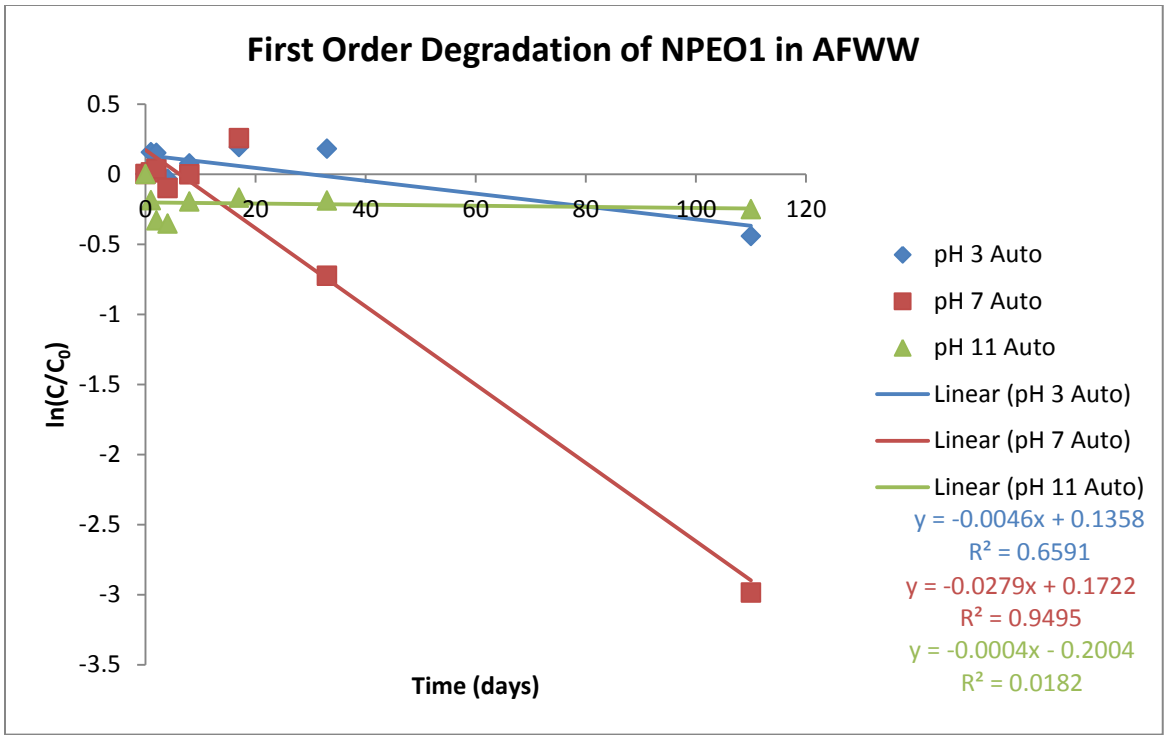

Figure 31: First order degradation of NPEO1 in sterilized (autoclaved) filtered wastewater at room temperature, stored in amber glass jars under three different pHs.

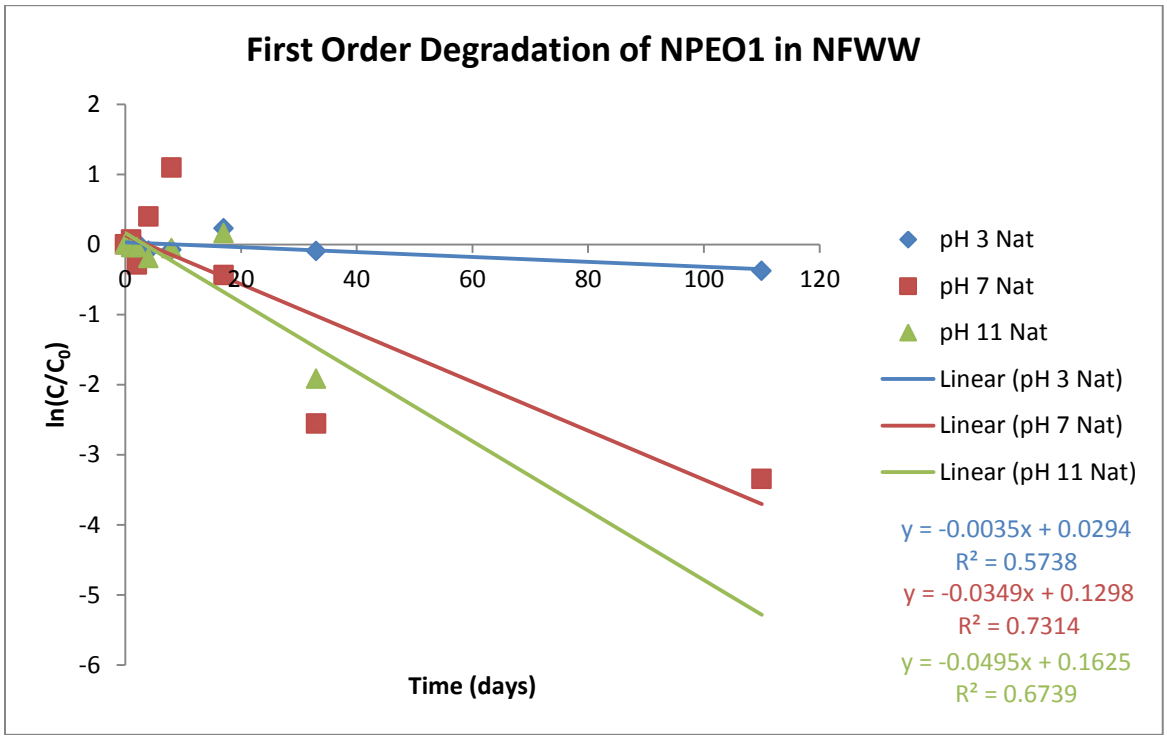

Figure 32: First order degradation of NPEO1 in non-sterilized (natural) filtered wastewater at room temperature, stored in amber glass jars under three different pHs.

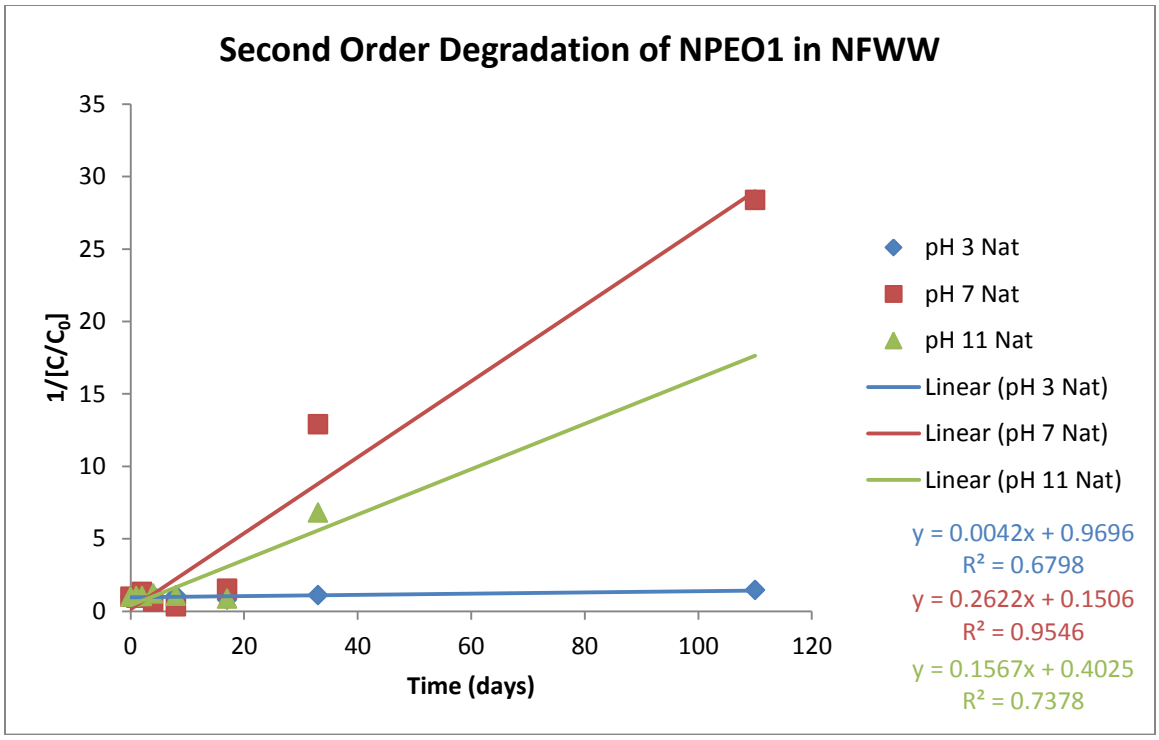

Figure 33: Second order degradation of NPEO1 in non-sterilized (natural) filtered wastewater a room temperature, stored in amber glass jars under three different pHs.

BPA

The change in the concentration of BPA stored in sterilized and non-sterile filtered wastewater for 110 and 33 days are shown in Figure 34 and Figure 35, respectively. Based on the ANCOVA analysis, all factors and interactions, except that of medium*pH, were significant, indicating a significant change in concentration. This shows that the change in concentration over time was different across all three pH levels as well as both storage conditions (autoclaved and natural) (appendix Table 36). The combination of medium and pH, however, did not give a significant difference in degradation, which means that the log concentration of BPA is changing under the different pH conditions at the same rate across the two media.

Looking now at only the natural (non-sterile) wastewater, the results are the same for the 33 day period as well as the 110 day period: there is a statistically significant change in concentration, and the slopes in time are statistically different between the three pHs. For the autoclaved wastewater, for the period of 110 days, there is a statistically significant change in

concentration over time, and the rate of change is statistically different according to pH (appendix Table 37). However, for the period of 33 day, there is still a statistically significant change in concentration over time, but the pH does not seem to have an effect on the rate of change over time (appendix Table 38). The rates of change for each can be seen in Table 11.

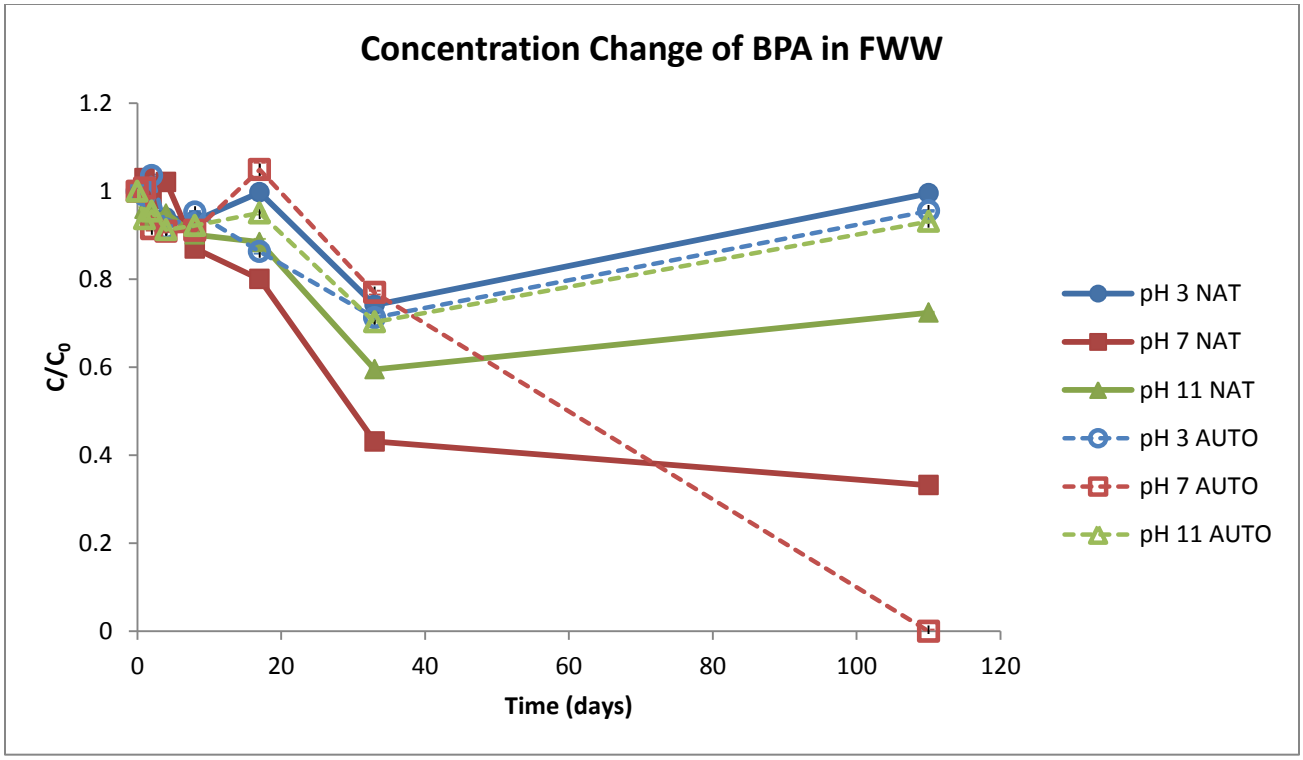

Figure 34: Concentration change of BPA stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature, and under three different pHs, for 110 days. Error bars represent standard error between sample replicates (n=3).

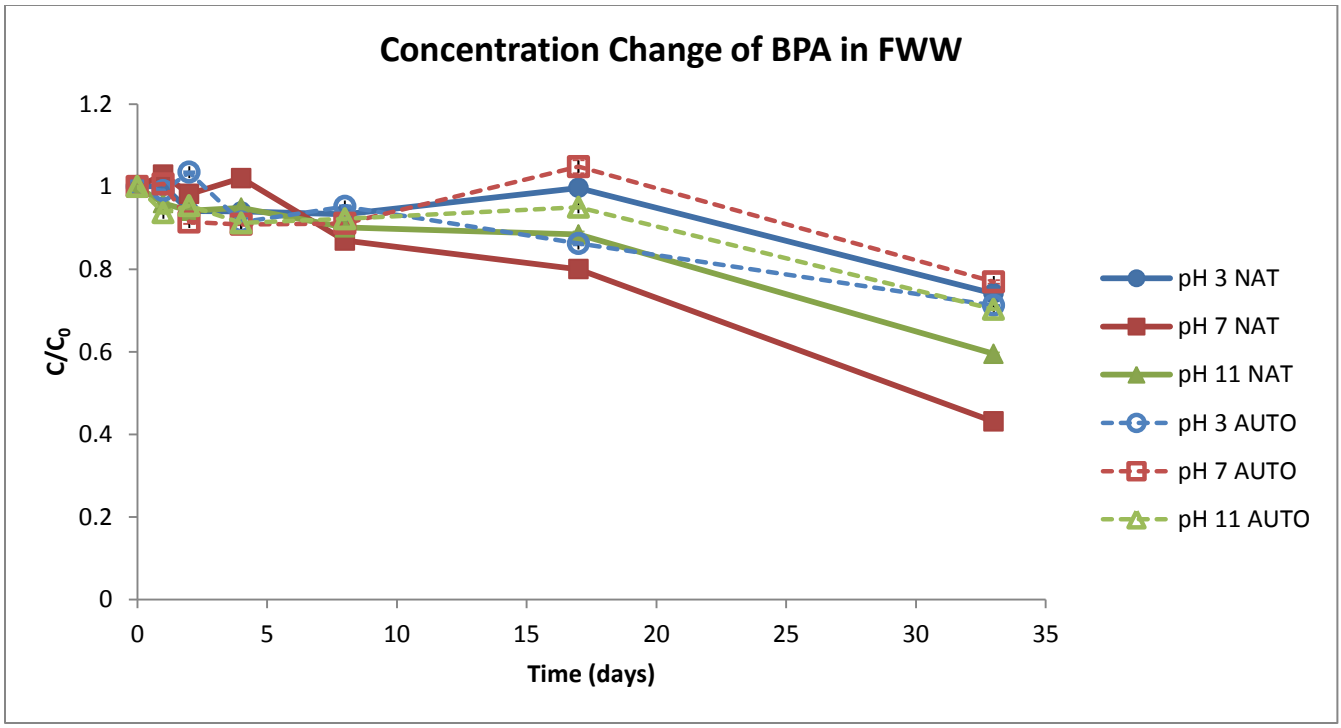

Figure 35: Concentration change of BPA stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature, and under three different pHs, for 33 days. Error bars represent standard error between sample replicates (n=3).

