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Degradation and biological assessment of aqueous micropollutant mixtures

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

DEGRADATION AND BIOLOGICAL ASSESSMENT OF AQUEOUS MICRO-POLLUTANT MIXTURES

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By

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Abstract

Presence of micropollutants in water is a global concern because of their ability to potentially cause adverse effects in organisms at concentrations as low as a few ng/L, particularly when present as a component of complex mixture. Most of the endocrine disrupting compounds (EDC) and pharmaceutical and personal care products (PPCP) are not removed well in traditional wastewater treatment processes and enter the environment and spread throughout the water ecosystem. Advance oxidation processes (AOPs) are a powerful technology for the treatment of water and wastewater contaminants. They are characterized by the production of highly reactive and non-selective hydroxyl radicals, and by mineralization of refractory pollutants. However, complete mineralization of organic contaminants is expensive, while partial mineralization may not produce desirable water quality both for ecosystem as well as for potable purposes. All these technologies require an efficient and powerful set of tools and assays in order to quantify the biological compatibility of treated water contaminated with micropollutants. Bioassays, which are powerful tools, can be used to screen the estrogencity and the toxicity of a complex chemical mixture. In this work, a full factorial design was applied to investigate the antagonistic-synergistic interactions of different concentrations and mixtures of the four compounds; 17-β estradiol (E2), sulfamethoxazole (SMX) and bisphenol A (BPA) and humic Acid (HA). The estrogenic activity was determined by using the yeast estrogencity screen (YES) assay, and the genotoxicity of the compounds and their intermediates was monitored by using the Ames test, before and after ozonation, UV/ O_3 and UV/ H_2O_2 which are very effective oxidative treatments for the degradation of various organic micropollutants in water. SMX showed ~ 100% removal in all the AOPs, the slowest removal occurred for only ozonation whereas the combination of UV with ozone and hydrogen peroxide produced much faster degradation rate. While E2 showed much higher degradation in ozonation and combination of UV increased the rate only by 18%. BPA also showed good removal with ozonation, by the addition of H_2O_2, the rate was reduced by 86% from that of UV/ozonation. Humic acid demonstrated the lowest degradation rate of all the compounds tested. The effect of the presence of humic acid on the degradation rate constant of pure compounds and mixtures varied depending on the micropollutants type and the mixture. TOC removal was reduced when HA was added to all solutions.

Humic acid and sulfamethoxazole had a synergistic interaction with 17-β estradiol that led to increase the estrogencity of water by 2.7- 4.7 times. BPA is a weak xenoestrogen that was able to create an impact upon E2 which is a strong estrogen by increasing the estrogencity of E2 by 2.4 times. Some mixtures showed an antagonistic interaction that resulted in dropping EEQ. No mutagenicity was shown by using the Ames test for all mixtures.
The work demonstrated that bioassays such as estrogencity and mutagenicity and total organic carbon (TOC) reduction can be used to determine the optimum AOP treatment without conducting detailed chemical analyses.

Keywords: 17-β estradiol, Sulfamethoxazole, Bisphenol A, Humic Acid, Advanced Oxidation Processes, FFD, Hydroxyl radicals, TOC, YES assay, Ames test.

Co-authorship

Chapter 3: Degradation of 17-β estradiol, sulfamethoxazole, bisphenol A in Water by various Advanced Oxidation Processes: Effect of Humic acid

Sura Ali, Lars Rehmann, Madhumita B. Ray. Sura Ali performed the major part of the experimental work. The manuscript to be submitted to Journal of Hazardous Materials was reviewed by Dr. Madhumita B. Ray and Dr. Lars Rehmann who provided valuable suggestions and recommendations for further improvement.

Chapter 4: A comparative study of the effect of different advance oxidation processes on the estrogencity and genotoxicity of 17-β estradiol, bisphenol A, sulfamethoxazole, and humic acid.

Sura Ali, Madhumita B. Ray, Lars Rehmann. Sura Ali performed the major part of experimental work. The manuscript to be submitted to Water Research was reviewed by Dr. Lars Rehmann and Dr. Madhumita B. Ray who provided valuable suggestions and recommendations for further improvement.

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Chapter One

1.1 Introduction

There has been an increasing concern in recent years about the occurrence, fate, and adverse effects of the micropollutants in aquatic systems including natural water resources and drinking water due to their potential harmful effects on human health, aquatic organisms and subsequent effects on the ecology (Fent et al. 2006; Jjemba 2006). Due to fast development in technology, industrialization and population growth, numerous harmful organic compounds are found in aquatic systems. Emerging contaminants (EC) such as pharmaceuticals and personal care products (PPCP) and endocrine disruptor compounds (EDCs), including antibiotics, fragrances, contraceptives, and many other personal care products at concentrations ranging from ng/L to µg/L are reported in Canada and elsewhere (Ternes et al. 1999; Cajthaml et al. 2009; Silva et al. 2011; Wu et al. 2012). The continuous input of the low concentrations of micropollutants may lead to important long-term consequences in aquatic ecosystems (Daughton et al. 1999). The endocrine disruptor compounds have recieved lately an increased attention in health care and water quality (Colborn et al. 1993), as they are able to mimic natural hormones in the endocrine system or interfere with the action of endogenous hormones by disrupting signal pathways as endocrine disrupters. For example, estrogens can stimulate the growth of human breast cancer cells (Soto et al., 1991).

These compounds are introduced to the environment as complex mixtures via many ways, mainly through the discharge of wastewater effluents due to their poor removal in traditional wastewater treatment processes. Recent literature reports the effet of EDCs on feminisation of the male fish due to the presence of estrogenic compounds in the WWTP effluent (Khanal et al., 2006. There are studies in Canada as well as in all over the world showing the presence of synthetic estrogen, 17α-ethinyl- estradiol (EE2), and the endogenous estrogens such as 17β-estradiol (E2), estrone, and estriol in the secondary effluents (Lee and Peart 1998; Ternes et al. 1999; Metcalfe et al. 2001). Sohoni and Sumpter (1998) indicated that BPA can leach from food can linings into the products and produce estrogenic activity. Since early nineties many reviews dealing with the elucidation and effect of pharmaceuticals and personal care products indicate them as toxic (Heberer, 2002; Petrovic et al., 2003; Larsen et al., 2004; Miège et al. 2009).

Hirsch et al. (1999); Kolpin et al. (2002); Martinez-Carballo et al. (2007). Tamtam et al. (2008) have reportd global occurrence of antibiotics in aqueous matrixes, including wastewater treatment plants (WWTPs), groundwater, surface water, and sediment. Especially, sulfamethoxazole which is a synthetic antibiotics that has been detected in ground-water, in effluents of WWTPs, and in rivers (Hirsch et al. 1999; Miège et al. 2009; Xu et al. 2011). In addition to all of these micropollutants, dissolved organic matter (DOM), a mixture of various organic compounds of humic substances can have a synergistic effect that can increase the estrogenic activity of other estrogenic compounds (Vigneault et al. 2000; Liu et al. 2012; Chen, et al., 2012) or antagonistic effect by decreasing the estrogenic activity (Muir et al. 1994; Qiao and Farrell 2002; Janošek et al. 2007).

Furthermore, runoff from the agricultural fields treated with biosolids contaminated with EDC can pollute the ground and surface water. At present, extensive research is being conducted on improving the degradation of the micropollutants both in wastewater as well as in the discharged effluents. In wastewater, research is being conducted mainly on the improvement of both aerobic and anaerobic biodegradation of the micropollutants whereas tertiary treatment methods such as various membrane processes including ultrafiltration, reverse osomosis, etc., adsorption, and advanced oxidation processes are being used for the removal of micropolluants in the effluents from wastewater (Esplugas et.al., 2007; Abdelmelek et al., 2011).

Advanced oxidation processes involving hydroxyl radicals OH•, the most powerful oxidizing agent, are found to degrade recalcitrant organic compounds have the potential to remove trace concentrations of micropollutants in water. OH• radical reacts with electron-rich sites on organic compounds and initiates complex radical chain reactions in aqueous phase (Klavarioti et al. 2009). In water treatment applications, AOPs can be used either alone or coupled with other biological or physiochemical processes. AOPs in water treatment refers to a specific subset of processes that involve O_3, H_2O_2, and/or UV light (Andreozzi et al. 1999; Eibes et al. 2011; Esplugas et al. 2007; Wu et al. 2012; Silva et al. 2012; Shemer et al. 2006). There are several studies about the application of AOPs to remove the endocrine disrupting chemicals and pharmaceuticals and personal care products in water and wastewater. UV coupled with H_2O_2 removed many micropollutants effectively (Chen et al. 2006; Staehelin & Hoigne 1982; Bolton et al. 2003; Chen et al. 2007; Irmak et al. 2005; Neamţu & Frimmel 2006; Rosenfeldt and

Linden, 2004). Esplugas et al. (2007) found that ozonation was the most studied processes with good removal of the target pollutants. In addition, the combination of UV with O_3 is an effective oxidation method in advanced water treatment for its destruction ability of various organics in water (Andreozzi et al. 1999).

Complete mineralization of organic contaminants is expensive, while partial mineralization may not produce desirable water quality. The residual presence and activity of intermediates are hard to assess due to their low concentrations and difficult chemical analysis. Bioassays such as AMES test and yeast estrogen screen (YES) assay which are powerful tools can be used (Rizzo, 2011) to screen the estrogencity and the toxicity of a complex chemical mixture as these compounds are never present as single compounds in ecosystems. Substantial theoretical challenges exist to assess the effect of exposures to xenobiotics, the synergisms, antagonist or additive responses of the individual mixture components (Silva et al., 2002). Rajapakse et al. (2001) have shown that the weak xenoestrogens are able to create an impact upon strong estrogens. Chen et al. (2007) reported that the estrogenic activity was additive. 17-β estradiol (E2) and 17∝ethinylestradiol (EE2) are the primary compounds driving estrogenic activity and that the concentrations of 4-nonylphenol (NP) and bisphenol A (BPA) used in the study had a negligible effect on estrogenic activity. Although, the importance of bioassays to determine the whole effluent toxicity after advanced oxidation is recognized in the scientific community, there is very limited information on the effect of dissolved organics (humic acids) on the intermediates and oxidation end products of various micropollutants.

1.2 Objectives of the Present Study

Base on the above, the objectives of the present study are:

- Determine the performance of three advance oxidation processes, commonly used in water and wastewater treatment plants including O3, UV/O3 and UV/H2O2 on the degradation of the model organics namely sulfamethoxazole (antibiotic**),** 17-β estradiol (estrogenic), bisphenol A (xenoestrogen) in a kinetic study.
- Evaluate the effect of different mixtures, concentrations and the presence of humic acid on the performance of different AOPs and the resultant water quality.

- Apply the bioassays to investigate the antagonistic-synergistic interactions of different concentrations and mixtures of the model compounds on the mutagenic and the estrogenic effects to determine possible health risks.

1.3 Overview of Dissertation

This thesis is divided into the following chapters:

- Chapter 1 provides the background and the objectives of the research.
- Chapter 2 presents a literature review of the present work and the theory behind it.
- Chapter 3 describes the first stage of the research, in which the effects of three different advance oxidation treatments on different concentrations and mixtures of the model compounds were studied.
- Chapter 4 discusses the results on the estrogenic activity determined by yeast estrogencity screen (YES) assay, and the genotoxicity monitored by using the Ames test, before and after different AOPs.
- Chapter 5 reports the conclusions and followed by recommendations for future work.

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Chapter Two

Literature review

2.1 Organic Micropollutants

A wide variety of synthetic and natural organic micropollutants is present in the aquatic environment. They are found at trace concentrations (μg- ng/L) and can cause adverse effects on human and ecosystem (Stangroom et al. 1998; Schwarzenbach, 2006; Murray, 2010). Usually, micropollutants are synthetic chemicals and an estimated 50,000- 100,000 are commercially available with increasing number every year (Worldwatch Institute, 2011). However, the environmental influence of all of these compounds and the toxicity are not yet well known (Schwarzenbach, et.al. 2003). There have been increasing concern as well as research interest about these compounds which is evident in the increasing number of publications on this subject over the last decade, as shown in Figure 2.1.

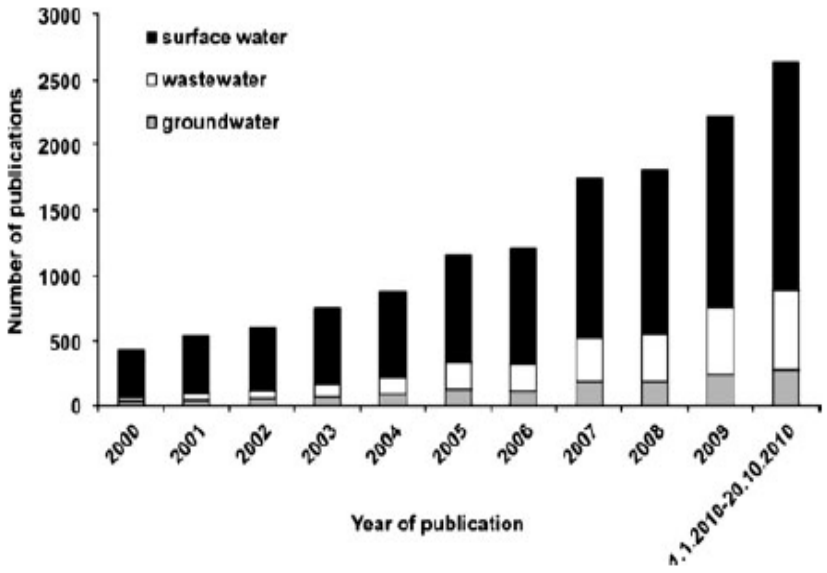


Figure 2.1: Publications on Micropollutants in the last decade (Fatta-Kassinos and Meric, 2011)

The pathways of emission and fate of organic micropollutants such as pharmaceutical residues, biocides, hormones and endocrine disruptive compounds are shown in Figure 2.2. Since many of these compounds are highly hydrophobic, a major fraction is partitioned into the solids in

wastewater, while a small fraction is removed in activated sludge plant. Finally, these compounds enter the environment through disposed effluent, sludge and biosolids.

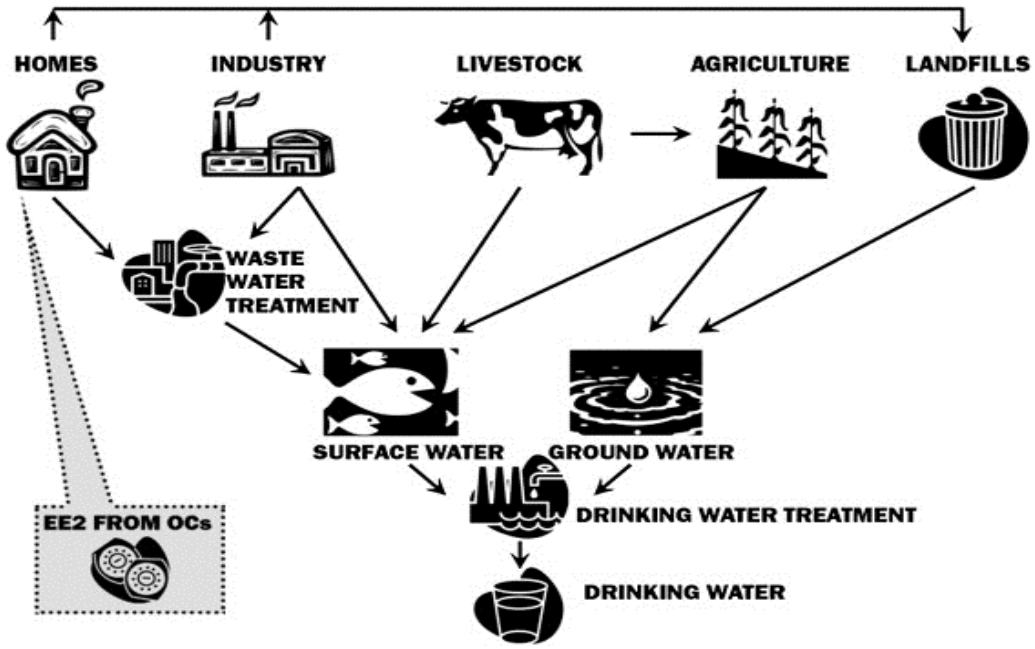


Figure 2.2: Exposure routes of micropollutants in the environment

(http://www.arhp.org/publications-and-resources/contraception-journal/august-2011)

2.1.1 Endocrine disruption compounds (EDCs):

EDCs are natural or synthetic agents which affect the synthesis, transport, secretion, binding, elimination or action of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior' according to US Environmental Protection Agency (USEPA) (Snyde, 2003; Caliman & Gavrilescu 2009**)**. EDCs interact indirectly with the endocrine systems that control the body's function leading to excessive amounts or suppression of hormones (Vogel &Vision, 2004) causing the following problems:

1- Sexual underdevelopment.
2- Infertility.
3- Attention deficit or hyperactivity.
4- Birth defects.
5- Altered or reduced sexual behavior.
6- Increased incidents of certain cancers.

7- Altered thyroid or adrenal cortical function, etc

EDCs are chemicals that have specific function in target receptors (Halling-Sorensen et al., 1998; Jones et al., 2005). However, they can cause adverse impacts to non-target receptors (Jones et al., 2005; Jasim et al., 2006), that interfere with endocrine (or hormone system) in animals and humans.

EDCs may have an agonistic effect, which means the hormone will act as mimic by binding to the receptor sites of the target cells and activating a response, and an antagonistic effect, which means the EDC will act as a hormone blocker and no response is produced as the chemical binds to the receptor and prevents natural hormones from interacting (Birklett, 2003), as shown in Figure 3.

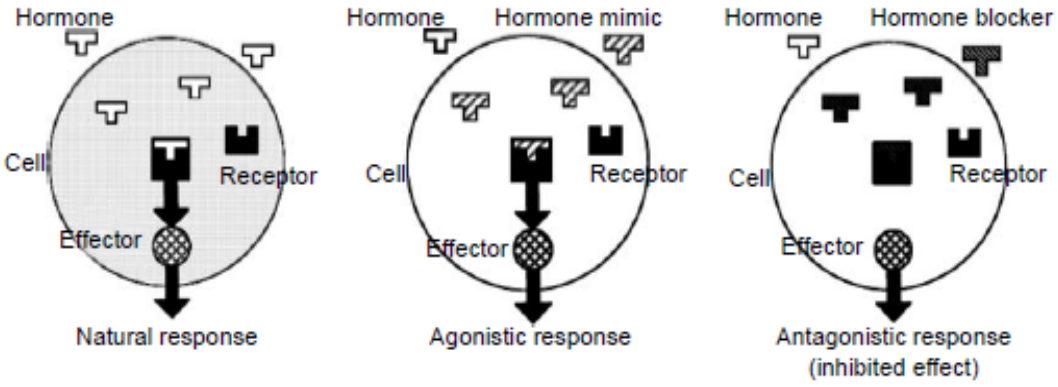


Figure 2.3: Endocrine disruption processes (Birklett, 2003)

2.1.2 Pharmaceutical and personal care products (PPCPs):

PPCPs are a group of compounds which include pharmaceutical drugs, ingredients in cosmetics, food supplements and other personal care products, as well as their respective metabolites and transformation products. PPCPs are continuously introduced into the environment and are prevalent at small concentrations, which can affect water quality and potentially impact drinking water supplies, ecosystem and human health. Some of the PPCPs that have been reported in the aquatic environment are analgesics and anti-inflammatory drugs, antibiotics/bacteriostatic (antibacterial drugs), antiepileptic drugs, oral contraceptives, antiseptics, musk fragrances, sun screen agents, and others. Pharmaceuticals are biologically active compounds and are designed

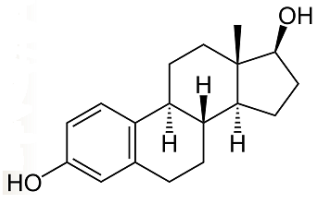
to be resistant to biodegradation in order to improve their desired pharmacological action, for this reason they have an environmental persistence, which makes them difficult contaminants to deal with (Fatta-Kassinos and Meric, 2011).

2.2 Model compounds

2.2.1 17-β estradiol (E2):

17-β estradiol is an important type of estrogenic compound; the physical characteristics are shown in Table 2.1. de Mes et al. (2005) & Jobling et al. (2006) mentioned that the main source of estrogens to the aquatic environment consist of the natural and synthetic steroidal hormones of the human and animal excretion.

Table 2.1: Physicochemical properties of 17-β estradiol (Silva et al. 2012)

Characteristics	17-β estradiol
Molecular formula	$C_{18}H_{24}O_2$
Molecular structure	
Molecular weight (g/mol)	272.38
Water solubility (mg/L)	3.6
pKa	10.4
log Kow	3.9-4.0
Vapour pressure (mm Hg)	2.3×10^{-10}
Sorption constant, Koc	3300
Henry's Law constant (Pa m^3/mol)	3.64×10^{-11}

E1, E2 and E3 are natural estrogens that are derived from cholesterol occur in human; they are important for the health of the reproductive tissue, skin, breast and brain (Silva et al. 2012). Average daily excretion rate of these three natural hormones is given in Table 2.2 (Johnson et al. 2000). EE2 is synthetic estrogen which is present in the contraceptive pill; it is also a major contributor to the total estrogencity of sewage effluent (Cargouet et al., 2004; Kidd et al., 2007).

Table 2.2: Estimation of estrogen excretion by humans (per person) in µg/day (Johnson et al. 2000)

	E1	E2	E3	Total
Males	1.6	3.9	1.5	7
Menstruating females	3.5	8	4.8	16.3
Menopausal females	2.3	4	1	7.3
Pregnant women	259	600	6000	6859

E1: estrogen; E2: 17β-estradiol; EE2: 17α-ethinylestradiol; E3: estriol.

The estrogens get deconjugated by fecal flora to form estrogenically active free form (Dray et al., 1972). Due to their relatively hydrophobic property, hormones are likely to be eliminated by sorption onto the solids (Lia et al., 2000; Yu and Huang, 2005), and this is a major challenge to extract the target compound from the sewage samples. Hernandez-Raquet and Combalbert(2010) proposed a degradation pathway of estrogens by bacteria as shown in Figure 2.4.

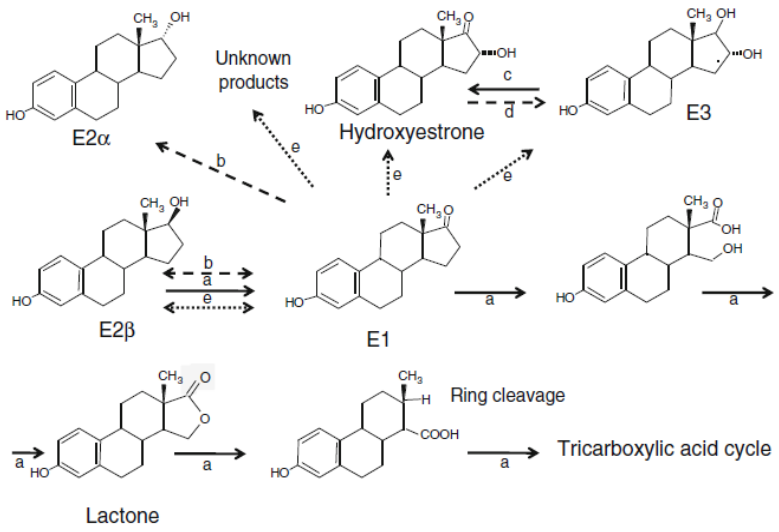
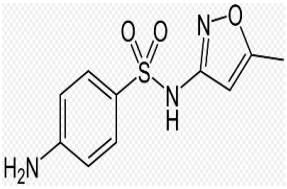


Figure 2.4: The degradation pathway of estrogens by bacteria under aerobic (solid line), anoxic or anaerobic conditions (dashed line), and by algae (dotted line). (a) Lee and Liu 2002, (b) Czajka and Londry 2006, (c) Ke et al. 2007, (d) Jarvenpaa et al. 1980, (e) Lai et al. 2002).

2.2.2 Sulfamethoxazole (SMX):

Sulfonamide is an antibiotic that is widely used in human therapy and livestock production. The physical characteristics are shown in Table 2.3. Recently there has been a concern about the antibiotics residue in the environment and their effects to various organisms as shown in Figure 2.5. Bacteria isolated from sewage bioreactors and the wastewater effluent has been shown to exhibit resistance to some antibiotics (Gulkowskaa, 2008; Shinwoo Yang, 2003). It functions by competitively inhibiting (i.e., by acting as a substrate analogue) enzymatic reactions involving para- aminobenzoic acid (PABA). PABA is needed in enzymatic reactions that produce folic acid, which acts as a coenzyme in the synthesis of purine, pyrimidine and other amino acids. Sulfonamide is also present in other medications that are not antimicrobials, and is also used in the treatment of inflammatory bowel diseases, skin and soft tissue infections or urinary tract infection of pets by bacteria (e.g., sulfadiazine, sulfamethazine etc.).

Table 2.3: Physicochemical properties of Sulfamethoxazole

Characteristics	**Sulfamethoxazole**
Molecular formula	**$C_{10}H_{11}N_3O_3S$**
Molecular structure	
Molecular weight (g/mol)	253.279
Water solubility (mg/L)	610
pKa	5.6-6.0
log Kow	0.5-0.9

In this study we will focus on sulfamethoxazole (SMX), which is one of the sulfanilamide compounds, that has been detected in surface water and wastewater (Larcher and Yargeau, 2012). Brown et al. (2006) found that sulfamethoxazole demonstrated poor removal (20%) in biological treatment process, and it forms several intermediates as shown in Table 2. Miao et al. (2004) and Xu et al. (2007) also indicated that sulfonamides could withstand different treatment processes in the WWTPs, and also it causes antibacterial resistance in biological wastewater treatment and the environment (Kümmerer, 2009; Reinthaler et al., 2003; Volkmann et al., 2004).

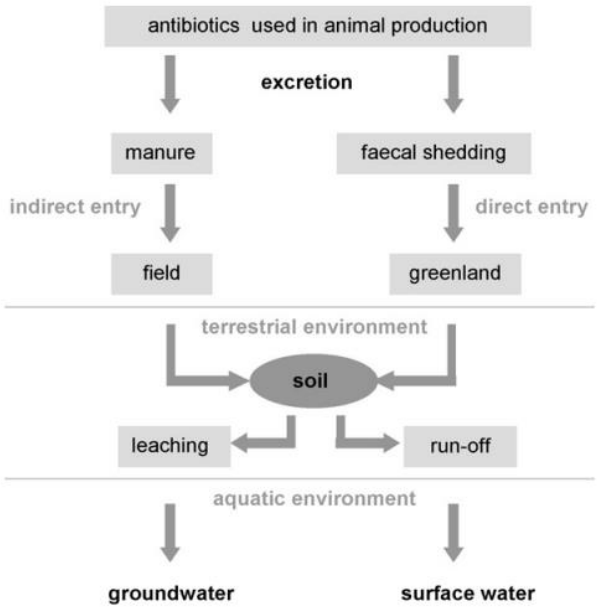


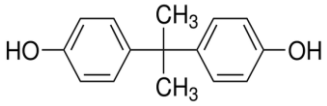
Figure 2.5: Veterinary antibiotics in the environment (Kemper 2008)

2.2.3 Bisphenol A (BPA):

Bisphenol A has been used extensively for the production of polycarbonates and epoxy resins over the past few decades (Metrzler, 2001). There is no clear consensus in the literature regarding the levels at which BPA can cause toxicity and the type of toxicity caused by it. Sohoni and Sumpter (1998) indicated that BPA can leach from food can linings into the products and produce estrogenic activity. Table 2.4 shows BPA properties, that it has solubility in water much greater than its EC50, and potentially toxic to the aquaticecosystem. Sajiki and Yonekubo

(2004) observed that BPA leached from polycarbonate tubes at 37C, suggesting that it can cause a problem when the temperature is elevated. There are other studies about the estrogenic potency and biodegradation of Bisphenol A (Lia et al., 2004). Figure 2.6 shows the biodegradation pathway of BPA (Ike et al., 2002).