Table 11: Rates of change in the log concentration of BPA over time, stored in sterilized (AUTO) and nonsterilized (NAT) filtered wastewater at room temperature and three different pHs. The rates displayed in red are those that were statistically different from zero.

	Rate of change between 0-33 days	**Rate of change between 0-110 days**
pH 3 AUTO	-0.00445	-0.00023
pH 7 AUTO	-0.00239	-0.03797
pH 11 AUTO	-0.00364	-0.00019
pH 3 NAT	-0.00317	-2.0668×10^{-5}
pH 7 NAT	-0.01103	-0.00468
pH 11 NAT	-0.00612	-0.00021

By examining the rates of change for the first 33 days, it was noticed that for the natural wastewater in particular, the water stored at pH 7 seemed to exhibit the fastest degradation of BPA. This shows that the pH adjustment does in fact eliminate, or at the very least deactivate, the bacteria capable of degrading BPA. Looking at the rates of change for the complete 110 day storage, the degradation rates for the natural pH 7 waters were again larger than that of pH 3 and 11. Furthermore, it was determined that the rates of change in log concentration of BPA stored at pH 3 and 11 were not different than zero, indicating only the samples stored at pH 7 (or an unadjusted pH) will undergo significant degradation. This seems to indicate that for BPA, adjusting the pH of the storage waters is enough to slow down degradation. Additionally, for the autoclaved wastewater, the degradation rates seemed to be relatively equal within the first 33 days. It can be attributed to adsorption to the walls of the storage container. After 110 days, however, only the rate of change in log concentration under pH 7's rate of change was different from zero, which is the same result that was shown for the nonsterile wastewater. For pH 7 in the autoclaved water, the rate of change dramatically increases, providing further evidence to support the idea that the autoclave doesn't kill off all the bacteria, and they may be able to gradually proliferate during longer storage periods, while the bacteria capable of biodegrading BPA were kept deactivated or eliminated under pH 3 and pH 11.

The actual changes in concentration between the two storage conditions are summarized in Table 12. It should be noted that the autoclave process does not seem to affect the starting concentration of BPA like it did for NP and NPEO1. In fact, it seemed to enrich the concentration slightly; however it is unknown if this was due to a contamination or some other factor. The degradation of BPA in both autoclaved and natural water was likely to follow a first order reaction, as can be seen in Figure 36 and Figure 37, respectively. Under both storage conditions, the degradation under pH 7 waters exhibited a much greater degree of degradation than that of the pH 3 and 11. Given that in the autoclaved water under pH 3 and pH 11 the degradation seems essentially straight, we are able to further prove that BPA does not undergo significant degradation in the pH adjusted autoclaved and natural wastewaters (Table 12) during

the 110 day storage period. The half-lives of BPA in filtered wastewater was calculated to be 65 days in natural wastewater, and 128 days in autoclaved wastewater.

Table 12: Change in BPA concentration throughout 33 and 110 days of storage in filtered wastewater at room temperature in amber glass jars. Concentrations are reported as ppb.

	[BPA] on Day 0	[BPA] on Day 33	[BPA] on Day 110	% Loss at Day 33	% Loss at Day 110
pH 3 AUTO	0.129	0.092	0.123	28.748	4.4793
pH 7 AUTO	0.135	0.104	0	23.039	100
pH 11 AUTO	0.139	0.098	0.129	29.721	6.891
pH 3 NAT	0.114	0.085	0.114	25.868	0.477
pH 7 NAT	0.114	0.049	0.0380	56.874	66.801
pH 11 NAT	0.127	0.075	0.121	40.482	4.460

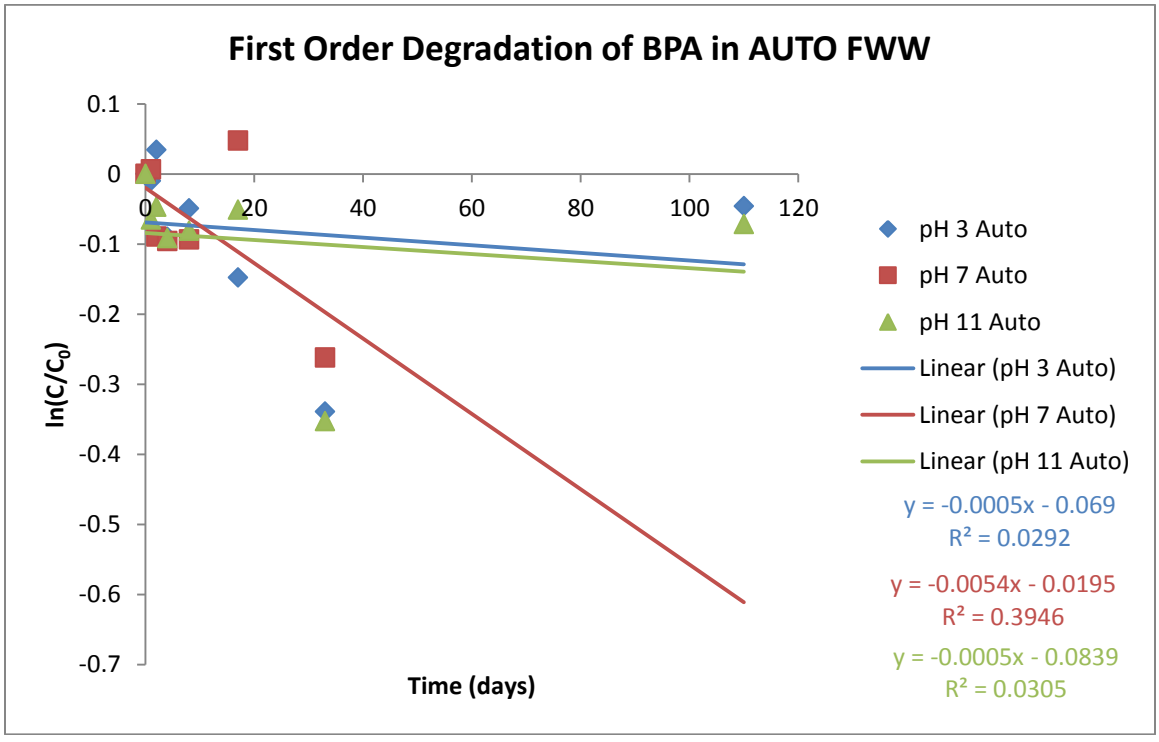

Figure 36: First order degradation of BPA in sterilized (autoclaved) filtered wastewater at room temperature, stored in amber glass jars under three different pHs.

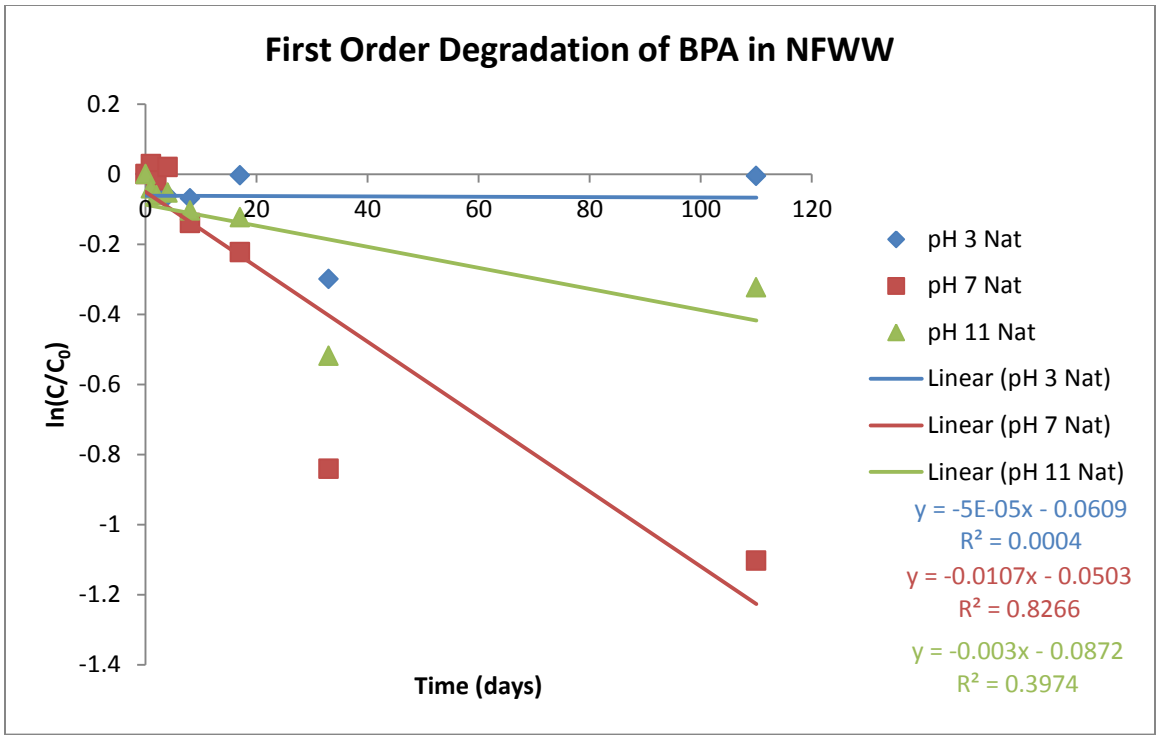

Figure 37: First order degradation of BPA in non-sterilized (natural) filtered wastewater at room temperature, stored in amber glass jars under three different pHs.

3.2.2 Degradation in Sterile and Non-sterile Unfiltered Wastewater

Since EDCs may be absorbed onto particles in wastewater, we hypothesized that filtration would reduce the degradation of NP and BPA. Influent wastewater was collected in the same manner as the previous experiment: wastewater samples were collected and stored in 1-L amber glass bottles, after which half were sterilized by an autoclave, while the other half remained natural. Sterile and nonsterile waters were then either pH adjusted to a pH of 3 and 11 through the addition of HCl and/or NaOH, or kept untreated. All treatments were held concurrently at room temperature. Since samples were not filtered and thus contained a certain amount of sediment, care was taken to not draw the sediment through the pipette when removing aliquots for SBSE. Two sets of the experiment were set up with wastewater collected on different dates. For the first replicate, samples were analyzed at days 0, 1, 2, 4, 8, 16, and 33. For the second replicate, analyses of samples were performed on days 0, 1, 2, 4, 9, 13, and 21.

NP

The concentration change for NP can be seen in Figure 38, for experimental replicate 1, and Figure 39, for experimental replicate 2. Visually speaking, in replicate 1 (Figure 38), the autoclaved samples seemed to perform similarly to the non-sterile (natural) samples when looking individually at each pH. However, across the three pHs, some differences started to surface. One of note is on days 1-4, we saw a steady increase in concentration of NP in the pH 7 wastewater samples. This could be due to the degradation of NPEOs into NP. A similar trend was seen in the second replicate of this experiment (Figure 39), although a lesser degree of increase than the first replicate was observed. It could be due to the different levels of NPEOs in wastewaters sampled on different dates. With reasons unknown, the concentration of the natural pH 11 waters increased in replicate 2 while the degradation of NP appeared to showed similar behavior between the two replicates.

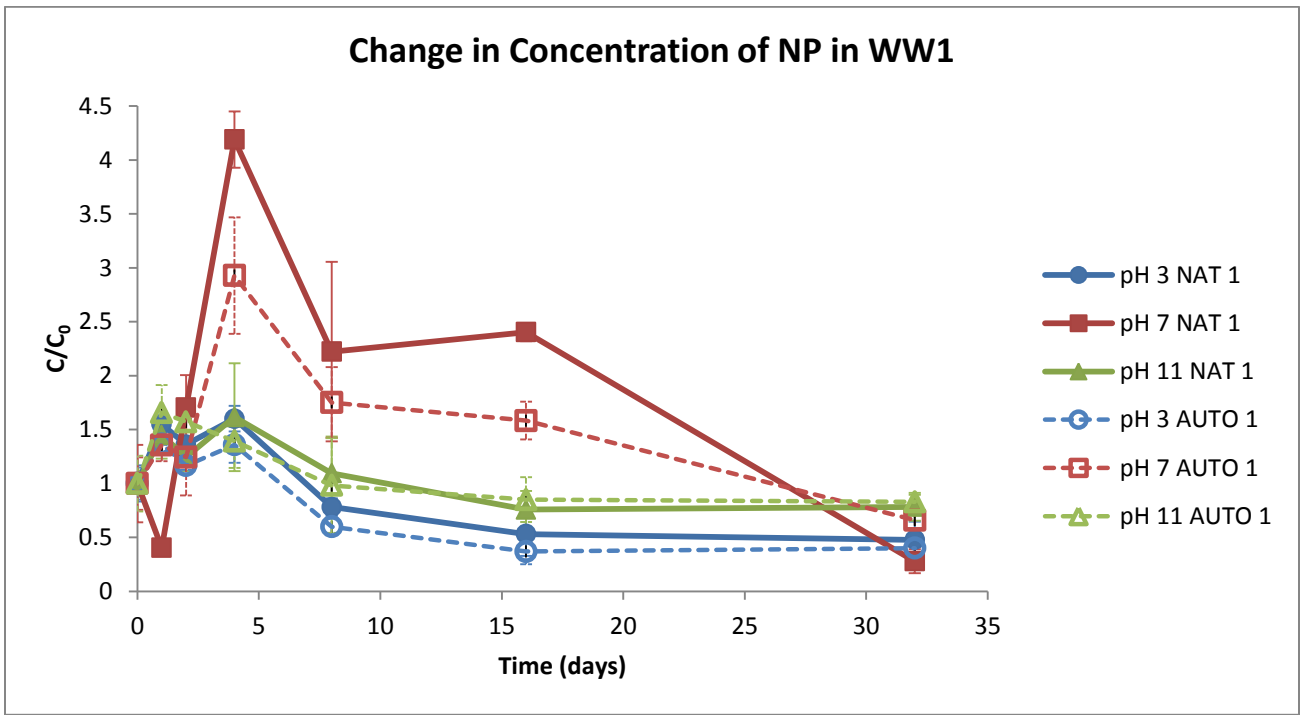

Figure 38: Concentration change of NP stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature during experiment replicate 1, and under three different pHs, for 32 days. Error bars represent standard error between sample replicates (n=3).

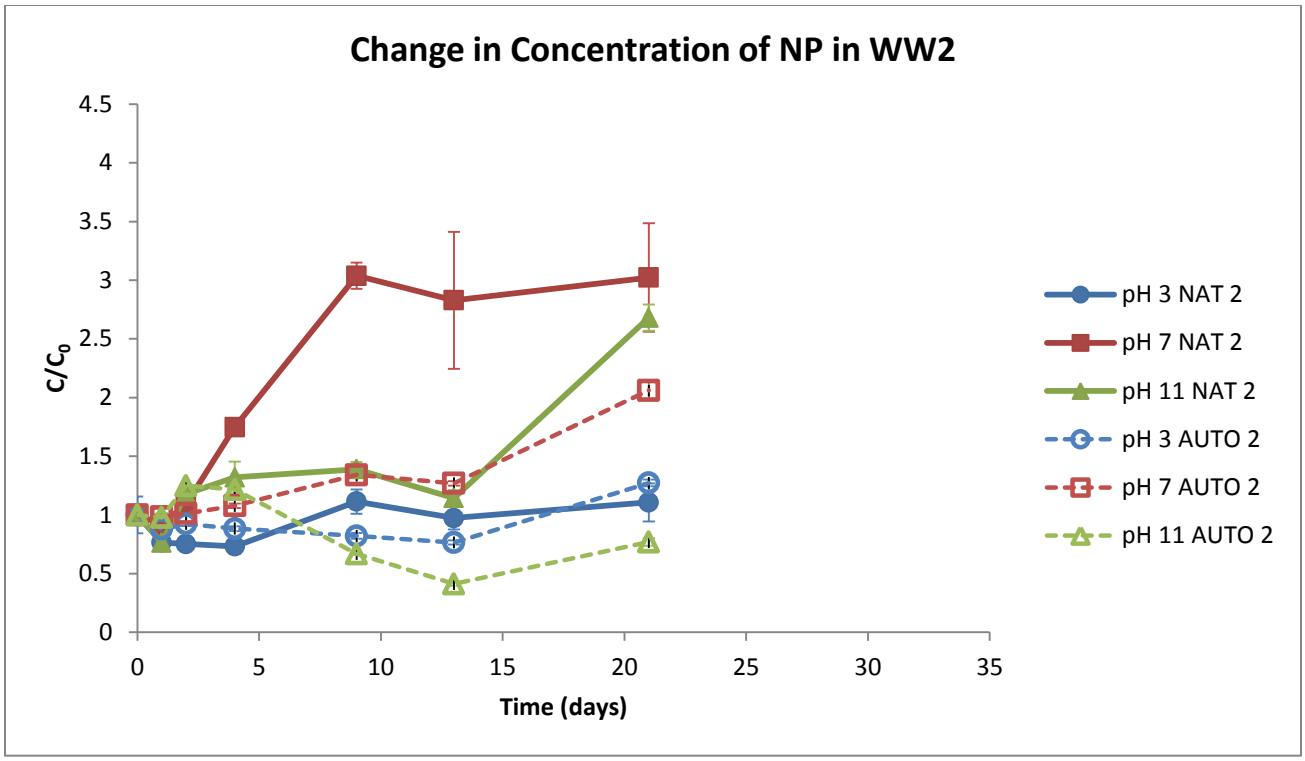

Figure 39: Concentration change of NP stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature during experiment replicate 2, and under three different pHs, for 21 days. Error bars represent standard error between sample replicates (n=3).

Statistically speaking, a significant change in NP concentration was observed (appendix Table 39), while there is no interaction between the change in concentration and the medium, meaning that the concentration change is the same across both storage mediums. Additionally, at 95% confidence, there is no interaction between the pH and the concentration change, indicating that the rate of change of log concentration is also the same across all three pHs. However, the p value for this test was 0.052, and therefore right on the cusp of statistical significance. It is also important to note that the random effect of the experimental replicates is also statistically significant, which means that some of the variation in the log concentration can be explained by variations between the experimental replicates.

Looking only at the autoclaved waters from both experimental replicates, there was an interaction between the pH and the rate of concentration change (appendix Table 40). In other words, the log concentration changed differently across all three pHs. The same conclusions were observed in both replicates. In replicate 1, there is a significant change in concentration (appendix

Table 41), and the effect of pH on degradation was significant. Over this 33 day storage period, the change in log concentration of NP decreased by 0.01810, 0.00893, and 0.00649 log[ppb] day^{-1} for pH 3, 7, and 11 respectively. The slopes of the change in log concentration of NP over time are larger in the current wastewater experiment than they were in the ultrapure water experiment (values ranged between 0.00327-0.00482 log[ppb] day^{-1}), we can infer that a certain extent of biodegradation has occurred. In replicate 2, we also see a statistically significant change in concentration, as well as the effect of pH on degradation being significant (appendix Table 42). There was also a significant interaction between the pH and the change in log concentration over time.

Contrary to the results obtained from the filtered wastewater, degradation of NP in autoclaved and unfiltered wastewater within this storage experiment's time period (33 days) was observed. This could be due to a couple of different factors. First, in the unfiltered wastewater, NP could be partitioning to the sediment rather than actually degrading. Second, the filtration process was capable of removing a certain amount of bacteria capable of degrading NP. Therefore it would be valuable to further study this effect by determining the concentration of NP in the sediment of the unfiltered wastewater. However, we can conclude that while it is not enough to stop degradation, filtration is a worthwhile step to slow degradation.

Next we look at the natural, non-sterile wastewaters. For both the experimental replicates, we see that there is a significant change in log concentration over time, as well as a significant interaction between the pH and the change in log concentration (appendix Table 43). This means that the concentration will change at different rates among the three pHs.

Additionally, the random effect of the replicate is statistically significant, which can also account for some of the variability in the log concentration due to variability between replicates. When we look individually at each replicate's natural waters, we see that for both replicates, there is a statistically significant change in concentration (appendix Table 44 & Table 45). However, in replicate 1, it appears as though there is no significant interaction between pH and the concentration change. Again, this means that the concentration will change at the same rate across all three pHs. For replicate 2, the interaction between the pH and the concentration change IS significant, however. We can see the various rates of change in non-sterile wastewater for each replicate in Table 13. In replicate 1, there did seem to be a decrease in log concentration over time, whereas in replicate 2, log concentration seemed to increase over time. Therefore we see that there is variability between the two replicates. Given that the wastewater for each replicate was collected on different days, a certain degree of variability is expected. The changes in concentration are shown in Table 14. If a negative loss in concentration is observed, it indicates that the concentration actually increased. We can see that during experiment replicate 2, the concentration of NP seemed to increase. However, we must also remember to consider the possibility that NP can adsorb onto the glass walls of the storage container and gradually be released back to the water, and that other NPEOs can break down in the water and turn into NP. This first phenomenon was observed in the ultrapure water experiments within around 30 days. Since replicate 2 was only extracted until day 21, there is the possibility that the equilibrium NP must reach in order to release from the storage container's wall occurs after that time period. Samples in replicate 1 were extracted at a time period 12 days longer and do in fact show a degradation. Therefore, we can conclude that in non-sterile wastewater, the biodegradation of NP will occur during time periods longer than approximately 33 days, with the possibility of adsorption onto glass walls (which can manifest as degradation) before that time point. Just as with the filtered wastewater, the degradation of NP in wastewater seems to follow a second order rate, as can be seen in Figure 40 and Figure 41. Contrary to the filtered wastewater experiment, however, the slopes of the lines seem to be relatively equal (recall that in the filtered wastewater

experiment, the slope of the pH 7 water was much steeper than the other two). Therefore, it can be postulated that perhaps filtration of the wastewater will affect the degradation of NP in wastewater. It appears that filtration of the wastewater, as well as pH adjustment, are the best conditions with which to slow down the degradation of NP.

Table 13: Rates of change (log_{10}ppb day^{-1}) for the log concentration change over time for NP in non-sterile (NAT) wastewater at various pHs (3, 7, and 11).

	Rate of change
pH 3 NAT 1	-0.01589
pH 7 NAT 1	-0.01980
pH 11 NAT 1	-0.00790
pH 3 NAT 2	+0.00675
pH 7 NAT 2	+0.026575
pH 11 NAT 2	+0.01836

Table 14: Change in NP concentration throughout storage in non-sterile (NAT) wastewater at room temperature in amber glass jars. Concentrations are reported as ppb.

	[NP] on Day 0	**[NP] on Day 33**	**[NP] on Day 21**	**% Loss at Day 33**	**% Loss at Day 21**
pH 3 NAT 1	2.478	1.186		52.149	
pH 7 NAT 1	4.189	1.174		71.977	
pH 11 NAT 1	5.8938	4.623		21.568	
pH 3 NAT 2	2.213		2.450		-10.715
pH 7 NAT 2	2.559		7.737		-202.316
pH 11 NAT 2	1.903		5.101		-168.075

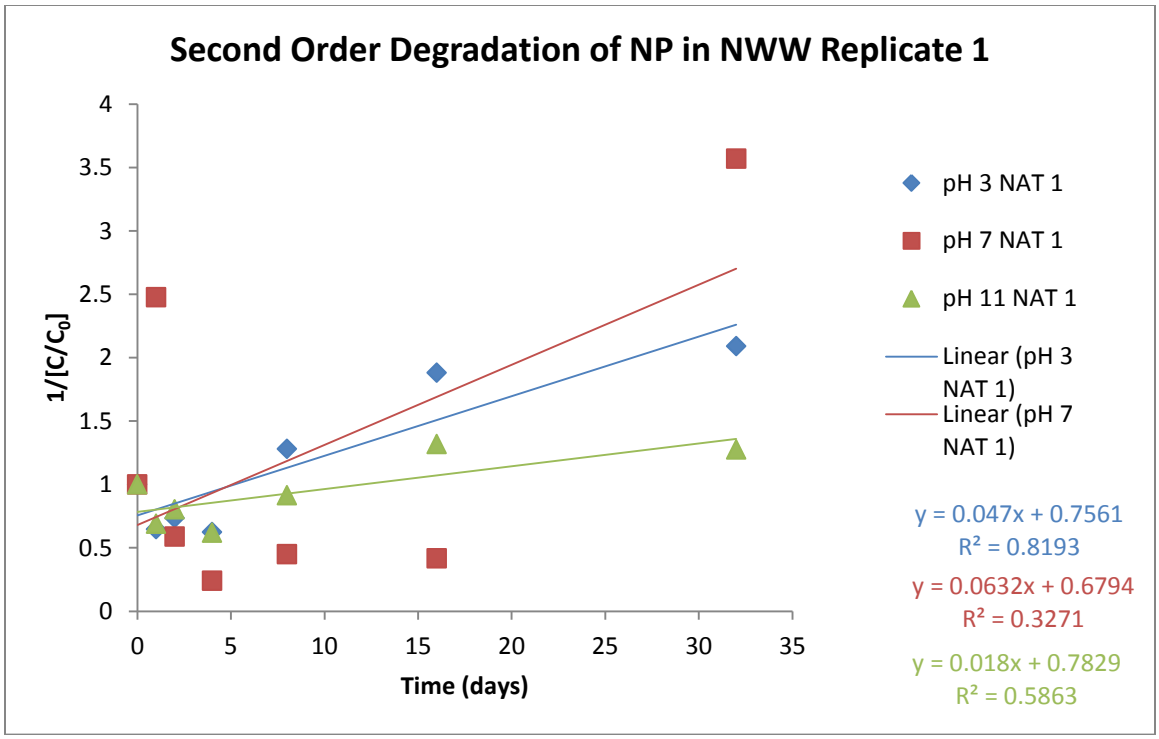

Figure 40: Second order degradation of NP in nonsterilized wastewater during replicate 1 at room temperature, stored in amber glass jars under three different pHs.

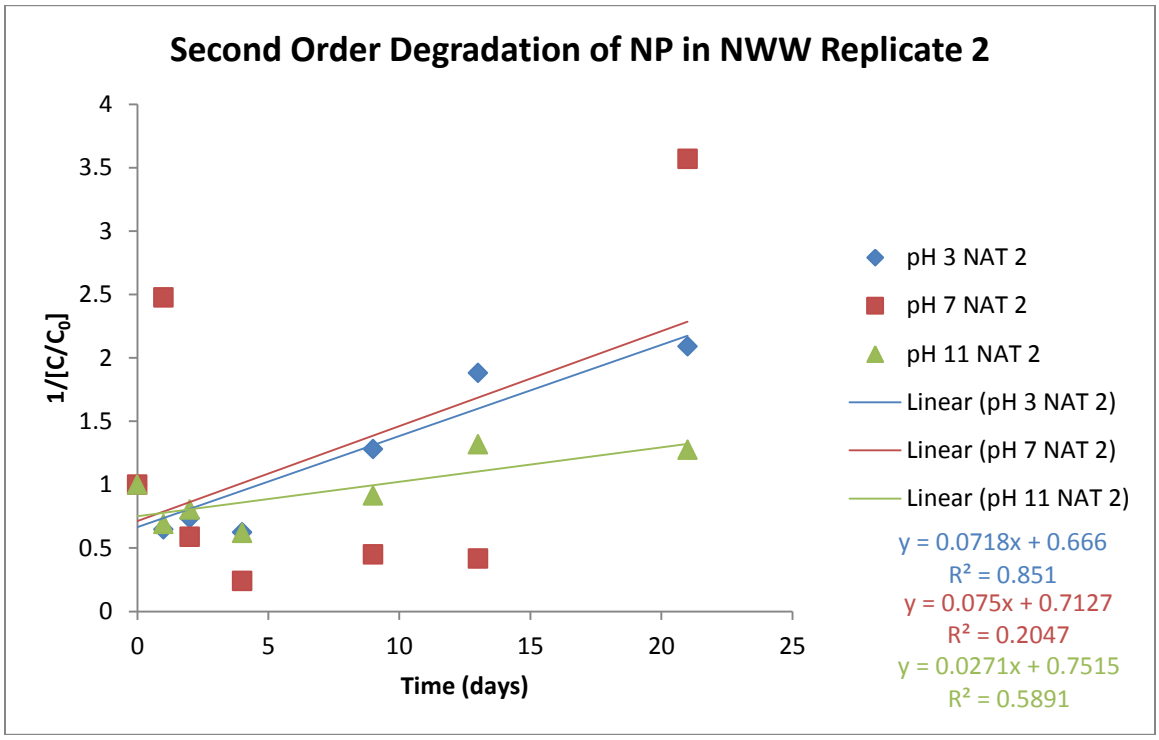

Figure 41: Second order degradation of NP in nonsterilized wastewater during replicate 2 at room temperature, stored in amber glass jars under three different pHs.

The change in the ratio of NP isomer was also studied and can be seen in Figure 42 and Figure 43, for the natural and autoclaved wastewater, respectively, for experimental replicate 1. It appears as though there are no major discrepancies in the degradation of each isomer until day 32 for the natural wastewater. At this time point, the isomer NP2 and NP10 seem to degrade to a greater extent than the previous day. Similar results are seen in the autoclaved wastewater.

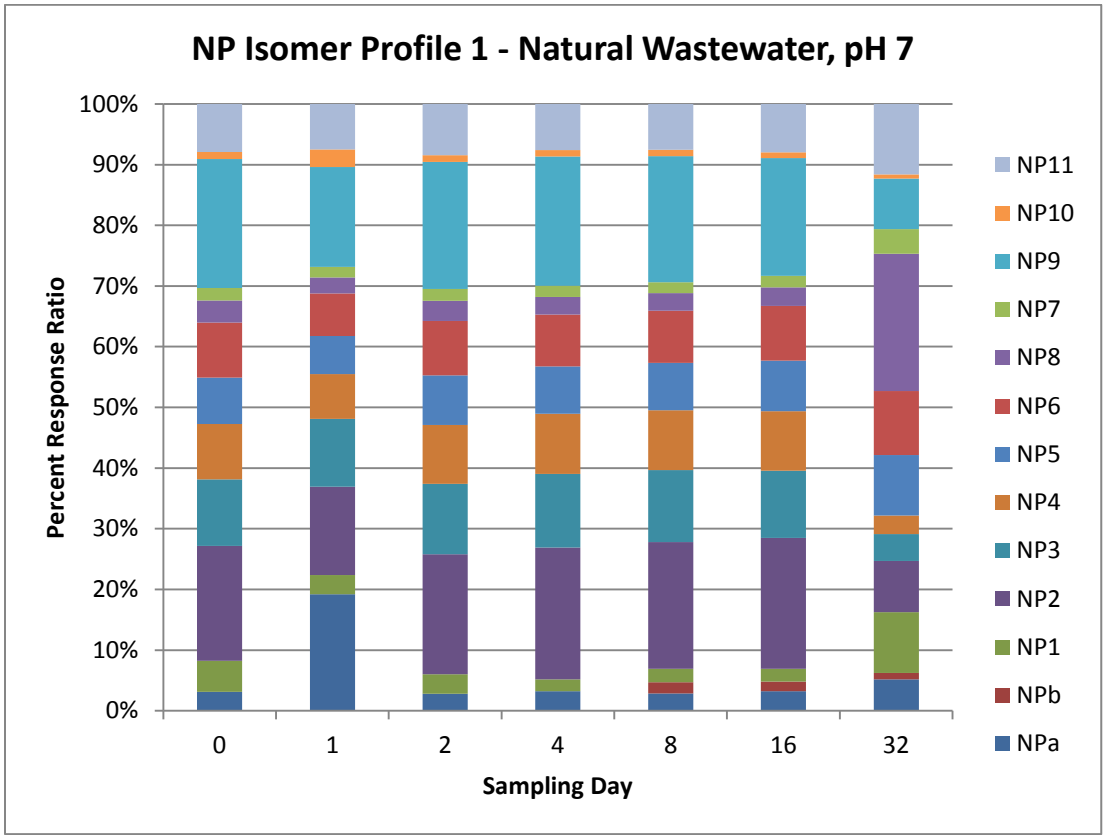

Figure 42: Response ratios of NP isomers in non-sterilized wastewater at pH 7. Ratios are reported as the instrument response of the NP isomer to the instrument response of the internal standard.