Table 2.4: Physicochemical properties of Bisphenol A

Characteristics	Bisphenol A
Molecular formula	$C_{15}H_{16}O_2$
Molecular structure	
Molecular weight (g/mol)	228.29
Water solubility (mg/L) at 289 K	120 ppm (21.5 °C)
pKa	9.6
log Kow	3.32
Melting point	330.4 K

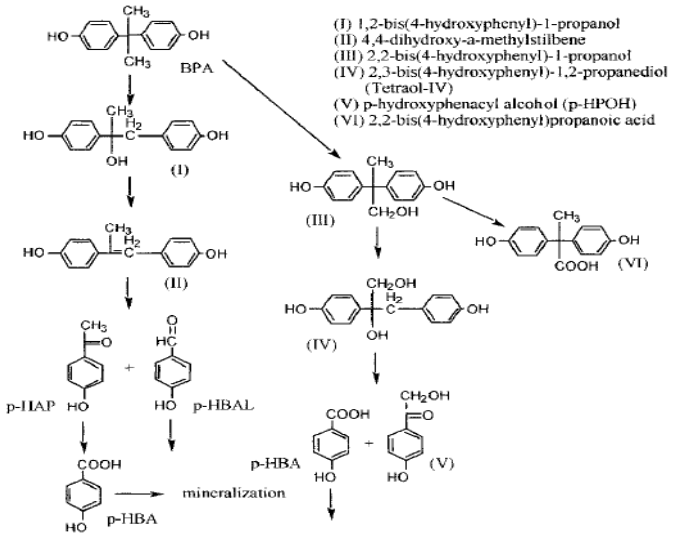
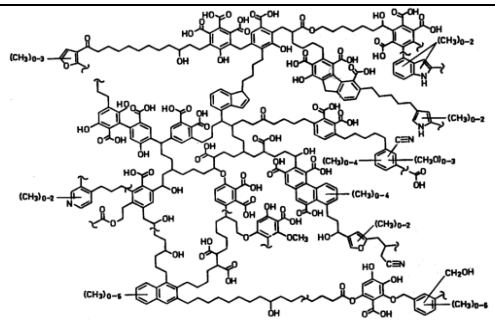


Figure 2.6: The biodegradation pathway of BPA (Ike et al., 2002)

2.2.4 Humic acid (HA):

Humic acid is a complex mixture of variety of different acids with concentrations ranging from several mg/L to several tens of mg/L; it is one type of natural organic matter (NOM) that present in ground water, lakes, streams and other water bodies, HA is of biological origin of aquatic plants and animals (Burges et al. 1964) HA is one type of mixture of various organic compounds in the humic substances, which represent 80% of the total organic carbon of natural waters (Buftle et al. 1978), and it also includes fulvic acids (FA), and humins which is known as the

Table 2.5: Physicochemical properties of humic acids

Characteristics	Humic acid
Molecular formula	Average chemical formula $\mathbf{C_{187}H_{186}O_{89}N_9S_1}$
Molecular structure	
Molecular weight (g/mol)	<1000 to >10,000[a]
Water solubility (mg/L)	more soluble in the aqueous phase [b&c], however with low pH leads to lower the solubility [e]
pKa	4.65[d]
log Kow	<-2.8[e]

a. (Shuang et al. 2014)

b. Lindstrom et al. 1988

c. (Tipping 1981)

d. (Berthat & Choppin 1978)

e. (Juckera & Clarkb 1994)

dissolved organic matter (DOM). Therefore, concentrations of humic acid are traditionally estimated as the concentrations of organic total organic carbon (TOC) or dissolved organic carbon (DOC).

Humic macromolecules form negative charges bound due to the presence of the carboxylic and phenolic groups that cross-linked carbon network of HA when dissociate in aqueous media, which make it interact with various organic pollutants with positive-charged group (Shuang et al. 2012). The phenolic and carboxylic groups, N-heterocyclic compounds, and lignin decomposition products in HA are preferred binding sites and responsible to site-specific sorption (Thiele-Bruhn et al. 2004). Therefore a serious environmental problem is caused by HA in water treatment due to the formation of disinfection by-products (DBPs) which affect the water quality as HA is present in the natural waters which leads to adsorption of other micropollutants to it (Shuang et al. 2014). Arnarson & Keil 2000 suggested six mechanisms to be involved in the adsorption of organic matter to mineral surfaces: ligand exchange, cation bridges, anion exchange, cation exchange, van der Waals interactions and hydrophobic effects.

2.3 The presence of the model compounds in the different water matrixes in the environment

In the aquatic environment, dissolved organic matter (DOM) found at concentrations of 0.5 to 50 mg/L, they are the decomposition products of dead organic matter, and approximately 50–70% of it is humic substances (HS). Molot et al. (1992), found that the DOM concentration in lakes of Ontario is 1.7mg/L to 5.2 mg/L and Bertilsson & Tranvik (2000) recorded 2- 22 mg/L in Ontario lakes. Bisphenol A concentration in activated sludge system effluent in Canada is 330- 680 ng/L (Melcer, H. and Klecˇka, G., 2011). In wastewater treatment plants WWTP influent the concentration of BPA is 2025- 2376 ng/L (Claraa, et al., 2005). Avila, et al.(2009) found that the influent for industrial effluent is 1920 to 11100 ng /L, for domestic is 2260 to 5370 ng /L and for mixed is 1320 to 7360 ng /L. The BPA concentration in groundwater is 70 to 1900 ng /L (Latorre et al., 2003). Sulfamethoxazole in wastewater treatment plants WWTP influent is in the range of 390- 1000 ng/L (Brown, K.D., 2006), and in surface water it is n.d. (not detected) - 470 ng/L (Hirscha, R., et al., 1999), and 400- 2100 ng/L (Brown, K.D., 2006). 17β-Estradiol in surface water is 9 ng/L (Kolpin et al. 2002). Furthermore <LOD <0.3- 0.9 ng/L (Belfroida, et al., 1999)

and LOD 1 ng/L (Stumpf et al., 1996). E2 present in aquatic environment through wastewater discharges at minimum detectable level (MOL) to 3.7 ng/L, and the environmental concentrations is less than detection to greater than 140 ng/L (Snyder et al., 1999). E2 is in river water of Germany is <30 ng/l to a maximum of 70 ng/L (Wiegel, s., et al., 2004).While the concentration in the primary effluent of WWTP is 2400 ng/L (Hartig C., et al., 1999).

According to the studies above the ratio of the concentrations of the model compounds chosen in this study is the environmental values of waste water treatment plant effluent is ~ **0.06: 1: 6.96: 6000** for 17β-Estradiol (E2)**:** Bisphenol A (BPA)**:** Sulfamethoxazole (SMX): Humic acid (HA) in the respectively as shown in Table 2.6.

Table 2.6: The concentration and the ratio of Bisphenol A, 17β-Estradiol, Sulfamethoxazole and Humic acid (HA) in waste water treatment plants WWTP effluent

Waste water treatment plants WWTP effluent			
Bisphenol A (BPA)	**17β-Estradiol (E2)**	**Sulfamethoxazole (SMX)**	**Humic acid (HA)**
26- 76 ng/L [Claraa, M., etal., 2005]	0.9 ng/L [Belfroida, A.C., et al., 1999]	400 ng/L [Hirscha, R., et al., 1999]	1.75- 5 mg/L [Molot, L.A., et al., 1992]
	2- 10 ng/L [Stumpf et al., 1996]	310 ng/L [Brown, K.D., 2006]	1.8- 4.8 mg/L (Hudson et al. 2003)
	Rarely detected [Kima , et al., 2007]	rarely detected [Kima , et al., 2007]	
	showed very rare detection and low concentration [Ternes et al., 1999a, b; Baronti et al., 2000; Huang and Sedlak, 2001; Kolpin et al., 2002]		
Average	**Average**	**Average**	**Average**
51 ng/L	3.45 ng/L	355 ng/L	300,0000ng/L

2.4 Synergy

This is a common phenomenon in aquatic biotests where the interaction of biological active agent produces a stronger effect than the additive calculation (Berendaum 1989). In a study where a mixture of 13 pharmaceuticals resulted in a 10–30% reduction in the growth of human

embryonic kidney cells after 2 days of exposure *in vitro*, while no effects were observed when the chemicals were present individually (Rice and Mitra, 2007; Carballa and Lema, 2006) showing the effect of background water quality on the effect of individual EDCs. In addition the weak xenoestrogens are able to create an impact upon strong estrogens (Rajapakse, et al., 2001),

Table 2.7: A comparison study from different references about the synergistic, additive or antagonistic effect when found in a mixture

Molar ratio E2: BPA	Notes	Presence of other compounds in the mixture	Effect on the estrogencity	Reference
1: 20000		------	The absorbed response were considerably higher than those of the hormone alone	Rajapakse, et al., 2001
1:5000		------	Indistinguishable from E2 alone	Rajapakseet al., 2001
1: 25000 of 11 xenoestrogens including BPA – 1: 100000	These xenoestrogens are at levels below individual absorbed effect (NOEC)	Another 10 xenoestrogens	Dramatic enhancement of mixture response, more than **doubling the effect of E2 alone**	Rajapakse, et al., 2002
Estradiol was used as reference compound	These xenoestrogens are at levels below individual absorbed effect (NOEC)	8 xenoestrogens mixed together	xenoestrogens are able to act together when combined at concentrations below their NOECs to produce significant effects **16 times increase in the estrogencity**	Silva, , et al, 2002
1: 60	EE2 has high estrogenic potency of the steroids. EE2 was approximately 11 to 27 times more potent than E2 in fish (Thorpe, K.L.,et al., 2003)	EE2 and NP, the ratio for E2: EE2: BPA: NP is 1:5: 60:200	The estrogenic activity was additive. E2 and EE2 are the primary compounds driving estrogenic activity and that the concentrations of NP and BPA used in this study have a negligible effect on estrogenic activity.	Chen, P. J.,et al., 2007
E2: EE2 (25:1)			E2 and EE2 are each able to contribute to the overall effect of the mixture, producing a mixture that is more potent than either of the individual chemicals	Thorpe, K.L.,et al., 2003

and the bioavailability of E2 was increased by low concentrations of humic acid (Chen et al. 2012), bioconcentration (Chen et al. 2012), furthermore changing the permeability of biological membranes (Vigneault et al. 2000). Table 2.7 shows the synergistic, additive or antagonistic effect when found in a mixture.

2.5 Removal of micropollutants in wastewater treatment plants (WWTPs) by advance oxidation processes (AOPs)

The quality of the treated effluent in WWTPs is measured by the removal of nitrogen and phosphate, pathogens, suspended solids, metals, and organic load. The micropollutants are poorly removed in conventional WWTP using physical and biological processes. In order to remove them, tertiary or advanced treatment step e.g. ultrafiltration, flocculation, ozonation, advanced oxidation, or reverse osmosis is needed, which is seldom used in standard WWTPs because of their high cost. However, recently many treatment plants are using UV-based disinfection processes for tertiary treatment. UV-oxidation is one of the advanced oxidation processes, which are good engineering solutions to eliminate the residual micropollutants and their metabolites derived from biological systems (Fatta-Kassinos and Meric 2011).

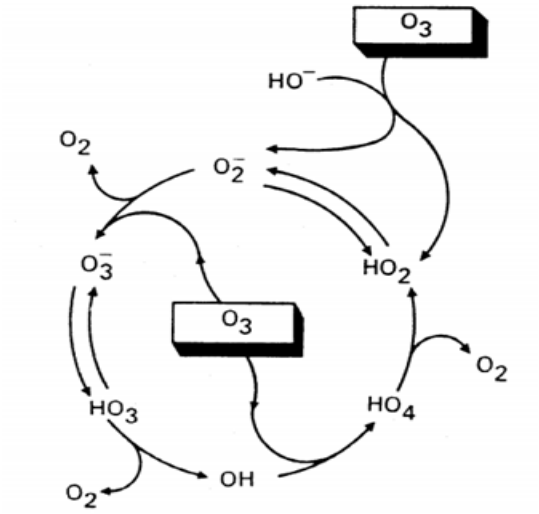


Figure 2.7: Scheme showing the principle species in the decomposition of ozone in pure water initiated by hydroxide ions (Glaze et al. 1987)

Advanced oxidation processes refer specifically to processes in which oxidation of organic contaminants occurs primarily through reactions with hydroxyl radicals. In water treatment applications, AOPs usually refer to a specific subset of processes that involve O_3 as shown in Figure 2.7, H_2O_2, and/or UV light (Kommineni etal., 2008) Figure 2.8. There are several studies about the application of AOPs to remove the endocrine disrupting chemicals and pharmaceuticals and personal care products in water and wastewater. Table 2.8 shows different AOPs for Bisphenol A, 17β-Estradiol, sulfamethoxazole.

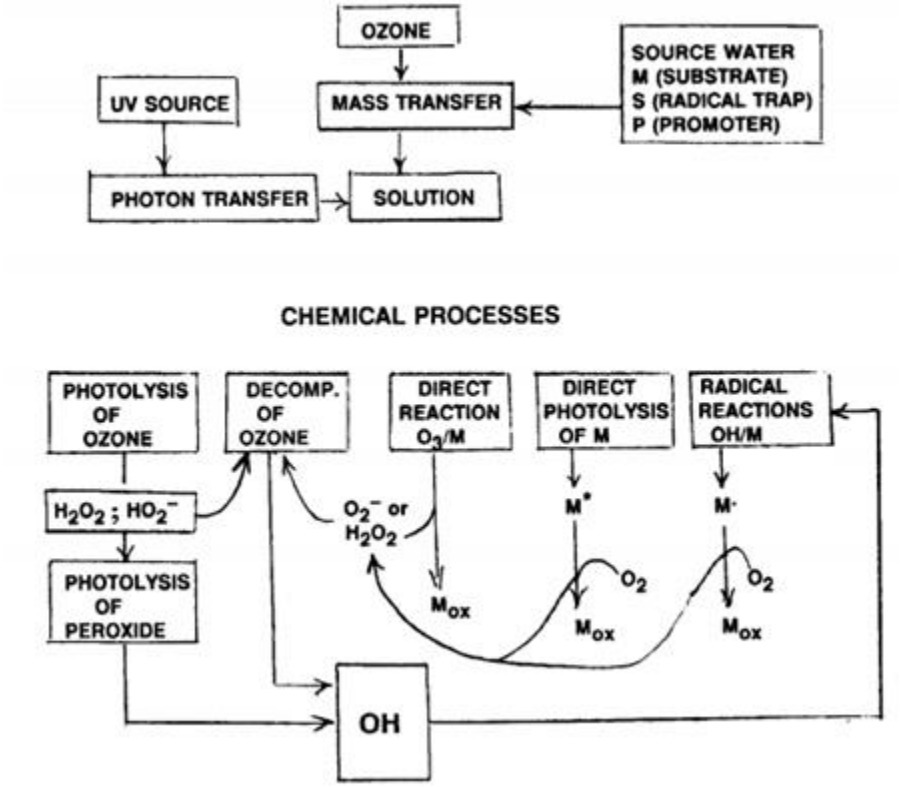


Figure 2.8: Schematic diagram of the element of mass and photon transfer, and chemical processes involved in the UV/O_3 process (Glaze et al. 1987)

Table 2.8: Different AOPs for Bisphenol A, 17β-Estradiol and Sulfamethoxazole

Bisphenol A					
Water Matrix type	**PH**	**AOPs Type**	**Concentration used**	**Results**	**Reference**
Milli-Q deionized water	5.3 - 4.3	UV and UV/ 10 ppm H_2O_2	13.7 ppm	UV alone did not effectively degrade BPA, were as UV/ AOP with adequate H_2O_2 and UV influence were highly effective for removing aqueous estrogenic activity to below detectable levels.	Chen et al. 2006
pure water surface water and wastewater effluents		UV/ and 25.5 ppmH2O2	118.7ppm	Presence of hydrogen peroxide. 17ppm H_2O_2 gave around 60% removal better results of degradation 45% removal after 90 min using 8.5 ppm	Neamtu 2006
aqueous samples	Adjusted to 7.0	10 mg /L of influent oz one gas	11.641ppm	Parent compound and complete min eralization of BPA may need extended ozonation.	**Garoma 2010**
Milli-Q deionized water		O3, UV- H_2O_2 and UV-TiO_2	11.643 ppm	The incomplete removal of TOC. BPA conversion was similar for all the experiments. 2 hours of treatment to reduce the TOC by 41% for O3 and UV/ H2O2	Gilmour, 2012
aqueous medium	5.25±0.03	Ozone and Ozone/UV	5.7- 91.3 mg/L	There was no significant difference in O3 amount consumed for complete conversion of BPA by O3 and O3/UV systems.	Irmak et al., 2005
17-β stradiol (E2)					
Wastewater samples		Ozonation		80% removal	Nakada 2007
aqueous medium	6.25±0.05	Ozone and Ozone/UV	5.4- 108 mg/L	UV decreased the O3 consumption by 22.5% in converting the same amount of E2	Irmak et al. 2005
Ultrapure water	buffered to 8.10	ozone	E2 was used to compared with their model compound	80% removal	Broseus et al. 2009
distilled water	7.5	Oxidation chlorination and ozonation	0.027 ppm	Both chlorination and ozonation removed from 75% to 99% and resulted in a similar estrogencity trend	Alum et al. 2004

Continue Table 2.8

Sulfamethoxazole (SMX)					
MQ and secondary treated wastewater	4.1	O3 (O3/UV)	10 ppm	After 7- 10 min Bellow detection limit. 10- 20 % TOC removal after 1 hr for O3 25- 35 % TOC removal UV- O3	Beltran et al., 2008 and Beltran et al. 2012
	different PH	Ozonation	200 ppm	After 15 min of Ozonation the complete antibiotic abatement was almost achieved; after 15 min of Ozonation just 10% of mineralization.	Dantas et al. 2008
activated sludge		sand filtration and Ozonation		Ozonation removed 80% or more of the, Sulfonamide	Nakada et al. 2007
the input and output of the secondary clarifier of Sewage Treatment Plant (STP)		Ozonation		Ozonation with doses lower than 90 mM allowed the removal of Sulfamethoxazole which exhibited removal efficiencies below 20% in the STP treatment.	Rosal et al. 2010
River water, received at the pilot plant had been prechlorinated		photo catalytic reactor UV/TiO2	5 mg of each compound as transferred to 3000 mgallon DI water.	Concentrations of all compounds Decreased following treatment. No estrogenically active transformation products were formed during treatment	Benotti et al. 2009
wastewater treatment plant effluent	6.6-7.1	Ozonation followed by biological activated carbon filtration		The non-specific toxicity of the by-products mixture was 30-40% lower than the parent compounds. Increasing the ozone dose further will not necessarily lead to substantive gain in water quality.	Reungoat et al. 2012

2.6 Bioassays

The bioassays which are powerful tools can be used to screen the estrogencity and the toxicity of a complex chemical mixture. It measures the response of organisms exposed to contaminants in comparison with a control. They have been used to establish the toxicity levels of target contaminants, genotoxicity of micropollutants and their degradation products and intermediates in aqueous matrices for aquatic organisms (Rizzo, 2011).

2.6.1. The yeast estrogen screen (YES assay):

Estrogenic activity is determined using the YES assay as described by Routledge and Sumpter (1996). This assay is based on a DNA recombinant strain of the yeast Saccharomyces *cerevisiae*, which contains a gene for the human estrogen receptor hER and expression plasmids carrying the reporter gene *lac-Z* encoding the enzyme β-galactosidase. Estrogenic active ligands induce the expression of the *lac-Z* gene followed by the synthesis of the enzyme β-galactosidase. This enzyme releases chlorophenol red from the chromogenic substrate chlorophenol red-β-d-galactopyranoside (CPRG) as shown in Figure 2.9. The absorbance resulting from the color change from yellow to red is a direct measure for the estrogenic activity of the test compound.

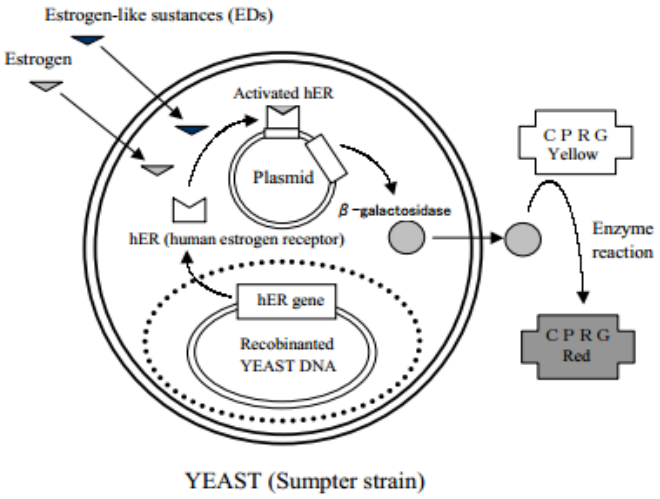


Figure 2.9: Schematic of the estrogen- inducible expression system in yeast (Tamaoto et. al., 2001)

2.6.2 The Ames test:

The Ames test is used to detect the genotoxicity of the compounds such as typical genotoxins like aromatic amines that can cause mutation *(*Guidance for Industry, 2012*)*, which can be defined as deleterious action on a cell's genetic material. Genotoxicity means damage to the genetic material of the cell compounds including genetic damage to DNA, fixation of damage to DNA, and mutation by various mechanisms. Several studies have been conducted to determine the effect of the micropollutant on the genotoxcity in water and wastewater (Crebelli et. al., 1995).

The mutagenic activity was determined by using the Ames test (Ames *et al.*, 1975) using *Salmonella typhimurium* strains, carrying mutation(s) in the operon coding for histidine biosynthesis. The assay is based on a bacterial reverse mutations occurring in histidine-deficiency mutants as shown in Figure 2.10, of five strains of *Salmonella typhimurium* strains (TA 97, TA98, TA100, TA102, TA1535) and two strains of *E.coli*.

Traditionally, reverse-mutation assays have been performed using agar plates, known as `pour plate', plate-incorporation' or `agar-overlay' assays. An alternate assay performed entirely in liquid culture is the `Fluctuation test', originally devised by Luria and Delbruck (1943) and was modified by Hubbard et al. (1984), and will be adopted in this work. The advantages of this test are the following:

1- It is more sensitive than the plate-incorporation assay, because it allows testing for higher concentration of samples (up to 75%v/v).
2- The concentration of bacteria remains constant during the auxotrophic growth phase.
3- It is a low cost and shorter time.

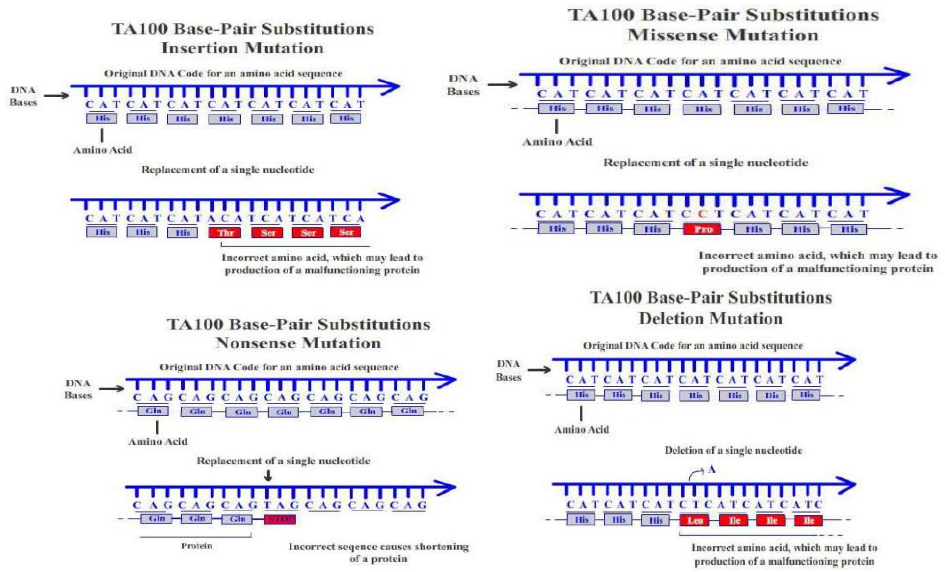


Figure 2.10: *Salmonella typhimurium* TA 100 carrying mutation (http://www.ebpi.ca)

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Chapter Three

Degradation of 17-β estradiol, Sulfamethoxazole, Bisphenol A in Water by various Advanced Oxidation Processes: Effect of Humic acid

3.1 Introduction

The existence of endocrine disrupting compounds (EDCs) as well as pharmaceuticals and personal care products (PPCPs) in drinking water supplies and wastewater treatment effluent raises concern over the removal of these compounds by common drinking water and wastewater treatment processes (Heberer, 2002; Westerhoff et al. 2005; Shemer et al. 2006; Rahman et al. 2009). Endocrine disrupter compounds (EDCs) are exogeneous agents that interfere with the secretion, synthesis, transport, binding, or elimination of natural hormones in the body that are responsible for the reproduction, development, maintenance and behavior (Irmak et al. 2005), by acting as receptor mimics, agonist/ antagonists, shifting the metabolism and the synthesis of natural hormones (Sonnenschein and Soto, 1998). The presence of large number of pharmaceuticals and personal care products and other micropollutants like EDCs in water bodies may have potential to produce additive harmful effects (Kolpin et al. 2002). Sulfamethoxazole (SMX) is an antibiotic that has been ranked within the top five mostly consumed sulfonamides and most common prescribed antibiotics (Nicolle, 2002). Bisphenol A has been used extensively for the production of polycarbonates and epoxy resins over the past few decades (Metrzler, 2001).

Humic acid (HA) is one type of natural organic matter (NOM) present in ground water, lakes, streams and other water bodies, which is of biological origin of aquatic plants and animals (Burgeset al. 1964). Although humic and fulvic acids are the most hydrophobic portion of DOM, they are relatively hydrophilic, as their octanol-water partition coefficients log K_{ow} is $\leq$2.8 (Juckera and Clarkb 1994) as shown in Table 1. In addition, the polar and ionic character affects the solubility and hydrophilicity of HA. HA carbonyl oxygen is polar and the hydroxyl is both polar and ionic (Howe and Clark 2002). HA has 3.5- 4.5 meq/g of carboxyl content (Thurman

1985), and 2.4- 2.9 meq/g of phenolic content (Juckera and Clarkb 1994) ; Burges et al. (1964) detected 30 phenolic groups in humic acid.

Humic macromolecules form negative charges bound cross-linked carbon network of HA when dissociated in aqueous media, which make it interact with various organic pollutants (Hayes et al. 1989). The phenolic and carboxylic groups, N-heterocyclic compounds, and lignin decomposition products in HA are preferred binding sites and responsible to site-specific sorption (Thiele-Bruhn et al. 2004). With the abundance of carboxyl acids in HA, the sorption of compounds like SMX to HA increases (Gao and Pedersen 2010). Kahle & Stamm (2007) showed that the sorption of SMX to HA increases with lower pH, and contact time. However, Pan et al. (2009); Zeng et al. (2006) found that there is a nonlinear relationship between the pH and the sorption of BPA to HA.Zhang & Zhou (2005) noted that HA influences the surface charge and the ionisation of chemicals; however the K_D for E2 did not change significantly within the pH range studied. Based on their abundance in natural water and wastewater effluents, the modle compounds chosen for this work are sulfamethoxazole, 17-β estradiol and bisphenol A. In addition humic acid also was used in the experiments to simulate the background organics concentration. With the relatively low water solubility and high log K_{ow} of sulfamethoxazole, 17-β estradiol and bisphenol A as shown in Table 3.1 promote association with biota and sedimentation (Birklett, 2003), therefore dictate partisan adsorption to humic acid. The high content of HA enhances the removal of sulfamethoxazole by coagulation (Vienoet al. 2006). On the other hand when HA is in low concentration (0.5–1.5 mg/ L) it enhances the photodegradation of the organics (Liu et al. 2012).

In the past decades, advanced oxidation processes (AOPs) have been used successfully in water and wastewater treatment (Legrini et al. 1993). The advantages of the AOPs are (Vilhunen 2010):

1- Fast reaction rate.
2- Permitting the treatment of multiple contaminants at the same time, due to the non-selective nature.
3- They also have the potential to reduce the toxicity of the contaminants.
4- Completely mineralize the target compounds.

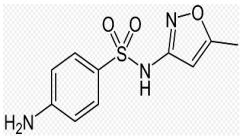
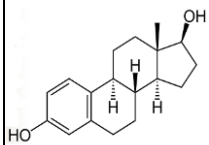
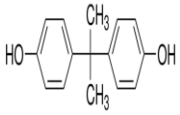
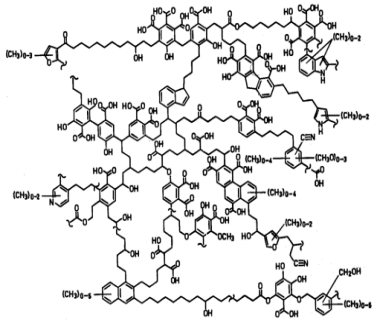
5- The majority of AOPs does not produce solid waste nor concentrate the waste with the subsequent requirement for the further treatment.
6- Removes unpleasant odour, colour of water due to the presence of NOM (Yildiz et al. 2007; Koparal et al. 2008).