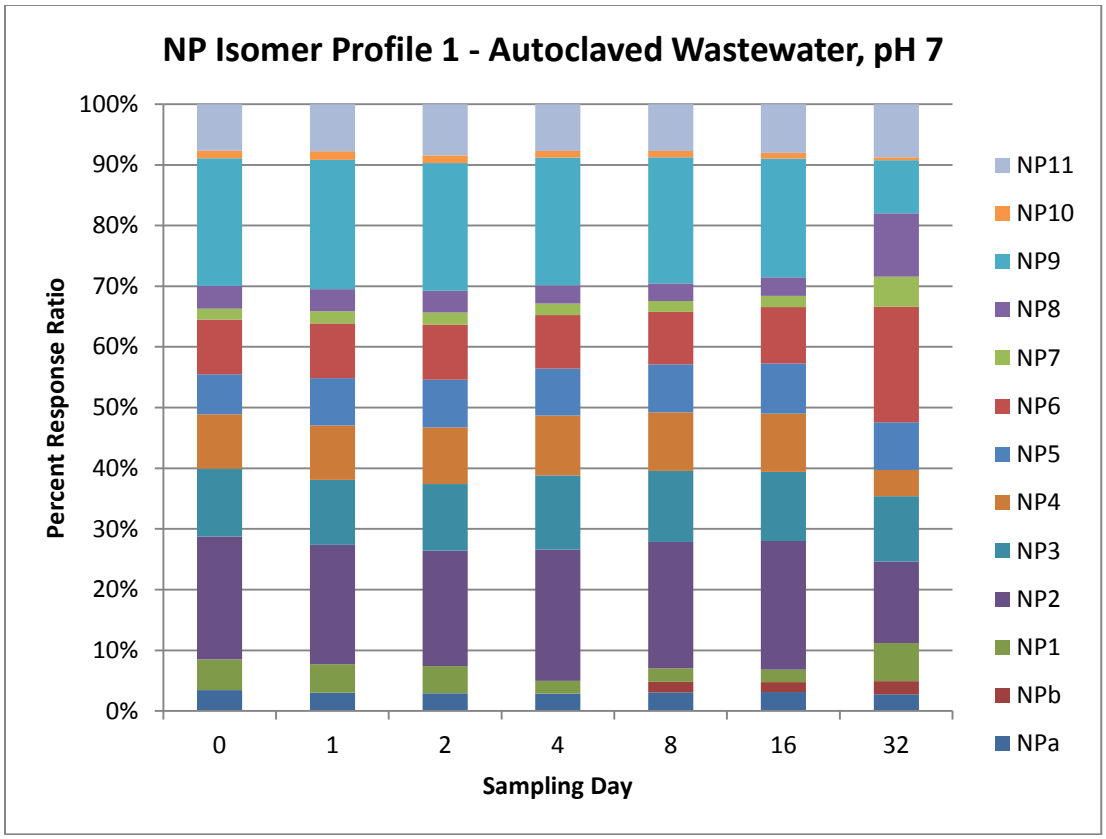

Figure 43: Response ratios of NP isomers in autoclaved wastewater at pH 7. Ratios are reported as the instrument response of the NP isomer to the instrument response of the internal standard.

NPEO1

The concentration change for NP can be seen in Figure 44, for experimental replicate 1, and Figure 45, for experimental replicate 2. Similar to what we saw with NP, it appears as though there is steady increase in the concentration of NPEO1 during the first 4 days of storage in pH 7 in both replicates. Again, the main difference is that the concentration of NPEO1 in replicate 2 doesn't seem to increase to as high a point as replicate 1. This increase could be due to the degradation of higher chain length NPEOs. And again, we see similar activity of the NPEO1 concentration between the natural and autoclaved wastewaters for each pH in experiment replicate 1.

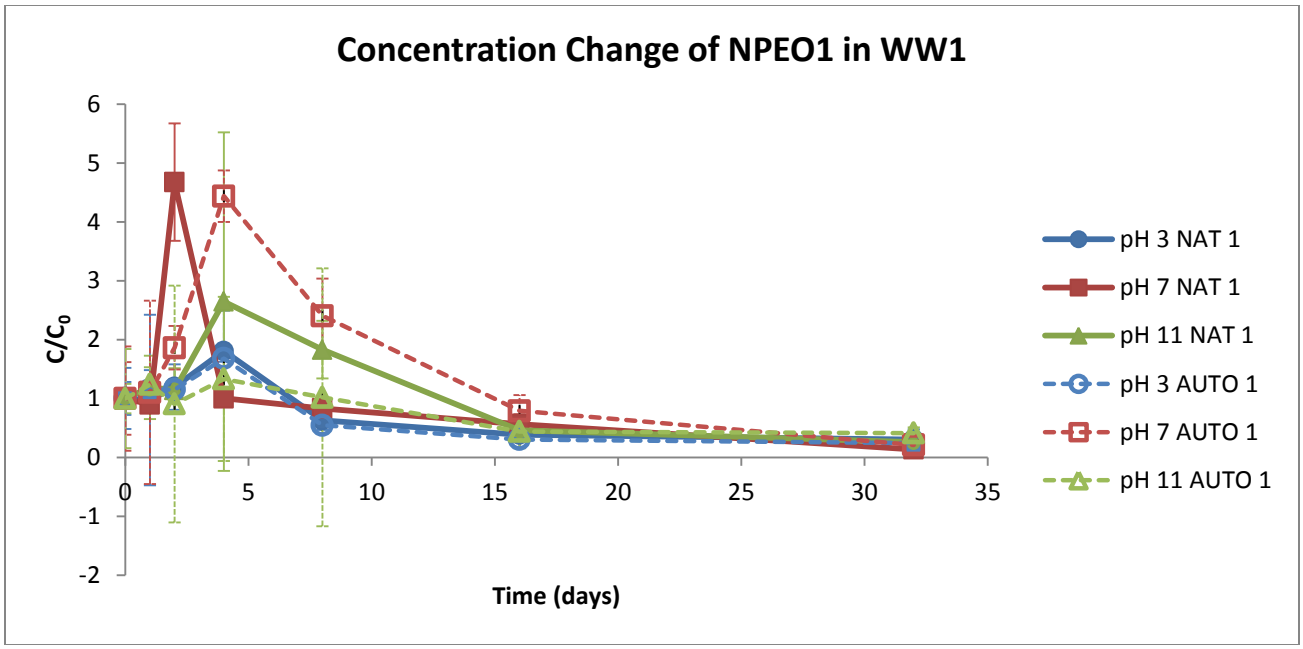

Figure 44: Concentration change of NPEO1 stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature during experiment replicate 1, and under three different pHs, for 32 days. Error bars represent standard error between sample replicates (n=3).

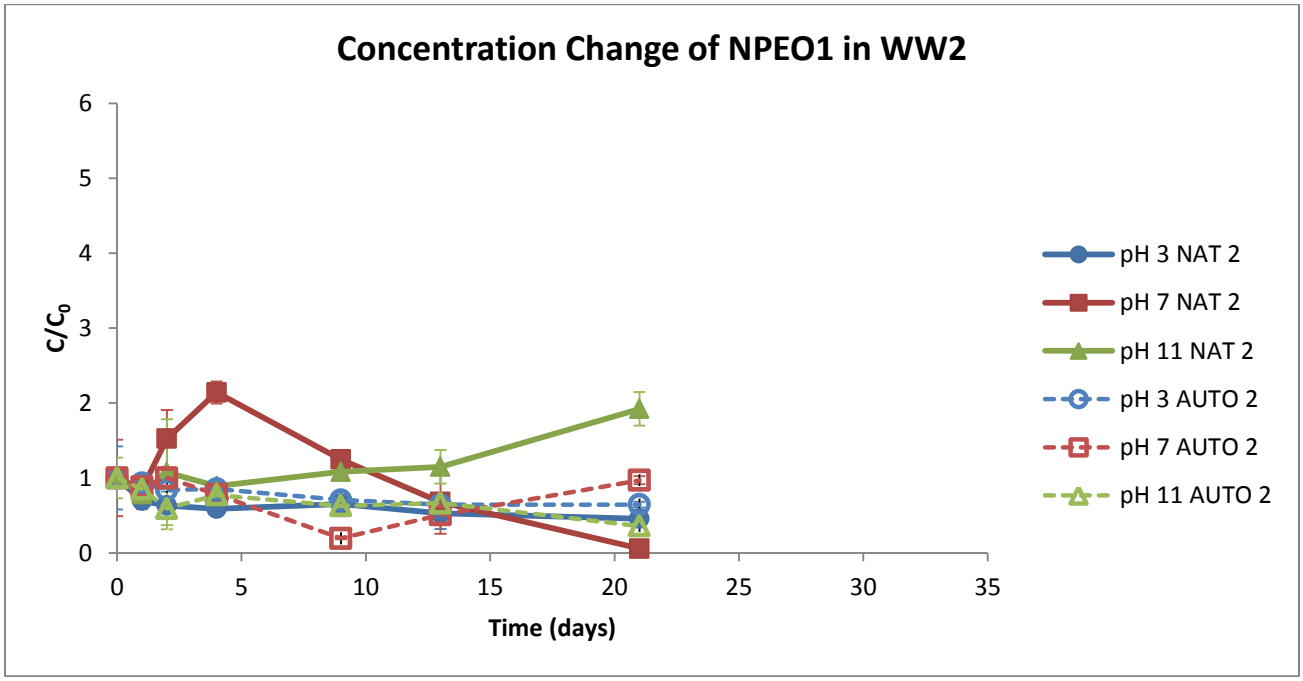

Figure 45: Concentration change of NPEO1 stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature during experiment replicate 2, and under three different pHs, for 21 days. Error bars represent standard error between sample replicates (n=3).

Statistically speaking, there is a significant change in log concentration over time, as well as a statistically significant difference between pHs and storage mediums (appendix Table 46). The interaction between pH and time is significant, indicating the concentration change of NPEO1 stored under various pHs will vary. However, the interaction between the storage medium and time is not significant, indicating that the concentration change of NPEO1 in the non-sterile and autoclaved wastewater will be statistically similar. The random effect of the experimental replicate is also statistically significant, which explains some of the variability of the log concentration in time as arising from the variability between experimental replicates.

Looking only at the autoclaved wastewater samples, it appears as though there is also a statistically significant change in log concentration over time (appendix Table 47). However, there is no interaction between pH and time, so the concentration of NPEO1 should change at the same rate, regardless of pH, in autoclaved wastewater. According to the data for each individual replicate, this is also true (appendix

Table 48 and Table 49). Therefore, it appears that there is a statistically significant concentration change of NPEO1 in autoclaved wastewater, and the log concentration will change at the same rate over time, regardless of the storage pH. The changes in concentration for NPEO1 are shown in Table 15. For experimental replicate 1, the degradation ranges between 59-79%, with the pH 11 storage exhibiting the least amount of degradation. For replicate 2, the results are quite different and the degradation ranges from 3-64%, with pH 7 exhibiting the least amount of degradation. It also appears that the degradation of NPEO1 in autoclaved wastewater follows a second order reaction rate, as shown in Figure 46 and Figure 47. Therefore, the differences in degradation between the two experimental replicates are not so alarming. Since the wastewater was analyzed for native concentrations of the EDCs, it is impossible to control the concentration of precursor compounds to NPEO1, and therefore degradation is expected to vary as the degradation of NPEO1 is likely to follow a second order reaction. Although these rates are

not statistically significantly different from each other, for the first replicate experiment, the log concentration of NPEO1 seems to degrade at a rate of 0.02448, 0.02763, and 0.01567 log[ppb] day^{-1} for pH 3, 7, and 11, respectively, while they were 0.00559, 0.00083, and 0.01060 log[ppb] day^{-1} for pH 3, 7, and 11, respectively, for the second replicate (although they were also not statistically significantly different from each other). Additionally, these rates are higher in these experiments using unfiltered wastewater, as compared to the rates in filtered wastewater (refer to Table 9). Therefore we can conclude that although it is not enough to prevent degradation, the filtration step of autoclaved wastewater will reduce the amount of degradation of NPEO1. Furthermore, it appears that the autoclave process is also not enough to prevent degradation of NPEO1 in wastewater, indicating that NPEO1 is more degradable than NP.

Table 15: Change in NPEO1 concentration throughout storage in autoclaved wastewater at room temperature in amber glass jars. Concentrations are reported as ppb.

	[NPEO1] Day 0	**[NPEO1] Day 33**	**[NPEO1] Day 21**	**% Loss at Day 33**	**% Loss at Day 21**
pH 3 AUTO1	8.435	2.03		75.851	
pH 7 AUTO 1	12.526	2.6443		78.891	
pH 11 AUTO 1	11.177	4.611		58.743	
pH 3 AUTO 2	3.165		2.037		35.642
pH 7 AUTO 2	3.055		2.962		3.0459
pH 11 AUTO 2	1.713		0.613		64.203

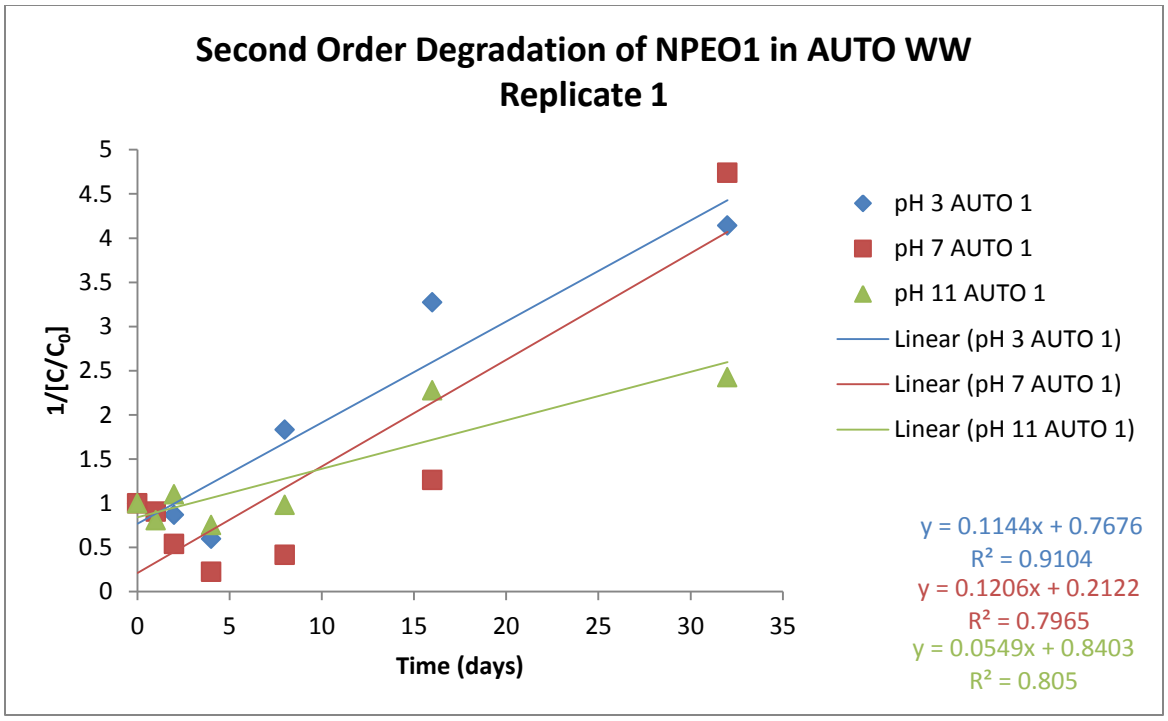

Figure 46: Second order degradation of NP in sterilized wastewater during replicate 1 at room temperature, stored in amber glass jars under three different pHs.

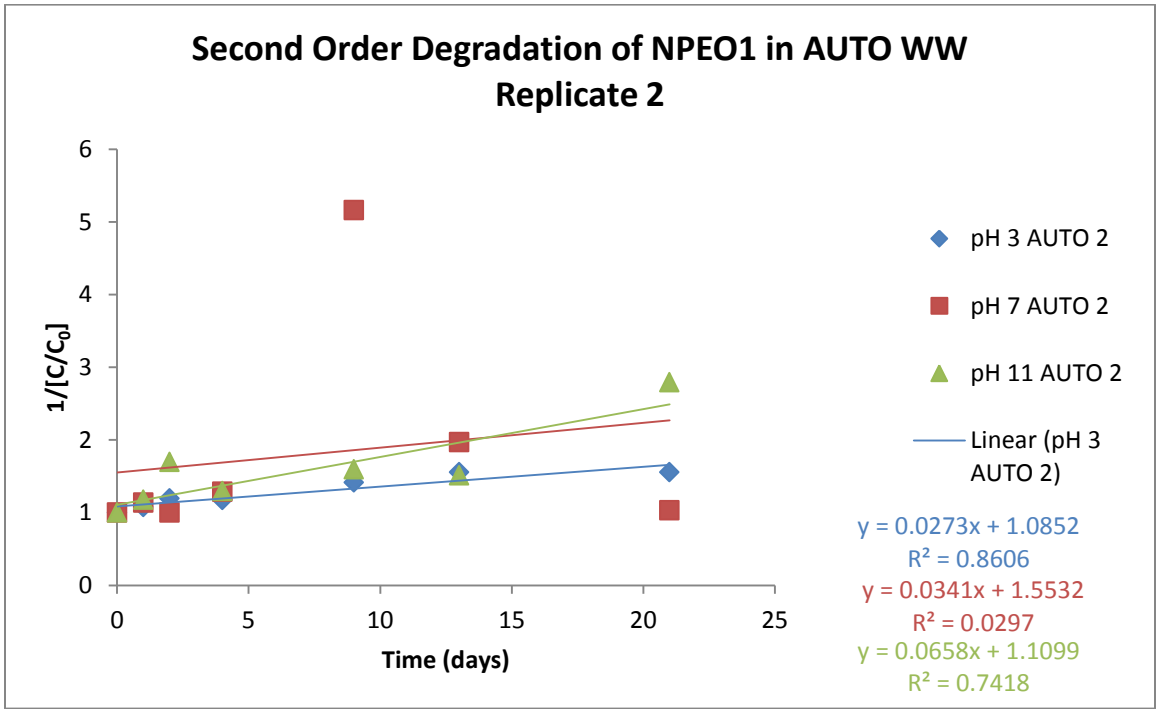

Figure 47: Second order degradation of NP in sterilized wastewater during replicate 2 at room temperature, stored in amber glass jars under three different pHs.

Looking at the concentration change of NPEO1 in natural (non-sterile) wastewater, there is a statistically significant change in concentration over time (appendix Table 50). Additionally, there is an interaction between concentration change and pH, indicating that the concentration of NPEO1 in natural wastewater will change at different rates across the three pHs. The random effect of replicate is also significant, accounting for some of the variation in concentration. Individually, the concentration change of NPEO1 in natural wastewater is different between replicates. For replicate 1, the interaction between pH and the concentration change is not significant (appendix Table 51); however, for replicate 2, it is, for reasons currently unknown (appendix Table 52). The changes in concentration of NPEO1 can be seen in Table 16. During the first replicate, the total loss in concentration ranged from 70-86%. In the second replicate, the losses are quite different. The pH 7 water experienced the highest degradation, at 94%. This result can also explain the observation in the concentration change for NP in the natural waters at pH 7 in which the concentration seems to increase and flatten out above the original NP concentration, perhaps due to NPEO1 degrading into NP. pH 3 seemed to degrade only about 54% in replicate 2, whereas pH 11 actually gained 92% of its original concentration (i.e. the concentration nearly doubled from day 0 to day 21). This same observation occurred in the samples analyzed for NP, in which there was less degradation, and even an increase in concentration, of the samples stored for 21 days as opposed to the samples stored for 33 days. This could be due to the higher chain NPEOs being more readily biodegradable in the beginning of the storage experiment, which would thus increase the concentration of NP and NPEO1, and during the storage period sometime between 21 and 33 days, NP and NPEO1 are able to degrade. This explains why the degradation of NPEO1 in non-sterile wastewater seems to follow more of a second order rate, as can be seen in Figure 48 and Figure 49. Additionally, the rates of degradation between the unfiltered wastewater and this filtered wastewater were very similar, indicating that the additional step of filtration may not be necessary. In addition, it was reported that bacteria that degrade NPEOs have different growth at different pH values (Ruiz et al., 2013).

Some degradation variation in two replicates could be the result of the difference in the bacterial population, which in turn caused NPEOs to degrade at different rates under different pH settings.

Table 16: Change in NPEO1 concentration throughout storage in wastewater at room temperature in amber glass jars. Concentrations are reported as ppb.

	[NPEO1] on Day 0	[NPEO1] on Day 33	[NPEO1] on Day 21	% Loss at Day 33	% Loss at Day 21
pH 3 NAT 1	8.134	2.476		69.556	
pH 7 NAT 1	11.3511	1.552		86.329	
pH 11 NAT 1	10.007	2.609		73.930	
pH 3 NAT 2	5.994		2.737		54.340
pH 7 NAT 2	6.335		0.359		94.328
pH 11 NAT 2	5.685		10.921		-92.097

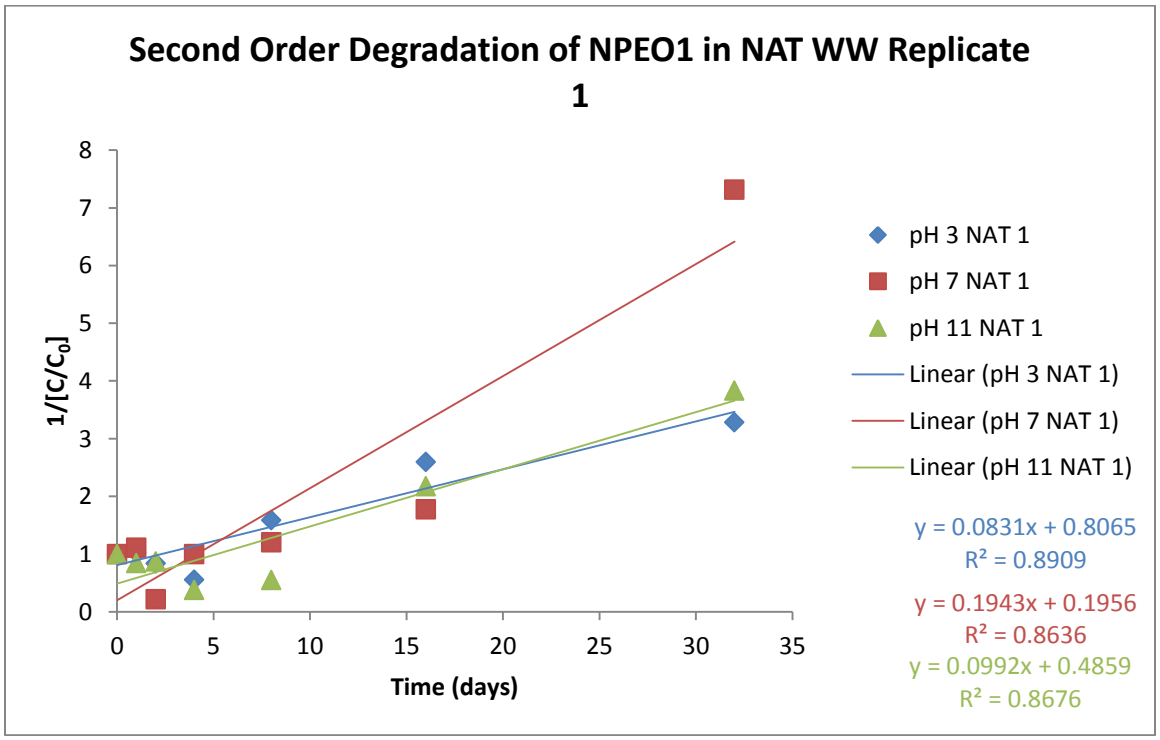

Figure 48: Second order degradation of NPEO1 in nonsterilized wastewater during replicate 1 at room temperature, stored in amber glass jars under three different pHs.

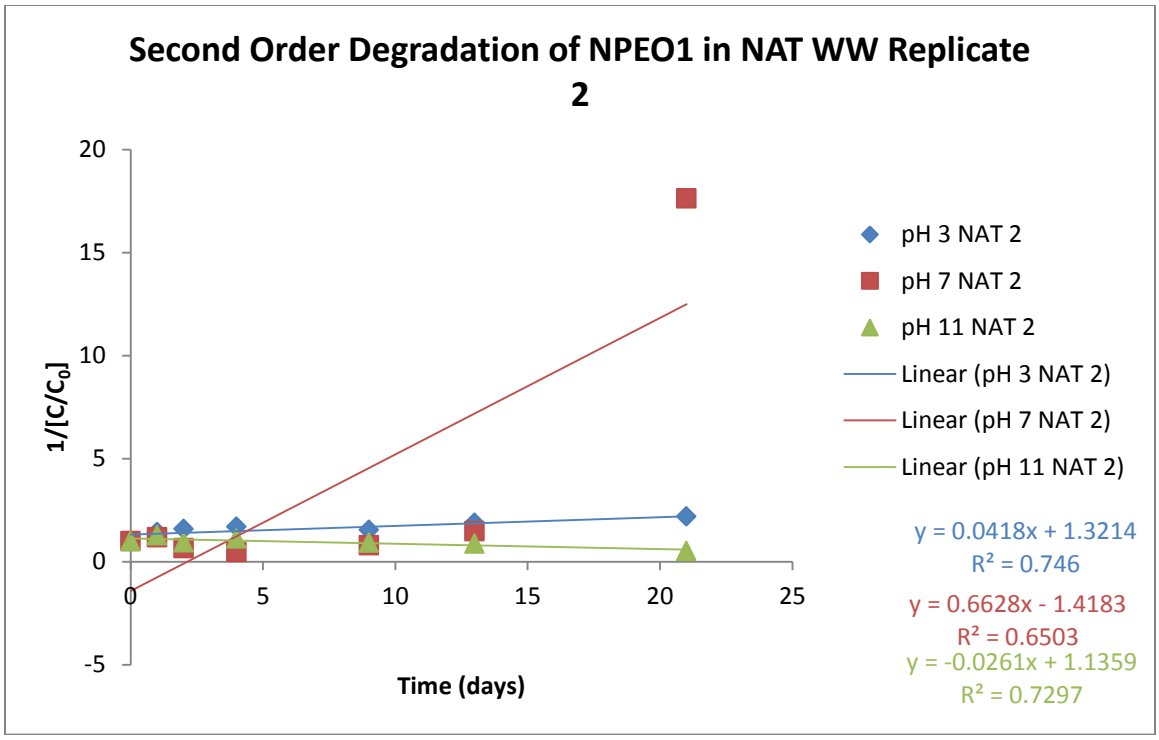

Figure 49: Second order degradation of NPEO1 in nonsterilized wastewater during replicate 2 at room temperature, stored in amber glass jars under three different pHs.

BPA

The concentration change for BPA can be seen in Figure 50 for experimental replicate 2, as the concentration of native BPA in replicate 1 was below the calibration levels, and thus unquantifiable.

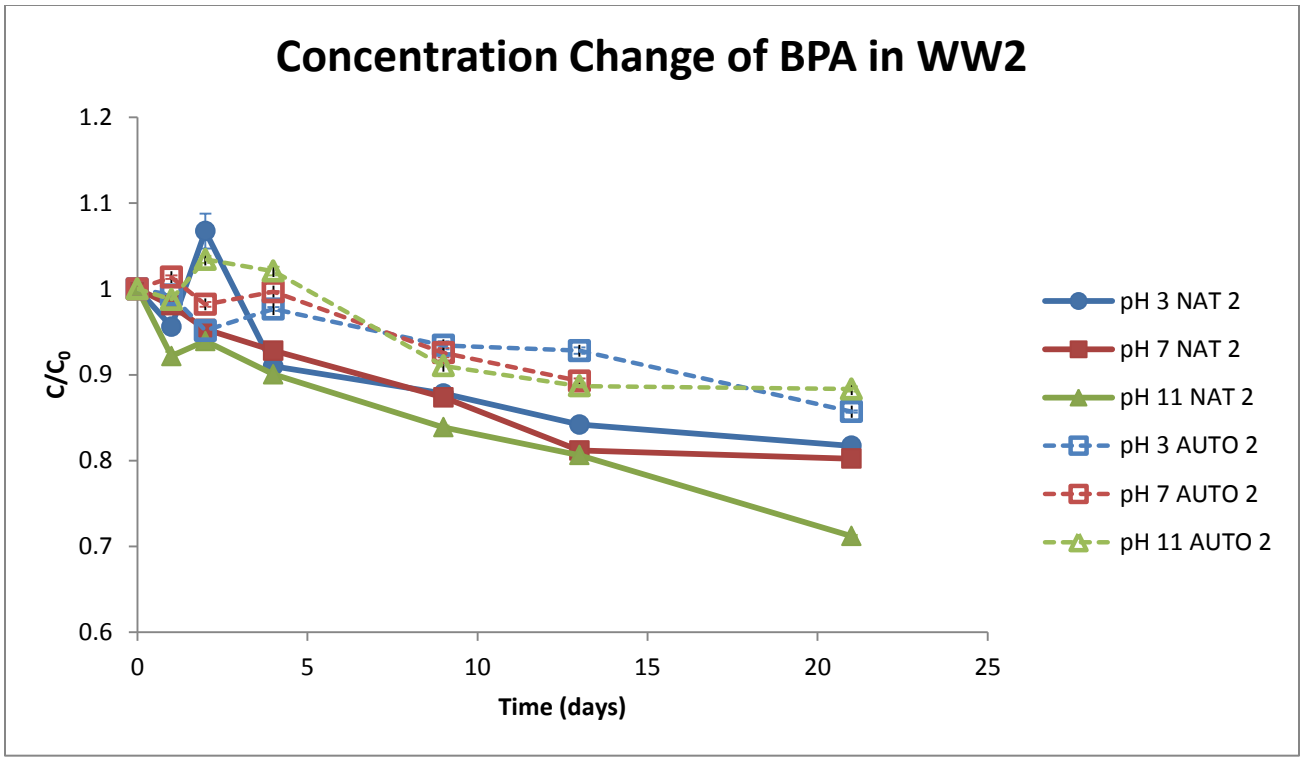

Figure 50: Concentration change of BPA stored in sterile (AUTO) and nonsterile (NAT) filtered wastewater (FWW) at room temperature during experiment replicate 2, and under three different pHs, for 21 days. Error bars represent standard error between sample replicates (n=3).

Statistically speaking, there is a significant change in the concentration of BPA over time (appendix Table 53). However, there is no interaction between the pH and the concentration change, indicating the concentration will change at the same rate across all pHs. By comparing the storage medium, e.g. sterile and non-sterile, the results are similar in that both exhibit a statistically significant change in concentration over time, however the factor of pH is not significant and there is no interaction between pH and concentration change (appendix Table 54 and

Table 55). The change in BPA concentration over the 21 day storage period can be seen in Table 17. It appears as though there is a greater amount of degradation in the non-sterile waters as compared to those that were autoclaved. The fact that there is still minor degradation in the autoclaved waters agrees with our results from the ultrapure water experiments, which demonstrated BPA is capable of chemical degradation in ultrapure water, such as hydrolysis. The concentration losses in the current experiment are similar to those seen in the ultrapure water experiment (refer to Table 7). In the ultrapure water experiment, BPA losses at 33 days ranged from 16-17%, whereas in the autoclaved wastewater of the current experiment, losses ranged from 11-14% at 21 days. However, these results are contrary to what we saw in the filtered wastewater (Table 12), where degradation of BPA in autoclaved wastewater ranged from 23-29% at 33 days. As of yet, the reason for this is unknown. For the non-sterile wastewater, degradation ranged between 18-29% at 21 days. In the filtered wastewater, however, degradation ranged between 25-56% (Table 12). Therefore it appears as though filtration of wastewater containing BPA seems to somehow enhance the amount of degradation.