The hydroxyl radical (HO•) is a strong oxidant that degrades many refractory organic pollutants by reacting with electron-rich sites on organic compounds and initiates complex radical chain reactions in aqueous phase advanced oxidation processes (AOPs) with high reaction rates (Chang et al. 2007; Goldstein et al. 2007; Minakata & Crittenden 2011).

AOPs categorize into a variety of groups including photochemical and photocatalytic AOPs in which UV irradiation is used, e.g., UV coupled with hydrogen peroxide (UV/H_2O_2) and photo-Fenton's reaction, O_3, UV and ozone (UV/O_3), and UV and titanium dioxide (UV/TiO_2), and microwave (MW) (Andreozzi et al. 1999; Beltra´n et al. 2012; Neamţu and Frimmel 2006; Shemer et al. 2006; Staehelin & Hoigne 1982; Stasinakis 2008; Irmak et al. 2005; Larcher and Yargeau 2013; Huber et al. 2003; Bolton et al. 2003; Ferrari et al. 2009).

AOPs have received extensive interest among scientific community, their benefits are indubitable, and however, in order to apply AOPs in large scale, bench and pilot scale studies are always required for target compounds as the rate of degradation is compound and AOP specific. The objective of this study is to evaluate the effect of the presence of humic acid on the efficiency of three advanced oxidation processes UV/H_2O_2, UV/O_3 and O_3 on the degradation and mineralization of different mixture of the three compounds (shown in Table 3.1) of increasing concern. The AOPs chosen in this study are commonly applied in water and wastewater treatment plants and can be easily retrofitted for the addition removal of the micropollutants.

Table 3.1: Physicochemical properties of Sulfamethoxazole, 17-β estradiol, Bisphenol A and Humic acid

Characteristics	Sulfamethoxazole	17-β estradiol	Bisphenol A	Humic acid
Molecular formula	$C_{10}H_{11}N_3O_3S$	$C_{18}H_{24}O_2$	$C_{15}H_{16}O_2$	Average chemical formula $C_{187}H_{186}O_{89}N_9S_1$
Molecular structure				
Molecular weight (g/mol)	253.279	272.38	228.29	<1000- >10,000[a]
Water solubility (mg/L)	610	3.6	20–300 ppm (21.5 °C)	more soluble in the aqueous phase [b&c], however with low pH leads to lower the solubility [e]
pKa	5.6-6.0	10.4	9.6	4.65[d]
log Kow	0.5-0.9	3.9-4.0	3.32	≤2.8[e]

a. (Shuang et al. 2014)
b. Lindstrom et al. 1988
c. (Tipping 1981)
d. (Berthat and Choppin 1978)
e. (Juckera and Clarkb 1994)

3.2 Experimental

3.2.1 Chemicals:

17-β estradiol (chemical formula: $C_{18}H_{24}O_2$, CAS: 50-28-2) was obtained from Sigma-Aldrich (Oakville, Ontario, Canada) of 98% purity. Sulfamethoxazole (chemical formula: $\mathbf{C}_{10}H_{11}N_3O_3S$, CAS: 723-46-6) was obtained from Fluka Analytical, bisphenol A (chemical formula: $C_{15}H_{16}O_2$, CAS: 80-05-7) was obtained from Sigma–Aldrich (Oakville, Ontario, Canada) of 99+% purity, and humic acid (Average chemical formula $C_{187}H_{186}O_{89}N_9S_1$**,** CAS: 1415-93-6) was obtained from Alfa Aesar. Hydrogen peroxide (H_2O_2, CAS: 7722-84-1) and catalase (CAS: 9001-05-2) were obtained from Sigma–Aldrich (Oakville, Ontario, Canada).
HPLC grade organic solvent acetonitrile (AcN) was purchased from Caledon Laboratories (Georgetown, Ontario, Canada). All reagents were used as received without further purification. Laboratory-grade Ultrapure (MiliQ) water (conductivity of 18M Ω) was obtained from a Millipore purification system (model Integral 5, EMD Millipore Corporation, Billerica, MA, USA).

3.2.2 HPLC Analysis:

17-β estradiol, sulfamethoxazole, bisphenol A and humic acid concentrations were measured by HPLC (ICS 300, Dionex), which included a DP pump, an AS auto sampler, a DC column oven, and PDA UV detector, connected to Chromeleon software. Separations were carried out with an Acclaim 120 C18 reversed-phase column (150 mm × 4.6 mm i.d., 5 μm particle size, Dionex, USA). The injection volume was 100 μL from 10 mL HPLC vials, capped and sealed with PTFE lids. The mobile phase used was a mixture of AN and Mili-Q water (60:40
v/v) at a flow rate of 1 mL/min by the HPLC pump at an isocratic mode. The column
temperature was maintained at 30°C and the detection wavelength was set at 200 nm for SMX, E2 and HA, 220 nm for BPA with a retention time of 2.27 min, 3.53 min, 0.8 to 1.00 min, and 3.27 min, respectively.

3.2.3 Other analyses:

Shimadzu TOC-VCPN analyser with an ANSI-V auto sampler was used to measure the TOC of the initial and treated samples. The pH was determined with a pH meter (model sympHony™ Benchtop Meters, B10P, obtained from VWR. The pH values of different mixtures are shown in Table 3.2. It can be seen that the pH varied in a narrow range of 5.2-6.3.

Table 3.2: The Natural pH of different compounds in solution at their environmental concentration

Solution	**pH**
SMX	5.2
BPA	6
E2	6.4
HA	6.2
BPA- SMX	5.6
BPA- E2	6.1
BPA- HA	6.1
E2- SMX	5.3
E2- HA	6.3
HA- SMX	5.5
BPA- SMX- HA	5.8
BPA- E2- HA	6.2
SMX- E2- HA	5.7
BPA- E2- SMX	5.4
BPA- SMX- E2- HA	6
0.5 conc. BPA+ E2 + SMX+ HA	6.3
0.5 conc. BPA+ E2 + SMX+ HA	6.3
0.5 conc. BPA+ E2 + SMX+ HA	6.3

3.2.4 AOPs experiments:

Pure solutions and mixtures of 17-β estradiol (0.7 mg/L), sulfamethoxazole (80 mg/L), bisphenol A (11.6 mg/L), and humic acid (1000 mg/L) were chosen as mentioned earlier according to the environmental values. They were prepared in Milli – Q water with stirring and heating at 80 °C to be used in the experiments with no further dilution.

All AOP experiments were performed in a bench-scale annular reactor with 750 mL reactor volume. Samples were taken in five different times for all the AOPs at t= 0 min, 10 min, 20 min, 50 min and 90 min.

a. *Ozonation* (O_3):

The experiments were performed in a bench-scale annular reactor. Ozone was produced by an ozone generator (model TG-40, Ozone Solutions, Hull, Iowa, USA) in which oxygen was fed to the generator from a compressed oxygen tank set at a pressure of 15 psi. The produced ozone was at a concentration of 2500 ppm, measured using an ozone analyzer (model UV-100, Eco Sensors, Newark, California, USA). The ozone was fed into 750 mL annular reactor. The corresponding aqueous concentration of ozone was calculated using Henry's constant and varied from 0.33-1.31 mg/L. The solutions had an ambient temperature with an initial pH as shown in Table 3.2. The experimental setup is shown in Figure 3.1.

a. Photolysis/ Hydrogen peroxide (UV/ H_2O_2):

The experiments were performed in a bench-scale annular reactor Figure 3.2. A 13W low-pressure Hg lamp (model Philips TUV PL-S, 1000Bulbs.com, Texas, USA), with monochromatic light at 253.7 nm was used as the light source. The UV intensity at 254 nm radiation on the quartz surface was measured to be 18 mW/cm^2. The reaction volume was 750 mL. A water cooling jacket was used to maintain the reaction temperature at 20°C; the initial pH of the solutions was measured, the reaction mixture was spiked with 33% H_2O_2 resulting in final H_2O_2 concentration of 10 ppm in the reaction media and the reactor contents were thoroughly

mixed using a magnetic stirrer. The samples were immediately quenched with catalase to decompose residual H_2O_2.

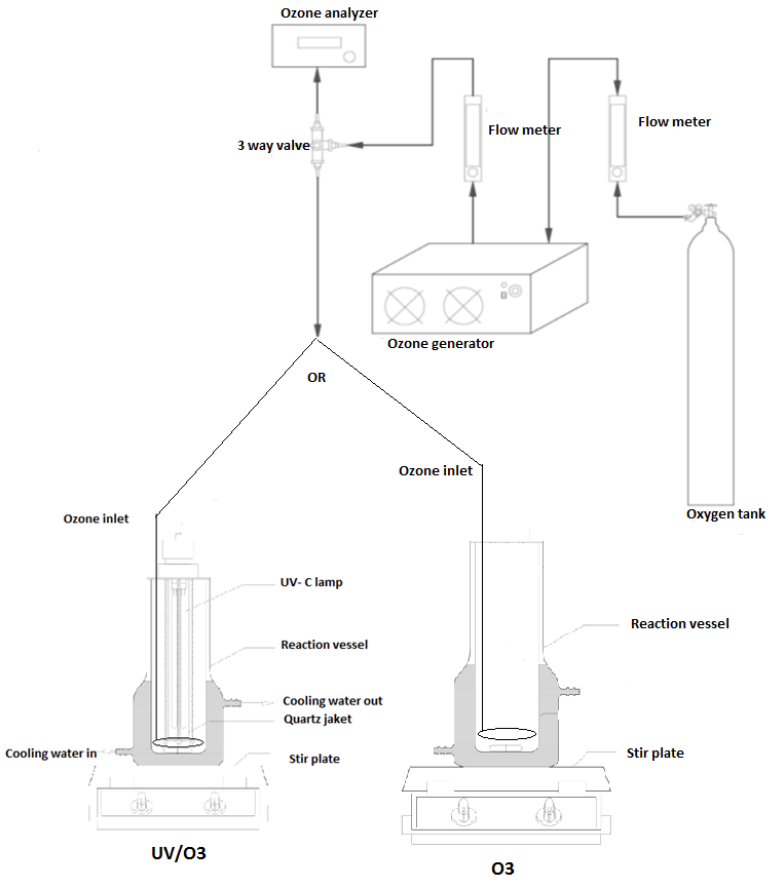


Figure 3.1: Experimental set-up for O_3 and UV/O_3

a. *Photolysis/ Ozonation* (UV/ O_3):

The experiments were performed in a bench-scale annular reactor as shown in Figure 3.1. A 13W low-pressure Hg lamp (model Philips TUV PL-S, 1000Bulbs.com, Texas, USA), with monochromatic light at 253.7 nm was used as the radiation source. The UV intensity on the quartz surface was measured to be 18 mW/cm^2. The reaction volume was 750 mL. A water cooling jacket was used to maintain the reaction temperature at 20°C. Ozone was produced by an ozone generator (model TG-40, Ozone Solutions, Hull, Iowa, USA) in which oxygen was fed to the generator from a compressed air tank set at a pressure of 15 psi. The produced

ozone concentration in gas phase was 2500 ppm, measured using an ozone analyzer (model UV-100, Eco Sensors, Newark, California, USA).

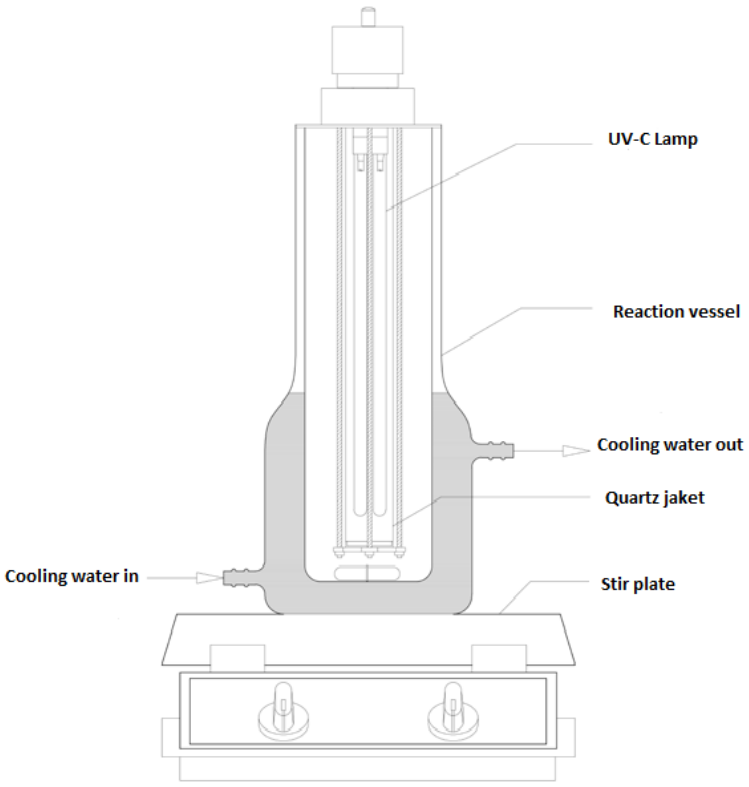


Figure 3.2: Experimental set-up for UV/H_2O_2

3.3 Results and Discussions

3.3.1 Kinetics of 17-β estradiol, Sulfamethoxazole, Bisphenol A and Humic acid Degradation in aqueous medium:

In this chapter, kinetics of degradation of the model compounds at their environmentally relevant concentrations are reported. The experiments were conducted using correlated environmental concentrations of 17-β estradiol (0.7 mg/L), sulfamethoxazole (80 mg/L), bisphenol A (11.6 mg/L) and humic acid (1000 mg/L) as pure compounds and as well in mixture. The interactions of the compounds were determined using full factorial design (FFD) (2^4; two levels and four factors), for three different advance oxidation treatments namely O_3, UV-O_3 and UV/ H_2O_2. Table 3.3 presents the coded values for high and low levels for the 2^4 full factorial design matrixes (Experiments 1–19). For the four-factor case, the response surface is given by the linear model (Myers and Montgomery, 1995**;** Ferreira et al. 2007).

Table 3.3: Full factorial design matrix (2^4)

Experiment sequence	BPA	SMX	E2	HA
1	**-1**	**-1**	**-1**	**-1**
2	**+1**	**-1**	**-1**	**-1**
3	**-1**	**+1**	**-1**	**-1**
4	**+1**	**+1**	**-1**	**-1**
5	**-1**	**-1**	**+1**	**-1**
6	**+1**	**-1**	**+1**	**-1**
7	**-1**	**+1**	**+1**	**-1**
8	**+1**	**+1**	**+1**	**-1**
9	**-1**	**-1**	**-1**	**+1**
10	**+1**	**-1**	**-1**	**+1**
11	**-1**	**+1**	**-1**	**+1**
12	**+1**	**+1**	**-1**	**+1**
13	**-1**	**-1**	**+1**	**+1**
14	**+1**	**-1**	**+1**	**+1**
15	**-1**	**+1**	**+1**	**+1**
16	**+1**	**+1**	**+1**	**+1**
17	**0.5**	**0.5**	**0.5**	**0.5**
18	**0.5**	**0.5**	**0.5**	**0.5**
19	**0.5**	**0.5**	**0.5**	**0.5**

Where **-1 is the low level** = 0, and +**1 is the high level** = (BPA C_0= 11.6 mg/L and/or SMX C_0= 80 mg/L and/or E2 C_0= 0.7 mg/L and/or HA C_0= 1000 mg/L)

a. Ozonation (O_3):

Ozonation is chosen in this study as it is widely used in drinking water and it is an advanced wastewater treatment. In addition, a large number of studies showed that it has the ability to oxidize compounds with structures containing carbon–carbon double bonds, aromatics, hydroxyl, and amino groups. All of the selected model compounds possess some of these structural characteristics (Irmak et al. 2005; Huber et al. 2003; Larcher and Yargeau 2013; Staehelin and Hoigne 1985).

Ozone follows two pathways when reacting with organic compounds, the first one is through hydroxyl radicals and the other one is the direct oxidation by molecular ozone (Irmak et al. 2005). In the radical pathway, it follows a chain of reaction which includes initiation, propagation and termination steps (Staehelin & Hoigne, 1985; Tomiyasu et al. , 1985)

- ***Initiation step***: It will start by OH^- ions yielding $^{\bullet}OH$ radicals.

$O_3 + OH^- \rightarrow O_2^{\bullet -} + HO_2^{\bullet}$ …….. (3.1)

$HO_2^{\bullet}$ is in acid-base equilibrium

$HO_2^{\bullet} = O_2^{\bullet -} + H^+$ ……..…….…….…. (3.2)

- ***Propagation step****:*

$O_3 + O_2^{\bullet -} \rightarrow O_3^{\bullet -} + O_2$ ……..……... (3.3)

$HO_3^{\bullet} = O_3^{\bullet -} + H^+$ ……..…….….. (3.4)

$HO_3^{\bullet} \rightarrow {}^{\bullet}OH + O_2$ ……..……..…… (3.5)

$O_3 + {}^{\bullet}OH \rightarrow HO_4^{\bullet}$ ……..…….....…....…...(3.6)

$HO_4^{\bullet} \rightarrow HO_2^{\bullet} + O_2$ ……..…….…... (3.7)

- ***Termination steps****:*

These steps include any recombination of $^{\bullet}OH$, $HO_2^{\bullet}$ and O_2.

b. Photolysis- hydrogen peroxide (UV/ H_2O_2):

Coupling UV irradiation with H_2O_2 is an effective technique for degradation of single and mixture of compounds, because it produces hydroxyl radical ($^{\bullet}OH$) (Chen et al. 2006; Chen et al. 2007; Rosenfeldt and Linden 2004), therefore UV/ H_2O_2 was applied in our study.

The hydroxyl radical is generated in UV/ H_2O_2 by photolysis of the peroxidic bond, when UV light is absorbed directly by hydrogen peroxide (Eq. (3.8)).

$H_2O_2 + h\nu \rightarrow 2^{\bullet}OH$ ……...(3.8)

Due to stronger absorption by the peroxide at lower wavelengths, the short-ultraviolet wavelength (200–280 nm) yields the highest hydroxyl radical (Shemer et al. 2006). Therefore, 254 nm UV wavelength was chosen in our study.

In UV/ H_2O_2 reaction the ultraviolet radiation cleavages the O-O bond in hydrogen peroxide in order to generate hydroxyl radical as described by (Buxton et al. 1988):

$H_2O_2 + h\nu \rightarrow 2^{\bullet}OH$ ……..…..... (3.9)

$H_2O_2 + HO^{\bullet} \rightarrow HO_2^{\bullet} + H_2O$……..... (3.10)

$H_2O_2 + HO_2^{\bullet} \rightarrow HO^{\bullet} + H_2O + O_2$ ……..…..... (3.11)

$2\ HO^{\bullet} \rightarrow H_2O_2$ ……... (3.12)

$2\ HO_2^{\bullet} \rightarrow H_2O_2 + O_2$ ……..……..... (3.13)

$HO^{\bullet} + HO_2^{\bullet} \rightarrow H_2O + O_2$ ……..…...…. (3.14)

The rate of reaction in Eq. (3.9) is the slowest one among all of the above reactions; therefore it is the rate limiting reaction.

c. *Photolysis- Ozone* (UV- O_3):

The combination of ultraviolet (UV) radiation with O_3 was used in our study because it is an effective oxidation method in advanced water treatment for its destruction ability of toxic organics in water. The extinction coefficient of O_3 at 254 nm is 3600 M^{-1} cm^{-1} which is much higher than that of H_2O_2 in UV/ H_2O_2 treatment (Andreozzi et al. 1999). UV/ O_3 provides much higher absorption cross section than UV-H_2O_2 (photochemical point of view), and inner filter effects (Legrini et al. 1993).

Coupling of UV with O_3 reduces the O_3 consumption requirement and transformation time compared to using only O_3. In addition due to the formation of additional H_2O_2 and $^{\bullet}OH$ radical via photolysis (Staehelin & Hoigne 1982), UV/ O_3 is more effective than O_3 alone for certain target materials (Irmak et al. 2005). $^{\bullet}OH$ radical in UV/ O_3 is produced via different reaction pathways; therefore it is more complex than other oxidation processes (Peyton & Glaze 1988). The general reactions that are involved (Staehelin & Hoigne 1982):

$O_3 + H_2O + h\nu \rightarrow H_2O_2 + O_2$ ……. (3.15)

$H_2O_2 + h\nu \rightarrow 2^{\bullet}OH$ ……..…….. (3.16)

$H_2O_2 \rightarrow HO_2^- + H^+$ ……..... (3.17)

This will react with further ozone by producing $O_3^{\bullet -}$ radicals.

$H_2O_2 + O_3 \rightarrow HO_2^{\bullet} + O_3^{\bullet -}$ ……..….. (3.18)

As it acts as a chain carrier (Staehelin & Hoigne 1985).

3.3.1.1 The kinetics of sulfamethoxazole degradation:

a. The kinetics of sulfamethoxazole degradation as a pure compound:

SMX showed ~ 100% removal in all the AOPs (O_3, UV/ O_3 and UV/ H_2O_2) as shown in Figure 3.3. The slowest removal occurred for only ozonation whereas the combination of UV with ozone and hydrogen peroxide produced much faster degradation rate. The kinetic data shown in Figure 3.4 exhibited exponential decay indicating possible first order kinetics.

$$\ln \frac{C}{C0} = -kt \quad \text{……} \quad (3.19)$$

Where C_0 is the concentration at zero time and t is the reaction time in min, k is the first order degradation constant in (min $^{-1}$).The kinetic data were plotted using pseudo-first order rate expression showed very good fitting with high values of correlation coefficient, R^2 as shown in Figure 3.4. UV/ O_3 showed the fastest degradation rate (0.264 min^{-1}), while the slowest degradation rate was found in ozonation with a rate constant of 0.036 min^{-1}. SMX in its non-ionized form in aqueous solution has UV absorption maximum at 268 nm which extends through the ultraviolet-B (UVB) region. With a molar extinction coefficient ε_{254} = 7345 M^{-1} cm^{-1}, it was found to be extremely susceptible to photodegradation with quantum yield as high as 0.47 at pH 3.0 and 0.084 at pH 9.0 (Moore & Zhou 1994). The lower quantum yield at pH 9.0 is due to the stability of SMX anion. SMX is a weak acid with a pKa value of 5.6, and therefore completely anionized at pH 9.0. The authors also reported a rate constant of 0.15 min^{-1} at pH 3.0 and incident intensity of 25 W/m^2. In this work, although a 7.2 times higher UV intensity (18 mW/cm^2=180 W/m^2), the rate constant was only 1.76 times higher than that of Moore and Zhou (1994). This is possibly due to higher pH of 5.3 used in this work where

SMX is almost 50% ionized. In addition, it is not the surface intensity rather than the illuminated volume is a more relevant factor affecting the photolysis rate constant. Different reactor size involved in this work compared to the work of Moore and Zhou would result in different illuminated volume. Ozone is expected to react with the NH_2 group on the SMX molecule at a much lower rate in the order of 20 M^{-1} s^{-1} with typical half-life of 90 hours. Therefore, the higher rate observed in the case of UV and ozone is predominantly due to the effect of UV. This was further proved in the experiments with UV+H_2O_2 when the rate of oxidation did not increase significantly even with the 10 fold increase in H_2O_2. Similar results were reported by (Giri et al. 2011) when hydrogen peroxide addition to ultraviolet photolysis was not very significant due to low molar absorption coefficient for hydrogen peroxide at ε_{254} nm (20.06 $M^{-1}cm^{-1}$) and acidic pH of reaction solution (< 5.7). Ozone with higher molar absorption coefficient 3300 $M^{-1}cm^{-1}$ than H_2O_2 produces 2 moles of reactive $OH^{\cdot}$ radicals per mole of incident photon, compared to 0.09 moles of $OH^{\cdot}$ for H_2O_2.

There is a significant difference between the degradation of the parent compounds and complete mineralization to carbon dioxide and mineral acids. Refractory compounds which are oxidized quite slowlyare known to form during the degradation of many micropollutants (Kusakabe et al. 1990). It can be seen in Figure 3.5, for all the three AOPs tested UV+O_3 degraded the total organic carbon (TOC) the most by 40% after 90 min of treatment. Dantas et al. (2008) achieved just 10% of mineralization with complete degradation of SMX after 15 min of ozonation. Beltran et al. (2012) achieved 10-20% TOC reduction after 1 hr of O_3 treatment and 25-35% TOC removal after UV-O_3 treatment; TOC reduction was the lowest for UV+H_2O_2.

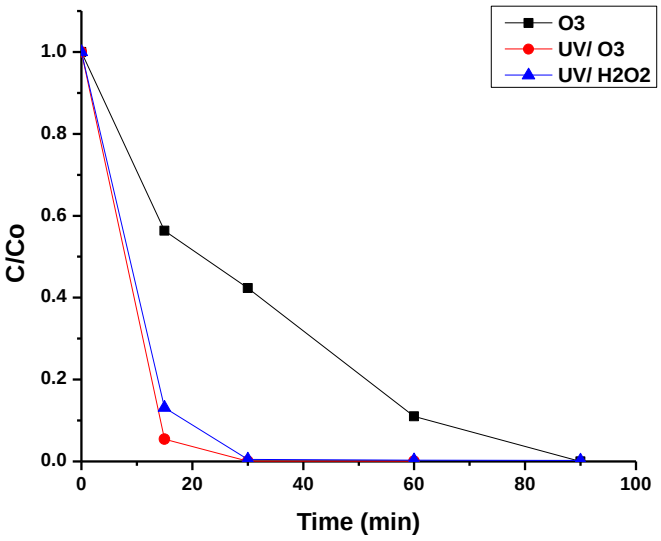


Figure 3.3: Degradation of SMX, C_0 = 80 mg/L, Ozone dosage is 1.31 mg/L, UV- intensity on the quartz surface was 18 mW/cm^2, H_2O_2 dosage is 10 mg/L, pH = 5.2, AOPs are O_3, UV/ O_3 and UV/ H_2O_2

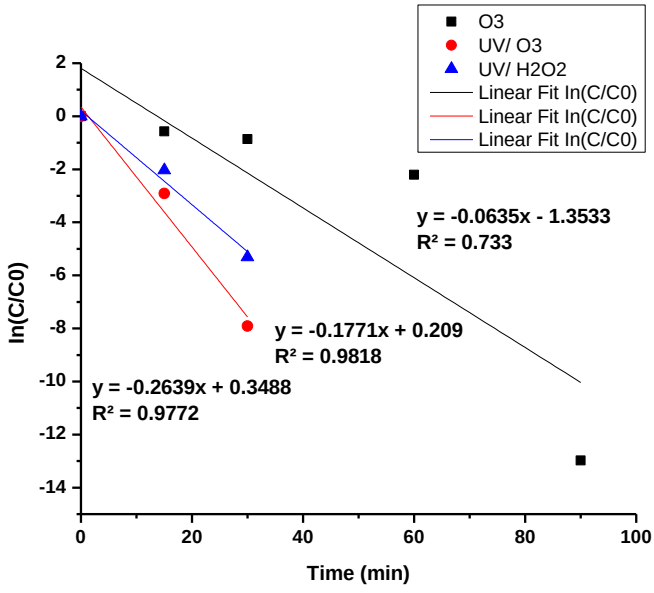


Figure 3.4: Determination of pseudo-first order rate constant, k (min^{-1}) of SMX C_0 = 80 mg/L, for O_3, UV/ O_3 and UV/ H_2O_2

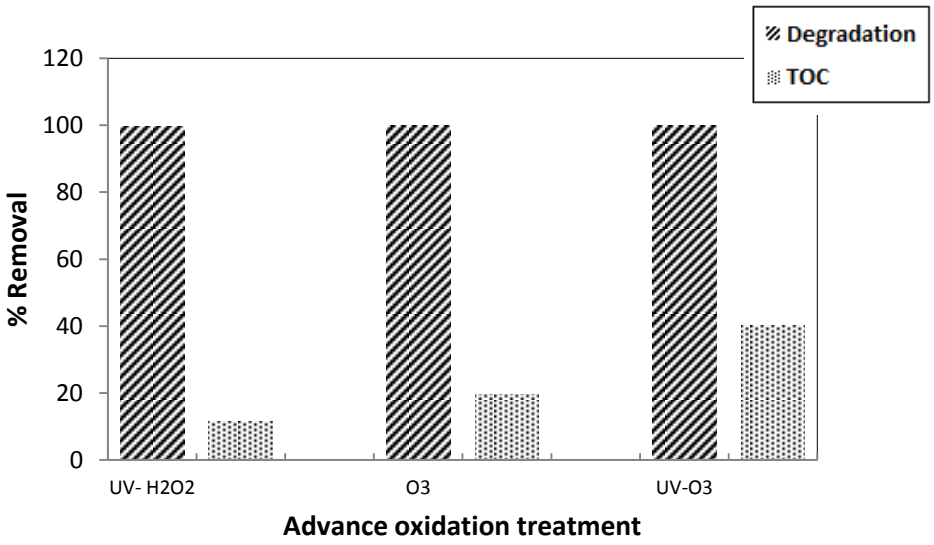


Figure 3.5: Comparison between mineralization and degradation efficiencies for SMX, C_0 (SMX) = 80 mg/L, pH = 5.2 and AOPs treatment time = 90 min

UV/ O_3 caused ~ 100% SMX removal in all the mixtures (SMX- E2, SMX- BPA and SMX-BPA- E2) after 90 min of treatment. O_3 also showed ~ 100% SMX removal in all the mixtures except SMX- BPA gave (~ 90 %) after 90 min. of treatment Although, in all mixtures SMX degraded, degradation rate constants as shown in Table 3.4 were affected negatively in presence of co-pollutants. The effect was more significant for the UV based processes as the rate constant decreased as high as 85% for UV/H_2O_2 compared to a drop of 23-31% in ozonation only. Since ozone concentration was kept constant at 1- 3 mg/L by passing ozone continuously through reactor, the competitive effect of the pollutants was not as pronounced as in the UV based processes. It was hard to determine the predominance of one compound over other due to their different initial concentrations and different molar absorption coefficient values. The increase in rate of SMX degradation in SMX-BPA mixture is probably due to experimental error.

Table 3.4: A comparison between the degradation rate constant, k (min^{-1}) of sulfamethoxazole in the mixture after O_3, UV/ O_3 and UV/ H_2O_2

Chemical	O_3 k (min^{-1})	UV/ O_3 k (min^{-1})	UV/ H_2O_2 k (min^{-1})
SMX	0.036	0.264	0.177
SMX- E2	0.028	0.096	0.095
SMX- BPA	0.043	0.143	0.029
SMX- BPA- E2	0.025	0.144	0.044

3.3.1.2 The kinetics of 17-β estradiol degradation:

Unlike SMX, E2 showed much higher degradation in ozonation and all three AOPs demonstrated comparable performance in degrading E2. Due to faster rate of reaction, only 2-3 samples could be collected for the entire duration of the experiment as shown in Figure 3.6. The pseudo first order rate constant was estimated based on the 90% degradation of E2 using different AOPs , and the rate constants varied in the following order: 0.189 min^{-1} ($UV+O_3$)> 0.160 min^{-1} (O_3)> 0.08 min^{-1} ($UV+H_2O_2$). This result is in agreement with our earlier work with estrone (E1) (Sarkar et al. , 2014). Unlike SMX, E2 showed much better removal in ozonation and combination of UV increased the rate only by 18%. The rate constant was the lowest with UV/H_2O_2. Ozone reacts with the phenolic group present in the structure of E2.

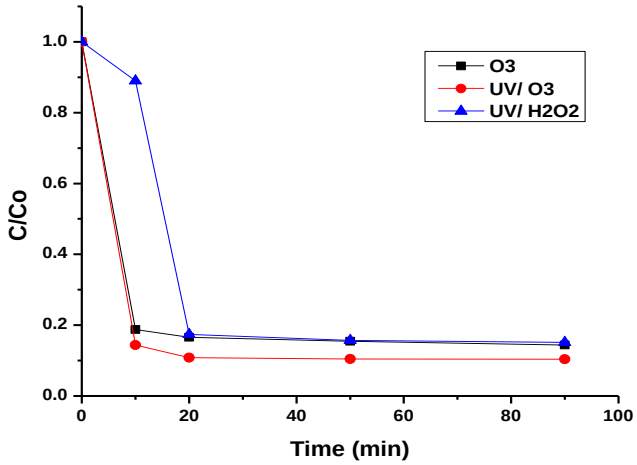


Figure 3.6: Degradation of E2, C_0 = 0.7 mg/L, Ozone dosage is 1.31 mg/L, UV intensity on the quartz surface was 18 mW/cm^2, H_2O_2 dosage is 10 mg/L, pH = 6.4, (a) UV/ H_2O_2, (b) UV/ O_3 and (c) O_3

Similar to SMX, reduction in TOC with pure E2 was low. For 85-90% removal of E2, only 25%, 29% and 38% TOC reduction occurred for UV/ H_2O_2, O_3, and UV/ O_3, as shown in Figure 3.7. Chowdhury et al. (2010) observed a difference between the rates of degradation and minerilazation for E2 after solar irradation, in which it was attributed to the breakage of the aromatic ring of E2 and the high stability of alicylic ring. Compared to SMX, TOC reduction of E2 was higher for all three AOPs. Although, SMX is lighter than E2, it is structurally more complicated than E2.

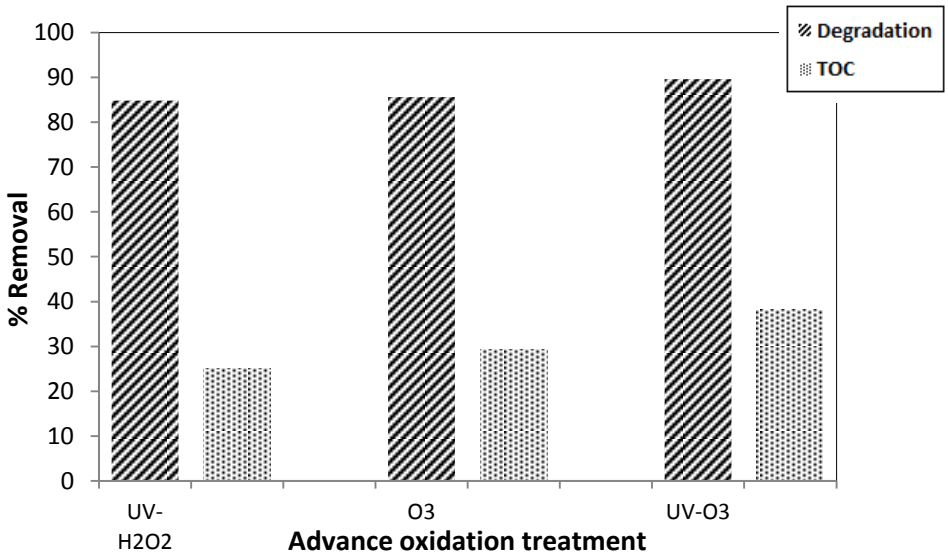


Figure 3.7: Comparison between mineralization and degradation efficiencies for E2, C_0 (E2) = 0.7 mg/L, pH = 6.4 and AOPs treatment time = 90 min

The effect of co-pollutant is always negative as has been the case for SMX. It is interesting to note that introducing SMX at a much higher concentration (114 times more than E2), the rate of degradation by ozonation only decreased by 23% when SMX was mixed with E2. However, the effect was more significant for UV-based processes, with 42% and 58% reduction for UV/O_3, and UV/ H_2O_2, respectively. These results also confirm that SMX degradation in UV based processes is higher than ozonation. The effect of mixture is much more complex, and can't be ascertained without determining reaction mechanism as shown in Table 3.5.

Table 3.5: A comparison between the degradation rate constant, k (min^{-1}) of 17-β estradiol in mixture after O_3, UV/ O_3 and UV/ H_2O_2

Chemical	O_3 k (min^{-1})	UV/ O_3 k (min^{-1})	UV/ H_2O_2 k (min^{-1})
E2	0.16	0.189	0.08
E2- SMX	0.13	0.11	0.034
E2- BPA	0.108	0.134	0.008
E2- SMX- BPA	0.074	0.086	0.01

3.3.1.3 The kinetics of Bisphenol A degradation:

As for E2, BPA also showed good removal capacity with ozonation, and UV/O_3 .By the addition of H_2O_2 the rate was reduced by 86% from that of UV/ozonation as shown in Figure 3.8 and Figure 3.9. BPA has much lower UV-C molar absorption coefficient (750 M^{-1} cm^{-1}), and H_2O_2 with 10 ppm concentration competes with BPA at 11 ppm for UV photon.

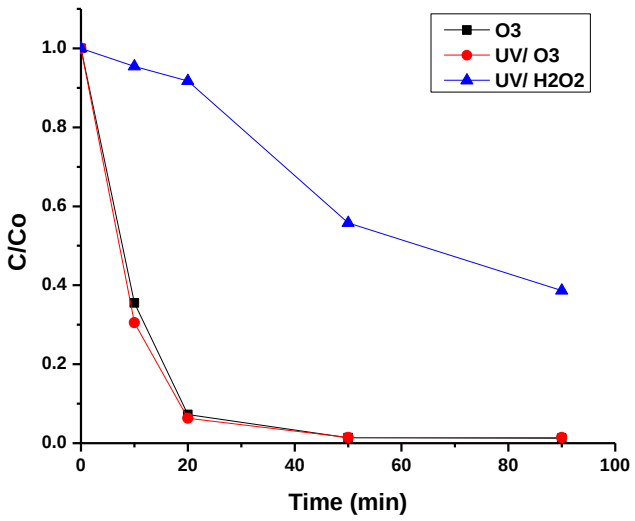


Figure 3.8: Degradation of BPA, C_0 = 11.6 mg/L, Ozone dosage is 1.31 mg/L, UV intensity on the quartz surface was measured to be 18 mW/cm^2, H_2O_2 dosage is 10 mg/L, pH = 6.0, AOPs: O_3, UV/ O_3 and UV/ H_2O_2

Toor & Mohseni (2007) found that UV photolysis (0–2500 mJ cm^{-2}) and H_2O_2 (2– 44 mg l^{-1}) treatments did not significantly reduce the formation of trihalomethanes (THM), disinfection

byproducts (DBPs) in drinking water. They found that UV-H_2O_2 at sufficiently high UV fluences (greater than 1000 mJ cm^{-2}) and initial H_2O_2 concentration of ≥23 mg l^{-1} is effective at reducing DBPs.

Once again, UV/O_3 performed the best for mineralization of BPA. With the lowest molecular weight of all three compounds tested, mineralization of BPA was the highest at >80% after 90 min as shown in Figure 3.10.

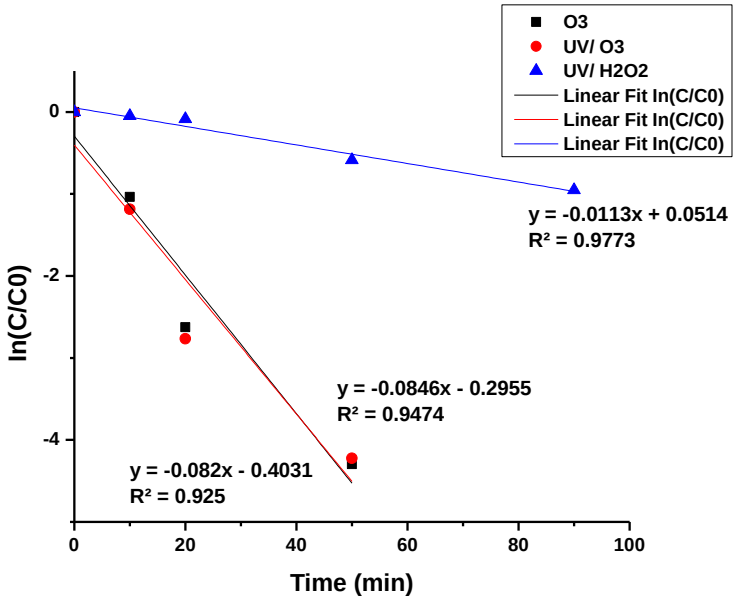


Figure 3.9: Determination of pseudo-first order rate constant, k (min^{-1}) of BPA C_0 = 11.6 mg/L, AOPs: O_3, UV/ O_3 and UV/ H_2O_2

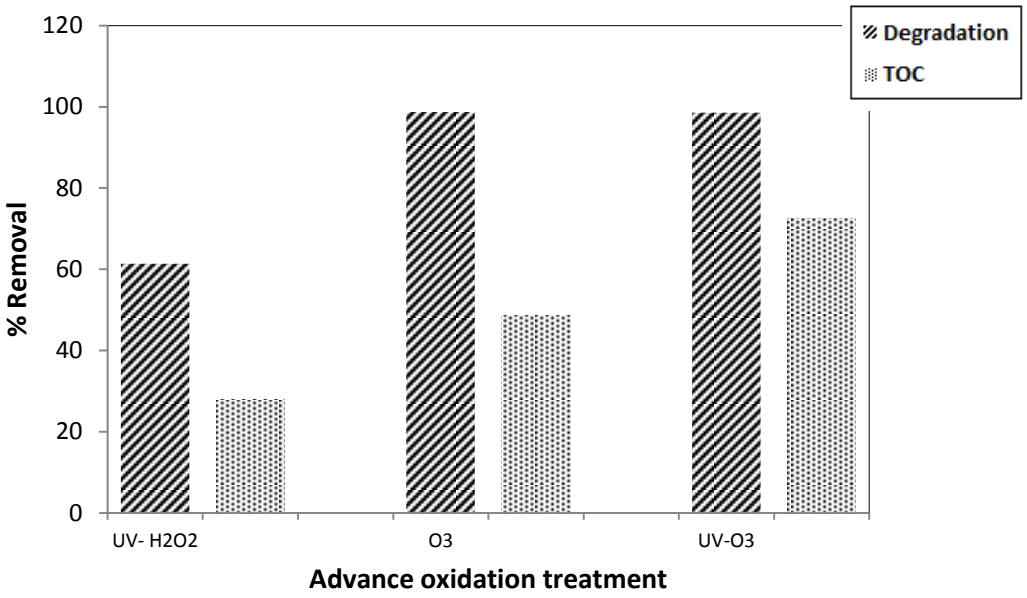


Figure 3.10: Comparison between mineralization and degradation efficiencies for BPA, C_0 (BPA) = 11.6 mg/L, pH = 6.0 and AOPs time = 90 min

Chen et al. (2006) achieved significant removal of BPA by coupling UV with H_2O_2 compared to using only UV. However, in this work, in general it was noted that UV/ H_2O_2 gave the lowest degradation, in addition to the slowest degradation rate of BPA in all the mixtures comparing with UV/ O_3 and O_3 as shown in Table 3.6. Andreozzi et al. (1999) mentioned that H_2O_2 has a small molar extinction coefficient ($18.6M^{-1}$ cm^{-1}) therefore only a relative small fraction of incident light is exploited, and in the presence of the other organic substrates they will act as inner filters for the UV light. Furthermore, H_2O_2 can become a scavenger for hydroxyl radicals when it exceeds 500 mM H_2O_2 (Neamţu & Frimmel 2006) due to formation of less reactive $HO_2^{\bullet}$ radicals. The effect of E2 on BPA degradation was minimal for UV based processes, whereas SMX affected the rate of UV degradation as it absorbs more UV-C radiation than BPA. UV/H_2O_2 gave higher rate of degradation for BPA-E2-SMX mixture, but this could be due to experimental errors.

Table 3.6: A comparison between the degradation rate constant, k (min^{-1}) of bisphenol A in mixture with O_3, UV/ O_3 and UV/ H_2O_2

Chemical	O_3 k (min^{-1})	UV/ O_3 k(min^{-1})	UV/ H_2O_2 k (min^{-1})
BPA	0.082	0.085	0.011
BPA- E2	0.067	0. 078	0.009
BPA- SMX	0.045	0.085	0.003
BPA- E2- SMX	0.072	0.02	-----

3.3.1.4 The kinetics of humic acid degradation:

The kinetics of humic acid degradation under different AOPs such as UV/ H_2O_2, UV/ O_3, and O_3 are shown in Figure 3.11, and the pseudo-first order rate constants are determined in Figure 3.12. At a very high concentration of 1000 mg/L, humid acid demonstrated the lowest degradation rate of all the compounds tested, and UV/ O_3 and UV/ H_2O_2 demonstrated comparable rates as shown in Table 3.7.

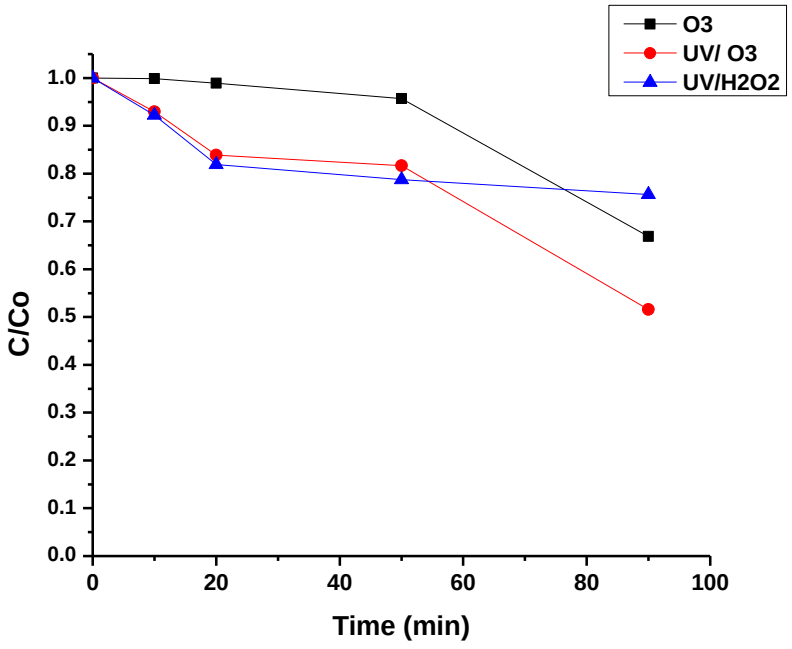


Figure 3.11: Degradation of HA, C_0 = 1000 mg/L, Ozone dosage is 1.31 mg/L, UV- intensity on the quartz surface was 18 mW/cm^2, H_2O_2 dosage is 10 mg/L, pH = 6.2, AOPs: O_3, UV/ O_3 and UV/ H_2O_2

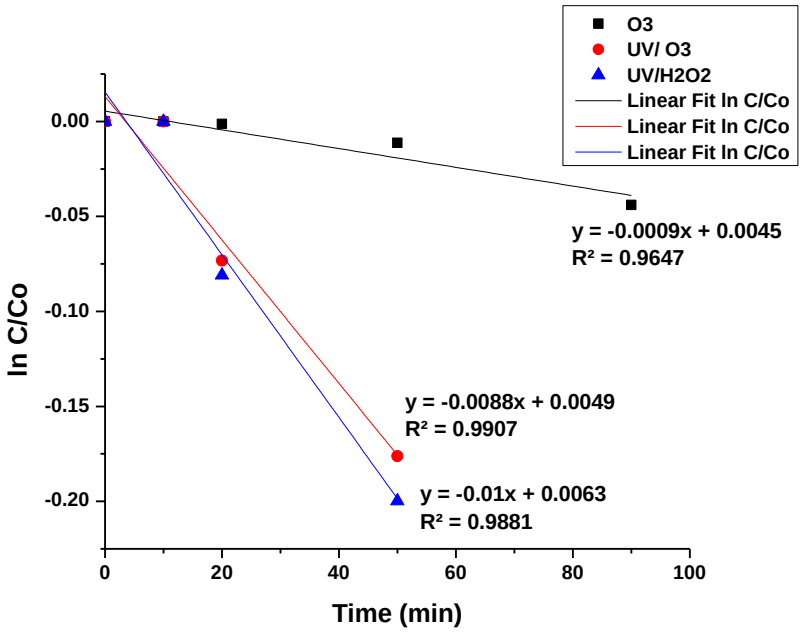


Figure 3.12: Determination of pseudo-first order rate constant, k (min^{-1}) of HA

Table 3.7: A comparison between the degradation rate constant, k (min^{-1}) of humic acid in mixture with O_3, UV/ O_3 and UV/ H_2O_2

Chemical	O_3 k (min^{-1})	UV/ O_3 k (min^{-1})	UV/ H_2O_2 k (min^{-1})
HA	0.0009	0.008	0.01
HA- E2	0.0007	0.006	0.008
HA- SMX	0.003	0.009	0.002
HA- BPA	0.002	0.004	0.002
HA- E2- SMX	0.004	0.017	0.001
HA- BPA- SMX	0.006	0.028	0.008
HA- BPA- E2	0.0007	0.006	0.008
HA- BPA- E2- SMX	0.009	0.005	0.009

UV/O_3 mineralizes HA by 19% and ~ 48% of degradation of HA after 90 min of treatment as shown in Figure 3.13. Chin and Bérubé (2005) observed a significant mineralization of DOC after UV/O_3 treatment. Ikemizu et al. (1987) mentioned that after UV/O_3 treatment a rapid reduction in the HA TOC; however it did not mineralize totally even after 5 hours. UV/ H_2O_2 and O_3 reduced the TOC of HA by 6% and 13% with reduction percentage of ~ 24% and ~ 33%, respectively. HA degradation in presence of the co-pollutants is mostly negatively

affected and there is no clear trend as to whether there is any synergy as some of the mixtures such as HA-BPA-SMX showed much higher than anticipated rates.

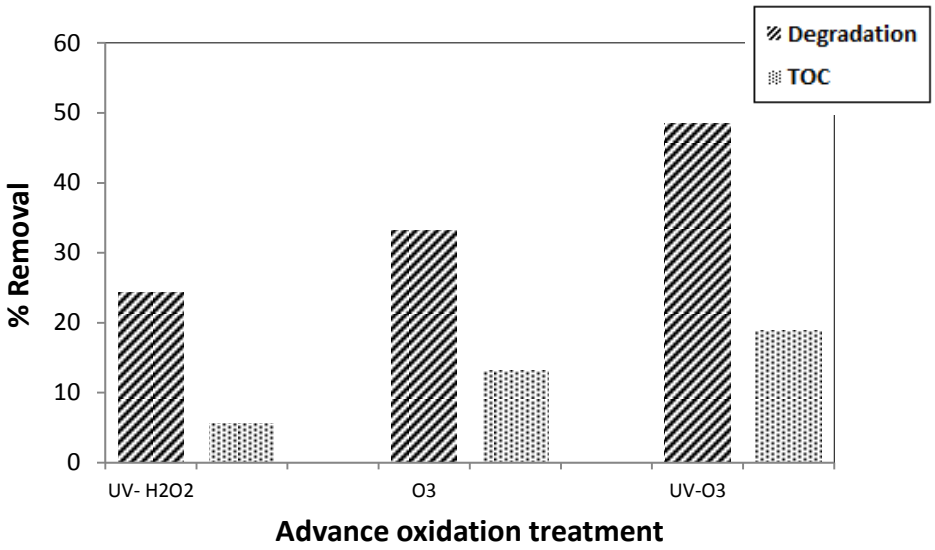


Figure 3.13: Comparison between mineralization and degradation efficiencies for HA, C_0 (HA) = 1000 mg/L, pH = 6.2 and AOPs treatment time= 90 min

Goslsn et al. (2006) and Toor and Mohseni (2007) showed that the combination of UV irradiation and H_2O_2 treatment promotes the $^{\bullet}OH$-radicals formation which will enhance NOM reduction. However, in this study, out of seven mixtures, four of them (HA- SMX, HA- BPA, HA- E2- SMX and HA- BPA- SMX) gave the lowest degradation in UV/ H_2O_2, and only one mixture (HA- E2- SMX) showed higher degradation rate of HA compared to the other two AOPs. Wang et al. (2000) found that when the HA concentration was increased the UV/ H_2O_2 rate constant was decreased. Furthermore Liao & Gurol (1995) found that at higher HA concentration and low H_2O_2 concentration; the scavenging effect of humic acid may influence the initial rate constant itself. At a short irradiation time, the effective OH radicals scavengers are humic acid and hydrogen peroxide, which is represented by these reactions (Brezonik & Fulkerson-brekken 1998):

$H_2O_2 + h\nu \rightarrow 2\ ^{\bullet}OH \quad \emptyset_{OH} FG_0/V$ ……..…….... (3.20)

$^{\bullet}OH$ + Humic acid → Humic acid radical + H_2O ……...…. (3.21)

$^{\bullet}OH + H_2O_2 \rightarrow HO_2^{\bullet} + H_2O$ …… (3.22)

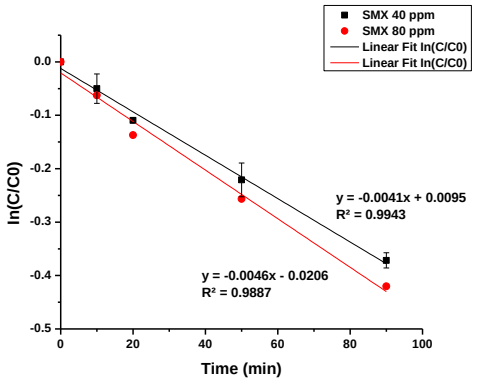
Ozone reacts with natural organic matters by a selective direct reaction by addition to an electrophilic double bond, and non-selective and fast reaction occurs with $^{\bullet}OH$-radicals which come from the decomposition of ozone in water (Matilainena & Sillanpää 2010).

HA in all the mixtures did not show a complete degradation and fluctuated between ~13 and 81%, due to very high concentration of HA used in our study (1000 mg/L) in order to correlate the concentration of the micropollutants used with the environmental values. The solution for better HA degradation is a longer time for AOPs treatment, and higher oxidant dosage, (Tuhkanen, 2004; Sarathy and Mohseni, 2007; Toor and Mohseni, 2007) and high UV intensity (Goslan et al., 2006; Huang et al., 2008), in order to generate enough $^{\bullet}OH$-radicals. However, excess H_2O_2 can cause scavenging of the $^{\bullet}OH$-radicals, making the process less effective (Tuhkanen, 2004; Rosenfeldt and Linden, 2007; Song et al., 2008).

3.3.2 Effect of initial concentration on the degradation of 17-β estradiol, sulfamethoxazole, bisphenol A and humic acid in a mixture:

Experiments were carried out at two different initial concentrations for E2, SMX, and BPA and HA mixtures, in order to study the effect of the initial concentration on the degradation rate constant. Initial tests were conducted at a concentration of 0.7 ppm for E2, 80 ppm for SMX, 11.6 ppm for BPA and 1000 ppm for HA. Next tests were conducted in a mixture with half of the concentrations mentioned above. All experiments produced a linear plot of t against $\ln(C/C_0)$ as shown in Figure 3.14 indication that the degradation of E2, SMX, BPA and HA in aqueous solution with UV/ H_2O_2, UV/ O_3 and O_3 treatments followed pseudo-first order kinetics. All the solutions in the three AOPs (UV/ H_2O_2, UV/ O_3 and O_3) showed faster degradation rate constant when the solution was in higher concentration as shown in Figure 3.14. The degradation rate constant of SMX in BPA- E2- SMX- HA mixture decreased by 46%, 26% and 11% for O_3, UV/ O_3 and UV/ H_2O_2, respectively when the concentration was reduced to half of the original concentration. The degradation rate constant of E2 in BPA- E2- SMX- HA mixture decreased by 60%, 23% and 40% for O_3, UV/ O_3 and UV/ H_2O_2, respectively. The degradation rate constant of BPA in BPA- E2- SMX- HA mixture decreased by 34%, 11% and 60% for O_3, UV/ O_3, and UV/

H_2O_2,respectively. The degradation rate constant of HA in BPA- E2- SMX- HA mixture decreased by 29%, 2% and 9% for O_3, UV/ O_3 and UV/ H_2O_2 respectively. These results are in line with (Sarkar 2013; Rozita Keyavoos 2012) where a significant reduction in the speed of the degradation was noticed at the end of the reaction than at the beginning where the concentration of the compounds in mg/L range, and changed to µg/L at the end of the kinetic experiments .