Table 17: Concentration change of BPA in unfiltered wastewater stored at room temperature in amber glass jars, in three pHs (3, 7, and 11). Concentrations are reported in ppb.

	[BPA] on Day 0	[BPA] on Day 21	% Loss at Day 21
pH 3 AUTO	0.192	0.165	14.298
pH 7 AUTO	0.188	0.160	14.738
pH 11 AUTO	0.191	0.169	11.672
pH 3 NAT	0.157	0.128	18.291
pH 7 NAT	0.153	0.123	19.772
pH 11 NAT	0.162	0.115	28.788

In both autoclaved and non-sterile wastewaters, the degradation of BPA follows a first order reaction rate. This is demonstrated in Figure 51 and Figure 52. For the autoclaved wastewater, degradation is expected to occur at 0.00189, 0.00247, 0.00233 log[ppb] day^{-1} for pH

3, 7, and 11, respectively, although the rates are statistically different. For the non-sterile wastewater, the degradation rates are 0.00286, 0.00301, and 0.00409 log[ppb] day^{-1} for pH 3, 7, and 11, respectively. Although they are also statistically different from each other, that minor difference between the rates of degradation of autoclaved wastewater and non-sterile wastewater are significant.

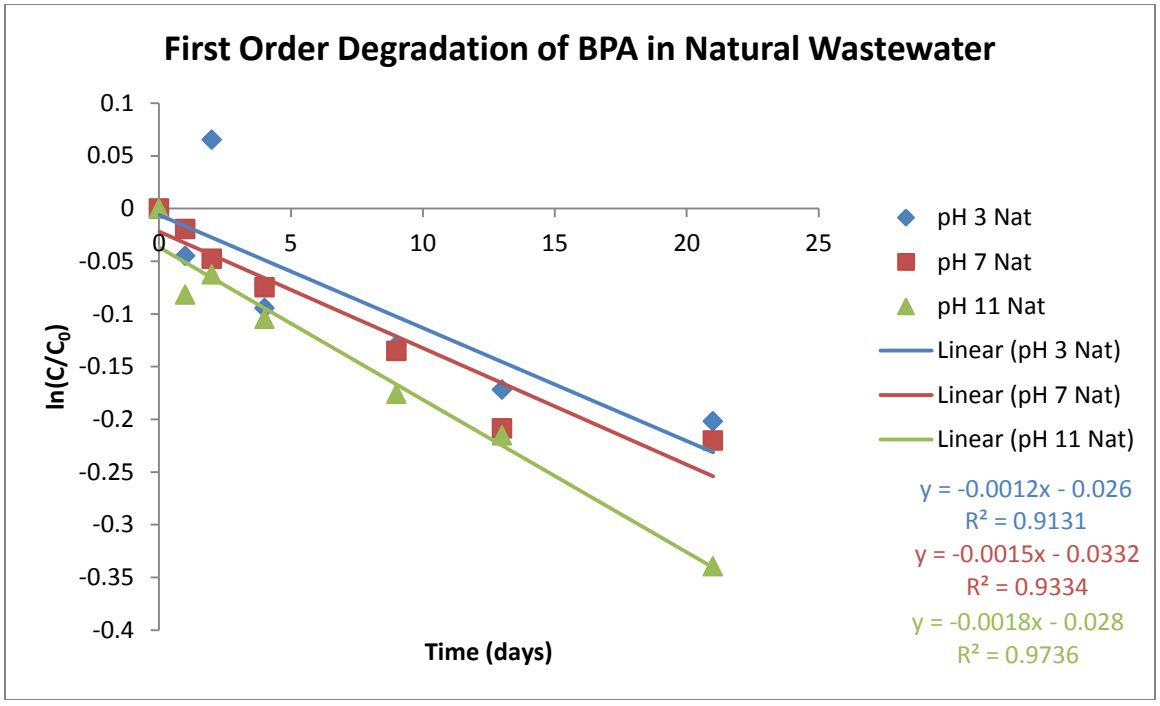

Figure 51: First order degradation of BPA in non-sterile wastewater stored in amber glass jars at room temperature and three pHs (3, 7, and 11) for 21 days.

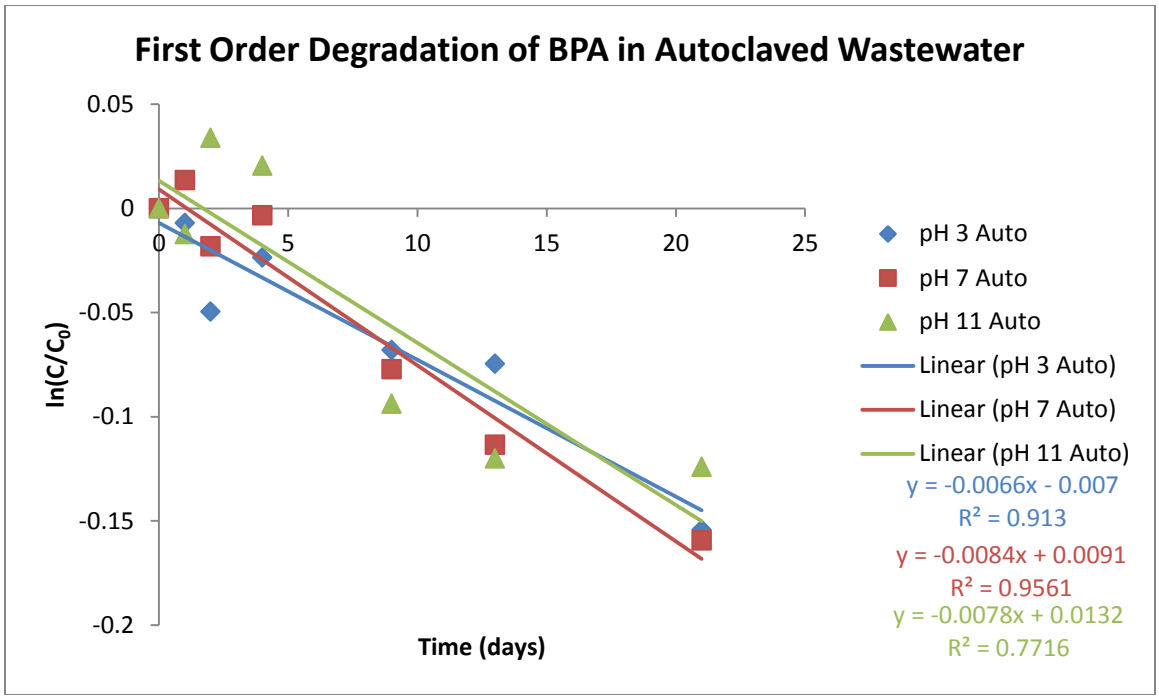

Figure 52: First order degradation of BPA in autoclaved wastewater stored in amber glass jars at room temperature and three pHs (3, 7, and 11) for 21 days.

3.3 FENTON DEGRADATION REACTION

The results presented previously show a slow degradation in water and wastewater. A significant amount of research has been done on finding other techniques to remove EDCs in those media. Advanced oxidation processes (AOPs) are of particular interest to be used to degrade organic constituents in water. In this study, we used the Fenton reaction, a chemical oxidation process, to briefly demonstrate the degradation of our target EDCs as compared to the natural degradations.

The Fenton reaction is an oxidative reaction that relies on the production of hydroxide radicals (•OH) through reaction of hydrogen peroxide with iron (II) sulfate heptahydrate (a catalyzing agent, commonly referred to as the Fenton reagent). The formation of the hydroxide radical is shown in Equation 1:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \bullet OH + OH^- \quad (1)$$

The Fenton reaction of alkylphenols and BPA in water has been extensively studied (Gültekin & Ince, 2007) and holds several benefits as an advanced oxidative process, namely because iron is an abundant and non-toxic element and peroxide is environmentally safe (Yongmei Li & Zhang, 2014). Additionally, the Fenton oxidation reaction is a relatively fast process, with typical reaction times reported within one hour.

The Fenton reagents used for this experiment were based on the reported procedure by Poerschmann, Trommler, & Górecki, 2010 with slight modification. Due to the presence of additional organic and inorganic materials, which will both compete for the •OH, in the influent wastewater, the concentrations of Fe^{2+} and H_2O_2 were increased.

3.3.1 NP

The degradation of standard NP in ultrapure water can be seen in Figure 53. It was evident that standard NP degradation by Fenton reagents occurs fast as the concentration was reduced to a level present in blank water within 32 minutes of reaction. Two different concentrations of Fenton reagents were used, 40 μM Fe^{2+} with 400 μM H_2O_2, and 80 μM Fe^{2+} with 800 μM H_2O_2, and it can be seen that the higher concentration of Fenton reagents produced a faster degradation. Note that the original concentration of NP in the ultrapure water was spiked to be 500 ppb. This higher concentration was used to account for the presence of additional reactants that would appear in the wastewater samples that are capable themselves of degradation by Fenton reagents.

The Fenton reaction was also capable of degrading the native NP in the influent wastewater, as is shown in Figure 54. A nearly complete removal of NP was accomplished within the 64 minute total reaction time. Remaining levels of NP were at levels similar to those seen in blank water samples.

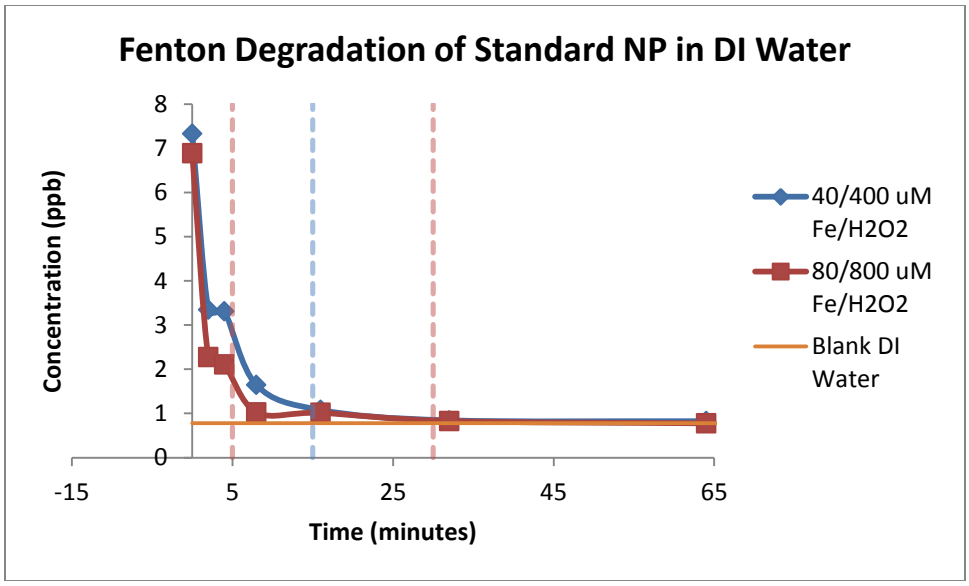

Figure 53: Fenton degradation of standard NP in DI water, at two different concentrations of Fe^{2+}/H_2O_2. Vertical lines represent the additions of Fenton reagents.

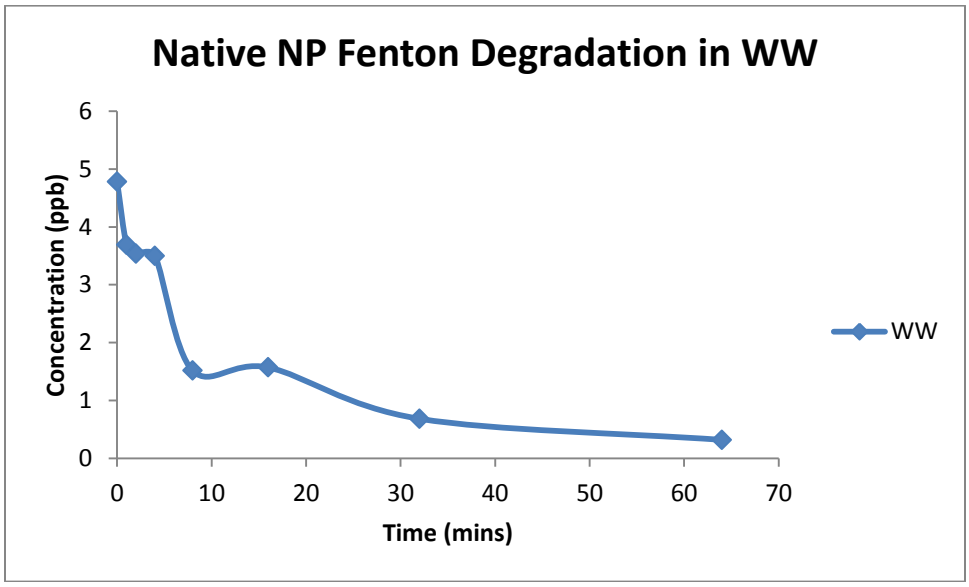

Figure 54: Fenton degradation of native NP in wastewater.

3.3.2 BPA

The degradation of standard BPA in ultrapure water did prove possible. This is demonstrated in Figure 55. As can be seen, BPA is readily degradable. The same two concentrations of Fenton reagent were used, 40 µM Fe^{2+} with 400 µM H_2O_2, and 80 µM Fe^{2+} with 800 µM H_2O_2, and it can be seen that the higher concentration of Fenton reagents produced

a faster degradation rate, however both concentrations were able to reduce the concentration of BPA to below the calibration level. The BPA was degraded to levels similar to blank DI water within 16 minutes of reaction. Note that, just as with NP, the original concentration of BPA in the ultrapure water was spiked to be 500 ppb. This higher concentration was used to account for the presence of additional reactants that would appear in the wastewater samples that are capable themselves of degradation by Fenton reagents. Microliter aliquots were taken from the Fenton reaction vessel at the various specified time points for extraction, and this data is shown in the figure.

For the degradation of BPA in wastewater, it was determined that the 80/800 µM Fe^{2+}/H_2O_2 was optimal. The concentration change can be seen in Figure 56. Native BPA in wastewater is capable of degradation by Fenton reagents, as complete degradation was seen within 8 minutes.

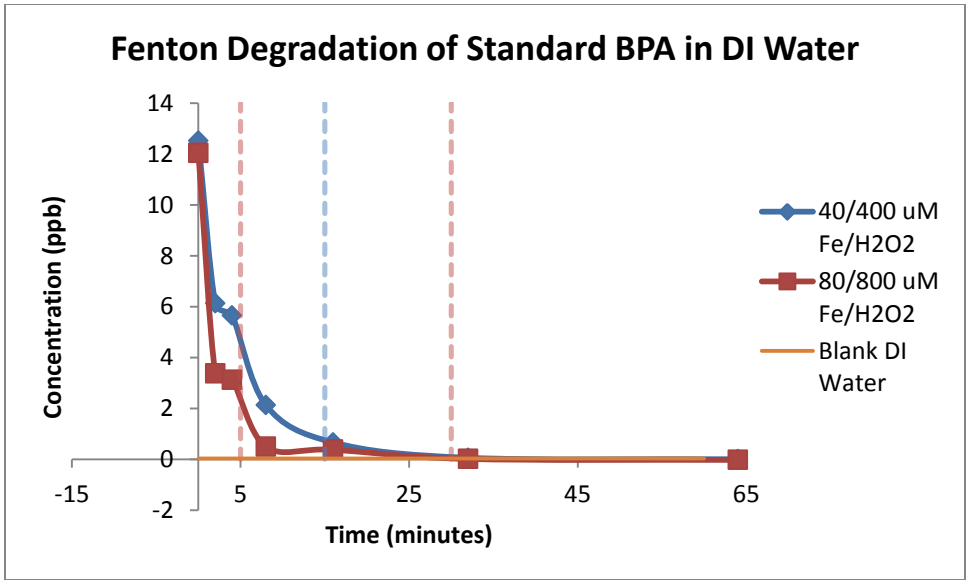

Figure 55: Fenton degradation of standard BPA in DI water, at two different concentrations of Fe^{2+}/H_2O_2. Vertical lines represent the additions of Fenton reagents.