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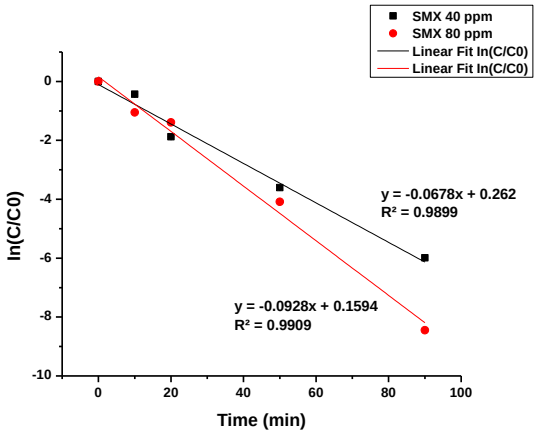
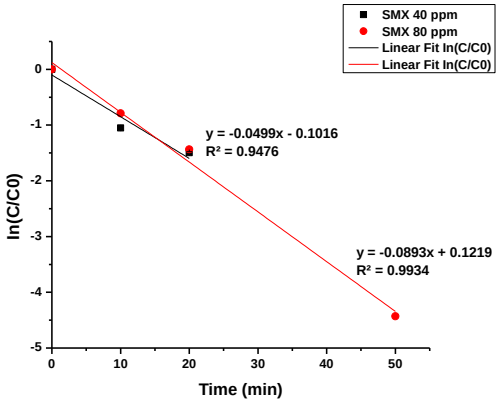


b)



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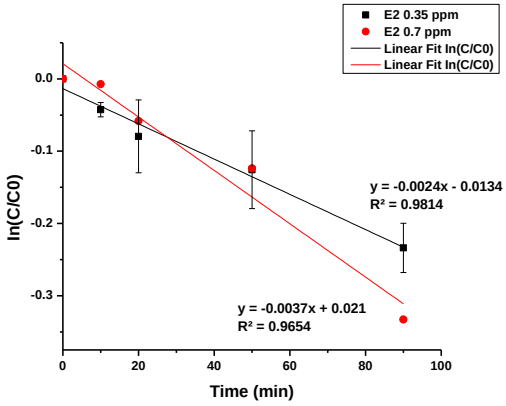

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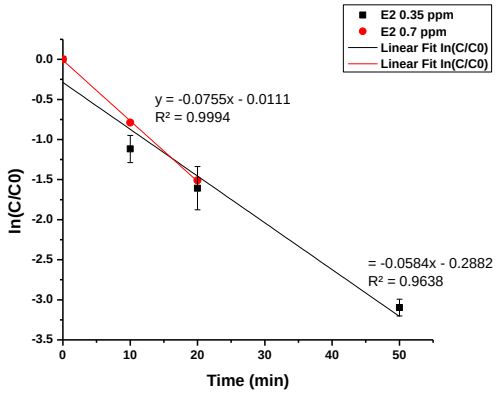
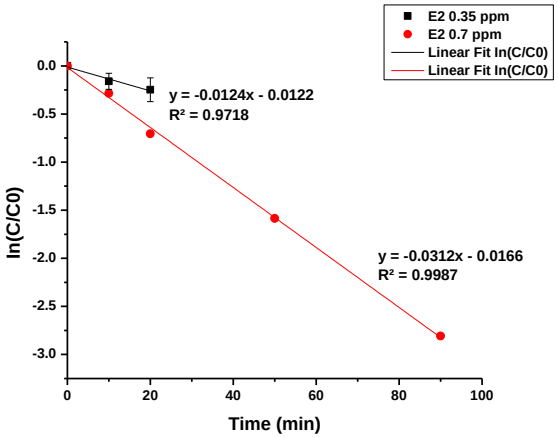
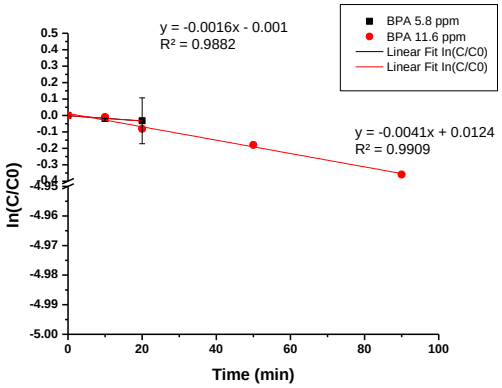

E2 0.35 ppm
E2 0.7 ppm
Linear Fit ln(C/C0)
Linear Fit ln(C/C0)
y = -0.0755x - 0.0111
R² = 0.9994
= -0.0584x - 0.2882
R² = 0.9638
ln(C/C0)
Time (min)

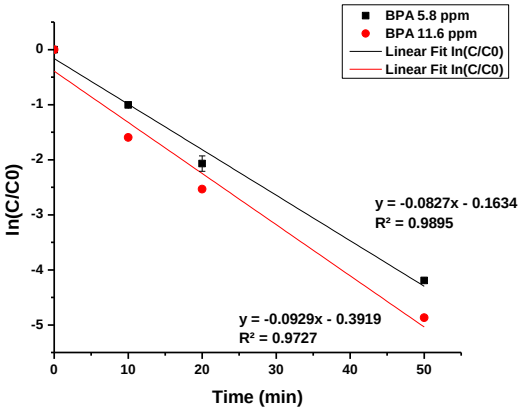
e)


E2 0.35 ppm
E2 0.7 ppm
Linear Fit ln(C/C0)
Linear Fit ln(C/C0)
y = -0.0124x - 0.0122
R² = 0.9718
y = -0.0312x - 0.0166
R² = 0.9987
ln(C/C0)
Time (min)

f)


BPA 5.8 ppm
BPA 11.6 ppm
Linear Fit ln(C/C0)
Linear Fit ln(C/C0)
y = -0.0016x - 0.001
R² = 0.9882
y = -0.0041x + 0.0124
R² = 0.9909
ln(C/C0)
Time (min)

g)


BPA 5.8 ppm
BPA 11.6 ppm
Linear Fit ln(C/C0)
Linear Fit ln(C/C0)
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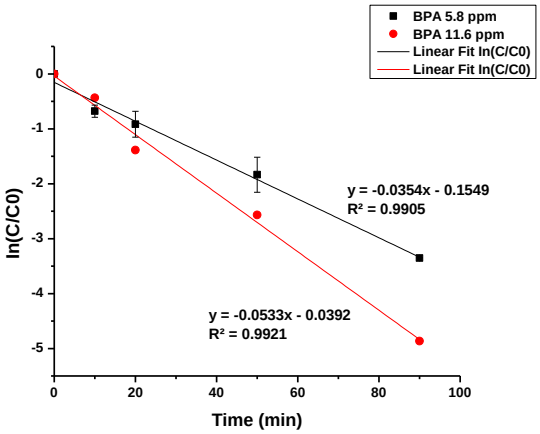
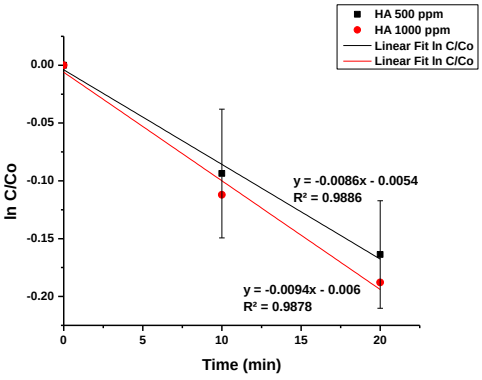
R² = 0.9895
y = -0.0929x - 0.3919
R² = 0.9727
ln(C/C0)
Time (min)

h)


BPA 5.8 ppm
BPA 11.6 ppm
Linear Fit ln(C/C0)
Linear Fit ln(C/C0)
y = -0.0354x - 0.1549
R² = 0.9905
y = -0.0533x - 0.0392
R² = 0.9921
ln(C/C0)
Time (min)

i)


HA 500 ppm
HA 1000 ppm
Linear Fit ln C/Co
Linear Fit ln C/Co
y = -0.0086x - 0.0054
R² = 0.9886
y = -0.0094x - 0.006
R² = 0.9878
ln C/Co
Time (min)

j)

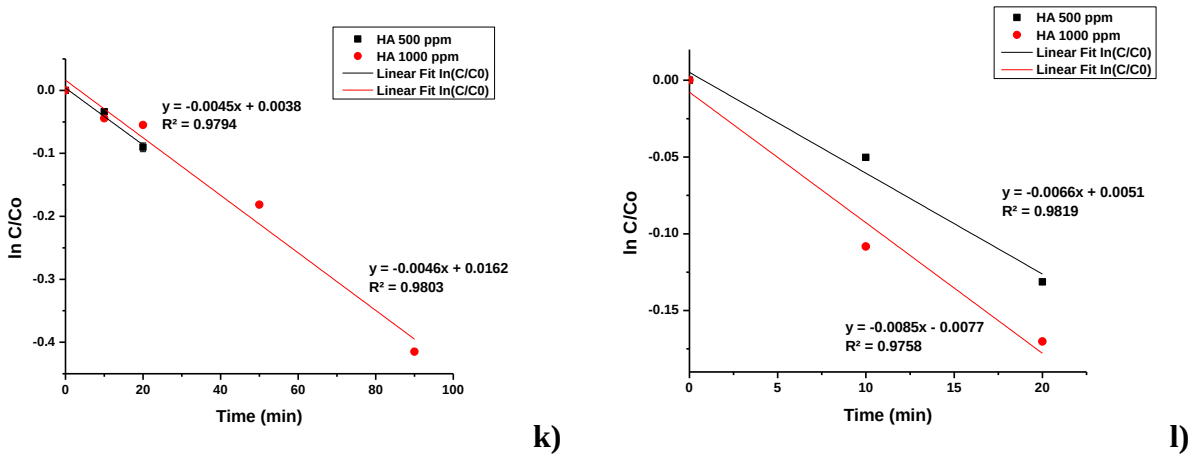


Figure 3.14: Effect of Initial Concentration of SMX, E2, BPA and HA in BPA- E2- SMX- HA mixture [**SMX** (a) UV/ H_2O_2, (b) UV/ O_3 and (c) O_3], [**E2** (d) UV/ H_2O_2, (e) UV/ O_3 and (f) O_3], [**BPA** (g) UV/ H_2O_2, (h) UV/ O3 and (i) O_3] and [**HA** (j) UV/ H_2O_2, (k) UV/ O_3 and (l) O_3], the half concentration is the average of three samples

3.3.3 Effect of Humic acid:

3.3.3.1 Effect of Humic acid on degradation rate constant of the model compounds:

Humic acid is an assembly of heterogeneous complex organic species, such as polymerized organic acids, phenol, carbohydrates, amino acids and hydrocarbons (Black and Christman, 1963), therefore it comprise sites that are involved in differing types of reactions which makes humic substances as radical initiators, promoters, as well as scavengers. The higher concentration of humic materials consumes the hydroxyl radicals – a scavenging effect which reduces the reaction rate (Staehelin and Hoigne, 1985)).

a. Effect of humic acid on degradation rate constant of sulfamethoxazole:

The effect of humic acid on degradation of SMX in the UV based AOPs is generally negative due to light absorption by the humic acid. As it was mentioned earlier that photolytic degradation rate of SMX is higher than by that of ozonation. Therefore, the effect of humic acid on SMX degradation is more pronounced in the UV-based processes. It is interesting to observe the rate

of degradation of SMX in ozonation increased in presence of HA as shown in Table 3.8, possibly due to the formation of reactive radicals. However, this can't be confirmed without knowing the mechanism of humic acid degradation by ozone. The nonhomogeniety in the HA structure makes it more difficult to determine the exact mechanism of degradation.

Table 3.8: The effect of humic acid on the degradation rate constant, k (min^{-1}) of sulfamethoxazole with O_3, UV/ O_3 and UV/ H_2O_2

Chemical	O_3		UV/ O_3		UV/ H_2O_2	
	Rate constant k (min^{-1})	Rate constant with HA k (min^{-1})	Rate constant k (min^{-1})	Rate constant with HA (min^{-1})	Rate constant k (min^{-1})	Rate constant with HA kmin^{-1})
SMX	0.036	0.042	0.264	0.053	0.177	0.0015
SMX- E2	0.028	0.056	0.096	0.102	0.028	0.0004
SMX- BPA	0.043	0.135	0.143	0.08	0.029	0.046
SMX- BPA- E2	0.025	0.09	0.144	0.092	0.044	0.043

b. Effect of Humic acid on degradation rate constant of 17-β estradiol:

As for SMX, humic acid reduced the rate of degradation of E2 in UV based processes due to the competition for UV photons, and the effect was minimal for ozonation as shown in Table 3.9. Ozone decomposition is catalyzed by the humic substances at low concentration (Ma & Graham 1999). Chowdhury et al (2010) observed that the rate of reaction of E2 was increased when the concentration of humic acid was elavated from 2-8 ppm, however due to the scavenging of reactive oxygen; the rate reached a plateau at 8 ppm as a result of increasing light attenuation with increasing humic acid concentration.

Table 3.9: The effect of humic acid on the degradation rate constant, k (min^{-1}) of 17-β estradiol in different mixtures with UV/ H_2O_2, UV/ O_3 and O_3

Chemical	O_3		UV/ O_3		UV/ H_2O_2	
	Rate constant k (min^{-1})	Rate constant with HA k (min^{-1})	Rate constant k (min^{-1})	Rate constant with HA k (min^{-1})	Rate constant K (min^{-1})	Rate constant with HA K (min^{-1})
E2	------	0.021	-----	0.062	-----	0.0078
E2- SMX	0.13	0.01	0.11	0.01	0.034	0.006
E2- BPA	0.108	0.021	0.134	0.005	0.008	0.003
E2- SMX- BPA	0.022	0.031	0.086	0.052	0.01	0.004

c. Effect of humic acid on degradation rate constant of bisphenol A:

The effect of humic acid on bisphenol A degradation was mixed as the rate increased in some of the mixtures while it was reduced in most of the experiments, especially in presence of UV/H_2O_2. The effect was minimal for ozonation as shown in Table 3.10.

Table 3.10: The effect of humic acid on the degradation rate constant, k (min^{-1}) of bisphenol A in different mixtures after UV/ H_2O_2, UV/ O_3 and O_3

Chemical	O_3		UV/ O_3		UV/ H_2O_2	
	Rate constant k (min^{-1})	Rate constant with HA k (min^{-1})	Rate constant k (min^{-1})	Rate constant with HA k (min^{-1})	Rate constant k (min^{-1})	Rate constant with HA k (min^{-1})
BPA	0.085	0.078	0.082	0.164	0.011	0.002
BPA- E2	0.149	0.16	0.165	0.14	0.009	0.003
BPA- SMX	0.045	0.052	0.085	0.12	0.003	0.002
BPA- E2- SMX	0.072	0.053	0.02	0.092	0.11	0.004

3.3.3.2 Effect of humic acid on the percentage of TOC removal (mineralization):

The % of TOC removal was reduced when HA was added to all solutions in the three AOPs except in BPA- SMX –E2 for all the AOPs. Refractory and complex intermediates are formed with the parent compounds, their intermediates and humic acid, which are hard to mineralize. In addition it was found that when the concentration was reduced by 50%, the TOC reduction was increased by 4%, 7% and 12% for UV/ H_2O_2, O_3, and UV/ O_3, respectively as shown in Figure 3.15, 3.17 and 3.16.

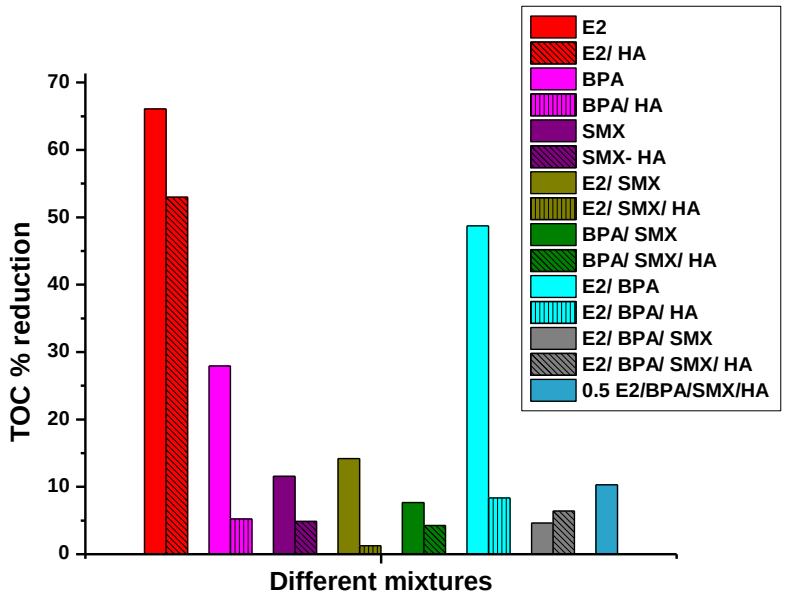


Figure 3.15: The effect of humic acid on the removal of TOC with UV/ H_2O_2

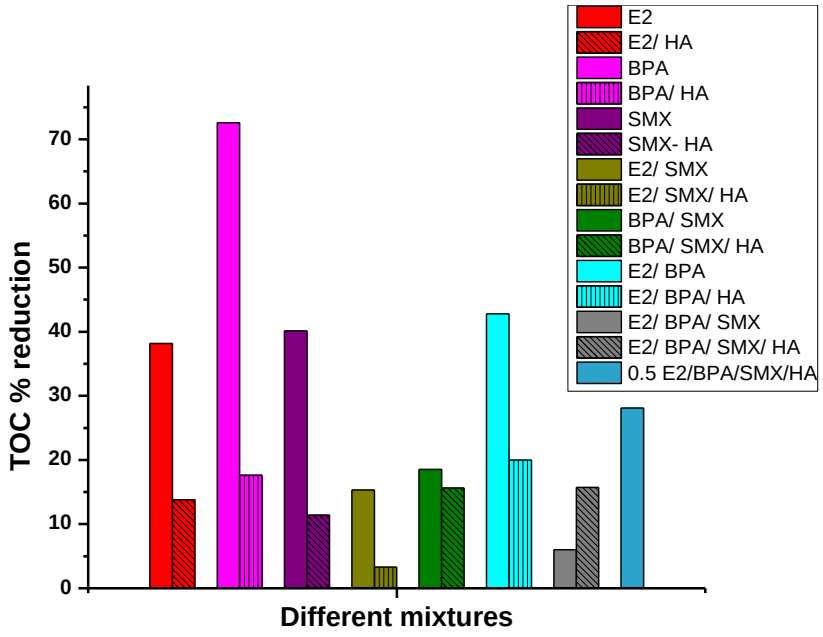


Figure 3. 16: The effect of humic acid on the removal of TOC with UV- O_3

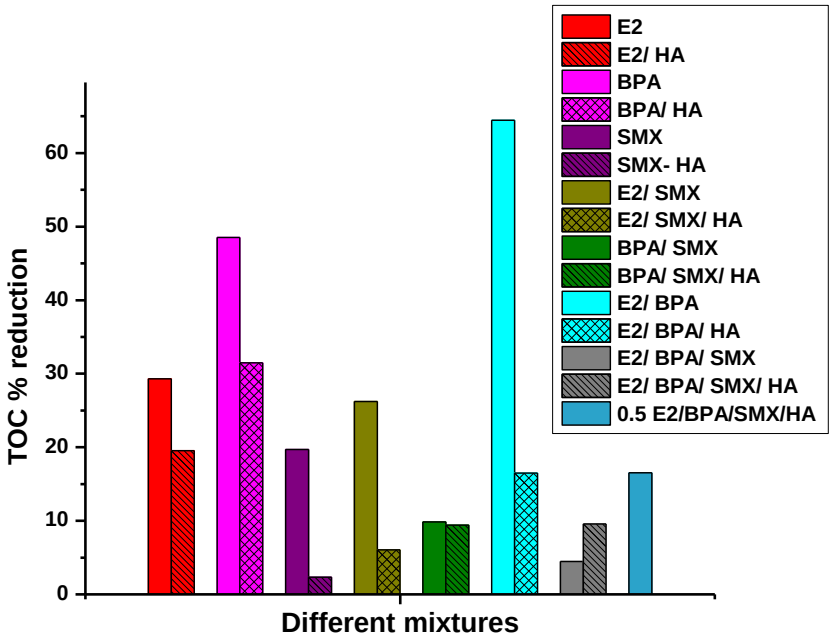


Figure 3.17: The effect of humic acid on the removal of TOC with O_3

3.3.4 Degree of Mineralisation using various AOPs:

Mineralisation of the micropollutants is important to obtain a good water quality, however one of the challenges is to obtain a complete mineralization of these complex organic compounds which is determined by the total organic compound (TOC) of the solution. The TOC removal was measured in all three AOPs (UV/ H_2O_2, UV/ O_3 and O_3) applied in this work as shown in Table 3.11.

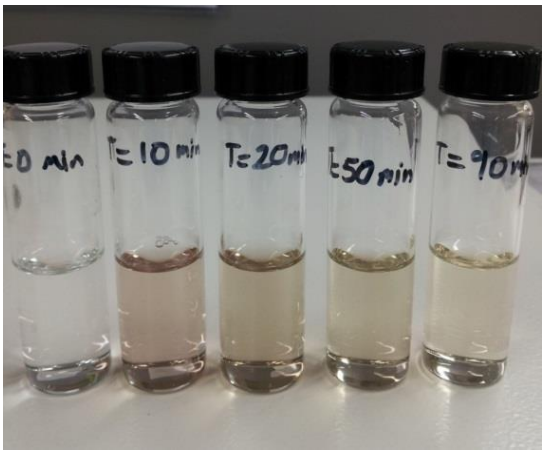
Table 3.11: Percentage of TOC removal after AOPs

Chemical	% TOC removal	% TOC removal	% TOC removal
	UV/ H_2O_2	UV/ O_3	O_3
E2	25	38	29
E2- HA	10	14	20
BPA	28	73	49
BPA- HA	5	18	31
SMX	12	40	20
SMX- HA	5	11	2
E2- SMX	14	15	26
E2- SMX- HA	1	3	6
BPA- SMX	4.2	18.5	9.9
BPA- SMX- HA	16.6	15.6	9.4
E2- BPA	48.7	42.8	64.4
E2- BPA- HA	8.4	20.0	16.5
E2- BPA- SMX	4.6	6.0	4.5
HA	5.7	13.2	18.8
E2-BPA- SMX- HA	6.4	15.7	9.6
0.5 E2-BPA- SMX- HA	10.0	28.8	16.4
0.5 E2-BPA- SMX- HA	10.4	30.6	15.2
0.5 E2-BPA- SMX- HA	10.5	24.8	18.0

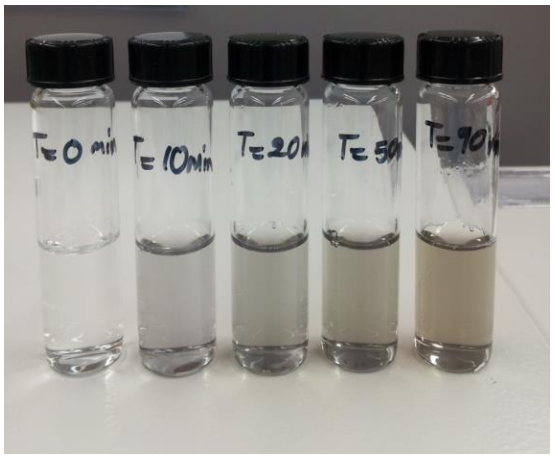
In most cases, ozonation and UV/ozonation performed the best for TOC reduction.

It was observed a change in the color of the aqueous solution of different mixtures after UV/ H_2O_2, UV/ O_3 and O_3 treatments at different times of exposure as shown in Figure 3.18, which indicates the formation of new and different intermediates. The solutions that contain HA were clearer with time. In addition Figure 3.19 reveals different peaks of E2, SMX, and BPA and HA

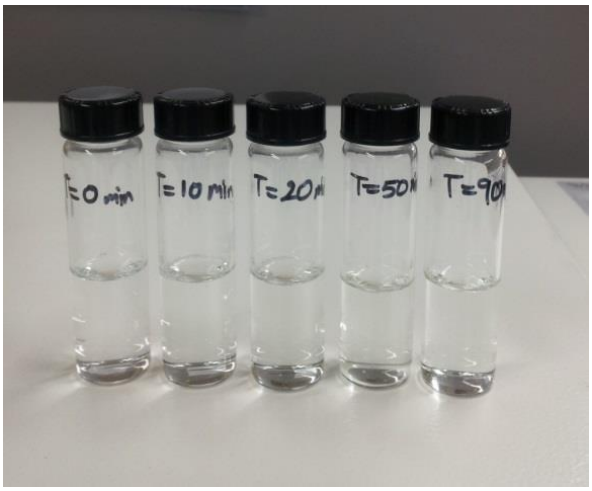
compounds and their intermediates after AOPs treatments, this explains the incomplete removal of these micropollutants because of the formation of these refractory compounds.



a)



b)



c)

3.18: Images of BPA E_2 SMX mixture, samples taken in five different times (A) UV- O_3 reactor (B) UV/ H_2O_2 reactor and (C) O_3 reactor

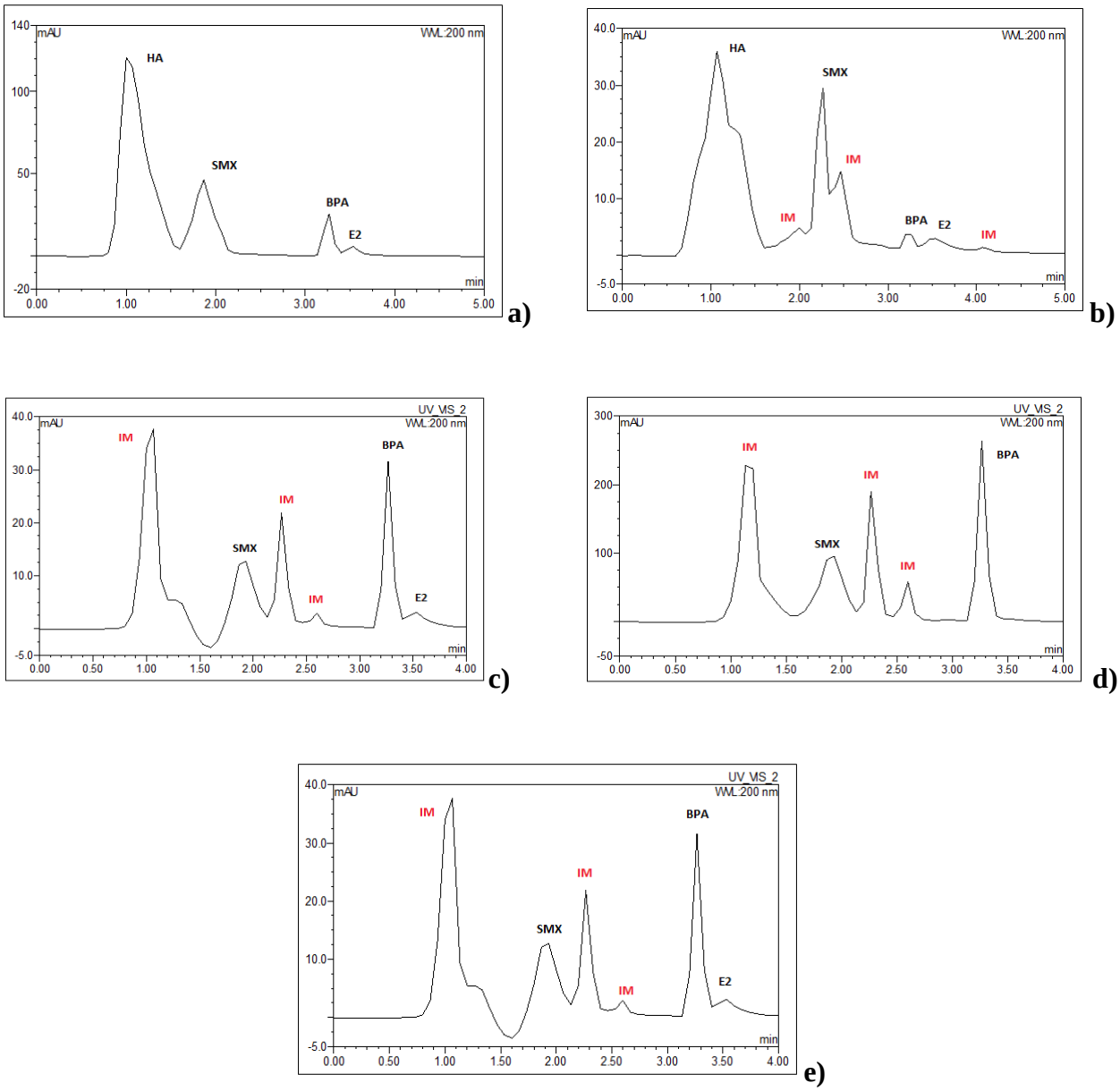


Figure 3.19: HPLC chromatogram of different mixtures degradation: **(a)** HA BPA E2 SMX t=0 min, **(b)** Half concentration of HA BPA E2 SMX with UV/ O_3 t=10 min, **(c)** BPA E2 SMX with UV/ H_2O_2 BPA E2 SMX t= 20 min, **(d)** BPA E2 SMX with UV/ H2O2 BPA E2 SMX t= 50 min and **(e)** BPA E2 SMX with UV/ O_3 BPA E2 SMX t= 10 min. IM (intermediate)

3.4. References

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Chapter Four

A comparative study of the effect of different advance oxidation processes on the estrogencity and genotoxicity of 17-β estradiol, Bisphenol A, Sulfamethoxazole, and Humic acid

4.1 Introduction

Earlier, the genotoxicity and estrogencity of several natural and synthetic organic compounds have been evaluated due to their potential adverse effect and the interference with the usual functioning of the endocrine system in humans and animals (Kaplan et al., 2004; Liehr 2000; Meier et al., 1986; Bridges et al., 1977; Chen et al., 2006; Aerni et al., 2004; Gagne & Blaise, 1998; Ikehata & El-Din, 2004; Bistan et al., 2011). Genotoxicity involves damage to the genetic material of the cell compounds including genetic damage to DNA, fixation of damage to DNA, and mutation by various mechanisms. The Ames test is used to detect the genotoxicity of the compounds such as typical genotoxins like aromatic amines that can cause mutation *(Guidance for Industry, 2012),* which can be defined as deleterious action on a cell's genetic material. Several studies have been conducted to determine the genotoxicity of the micropollutants in water and wastewater (Crebelli et al., 1995; Shishida et al., 2000; Rizzo, 2011; Whatley & Cho, 2010). The mutagenic activity is determined by using the Ames test (Ames et al., 1975) using *Salmonella typhimurium* strains, carrying mutation(s) in the operon coding for histidine biosynthesis, that leads to the need of histidine for survival, but when the mutagen is present it will cause reverse mutation in which the bacteria will be able to survive without histidine.