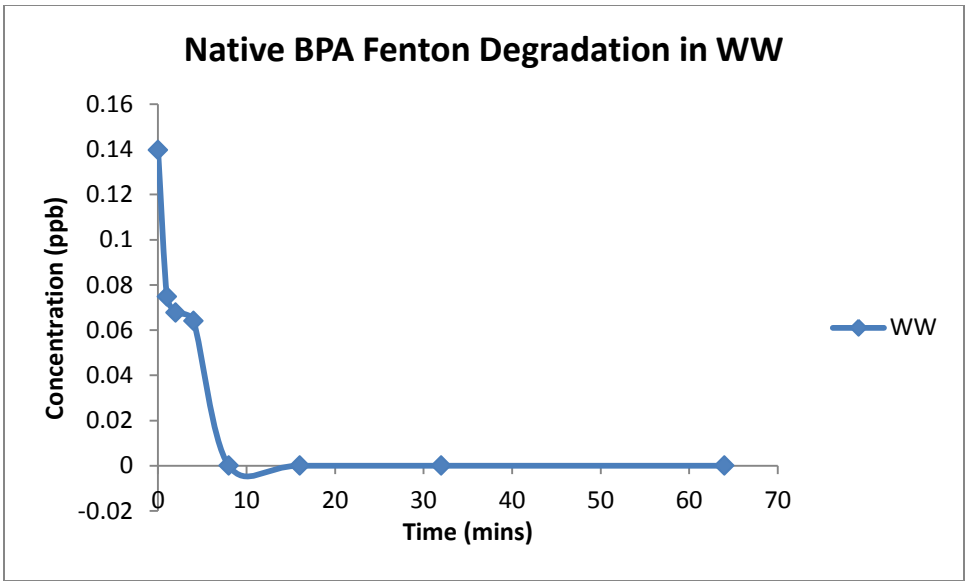

Figure 56: Fenton degradation of native BPA in wastewater.

Chapter 4: Conclusions

The degradation of NP and BPA in various aqueous media was investigated. The rates of degradation were determined under three pHs (3, 7, and 11) as well as three storage conditions (room temperature, 4 ºC, and room temperature with exposure to ambient light) in ultrapure water to determine the degree of abiotic degradation (i.e. photolysis and hydrolysis). In influent wastewater, degradation was compared at room temperature under the same three pHs, as well as under sterile and non-sterile conditions. Storage periods ranged from 21 to 110 days.

In ultrapure water exposed to ambient light, it was found that NP did not degrade within a period of 91 days. In contrast, BPA did degrade at a rate of 0.00347 log[ppb] day^{-1}, due primarily to photodegradation, resulting in removal ranging from 55-61%. This rate of decrease was independent of pH. Additionally, the degradation followed that of a first order reaction. It was also found that NP and BPA remained stable in ultrapure water regardless of the storage temperature or pH in amber glass jars. At longer periods of time (e.g. 196 days of storage), no significant degradation of NP was observed; while for BPA degradation occurred at 0.00063 log[ppb] day^{-1}, indicating BPA is perhaps capable of undergoing both photodegradation as well as hydrolysis.

In untreated influent wastewater, NP and BPA both underwent biodegradation. Nonylphenol monoethoxylate (NPEO1), which will degrade into NP, was also capable of biodegradation. In filtered wastewater for a period of 110 days, NP was degraded in both the autoclaved (sterile) and nonsterile samples, but only in non-pH adjusted waters. During the first 33 days of storage, NP did not degrade in the autoclaved filtered wastewater, however during longer storage periods (e.g. 110 days), it degraded at a rate of 0.05327 log[ppb] day^{-1}, indicating a possible lag phase in bacterial growth, which resulted in 100% removal of NP. For the nonsterile filtered wastewater, the degradation rate of NP increased from 0.0129 log[ppb] day^{-1} during the first 33 days to 0.05722 log[ppb] day^{-1} for the entire 110 day period, resulting in 67% removal at 33 days and 100% removal at 110 days. In unfiltered wastewater, degradation of NP

was seen in both the autoclaved and the nonsterile waters at storage periods of 21-33 days and across all three pHs, indicating the filtration process in fact slowed the degradation of NP. An increase in NP concentration was also seen at a storage period of 21 days, indicating that in the unfiltered wastewater, the degradation of NPEO1 was more dominate than that of NP. For NPEO1, degradation occurred in the filtered wastewater in the autoclaved samples stored at an unadjusted pH of approximately 7. For the first 33 days, the concentration of NPEO1 decreased by 0.00761 log[ppb] day^{-1}. At 110 days, NPEO1 degraded in the autoclaved filtered wastewater at both pH 3 and the unadjusted pH at rates of 0.00198 and 0.01217 log[ppb] day^{-1}, respectively. For the nonsterile filtered wastewater, degradation occurred in the unadjusted pH as well as pH 11 at rates of 0.01515 and 0.06077 log[ppb] day^{-1}, respectively. Removals ranged from 22-100%. The degradation of both NP and NPEO1 followed second order degradation. In the unfiltered wastewater, the removals and rates of degradation were very similar to the filtered wastewater, indicating that in the case of NPEO1, filtration did not affect degradation.

BPA was also capable biodegradation in the filtered wastewater, under both sterile (autoclaved) and nonsterile conditions. During the first 33 days, the rates of degradation were similar across all three pHs in the autoclaved filtered wastewater, ranging from 0.00239-0.00445 log[ppb] day^{-1}, the rates were 0.00023, 0.03797, 0.00019 log[ppb] day^{-1} for pH 3, 7, and 11, respectively. In the nonsterile filtered wastewater, the degradation was significant in only the waters at the unadjusted pH 7, with faster degradation occurring during the beginning of the storage period (rates of 0.01103 and 0.00468 log[ppb] day^{-1} for 33 days and 110 days, respectively). Removals ranged from 67-100%, however the rates of decrease indicate the autoclave process actually served to enhance the biodegradation of BPA. The degradation of BPA in filtered wastewater also followed a first order rate. For BPA, filtration of the water also seemed to enhance degradation as the degradation rates were lower in the unfiltered wastewater (ranging from 0.00189-0.00247 log[ppb] day^{-1} in autoclaved wastewater, and 0.00286-0.00409 log[ppb] day^{-1} in nonsterile wastewater). In all three cases, one autoclave cycle of 60 minutes did not effectively sterilize the wastewater, as all three compounds were underwent biodegradation.

4.1 FUTURE WORK

These results represent preliminary tests in determining the stability of NP and BPA in water. Further tests are required to gain a better understanding of the results obtained herein. Given that additional chemicals were not added to the storage waters to reduce adsorption of the compounds to the walls, the degree to which either compound adsorbs is unknown. Therefore, it would be useful to conduct the experiments in ultrapure water by adding increasing percentages of methanol.

For the wastewater studies, it would be useful to culture the bacteria present in the autoclaved wastewater at each pH to further confirm the strains of bacteria as well as their survival rate after an autoclave cycle of 60 minutes. This would allow us to determine whether in the autoclaved samples, the EDCs do actually undergo biodegradation as opposed to abiotic degradation. Additionally, this would also elucidate the idea that pH adjustment to an extreme acidic or basic pH will also help to eliminate bacteria. To determine the extent of autoclaving needed to eliminate all bacteria, it would be helpful to run storage experiments concurrently in wastewater samples that were autoclaved for an increasing number of cycles. Again, this is to ensure that if a concentration change is seen, we can be clear whether it is due to abiotic or biotic degradation. Additionally, it would be useful to perform wastewater storage experiments in the same way as the ultrapure water, i.e. in clear glass jars and at 4 ºC, to determine if storage temperature as well as exposure to ambient light will affect the growth of bacteria, or the biodegradation of NP, NPEO1, and BPA. Finally, it is also necessary to determine the concentration of the EDCs partitioning to the sediment in the unfiltered wastewater to determine the degree to which biodegradation occurs versus adsorption to the sediment. Given that the degradation across all mediums took a very long time, we can speculate that the concentrations of these EDCs will remain persistent in the environment. Furthermore, relying on natural processes as the sole source of removal of these compounds from water is not recommended.

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Appendix

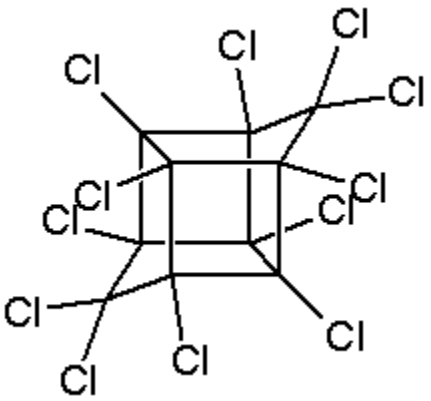

Figure 57: Structure of mirex, used as an internal standard.

Table 18: Standard error between replicates, where n=3. A * indicates only one replicate was analyzed, and therefore no standard deviation with which to calculate the standard error was available. N/A represents data that is unavailable due to loss of samples.

	Day 0	**Day 3**	**Day 6**	**Day 9**	**Day 12**	**Day 21**	**Day 36**	**Day 56**	**Day 91**
pH 3 RTUV	0.594	0.396	0.079	1.058	0.631	1.530	0.146	0.416	0.160
pH 7 RTUV	4.174	1.640	0.646	1.220	0.642	0.616	*	2.011	0.331
pH 11 RTUV	1.531	0.612	1.185	0.441	1.507	1.502	N/A	*	1.421
pH 3 RT	2.803	1.443	0.098	1.929	0.972	1.409	1.297	0.890	0.145
pH 7 RT	3.172	1.300	0.826	2.357	0.987	1.108	0.031	1.186	1.127
pH 11 RT	1.509	1.482	1.305	2.356	0.747	0.023	0.487	1.635	0.782

Table 19: Standard error between replicates, where n=3. A * indicates only one replicate was analyzed, and therefore no standard deviation with which to calculate the standard error was available. N/A represents data that is unavailable due to loss of samples.

	Day 0	Day 3	Day 6	Day 9	Day 12	Day 21	Day 36	Day 56	Day 91
pH 3 RTUV	0.051	0.187	1.011	0.777	0.316	0.035	0.011	0.403	0.108
pH 7 RTUV	0.730	0.624	0.008	0.175	0.579	0.543	*	1.414	0.064
pH 11 RTUV	0.593	0.494	0.912	0.235	0.684	1.496	N/A	*	0.309
pH 3 RT	0.588	0.748	0.247	0.958	0.221	1.503	0.750	3.162	0.090
pH 7 RT	1.157	0.844	0.426	1.088	0.602	1.192	0.123	1.228	0.344
pH 11 RT	0.219	0.486	0.843	1.221	0.419	0.054	0.236	0.342	0.263

Table 20: ANCOVA results for the concentration change of BPA stored in ultrapure water at room temperature in clear glass jars, at three pHs (3, 7, and 11) over a period of 91 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
UV pH	2	0.00137	0.01375	0.00687	0.51	0.603
UV Day	1	0.74324	0.73970	0.73970	54.84	0.000
UV pH*UV Day	2	0.01928	0.01928	0.00964	0.71	0.493
Error	62	0.83631	0.83631	0.01349		
Total	67	1.60019				

Table 21: ANCOVA results for the concentration change of BPA stored in ultrapure water at room temperature in amber glass jars, at three pHs (3, 7, and 11) over a period of 91 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
RT pH	2	0.05832	0.02036	0.01018	1.02	0.366
RT Day	1	0.00239	0.00201	0.00201	0.20	0.655
RT pH*RT Day	2	0.06110	0.06110	0.03055	3.06	0.053
Error	74	0.73899	0.73899	0.00999		
Total	79	0.86080				

Table 22: ANCOVA results for the concentration change of BPA stored in ultrapure water at room temperature in clear glass jars, at three pHs (3, 7, and 11) over a period of 36 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
RTUV pH	2	0.00718	0.04315	0.02158	1.28	0.288
RTUV Day	1	0.28923	0.23523	0.23523	13.91	0.000
UV pH*UV Day	2	0.06283	0.06283	0.03142	1.86	0.167
Error	49	0.82883	0.82883	0.01691		
Total	54	1.188				

Table 23: ANCOVA results for the concentration change of BPA stored in ultrapure water at room temperature in amber glass jars, at three pHs (3, 7, and 11) over a period of 36 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
RT pH	2	0.007297	0.007805	0.003902	0.53	0.593
RT Day	1	0.000762	0.000762	0.000762	0.10	0.749
RT pH*RT Day	2	0.009898	0.009898	0.004949	0.67	0.516
Error	56	0.413666	0.413666	0.007387		
Total	61	0.431623				

Table 24: ANCOVA results for the concentration change of NP stored in ultrapure water in amber glass jars at room temperature and 4 ºC ("Storage" condition), at three pHs (3, 7, and 11) over a period of 48 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	0.00161	0.00026	0.00013	0.01	0.990
Day	1	0.04670	0.04522	0.04522	3.48	0.064
Storage	1	0.00065	0.00199	0.00199	0.15	0.696
pH*Day	2	0.00394	0.00438	0.00219	0.17	0.845
pH*Storage	2	0.00895	0.01080	0.00540	0.42	0.661
Storage*Day	1	0.01065	0.01062	0.01062	0.82	0.368
pH*Storage*Day	2	0.03759	0.03759	0.01879	1.44	0.239
Error	165	2.14676	2.14676	0.01301		
Total	176	2.25688				

Table 25: ANCOVA results for the concentration change of BPA stored in ultrapure water in amber glass jars at room temperature and 4 ºC ("Storage" condition), at three pHs (3, 7, and 11) over a period of 48 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	1	0.00030	0.00043	0.00043	0.68	0.412
pH	2	0.00632	0.00128	0.00064	1.01	0.368
Storage	1	0.00048	0.00200	0.00200	3.14	0.078
pH*Storage	2	0.00203	0.00170	0.00085	1.33	0.267
pH*Day	2	0.00160	0.00155	0.00078	1.21	0.300
Storage*Day	1	0.0019	0.00196	0.00196	3.08	0.081
pH*Storage*Day	2	0.00049	0.00049	0.00024	0.38	0.682
Error	165	0.10517	0.10517	0.00064		
Total	176	0.11835				

Table 26: ANCOVA results for the concentration change of NP stored in ultrapure water in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period of 32 days. The experiment was repeated for a total of two experimental replicates ("Replicate" condition).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	0.02509	0.01221	0.00610	2.12	0.125
Day	1	0.20840	0.21875	0.21875	75.82	0.000
pH*Day	2	0.00108	0.00180	0.00090	0.31	0.732
Replicate	1	0.56100	0.56100	0.56100	194.46	0.000
Error	111	0.32023	0.32023	0.00288		
Total	117	1.11580				

Table 27: ANCOVA results for the concentration change of NP stored in ultrapure water in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period of 196 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
1 - pH	2	0.021186	0.021866	0.010933	2.49	0.091
1 - Day	1	0.000262	0.000144	0.000144	0.03	0.857
1 - pH*1 - Day	2	0.001899	0.001899	0.000949	0.22	0.806
Error	61	0.267369	0.267368	0.004383		
Total	66	0.290715				

Table 28: ANCOVA results for the concentration change of BPA stored in ultrapure water in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period of 32 days. The experiment was repeated for a total of two experimental replicates ("Replicate" condition).