Endocrine disruptors compounds (EDCs) such as estrogens demonstrated altered sexual development such as feminization of male fish (Rodgers-Gray et al., 2000). The EDC compounds can be grouped as following (Caliman and Gavrilescu, 2009; Burkhardt-Holm, 2010):

1- Natural estrogenic/androgenic hormones: E2, E1, testosterone etc.
2- Synthetic hormones: EE2, diethylstilbestrol, 19- norethindrone etc.
3- Phyto- and mycoestrogens: daidzein, genistein, zear- alenone etc

EE2 and E2 are the most potent estrogenic compounds, followed by E1 and E3 (Folmar et al., 2002). Estrogenic activity is determined using the YES assay as described by Routledge and

Sumpter (1996), where a human estrogen receptor engineered with a beta-galactosidase and recombinant with Saccharomyces *cerevisiae's* DNA is used.

In the environment and aquatic system the estrogenic and non-estrogenic chemicals exist together as complex environmental samples not as a single compound. The non-estrogenic chemicals may mimic and/or interrupt the real estrogenic activity.

Mixtures of chemicals are expected to induce greater biological effects (European Inland Fisheries Advisory Commission 1987; Frische et al., 2009; Thorpe et al., 2005). However, using mere summation of individual components to predict the cumulative behavior of mixture of compounds which called the concentration additive (CA) led to strongly confusing in-vitro observations (Silva et al., 2002; Frische et al., 2009; Thorpe et al., 2006). Hence, there are uncertainties if the CA can be a trustworthy method (Berenbaum, 1985; Greco et al., 1995) to evaluate the estrogenicity of mixtures.

There are three significant major types of interference to estrogenicity (Frische et al. 2009):

1- Toxic masking: Occurs if toxic chemical but non- estrogenic compounds are present in a mixture, it will cause reduction of the apparent estrogencity of both single estrogens and their mixtures due to the high toxic effect (Frische et al., 2009).

2- Antagonistic modulation: It happens when a chemical confounder impairs the estrogencity through decreasing the bioavailability of E2 (L. Chen et al. 2012) or blocking membrane transport (Janosek et al. 2007). Tanghe et al. (1999) mentioned that humic acid causes reduction of bioavailability of the estrogenic compound.

3- Synergistic modulation: This is a common phenomenon in aquatic biotests where some non-estrogenic chemicals can increase the apparent estrogenic activity. In addition, the weak xenoestrogens are able to create an impact upon strong estrogens (Rajapakse, N., et al., 2001), and the bioavailability of E2 was increased by low concentrations of humic acid (L. Chen et al. 2012), furthermore changing the permeability of biological membranes (Vigneault et al. 2000).

While the removal of estrogens and the genotoxins in wastewater treatment plant is incomplete, some transformation processes may produce more harmful by-products or transformation products. (Bila et al., 2007; Nakamura et al., 2006; Shappell et al., 2008). During tertiary treatment using UV based advanced oxidation processes are able to reduce the concentration of micropollutants in wastewater effluents to some extent. However, intermediates formed during treatment may have higher toxicity; for example, UV/H_2O_2 was found to increase mutagenicity of water sample (Heringa et al. 2011). On the other hand, some studies have demonstrated the efficiency of AOPs to reduce estrogencity and/or the genotoxicity after ozonation, (Beltrán et al. 2008; Esplugas et al., 2007; Gunten, 2003), UV, UV/O_3 or H_2O_2, and TiO_2 (Irmak, Erbatur, and Akgerman 2005; P.-J. Chen et al. 2007; Bolton, Linden, and Asce 2003). However, the effects are very system and compound specific. The background water quality such as the effect of dissolved organic compounds also can be very different for different compounds.

In this study four compounds of increasing concern, sulfamethoxazole an antibiotic, estrogenic compound 17-β estradiol, and industrial chemical BPA, which is also an endocrine disrupting compound (EDC), and humic acid (NOM) have been used as model compounds. The estrogenic activity is determined by the yeast estrogencity screen (YES) assay, and the genotoxicity is monitored by using the Ames test, before and after three different three advanced oxidation processes UV/H_2O_2, UV/O_3 and O_3. The effects of different concentrations and mixtures of the model compounds, oxidant type, and background water quality have been studied.

4.2 Experimental

4.2.1 Chemicals:

17-β estradiol (chemical formula: $C_{18}H_{24}O_2$, CAS: 50-28-2) was obtained from Sigma- Aldrich (Oakville, Ontario, Canada) of 98% purity. Sulfamethoxazole (chemical formula: **C**$10H_{11}N_3O_3S$, CAS: 723-46-6) was obtained from Fluka Analytical, bisphenol A (chemical formula: $C_{15}H_{16}O_2$, CAS: 80-05-7) was obtained from Sigma–Aldrich (Oakville, Ontario, Canada) of 99+% purity, and humic acid (Average chemical formula $C_{187}H_{186}O_{89}N_9S_1$, CAS: 1415-93-6) was obtained from Alfa Aesar.

All reagents were used as received without further purification. Laboratory-grade Ultrapure (MiliQ) water (conductivity of 18M Ω) was obtained from a Millipore purification system (model Integral 5, EMD Millipore Corporation, Billerica, MA, USA).

4.2.1.1 Chemicals for the YES assay:

Minimal Medium:

Contaminated glassware, spatulas, stirring bars, etc. with an estrogenic chemical will lead to elevated background expression; therefore, they were scrupulously cleaned, and had no prior contact with steroids. The glassware, spatulas and stirring bars were rinsed twice with absolute ethanol, and left to dry.

The following chemicals, shown in Table 4-1, were added to prepare minimal growth media. All

Table 4.1: List of chemicals for minimal media preparation for the YES assay

Chemical	**Amount**	**Supplier/ Location**	Purity
KH_2PO_4	13.61 g	Caledon/ Canada	-----
$(NH_4)_2SO_4$	1.98 g	Caledon/ Canada	-----
$MgSO_4$	0.2 g	Caledon/ Canada	-----
$Fe_2(SO_4)_3$	1ml of (40 mg/50 ml H_2O) solution	Alfa Aesar/ Canada	-----
L-Ieucine	50 mg	Alfa Aesar/ Canada	99%
L-histidine	50 mg	Alfa Aesar/ Canada	98%
Adenine	50 mg	Alfa Aesar/ Canada	99%
L-arginine-H	20 mg	Alfa Aesar/ Canada	98%
L-methionine	20 mg	Alfa Aesar/ Canada	98+%
L-tyrosine	30 mg	Alfa Aesar/ Canada	99%
L-isoleucine	30 mg	Calbiocheem/ Canada	99%
L-lysine-HCI,	30 mg	Calbiocheem/ Canada	99.6%
L-phenylalanine	25 mg	Alfa Aesar/ Canada	99%
L-glutamic acid	100 mg	Alfa Aesar/ Canada	99%

the chemicals were dissolved separately in Milli-Q water and stirred on a hot plate.
KOH pellets were dissolved in 5 ml Milli-Q water and added gradually to the above mixture of chemicals to obtain a pH of 7.0 (± 0.1). The final volume of the solution was adjusted to 1 L using Milli-Q water. The media was sterilized at 121°C for 10 min to avoid any bacterial contamination. Thereupon, it was stored in glass bottles at room temperature.

Chemicals for preparation of yeast for assay:

The growth medium was prepared by adding 5 ml glucose solution, 1.25 ml L-aspartic acid solution, 0.5 ml vitamin solution, 0.4 ml L-threonine solution, and 125 ul copper (II) sulfate solution to 45 ml minimal medium. Then, it was transferred to a sterile conical flask (final volume of approximately 50 ml). A 125 µl of 10X concentrated yeast stock from cryogenic vial was added and incubated at 28°C on an orbital shaker for approximately 24 hours or until turbid.
D-(+)-Glucose (Alfa Aesar, CA**)** A 20% w/v solution was prepared and sterilized in 20 ml aliquots at 121°C for 10 min in distilled water.
L-Aspartic Acid (Alfa Aesar, CA) **-** A stock solution of 4 mg/ml of aspartic acid was prepared in distilled water and sterilized in 20 ml aliquots at 121°C for 10 min.

Vitamin solution was prepared by adding 8 mg thiamine, 8 mg pyridoxine (Sigma Aldrich, USA, 8 mg pantothenic acid (98%, Alfa Aesar, Canada), 40 mg inositol (Himedia/ India), and 20 ml biotin solution (2 mg/100 ml H_2O) (Fluka, USA) to 180 ml 71 double-distilled water. The solution was sterilized by filtering through a 0.2 µm pore size disposable filter (VWR international, CA) into sterile glass bottles and stored at 4 °C for further use.

L-Threonine (Alfa Aesar, CA**)** solution of 24 mg/ml was prepared in distilled water. The solution was sterilized at 121°C for 10 min and stored at 4 °C prior to use.

Copper (II) Sulfate (VWR BDH Prolabo**)** solution of 20 mM was prepared in distilled water. The solution was sterilized by filtering through a 0.2 µm pore size filter (Cellulose acetate, VWR, CA) in sterile glass bottles in 5 ml aliquots and stored at room temperature.

Chlorophenol red-β-D-galactopyranoside (CPRG) (Sigma- Aldrich**) –** a 10 mg/ml stock solution of CPRG was prepared in distilled water. It was further sterilized by filtering through a 0.2 µm pore size filter (Cellulose acetate, VWR, CA) into sterile glass bottles in a laminar flow cabinet and stored at 4 °C.

4.2.1.2 Chemicals for the Ames:

Standard Mutagens:

9-Aminoacridine (Alfa Aesar, Canada) and *Sodium azide* (Caledon/ Canada).

Concentrate Davis-Mingioli salts consist of 38.5 g of dipotassium phosphate (Caledon/ Canada), 11 g of monopotassium phosphate (Caledon/ Canada), 2.75 g of sodium citrate (Caledon/ Canada), 0.55 g magnesium sulphate (Caledon/ Canada) and 5.5 g of ammonium sulphate (Caledon/ Canada).

Reaction Mixture (RXM) consists of Davis-Mingioli salts (concentrate) 43.24 ml, 9.5 ml of 40% D-glucose (Alfa Aesar, Canada), 4.76 ml of 2 mg/L Bromocresol Purple (Caledon/ Canada), 2.38 ml of 0.1 mg/L D-Biotin (Sigma Aldrich/ Canada and 0.12 ml of 0.1 mg/L Histidine (Alfa Aesar / Canada).

4.2.2 The toxicity experiment of sulfamethoxazole (SMX) for the *Saccharomyces cerevisiae* and the *Salmonella typhimurium* TA 97 and TA 100:

The toxicity experiment of sulfamethoxazole (SMX) for the yeast of the YES assay (*Saccharomyces cerevisiae*) was performed by diluting 100 ppm and 80 ppm of SMX using a twofold serial dilution, then 100 µL of each dilution was added to 100 µL of the yeast. The dose effect of SMX was monitored after 24 hrs of incubation at 30 ^{o}C by measuring the growth of the yeast at absorbance 540 nm. Two rows of each concentration were used in addition to a three rows of the positive control which contain the 100 µL of the yeast plus 100 µL of milli-Q water. Two rows of the negative control were prepared by adding 100 µL of the yeast, 10 µL of ethanol and 90 µL of milli-Q water.

4.2.3 A comparison between the YES assay with GCMS analysis:

The comparison between 17-β estradiol (E2) equivalents (EEQs) in the YES assay versus the actual concentrations measured by the GCMS was done. These tests were conducted with known

concentration of E2 dissolved in methanol and then diluted in mili-Q water in the concentration range of E2 at 5-50 μg/L.

4.2.4 Yeast Estrogen Screen:

Estrogenic activity was determined using the YES assay as described by Routledge and Sumpter (1996). A recombinant yeast strain (*Saccharomyces cerevisiase)* was obtained from Trojan UV (Ontario, Canada). A 250 μL concentrated yeast stock from cryogenic vial was added to the conical flask containing the growth medium. The flask was incubated at 28°C, 180 rpm for approximately 24 hours or until turbid with an optical density of ~1 on an orbital shaker. A standard solution (50 μg/L) was prepared using 17-β estradiol (E2) and was diluted using a twofold serial dilution in absolute methanol; 12 dilutions in the range of 24.41 ng/L - 50,000 ng/L of E2 were prepared. For standard tests, 10 μL of the E2 standard dilutions were added to three rows of wells in a 96-microtitre plate (Corning Costar, USA) and allowed to dry completely. The blank was prepared by adding 10 μL of absolute methanol to 190 μL of the assay media (growth medium containing the dye, chlorophenol red-β-D-galactopyranosid (CPRG), and yeast) to two rows of the same 96-microtitre plate. The samples were treated differently depending on the micropollutants type. For the preliminary study of BPA, samples of 10 mL from each AOP reactor were collected and freeze-dried overnight and re-dissolved in 1 mL of methanol with a recovery of 87-100%. Thereafter, 60 μL of the concentrated samples were further two-fold serially diluted in two rows of the 96-microtitre plate using methanol, and were left to fully evaporate. Subsequently, 200 μL of the seeded assay medium was added to each well. Due to the high cost of the freeze drying, the rest of pure BPA and BPA mixtures were used by adding 10 times of the regular amount of sample (100 μL) to a 100 μL of 10 times concentrated assay media. Two rows of the sample were prepared in 96-microtitre plate by using twofold serial dilution with Milli-Q water. For the rest of the samples two rows of each sample were prepared by diluting the sample with Milli-Q water using a two-fold serial dilution in the 96-microtitre plate. Thereafter, 10 μL of each dilution was transferred to the assay plate in which 190 μL of the assay media was added.

The plates were sealed with sterile adhesive film and shaken vigorously for 2 min in a plate shaker (VWR). Subsequently, the plates were incubated at 30°C in a naturally ventilated heating cabinet for 3 days. After the incubation, the plates were shaken at 240 rpm for 2 min, and left for

approximately 1 hour to allow the yeast to settle. The YES assay was done in duplicate for the samples and the blank, and in triplicate for E2 standard. A typical dose-response curve for E2 is shown in Fig. 4.1.

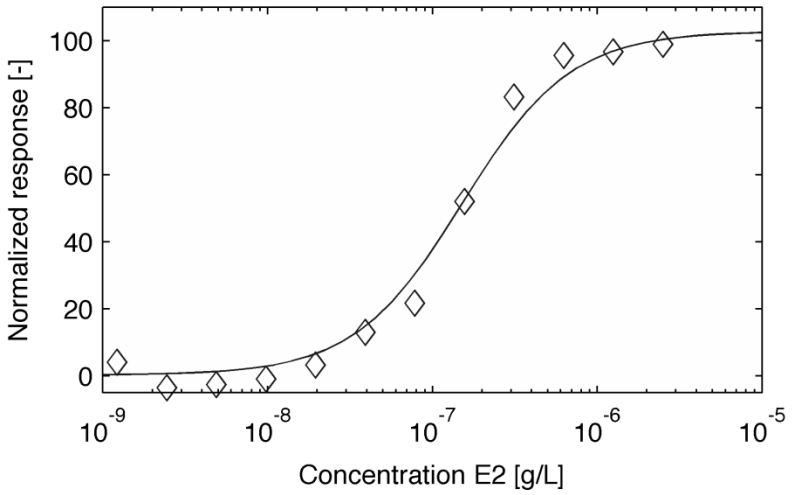


Figure 4.1: The log concentration of 17β estradiol serially diluted from 24.41ng/L - 50,000 ng/L versus the absorbance after three days of incubation. The diamonds are the average of standard triplicates, and the line is the best fits using the Hill equation

The absorbance of the sample, standard and blanks were read at an absorbance of 540 nm (optimum absorbance for CPRG 575 nm) and 620 nm (for turbidity) using a plate reader (Infinite® 200 PRO, Tecan, USA) as shown in schematic of Figure 4.2.

YES assay calculations:

The absorbance data at 540 nm was used to evaluate the response of the yeast strain. A control experiment with known estrogen concentration was run with each plate. Plotting the response at 540 nm vs. the E2 concentration results in a sigmoidal plot. The resulting sigmoidal dose-response curve was analyzed using the Hill equation (4.1) following the method described by Huber (2004).

$$OD_{540} = a + \frac{a-b}{1+\left({}^{X_{E2}}/_{EC_{50}}\right)^{-m}} \quad \text{..... (4.1)}$$

where OD_{540} is the optical density at 540 nm, X_{E2} is the E2 concentration [mg/L] and a, b, m and EC_{50} are fitting parameters representing the low response, high response, Hill-slope, and the half maximal effective concentration [mg/L].

The parameters were estimated using nonlinear regression analysis implemented in Matlab (Matlab 2013b MathWorks, Natick, MA), see Appendix (6.1) for code. Experimental dataset with an unknown amount of estrogenic compounds(s) were analyzed with a modified Hill equation (4.2):

$$OD_{540} = a + \frac{a-b}{1+\left(D/_{EC_{50}}\right)^{-m}} \quad \text{..... (4.2)}$$

Where D is the dilution factor of the original sample [-] (dilution in the well) resulting in a dimension of EC_{50}. The estrogen equivalent concentration (EEq) [mg/L] can be estimated as the ratio of the sample's EC_{50} and the standard's EC_{50} equation (4.3):

$$EEq\ [mg/L] = \frac{EC_{50}^{standard}\ [mg/L]}{EC_{50}^{sample}\ [-]} \quad \text{..... (4.3)}$$

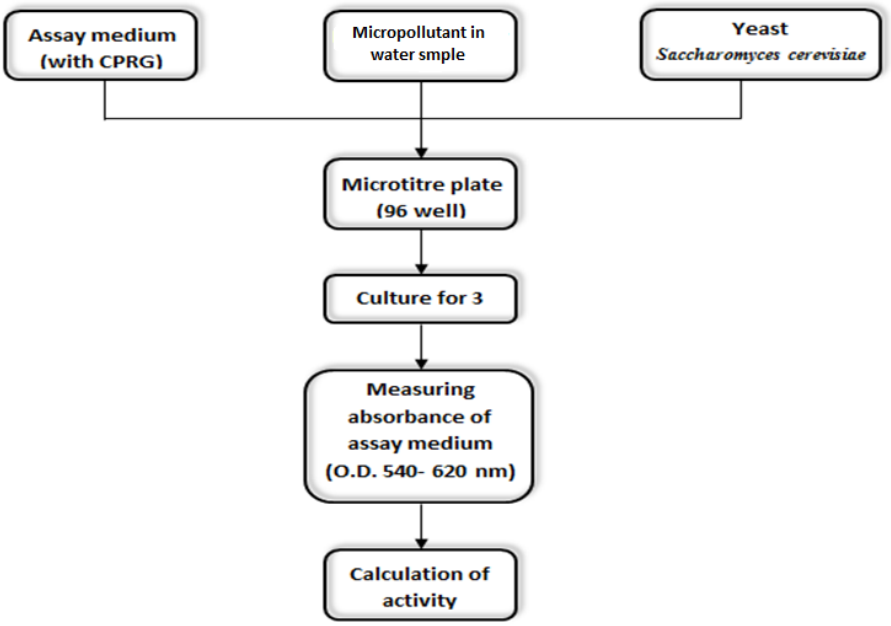


Figure 4.2: The schematic of the YES assay

4.2.5 The Ames test:

The mutagenic activity and mutagenic material in water samples were determined by using the Ames test (Ames *et al.*, 1975). Reverse-mutation assays have been performed using the `Fluctuation test' originally devised by Luria and Delbruck (1943) and modified by Hubbard *et al.* (1984). In this test two strains of *Salmonella typhimurium* TA97a and *Salmonella typhimurium* TA100 (obtained from EBPI- Environmental bio- detection product inc.) were used. They carry mutation(s) in the operon coding for histidine biosynthesis. Standard mutagens *9-aminoacridine* (0.4 mg/ml) was used for *S. typhimurium* TA97a and *sodium azide* (0.1mg/ml) was used for *S. typhimurium* TA100.

A sample of 5 ml was filtered through 0.22 µm membrane PTFE filter. Then it was mixed with 2.5 ml of the reaction mixture RxM (Davis-Mingioli salts, D-glucose, Bromocresol Purple, D-Biotin and L-Histidine), 12.5 ml of distilled water, and 10 µl of the bacteria with an optical density of 0.5 \. The positive control was prepared by adding 0.1 ml of the standard mutagen to 2.5 ml of the RxM, 17.4 ml distilled water and 10 µl of the bacteria. The background was prepared by adding 17.5 ml distilled water to 2.5 ml of the RxM and 10 µl of the bacteria. The blank (the sterility check) was prepared by adding 17.5 ml distilled water to 2.5 ml of the RxM only.

Afterward 200 µl of the mixtures was dispensed into 96-well micro-titration plate (Corning Costar, USA). The plates were covered with a lid and put into a plastic bag to prevent evaporation, then transferred to a 37°C incubator for five days.

Analysis of the Ames results:

The response of Ames test to BPA, E2, and SMX and HA as pure compounds and mixtures after different oxidation times, by using two *Salmonella* strains was determined visually. After five days of incubation at 37°C the number of positive reaction was monitored by changing the color from purple to yellow as a positive reaction as shown in Figure 4.3. The `Background' showed the level of spontaneous or background mutation of the assay organism. The results for each treatment plate refers to positive responses in the sample plate vs. positive responses in the background plate, and the number of positive wells scored in a 96-well microtitre plate leading to clear significance in the fluctuation test by using Table 4.2.

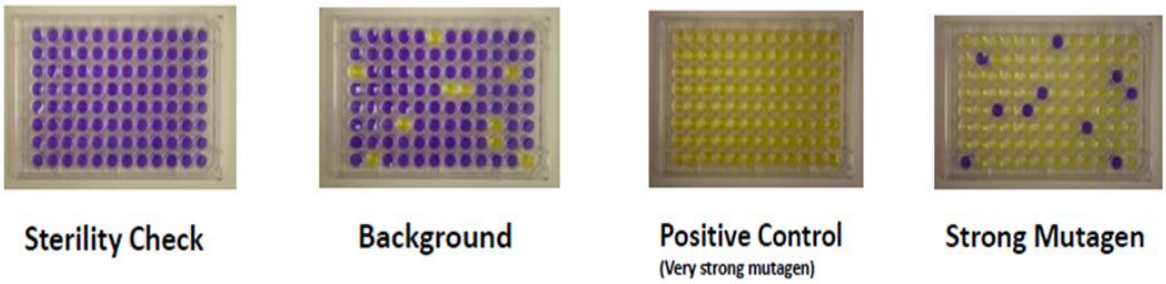


Figure 4.3: The Ames plates showing the reverse mutation

Table 4.2: The number of positive wells scored in a 96- well microplate leading to clear significance (The Muta-ChromoPlateTM Bacterial Strain Kit, Version 3.3, 2009)

No. Wells Positive in Background Plate	No. Wells Positive in Treatment Plate			No. Wells Positive in Background Plate	No. Wells Positive in Treatment Plate		
	0.05	0.01	0.001		0.05	0.01	0.001
0	3	6	10	36	48	53	58
1	5	8	12	37	49	54	59
2	7	10	14	38	50	55	60
3	9	12	16	39	51	56	61
4	10	14	19	40	52	57	62
5	12	15	20	41	53	58	63
6	13	17	21	42	54	59	64
7	15	18	23	43	55	60	65
8	16	20	25	44	56	61	66
9	17	21	26	45	57	62	67
10	19	23	27	46	58	63	68
11	20	24	29	47	59	64	69
12	21	25	30	48	60	63	70
13	22	27	32	49	61	66	70
14	24	28	33	50	62	67	71
15	25	29	34	51	63	67	72
16	26	30	36	52	64	68	73
17	27	32	37	53	65	69	74
18	28	33	38	54	66	70	75
19	30	34	39	55	67	71	76
20	31	35	40	56	68	72	77
21	32	36	42	57	68	72	77
22	33	38	43	58	69	74	78
23	34	39	44	59	70	75	79

The number of positive wells scored in a 96- well microplate leading to clear significance

The Muta-ChromoPlate™ Bacterial Strain Kit, Version 3.3

24	35	40	45	60	71	75	80
25	36	41	46	61	72	76	81
26	37	42	47	62	73	77	71
27	39	43	49	63	74	78	82
28	40	44	50	64	75	79	83
29	41	45	51	65	76	80	84
30	42	47	52	66	77	80	84
31	43	48	53	67	78	81	85
32	44	49	54	68	78	82	86
33	45	50	55	69	79	83	87
34	46	51	56	70	80	84	87
35	47	52	57	71	81	84	88
72	82	85	89	84	91	94	95
73	83	86	89	85	92	94	96
74	83	87	90	86	93	94	96
75	84	87	90	87	93	95	-
76	85	88	91	88	94	95	-
77	86	89	92	89	94	96	-
78	87	89	92	90	95	96	-
79	87	90	93	91	96	-	-
80	88	91	93	92	96	-	-
81	89	91	94	93	96	-	-
82	90	92	94				
83	90	93	95				

4.3 Results and Discussions:

4.3.1 Preliminary studies for estrogencity, toxicity and mutagenicity of model compounds:

4.3.1.1 Toxicity experiment of sulfamethoxazole (SMX) for *Saccharomyces cerevisiae* and *Salmonella typhimurium* TA 97 and TA 100:

Sulfamethoxazole (SMX) being an antibiotic, toxicity of SMX for the yeast used in the YES assay (*Saccharomyces cerevisiae*) was evaluated by using two fold serial dilutions of 100 ppm and 80 ppm SMX. Optical density measurements after 24 hrs of incubation at 30 ^{o}C by (540 nm), showed that SMX did not affect the growth of the yeast.

Similar toxicity experiment was conducted with the bacteria of the Ames test (*Salmonella* TA 97 and TA 100). Optical density measurements after 24 hrs of incubation at 37 ^{o}C by using the reader at 600 nm showed that SMX did not affect the growth of the *S.* ***typhimurium*** TA 97 and TA 100. The toxicity tests are summarized in the diagram shown in Figure 4.4.

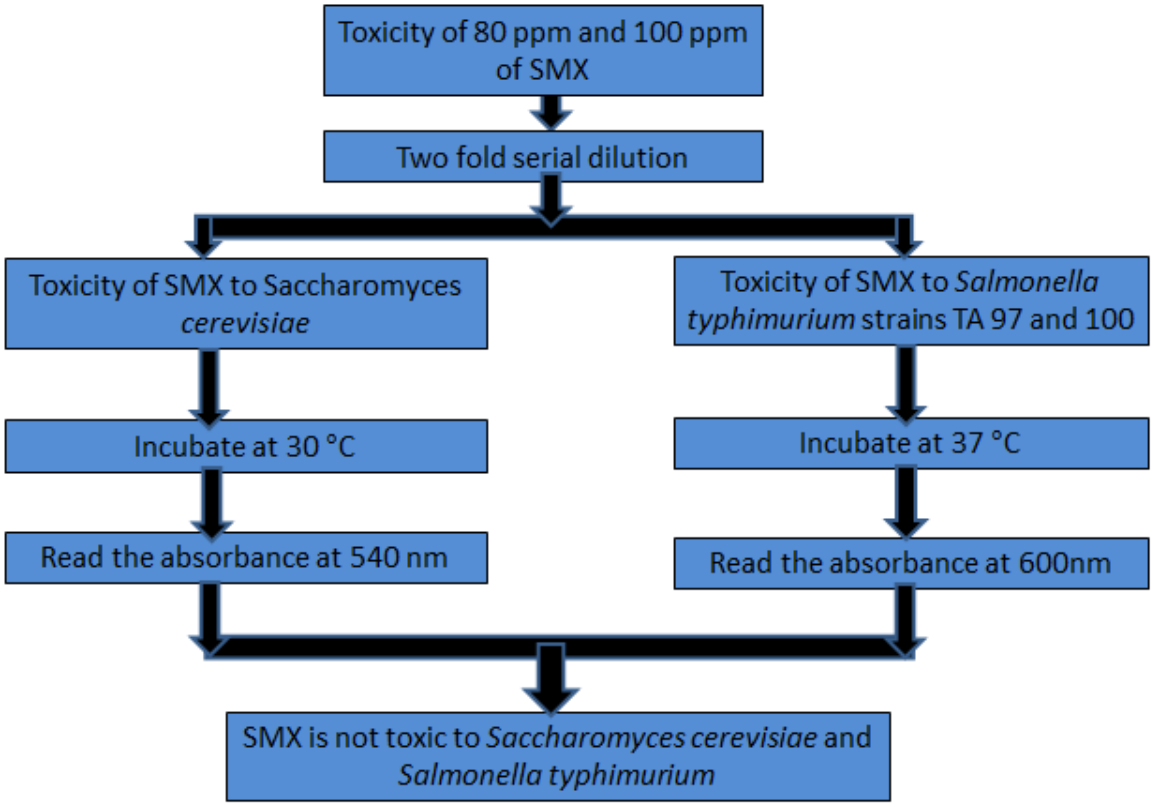


Figure 4.4: Toxicity experiment of sulfamethoxazole for yeast of the YES assay and the bacteria of the Ames test

4.3.1.2 YES assay vs GCMS analysis:

The 17-β estradiol (E2) equivalents (EEQs) of known E2 concentrations were measured via the YES assay and compared to what was measured via GCMS. These tests were conducted with known concentration of E2 dissolved in methanol and then diluted in mili-Q water in the concentration range of 5 µg/L-50 µg/L. There is a linear relationship between the EEQ and the GCMS response with the original concentration as shown in Figure 4.5, and the EEQs are always

within 80% of the original concentration. The purpose of this quality control study was to ensure that the sample preparation was not introducing a bias or rendering the assay non-suitable for the desired concentration range.

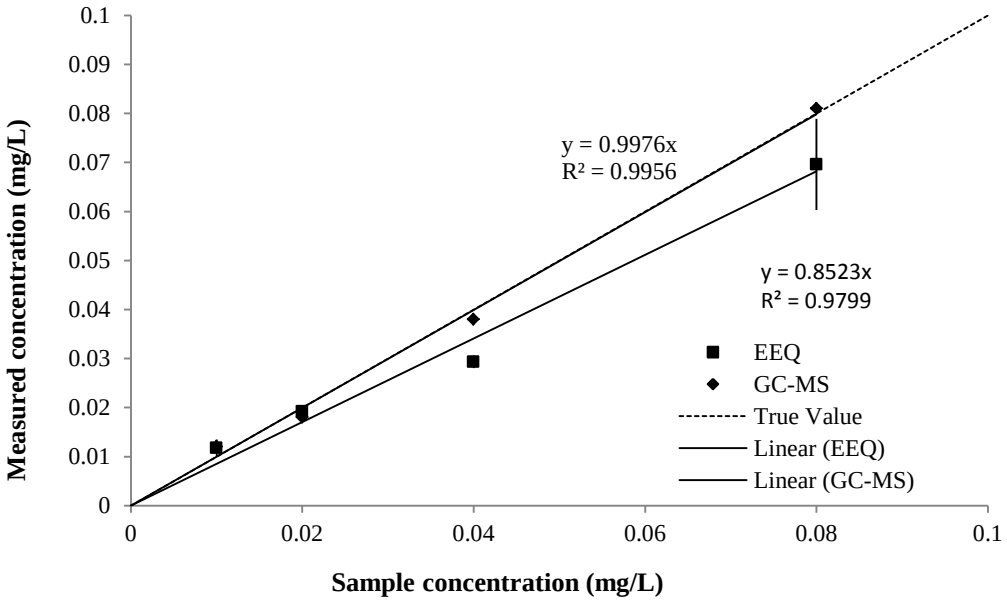


Figure 4.5: Comparison of YES assay with the GC-MS analysis
(GCMS data were the average of two samples)

4.3.1.3 The estrogencity and the mutagenicity of different concentrations of the model compounds:

The results of the Ames test for 17-β estradiol, bisphenol A, sulfamethoxazole, and humic acid by using different concentrations showed some reverse mutation of *Sallmonella* TA 97 and *Sallmonella* TA 100: however, none of them were statistically significant as shown Table 4.3. The existing literature shows contradictory results on the mutagenicity of SMX. Isidori et al. (2005) mentioned that SMX is mutagenic, on the other hand Nakmura et al. (1995), found that SMX didn't show mutagenicity to *Sal*. strain TA 98 and TA 100. Humic acid is not mutagenic

by itself; however, it can result in mutagenic actions detected by the Ames test after chlorination (Meier et al. 1986).

17-β estradiol is not mutagenic; although Lieher (2000) mentioned that E2 is a weak carcinogen and a weak mutagen. Bisphenol A is not mutagenic and this agrees with the result of Ike et al. (2002).

The results of the YES assay for 17-β estradiol showed a strong estrogencity as E2 has the highest estrogenic potential amongst the natural estrogens (Routledge & Sumpter 1997).

Bisphenol A showed weak estrogencity as it is considered as a weak estrogen known as xenoestrogens (Rajapakse et al. 2002; Ike et al. 2002). SMX exhibited no estrogencity and this agrees with (Esaher, et al. 2005). Humic acid by itself had shown no estrogencity as presented in Table 4.3.

4.3.2 The estrogencity of 17-β estradiol, Bisphenol A, Sulfamethoxazole, and Humic acid: Effect of different AOPs:

In this chapter, the estrogenic activity was determined using the YES assay as described by Routledge and Sumpter (1996). This assay is based on a DNA recombinant strain of the yeast *Saccharomyces cerevisiae*, as shown in Figure 4.6 a, which contains a gene for the human estrogen receptor hER and expression plasmids, which is encoding the enzyme β-galactosidase that results in changing the color of chlorophenol red-β-d-galactopyranoside (CPRG) from yellow to red, as shown in Figure 4.6 b.

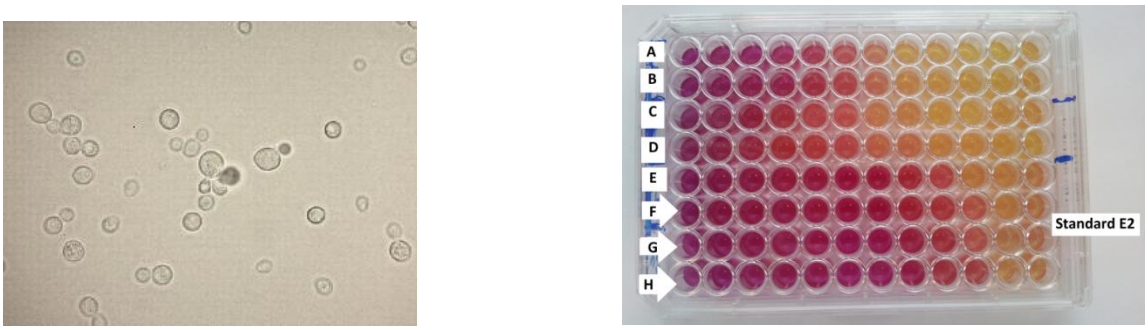


Figure 4.6 a) *Saccharomyces cerevisiae* X100 **b)** Assay plate showing the change in the color from yellow to pink as a response of the yeast screen to 12 dilutions of the standard E2 in the range 24.41 ng/L-50,000 ng/L (row F,G and H) and the samples (row A, B, C, D and E)

Table 4.3: The estrogencity and the mutagenicity of the model compounds

Compound (mg/L)	Mutagenicity in the Ames test	(EEQ mg/L) in the YES assay
BPA	Non mutagenic	Estrogenic
100	-	0.00437
50	-	0.00183
25	-	0.00064
11.6	-	0.00049
5	-	6.21E-23
1	-	4.54E-25
E2	Non mutagenic	Estrogenic
50	-	50
25	-	22.8
12.5	-	10.5
6.25	-	6.69
3.125	-	3.5
1	-	0.93
SMX	Non mutagenic	Non estrogenic
100		
50	-	-
25	-	-
12.5	-	-
6.25	-	-
3.125	-	-
1	-	-
HA	Non mutagenic	Non estrogenic
1000	-	-
500	-	-
750	-	-
250	-	-

The estrogencity of 17-β estradiol (C_0 = 0.7 mg/L), bisphenol A (C_0 = 11.6 mg/L), sulfamethoxazole (C_0 = 0.7 mg/L), and humic acid (C_0 = 1000 mg/L) was measured as pure compounds as well in mixture by calculating the estrogenic equivalent concentration (EEQ) see

Appendix (6.2) for all the mixtures of these micropollutants after the exposure time to three advance oxidation processes (O_3, UV/O_3 and UV/H_2O_2) as shown in Figure 4.7.

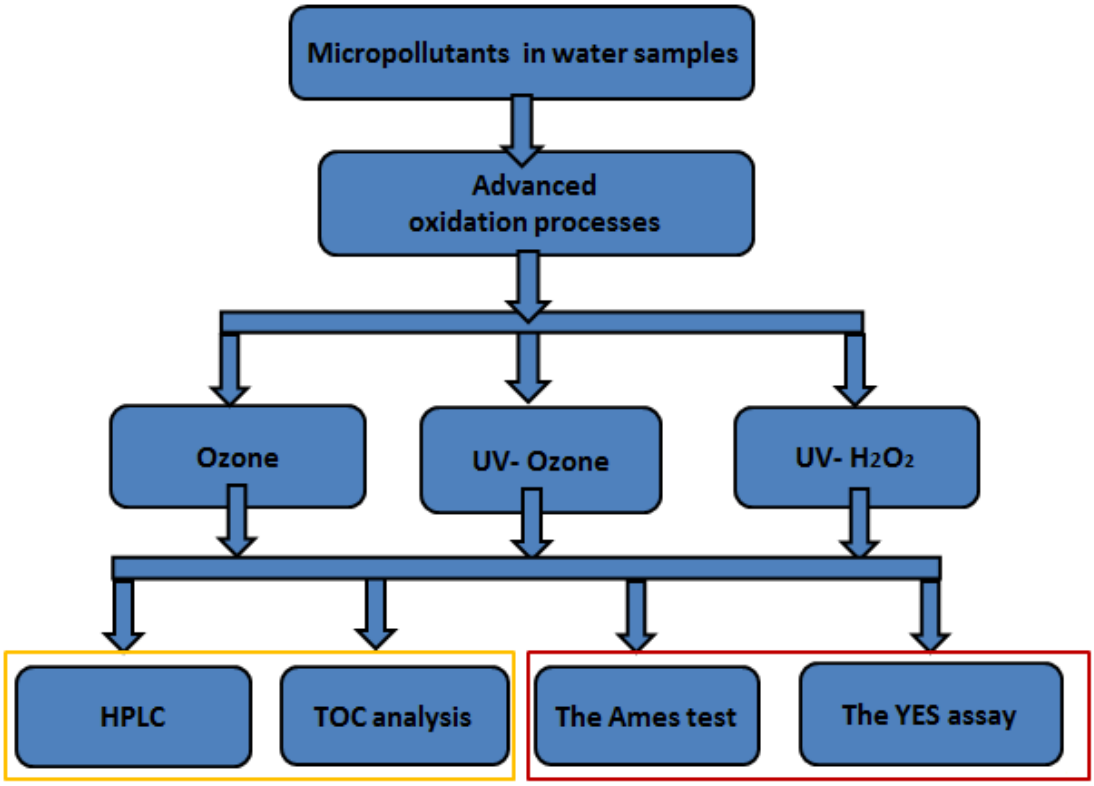


Figure 4.7: The schematic of the experimental procedure

4.3.2.1 The estrogencity of pure 17-β estradiol with exposure to advance oxidation processes:

UV/O_3 led to reduce the estrogencity by 100% after only 10 min of treatments as shown in Figure 4.8 b and Figure 4.10. This result is in line with the chemical analysis by HPLC which showed fast degradation of E2 after 10 min of treatment. O_3 and UV/ H_2O_2 showed ~ 100% of measured EEQ after 50 min of treatment as shown in Figure 4.8 a. and c. 4. 8, Figure 4.9 and Figure 9.11.

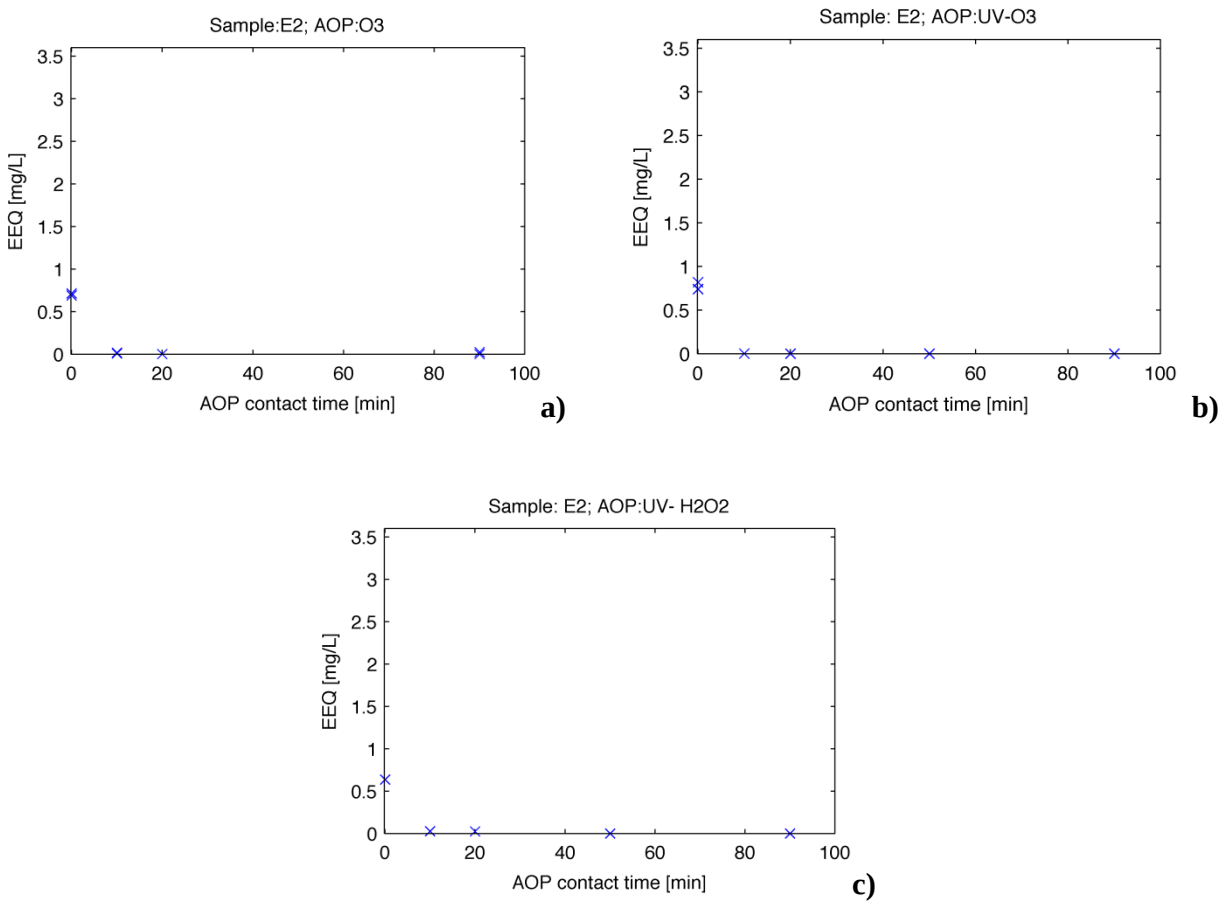


Figure 4.8: The EEQ of E2 C_0 = 0.7 mg/L and pH= 6.4 after different AOPs (a) O_3 (b) UV/ O_3, (c) UV/ H_2O_2 (X) sample number one and two

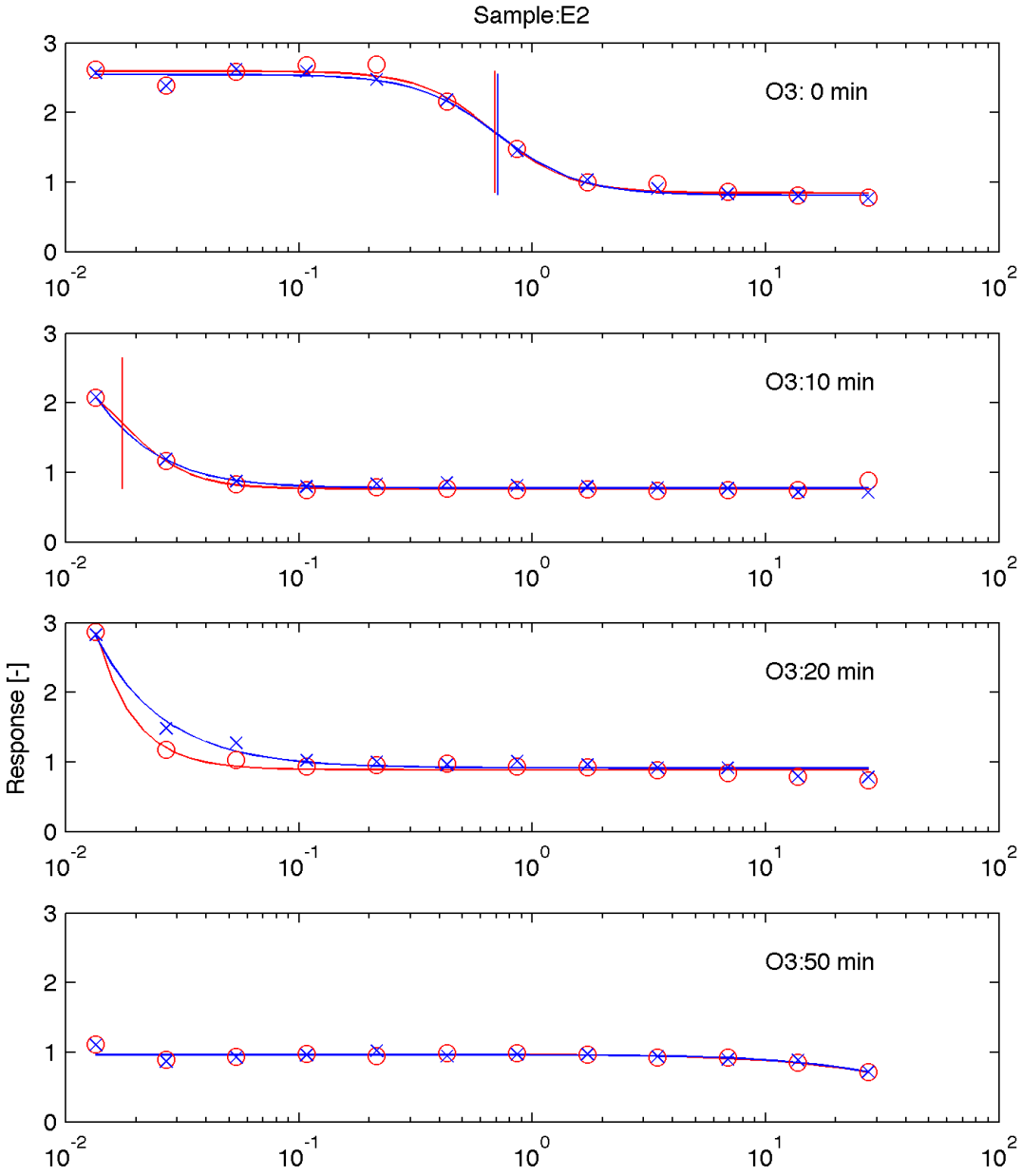


Figure 4.9: Reduction in the estrogencity of E2 C_0 = 0.7 mg/L and pH= 6.4 after different treatment times with ozone; Ozone dosage is 1.31 mg/L. (x) sample number one, (o) sample number two

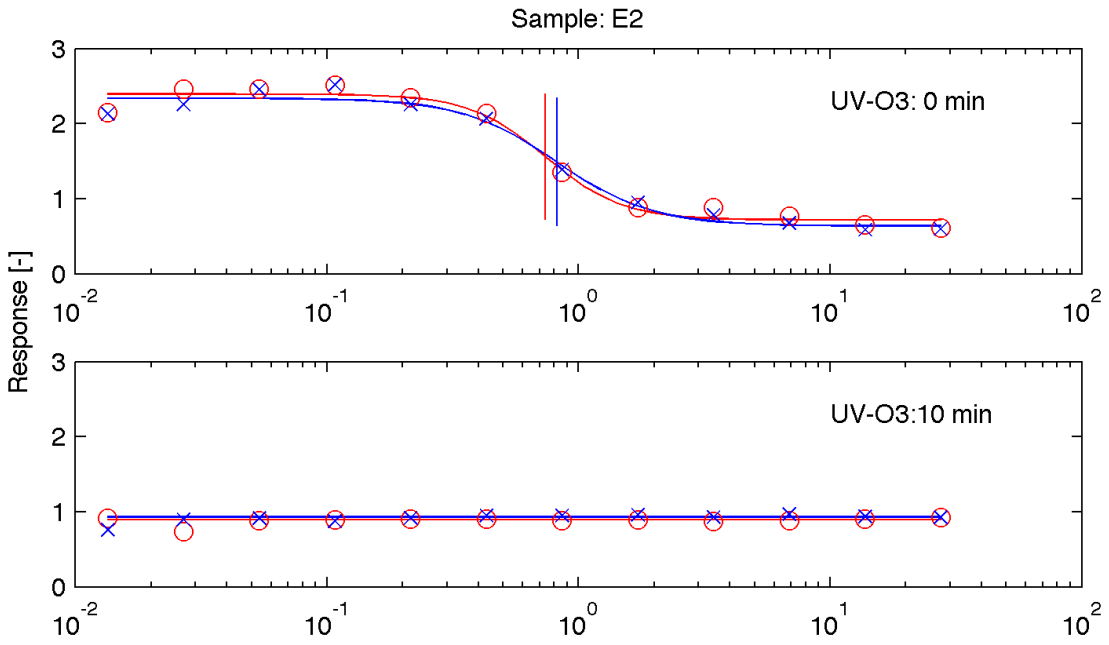


Figure 4.10: Reduction in the estrogencity of E2 C_0 = 0.7 mg/L and pH= 6.4 after different treatment times with UV- O_3; UV- intensity on the quartz surface was measured to be 18 mW/cm^2, Ozone dosage is 1.31 mg/L, (x) sample number one, (o) sample number two

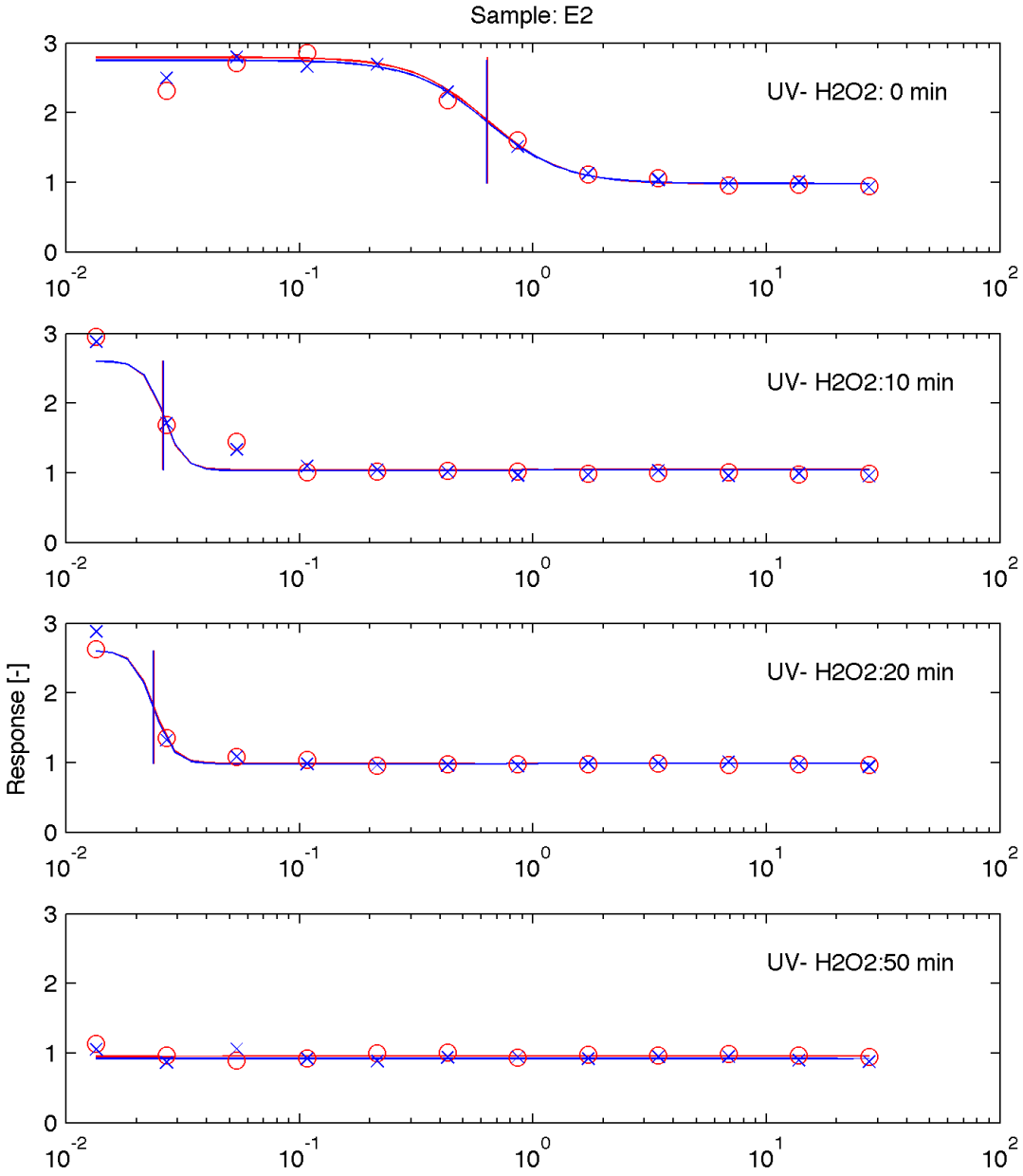


Figure 4.11: Reduction in the estrogencity of E2 C_0 = 0.7 mg/L and pH= 6.4 after different treatment times with UV- H_2O_2; UV- intensity on the quartz surface was measured to be 18 mW/cm^2, H_2O_2 dosage is 10 mg/L, (x) sample number one, (o) sample number two

4.3.2.2 The estrogencity of pure and mixtures of sulfamethoxazole, and humic acid with exposure to advance oxidation processes:

Sulfamethoxazole showed no estrogencity in all three AOPs as shown in Figure 4.12. This result is in line with (Esaheret al. 2005). Humic acid also showed no estrogencity as it binds to the estrogen receptor and blocks the access for estrogenic compounds (Tanghe, Tom; Devriese, Greet; Willy 1999) as shown in Figure 4.13. The combination of SMX and HA showed no estrogencity after different AOPs treatment times as shown in Figure 4.14.