Source	DF	Seq SS	Adj SS	Adj MS	F	P
pH	2	0.038277	0.025504	0.012752	23.06	0.000
Day	1	0.028286	0.032496	0.032496	58.77	0.000
pH*Day	2	0.000256	0.000176	0.000088	0.16	0.853
Replicate	1	0.187257	0.187257	0.187257	338.68	0.000
Error	115	0.063583	0.063583	0.000553		
Total	121	0.317658				

Table 29: ANCOVA results for the concentration change of BPA stored in ultrapure water in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period of 196 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
1 - pH	2	0.100705	0.065516	0.032758	64.64	0.000
1 - Day	1	0.111392	0.111481	0.111481	219.98	0.000
1 - pH*1 - Day	2	0.002436	0.002436	0.001218	2.40	0.099
Error	63	0.031927	0.031927	0.000507		
Total	68	0.246460				

Table 30: ANCOVA results for the concentration change of NP stored in filtered wastewater, either sterilized or nonsterile ("Medium" condition) in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period of 110 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Medium	1	4.35209	3.12250	3.12250	191.31	0.000
Time	1	0.80356	0.80465	0.80465	49.30	0.000
pH	2	0.04363	0.06210	0.03105	1.90	0.153
Medium*pH	2	0.04082	0.03997	0.01999	1.22	0.297
Medium*Time	1	0.00019	0.00016	0.00016	0.01	0.922
pH*Time	2	0.12991	0.12991	0.06495	3.98	0.021
pH*Medium*Time	2	0.38162	0.38162	0.19081	13.90	0.00
Error	133	2.17079	2.17079	0.01632		
Total	142	7.54099				

Table 31: ANCOVA results for the concentration change of NP stored in autoclaved (sterilized) filtered wastewater in amber glass jars at room temperature at three pHs over a period of 110 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO - Time	1	35.745	35.627	35.627	293.63	0.000
AUTO - pH	2	8.188	1.595	0.798	6.57	0.003
AUTO - pH*AUTO - Time	2	47.690	47.690	23.845	196.53	0.000
Error	65	7.886	7.886	0.121		
Total	70	99.509				

Table 32: ANCOVA results for the concentration change of NP stored in autoclaved (sterilized) filtered wastewater in amber glass jars at room temperature at three pHs over a period of 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO - Time	1	0.01133	0.01123	0.01123	0.89	0.348
AUTO - pH	2	0.01198	0.00053	0.00026	0.02	0.979
AUTO - pH*AUTO - Time	2	0.01835	0.01835	0.00918	0.73	0.486
Error	56	0.70284	0.70284	0.01255		
Total	61	0.74450				

Table 33: ANCOVA results for the concentration change of NP stored in nonsterilized filtered wastewater in amber glass jars at room temperature at three pHs over a period of 110 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
NAT - Time	1	35.985	35.916	35.916	383.53	0.000
NAT - pH	2	9.919	2.107	1.053	11.25	0.000
NAT - pH*NAT - Time	2	59.871	59.871	29.935	319.67	0.000
Error	65	6.087	6.087	0.094		
Total	70	111.862				

Table 34: ANCOVA results for the concentration change of NP stored in nonsterilized filtered wastewater in amber glass jars at room temperature at three pHs over a period of 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
NAT 2 - Time	1	0.18217	0.18784	0.18784	20.06	0.000
NAT 2 - pH	2	0.09662	0.08647	0.04323	4.62	0.014
NAT - pH*NAT - Time	2	0.26840	0.26840	0.13420	14.33	0.000
Error	56	0.52448	0.52448	0.00937		
Total	61	1.07166				

Table 35: ANCOVA results for the concentration change of NPEO1 stored in filtered wastewater, either sterilized or nonsterile ("Medium" condition) in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period of 110 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Medium	1	0.072	4.221	4.221	13.29	0.000
Time	1	40.953	41.056	41.056	129.29	0.000
pH	2	5.390	0.527	0.264	0.83	0.438
Medium*Time	1	19.452	19.157	19.157	60.33	0.000
Medium*pH	2	5.563	5.758	2.879	9.07	0.000
pH*Time	2	24.456	24.456	12.228	38.51	0.000
Error	133	42.234	42.234	0.318		
Total	142	138.121				

Table 36: ANCOVA results for the concentration change of BPA stored in filtered wastewater, either sterilized or nonsterile ("Medium" condition), in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period of 110 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Medium	1	0.0072070	0.0084702	0.0084702	40.09	0.000
Time	1	0.0214950	0.0215348	0.0215348	101.93	0.000
pH	2	0.0084414	0.0013661	0.0006831	3.23	0.043
Medium*Time	1	0.0013849	0.0013550	0.0013550	6.41	0.012
Medium*pH	2	0.0003094	0.0003210	0.0001605	0.76	0.470
pH*Time	2	0.0335719	0.0335719	0.0167860	79.45	0.000
Error	133	0.0280985	0.0280985	0.0002113		
Total	142	0.1005082				

Table 37: ANCOVA results for the concentration change of BPA stored in nonsterilized filtered wastewater in amber glass jars at room temperature at three pHs over a period of 110 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO - Time (days)	1	14.3272	14.2703	14.2703	104.24	0.000
AUTO - pH	2	4.2172	1.1523	0.5761	4.21	0.019
AUTO - pH*AUTO - Time (days)	2	27.6842	27.6842	13.8421	101.11	0.000
Error	65	8.8983	8.8983	0.1369		
Total	70	55.1269				

Table 38: ANCOVA results for the concentration change of BPA stored in nonsterilized filtered wastewater in amber glass jars at room temperature at three pHs over a period of 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO - Time (days)	1	0.092833	0.093394	0.093394	85.41	0.000
AUTO - pH	2	0.009530	0.001927	0.000964	0.88	0.420
AUTO - pH*AUTO - Time (days)	2	0.005575	0.005575	0.002788	2.55	0.087
Error	56	0.061233	0.061233	0.001093		
Total	61	0.169172				

Table 39: ANCOVA results for the concentration change of NP in two experimental replicates stored in wastewater, either sterilized or nonsterile ("Medium" condition), in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Medium	1	1.7714	1.1899	1.1899	23.47	0.000
Time (days)	1	0.3238	1.0794	1.0794	21.29	0.000
pH	2	2.3863	0.7489	0.3745	7.39	0.001
Medium*Time (days)	1	0.0040	0.0069	0.0069	0.14	0.714
Medium*pH	2	0.3807	0.3678	0.1839	3.63	0.028
pH*Time (days)	2	0.2792	0.3030	0.1515	2.99	0.052
Replicate	1	16.4449	16.4449	16.4449	324.37	0.000
Error	234	11.8635	11.8635	0.0507		
Total	244	33.4539				

Table 40: ANCOVA results for the concentration change of NP in two experimental replicates stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO - pH	2	1.2124	0.3083	0.1542	4.10	0.019
AUTO - Time (day)	1	0.1243	0.6437	0.6437	17.12	0.000
AUTO - pH*AUTO - Time (day)	2	0.1322	0.1530	0.0765	2.04	0.035
AUTO - Replicate	1	15.4251	15.4251	15.4251	410.29	0.000
Error	115	4.3235	4.3235	0.0376		
Total	121	21.2174				

Table 41: ANCOVA results for the concentration change of NP in experimental replicate 1 stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO1 - pH	2	2.84081	1.02115	0.51058	25.03	0.000
AUTO1 - Time (day)	1	0.86018	0.83141	0.83141	40..76	0.000
AUTO1 - pH*AUTO1 - Time (day)	2	0.16501	0.16501	0.08250	4.04	0.023
Error	55	1.12200	1.12200	0.02040		
Total	60	4.98800				

Table 42: ANCOVA results for the concentration change of NP in experimental replicate 2 stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 21 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO2 - pH	2	0.87918	0.12019	0.06009	9.59	0.000
AUTO2 - Time	1	0.04989	0.02912	0.02912	4.65	0.036
AUTO2 - pH*AUTO2 - Time	2	0.27680	0.27680	0.13840	22.09	0.000
Error	55	0.34467	0.00627			
Total	60	1.55054				

Table 43: ANCOVA results for the concentration change of NP in two experimental replicates stored in non-sterile wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
NAT Time (days)	1	0.19698	0.44216	0.44216	9.95	0.002
NAT pH	2	1.56119	0.50612	0.25306	5.69	0.004
NAT Replicate	1	3.27218	3.29969	3.29969	74.26	0.000
NAT pH*NAT Time (days)	2	0.28025	0.28025	0.14012	3.15	0.046
Error	116	5.15451	5.15451	0.04444		
Total	122	10.46510				

Table 44: ANCOVA results for the concentration change of NP in experimental replicate 1 stored in non-sterile wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
NAT1 pH	2	2.50985	1.31461	0.65731	14.68	0.000
NAT1 Time (days)	1	1.44812	1.47144	1.47144	32.86	0.000
NAT1 pH*NAT1 Time (days)	2	0.17207	0.17207	0.08603	1.92	0.156
Error	54	2.41794	2.41794	0.04478		
Total	59	6.54799				

Table 45: ANCOVA results for the concentration change of NP in experimental replicate 2 stored in non-sterile wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 21 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
NAT2 - pH	2	0.08438	0.00020	0.00010	0.02	0.983
NAT2 - Time	1	0.36017	0.36017	0.36017	63.49	0.000
NAT2 - pH*NAT2 - Time	2	0.09578	0.09578	0.04789	8.44	0.001
Error	57	0.32333	0.32333	0.00567		
Total	62	0.86366				

Table 46: ANCOVA results for the concentration change of NPEO1 in two experimental replicates stored in wastewater, either sterilized or nonsterile ("Medium" condition), in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Medium	1	2.0542	2.1204	2.1204	30.32	0.000
Time (days)	1	7.5591	7.9147	7.9147	113.17	0.000
pH	2	0.9633	1.9627	0.9814	14.03	0.000
Replicate	1	14.1558	14.1162	14.1162	201.84	0.000
Medium*Time (days)	1	0.2299	0.2320	0.2320	3.32	0.070
Medium*pH	2	1.1128	1.0972	0.5486	7.84	0.001
pH*Time (days)	2	1.3014	1.3014	0.6507	9.30	0.000
Error	235	16.4354	16.4354	0.0699		
Total	245	43.8119				

Table 47: ANCOVA results for the concentration change of NPEO1 in two experimental replicates stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Auto Time (days)	1	2.6071	2.8175	2.8175	48.41	0.000
Auto pH	2	1.2512	0.7816	0.3908	6.72	0.002
Auto Rep	1	15.8514	15.8425	15.8425	272.22	0.000
Auto pH*Auto Time (days)	2	0.0133	0.0133	0.0067	0.11	0.892
Error	116	6.7509	6.7509	0.0582		
Total	122	26.4739				

Table 48: ANCOVA results for the concentration change of NPEO1 in experimental replicate 1 stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO1 - Time (days)	1	3.6935	3.6054	3.6054	96.05	0.000
AUTO1 - pH	2	1.6627	1.1878	0.5939	15.82	0.000
AUTO1 - pH*AUTO1 - Time (days)	2	0.1815	0.1815	0.0907	2.42	0.099
Error	55	2.0644	2.0644	0.0375		
Total	60	7.6021				

Table 49: ANCOVA results for the concentration change of NPEO1 in experimental replicate 2 stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 21 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
AUTO2 - pH	2	1.35100	0.58394	0.29197	10.71	0.000
AUTO2 - Time	1	0.22668	0.22953	0.22953	8.42	0.005
AUTO2 - pH*AUTO2 - Time	2	0.11309	0.11309	0.05654	2.07	0.135
Error	56	1.52657	1.52657	0.02726		
Total	61	3.21734				

Table 50: ANCOVA results for the concentration change of NPEO1 in two experimental replicates stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Nat Time (days)	1	5.1710	5.3430	5.3430	121.79	0.000
Nat pH	2	0.8856	1.5300	0.7650	17.44	0.000
Nat Rep.	1	1.7677	1.8299	1.8299	41.71	0.000
Nat pH*Nat Time (days)	2	2.3708	2.3708	1.1854	27.02	0.000
Error	116	5.0889	5.0889	0.0439		
Total	122	15.2839				

Table 51: ANCOVA results for the concentration change of NPEO1 in experimental replicate 1 stored in non-sterile wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 33 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Nat1 Time (days)	1	4.7764	4.8109	4.8109	117.49	0.000
Nat1 pH	2	0.4760	0.6018	0.3009	7.35	0.002
Nat1 pH*Nat1 Time (days)	2	0.2290	0.2290	0.1145	2.80	0.070
Error	54	2.2112	2.2112	0.0409		
Total	59	7.6926				

Table 52: ANCOVA results for the concentration change of NPEO1 in experimental replicate 2 stored in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 21 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
NAT2 - pH	2	0.47178	1.12627	0.56314	27.87	0.000
NAT2 - Time	1	1.15135	1.15135	1.15135	56.98	0.000
NAT2 - pH*NAT2 - Time	2	3.14530	3.14530	1.57265	77.83	0.000
Error	57	1.15170	1.15170	0.02021		
Total	62	5.92012				

Table 53: ANCOVA results for the concentration change of BPA stored in wastewater, either sterilized or nonsterile ("Medium" condition), in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 21 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Medium	1	0.387613	0.188747	0.188747	429.60	0.000
Time (days)	1	0.100481	0.100381	0.100381	228.47	0.000
pH	2	0.002316	0.001886	0.000943	2.15	0.122
Medium*Time (days)	1	0.003878	0.003858	0.003858	8.78	0.004
Medium*pH	2	0.000442	0.000417	0.000209	0.47	0.623
pH*Time (days)	2	0.001999	0.001999	0.001000	2.28	0.107
Error	114	0.050086	0.050086	0.000439		
Total	123	0.546816				

Table 54: ANCOVA results for the concentration change of BPA in autoclaved wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 21 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Auto - Time (days)	1	0.0331133	0.0319048	0.0319048	69.02	0.000
Auto - pH	2	0.0015858	0.0006741	0.0003371	0.73	0.487
Auto - pH*Auto - Time (days)	2	0.0004312	0.0004312	0.0002156	0.47	0.630
Error	55	0.0254237	0.0254237	0.0004622		
Total	60	0.0605540				

Table 55: ANCOVA results for the concentration change of BPA stored in non-sterile wastewater in amber glass jars at room temperature at three pHs (3, 7, and 11) over a period between 0 and 21 days.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Nat Time (days)	1	0.078995	0.078995	0.078995	105.12	0.000
Nat pH	2	0.002829	0.001815	0.000908	1.21	0.306
Nat pH*Nat Time (days)	2	0.002163	0.002163	0.001081	1.44	0.246
Error	57	0.042833	0.042833	0.000751		
Total	62	0.126820				

Vita

Toni Carrick was born in July of 1987 to David and Tina Carrick, the second youngest of four daughters. After graduating from Coronado High School in El Paso, Texas in 2005, she went on to pursue her bachelor's degree at the University of Texas at El Paso (UTEP). Initially studying to become a nurse with a strong desire to help her community, it wasn't until she became actively involved in scientific research under the mentorship of Dr. Wen-Yee Lee that she realized she could still accomplish her humanitarian goals as a chemist. During her final year as an undergraduate, she participated in the NSF-sponsored Pathways to the Geosciences Undergraduate Research Experience, where she worked on detecting endocrine disruptors in wastewater in the El Paso, Texas/Juarez, Mexico border region. After receiving a Bachelor of Science in Chemistry from UTEP in August of 2011, she participated in two consecutive internships at Lawrence Berkeley National Laboratory. Soon after, she began her pursuit of a Master of Science in Chemistry at UTEP. During this time, she presented her research at departmental symposia, university expos, and national conferences, including the Society of Environmental Toxicology and Chemistry national meetings. Additionally, she served as a graduate teaching assistant in the department of chemistry. In 2012, she was awarded the NSF Graduate STEM Fellowship in K-12 Education, where she worked in the chemistry classroom at Valle Verde Early College High School, mentoring high school students on their science fair projects, as well as developing and leading original inquiry-based classroom and laboratory activities. After receiving her Master's degree, she hopes to pursue a career as a research scientist, specializing in environmental and analytical chemistry.

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