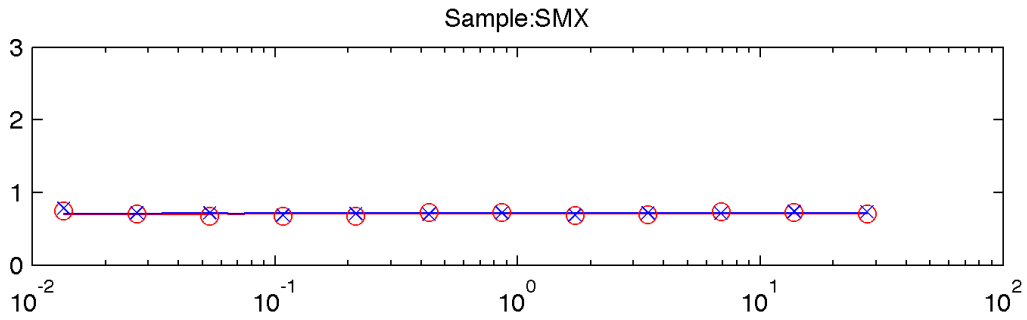


Figure 4.12: SMX C_0 = 80 mg/L and pH = 5.2 showed no estrogencity in all AOPs (x) sample number one, (o) sample number two

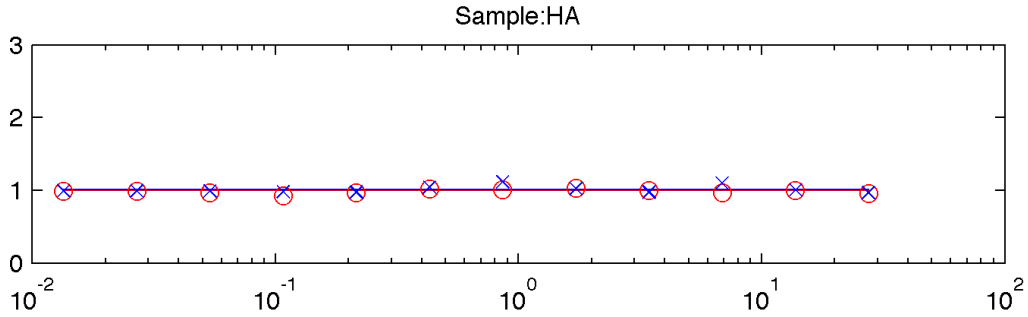


Figure 4.13: HA C_0 = 1000 mg/L and pH = 6.2 showed no estrogencity in all AOPs (x) sample number one, (o) sample number two

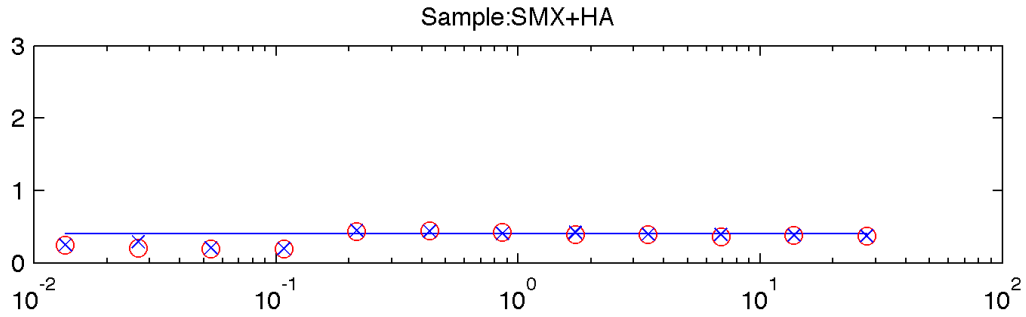


Figure 4.14: SMX and HA mixture showed no estrogencity in all the AOPs, SMX C_0 = 80 mg/L and pH= 5.2, HA C_0 = 100 mg/L and pH= 6.2 (x) sample number one, (o) sample number two

4.3.2.3 The synergistic or antagonistic effect of non-estrogenic compounds on the estrogencity of 17-β estradiol:

As mentioned earlier SMX is not estrogenic: however, in our study it was found that SMX has a synergistic interaction with E2 which led to increase in the measured EEQ by 2.7 times. In addition, it took longer to reduce the estrogencity of SMX and E2 mixture by all the AOPs. For example E2- SMX mixture showed ~ 71% drop in estrogencity in UV/H_2O_2 after 90 min of exposure while pure E2 showed ~ 100% reduction of EEQ after 50 min of UV/H_2O_2 treatment. Ozone showed ~ 98% reduction in estrogencity after 90 min of treatment for E2- SMX mixture as shown in Figure 4.15 a. However, pure E2 had ~ 100% reduction in EEQ after 50 min of treatment. While UV/O_3 showed ~ 100% reduction in estrogencity after 90 min of treatments for E2- SMX mixture as showed in Figure 4.15 b. However, pure E2 gave ~ 100% of reduction measured EEQ after 10 min of treatment.

Of all the AOPs tested UV/O_3 had the best performance in the removal of estrogenicity both for pure E2 and the mixture of E2 and SMX.

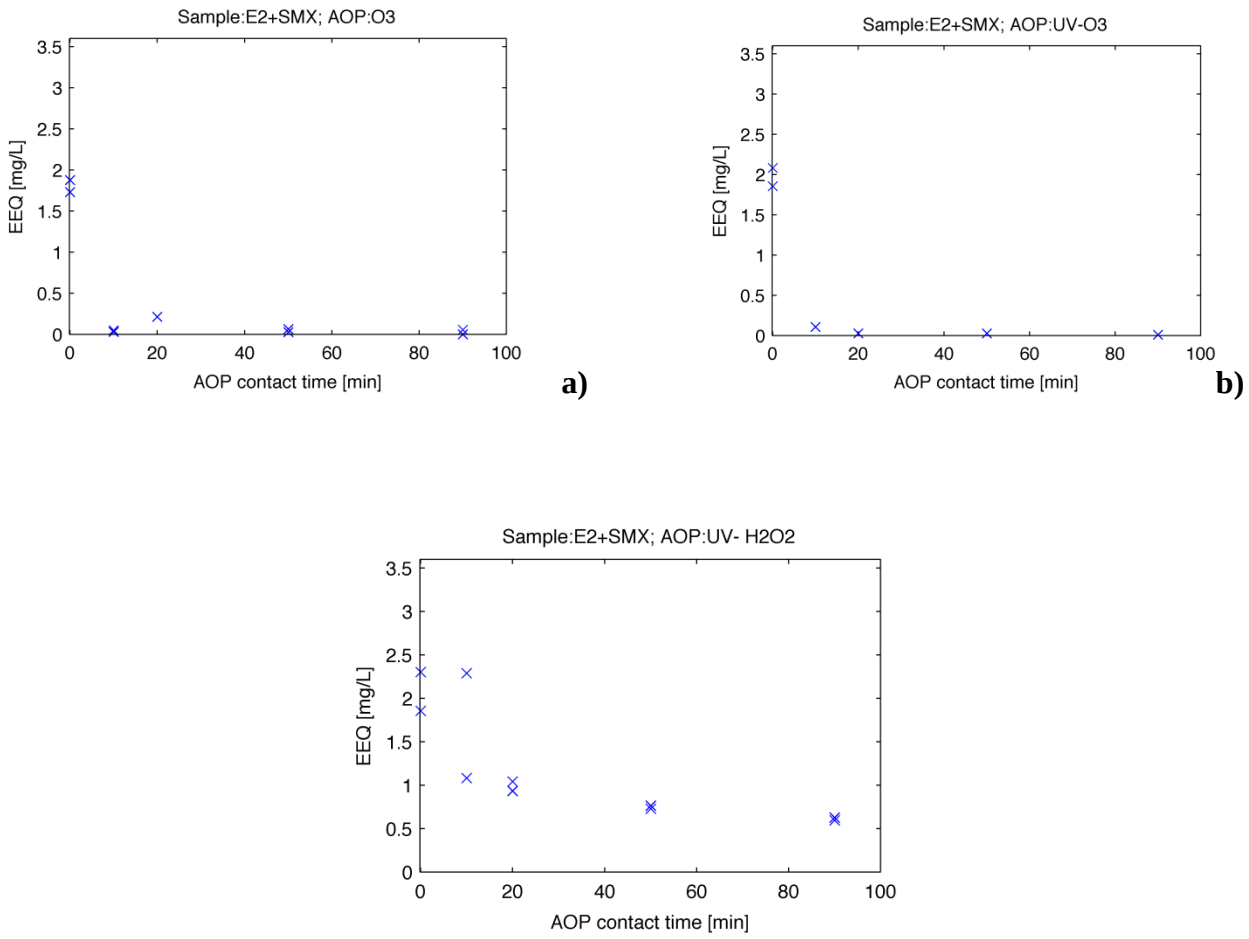


Figure 4.15: The EEQ of E2 and SMX mixture after (a) O_3 (b) UV/O_3 (c) UV/ H_2O_2, SMX C_0 = 80 mg/L and pH= 5.2, E2 C_0 = 0.7 mg/L and pH= 6.4, (x) sample number one and two

Humic acid also showed a synergistic effect on the estrogencity of E2 which led to increase the measured EEQ by 4.4 times, and also it affected the percentage of reduction. As E2-HA mixture gave ~ 98%, 99%, 61% of reduction in the measured EEQ for O_3, UV/O_3 and UV/H_2O_2, respectively after 90 min of treatments whereas it took only 10-15 minutes of AOP treatment for pure E2. UV/H_2O_2 treatment was the least effective of the three AOPs tested as it took longer to reduce the estrogencity. Since very high concentration of humic acid (1000 mg/L) was used in the experiment, adsorption of relatively hydrophobic E2 to carbon-rich HS seems to be insignificant, as shown in Figure 4.16 a, b and c.

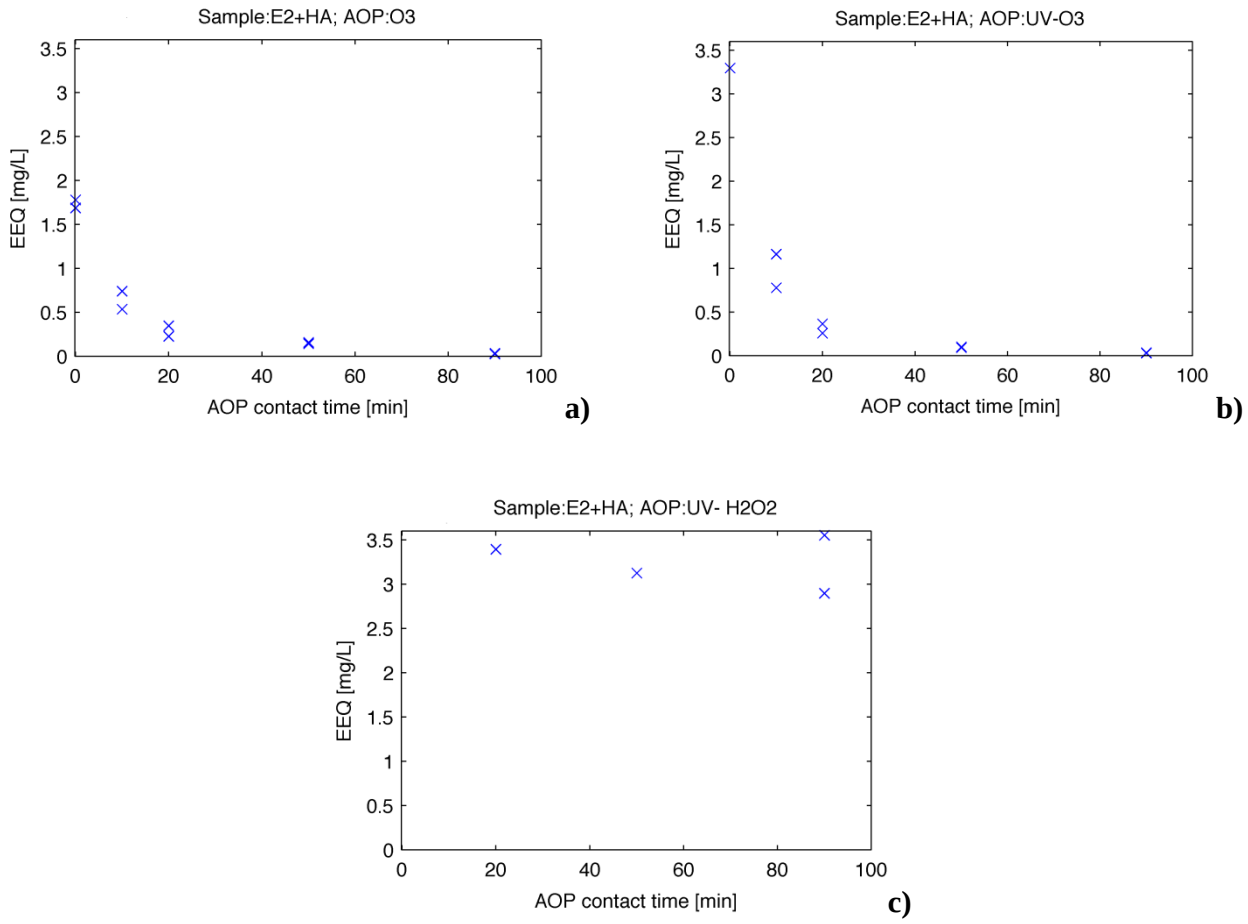


Figure 4.16: The EEQ of E2 and HA mixture after (a) O_3 (b) UV/ O_3, and (c) UV/ H_2O_2, E2 C_0 = 0.7 mg/L and pH= 6.4, HA C_0 = 1000 mg/L and pH= 6.2, (x) sample number one and two

4.3.2.4 The synergistic effect of xenoestrogens compound on the estrogencity of 17-β estradiol:

There was no concentration addition of the EEQ of BPA and E2 when they were mixed together; rather a synergistic interaction between the strong estrogen E2 and a weak xenoestrogen BPA was observed. BPA and E2 mixture showed ~ 100% reduction in the EEQ after 50 min and 20 min for O_3 and UV/O_3 treatment, respectively, as shown in Figure 4.17 a and b. While UV/ H_2O_2 showed only ~ 71% reduction after 90 min of treatment as shown in Figure 4.17 c. Rajapakse et al. (2002) and Silva et al (2002) reported that xenoestrogens are able to act together when combined at concentrations below their no-observed-effect concentration (NOECs) to produce

significant effects. Bliss (1939) mentioned that when the compound is present at the sub threshold doses the individual compound is not assumed to contribute to the overall mixture. It was found that the removal rates of in vitro estrogenic activity of the EDC mixtures were lower than that observed for single compounds for E2 and BPA and in a mixture with 17α-ethinyl estradiol (EE2) and nonylphenol (NP) (Chen et al. 2007). As for the other cases, UV/H_2O_2 treatment took longer compared to simple ozonation and UV/O_3 process.

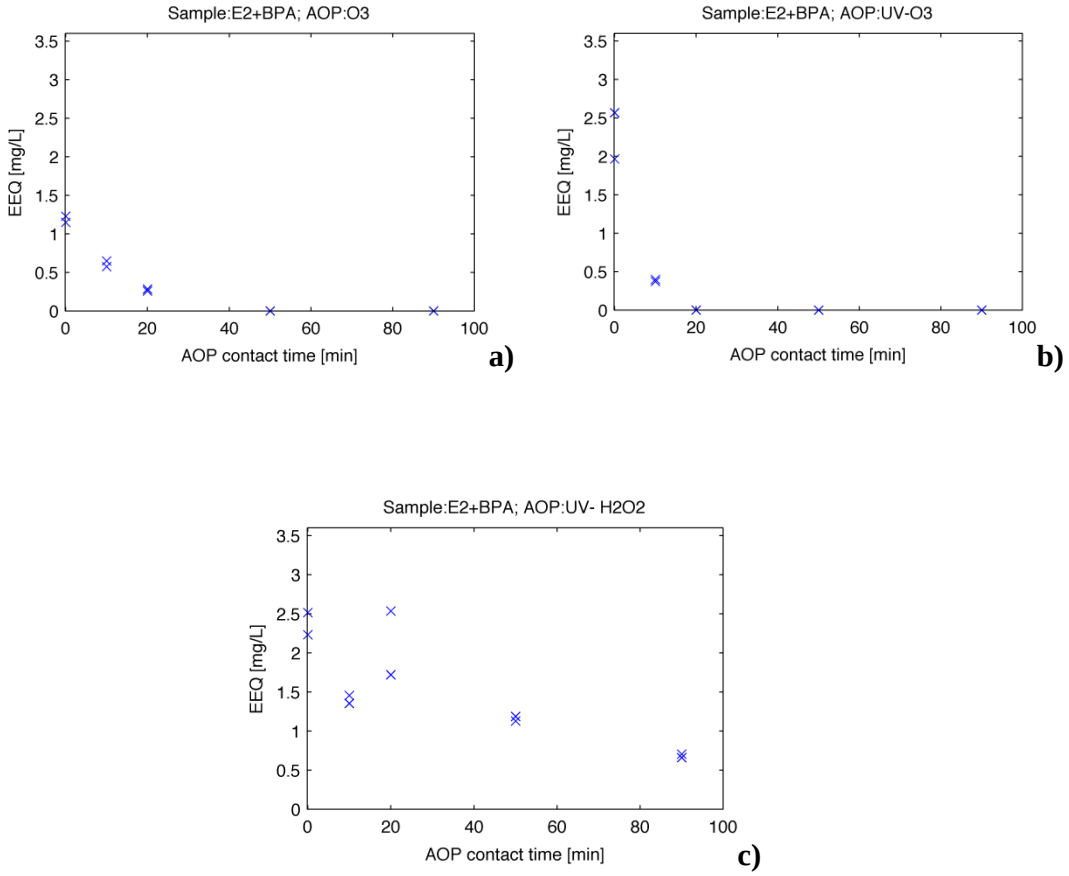


Figure 4.17: The EEQ of E2 and BPA mixture after (a) O_3 (b) UV/ O_3, and (c) UV/ H_2O_2, E2 C_0 = 0.7 mg/L and pH= 6.4, BPA C_0 = 11.6 mg/L and pH= 6, (x) sample number one and two

4.3.2.5 The synergistic - antagonistic interaction of SMX BPA HA mixture on the estrogencity of 17-β estradiol:

It was found that SMX BPA HA E2 mixture gave a ~ 98 % and ~ 99 % reduction after 90 min of treatments in O_3 and UV/O_3 as shown in Figure 4.18 a and b, respectively. However, UV/H_2O_2 showed only ~ 11% reduction after 20 min of exposure as shown in Figure 4.18 c. Then it showed an increase in the measured EEQ by ~ 50 % and 25 % comparing with the original value after 50 min and 90 min, of time, respectively. All this indicates intermediate formation that is more estrogenic than the parent compound. This warrants further chemical and biassays to confirm this result.

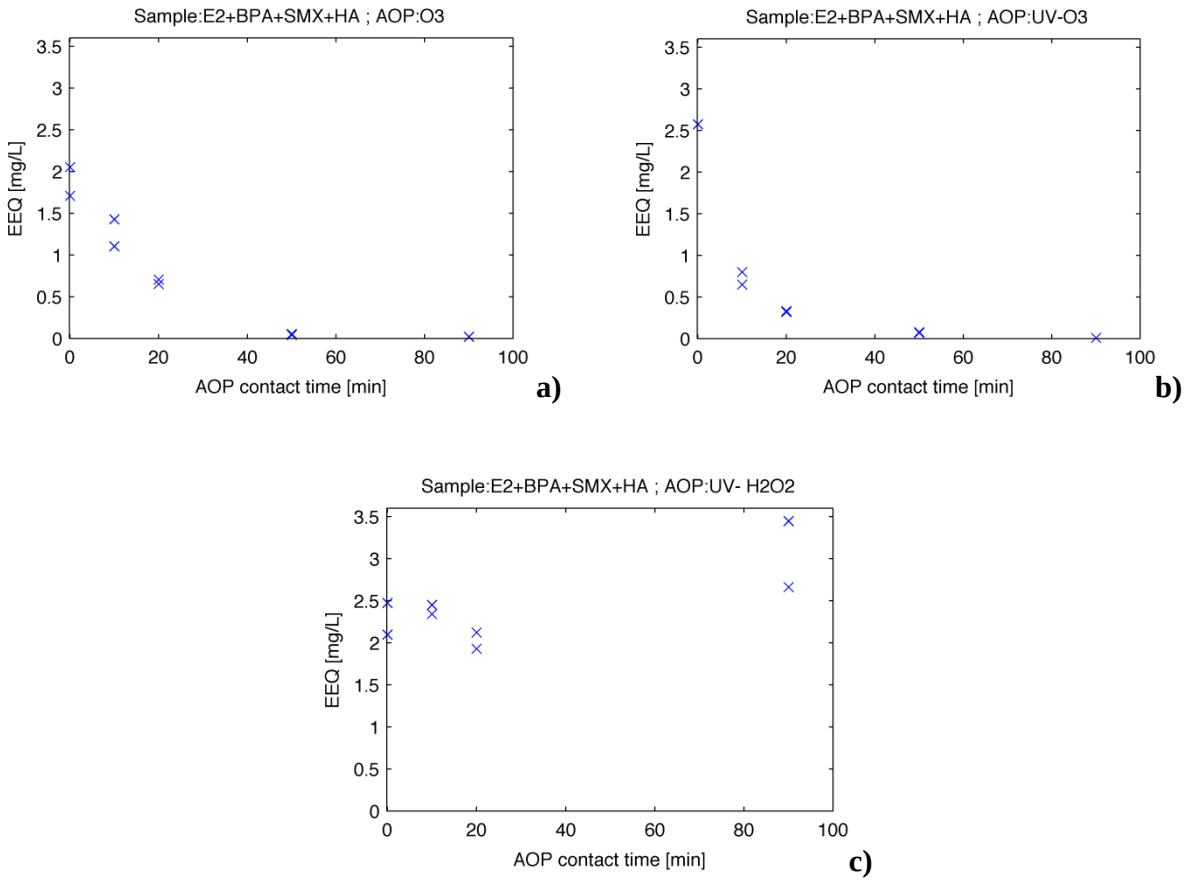


Figure 4.18: The EEQ of E2, BPA, and SMX and HA mixture after (a) O_3 (b) UV/ O_3, and (c) UV/ H_2O_2, E2 C_0 = 0.7 mg/L and pH= 6.4, BPA C_0 = 11.6 mg/L and pH= 6, (x) sample number one and two

4.3.2.6 The antagonistic-synergistic interactions of different mixtures on the estrogencity of 17-β estradiol:

As mentioned earlier, although SMX and humic acid are non-estrogenic compounds; they have a synergistic effect on the estrogencity of E2. HA showed synergistic effect on E2 by increasing the EEQ by 4.4 times, when it was in a mixture with E2. While the addition of SMX showed a lower synergistic effect than HA on the estrogencity of E2 by increasing the EEQ 2.7 times as shown in Table 4.4 and Figure 4.19. The combination of E2 SMX and HA gave the highest synergistic effect; a 4.7 times increase in the EEQ of E2. Chen et al. (2012) & Steinberg et al. (2006) showed that the bioavailability of E2 was increased in the presence of humic acid. Vigneault et al. (2000) mentioned that HA can cause some change in the permeability of biological membranes which could increase the uptake of E2. It is possible that the blocking and inhibition of the modification of multixenobiotic resistance transporter (MXR) activity by direct interaction of DOM with organisms can cause intracellular accumulation of E2 and lead to the increase in estrogenic effects of E2, this could also increase the bioconcentration (Chen et al. 2012).

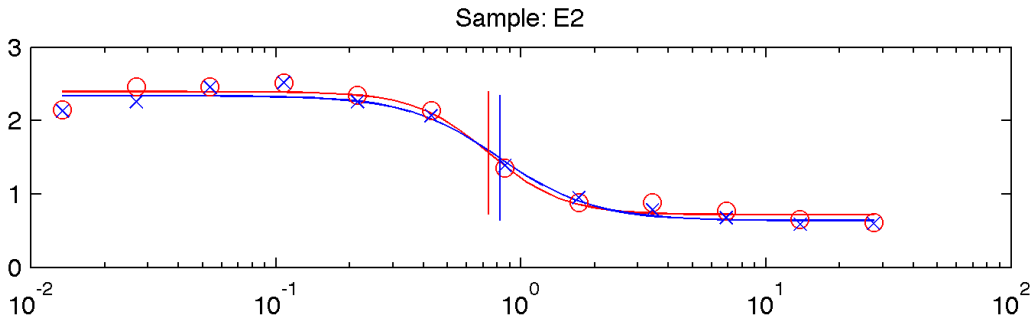
The relative contribution of different compounds on the estrogenicty of E2 was quantified using factorial fit. Of all the different combinations, BPA E2 mixture showed a 2.4 times increase of the EEQ than pure E2 .Thorpe et al. (2003) reported that E2 and BPA when present in a mixture are each able to contribute to the overall effect of the mixture, producing a mixture that is more potent than either of the individual chemicals. E2- BPA- SMX- HA mixture showed 3.4 times increase in the EEQ. The synergistic effect of different compounds with E2 is rather complex and is never additive. Therefore, it may never be possible to estimate the estrogenicity of a mixture a-priori, but YES assay is a powerful tool to determine estrogenicity of a mixture quantitatively without knowing the complex molecular and bio-chemistry. Silva, et al. (2002) mentioned that there is a large difference between the additive estrogenicity by simple concentration addition and independent action (IA) which means that compounds may work on different systems within the organisms (Bliss, 1939). However, Chen, et al., (2007) reported that the estrogenic activity was additive.

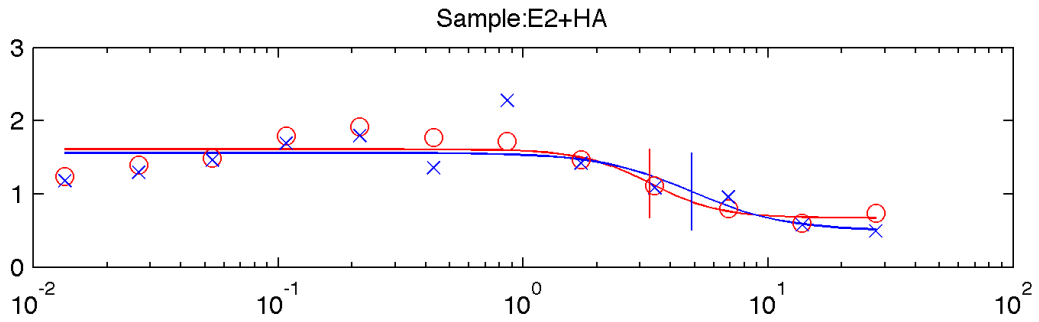
Table 4.4: The EEQ of different mixtures of E2

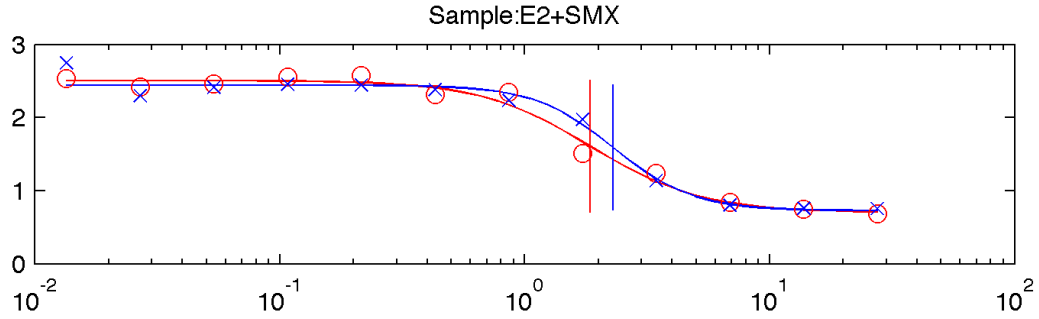
Sample ID	EEQ [mg/L]	±Error [mg/L]	Increase X	Reduction X
E2	0.7	0.1	----	----
E2+ HA	3.1	2.1	4.4	----
E2 SMX	1.9	0.2	2.7	----
E2 SMX HA	3.3	0.7	4.7	----
E2BPA	1.7	0.7	2.4	----
E2 BPA HA	0.2	0.3	----	3.5
E2 BPA SMX	0.2	0.1	----	3.5
E2 BPA SMX HA	2.4	0.8	3.4	----
0.5 E2 BPA SMX HA	0.5	0.7	----	1.4
HA	n.d	n.d		
BPA	n.d	n.d		
SMX	n.d	n.d		
SMX HA	n.d	n.d		
BPA HA	n.d	n.d		
BPA SMX	n.d	n.d		
BPA SMX HA	n.d	n.d		

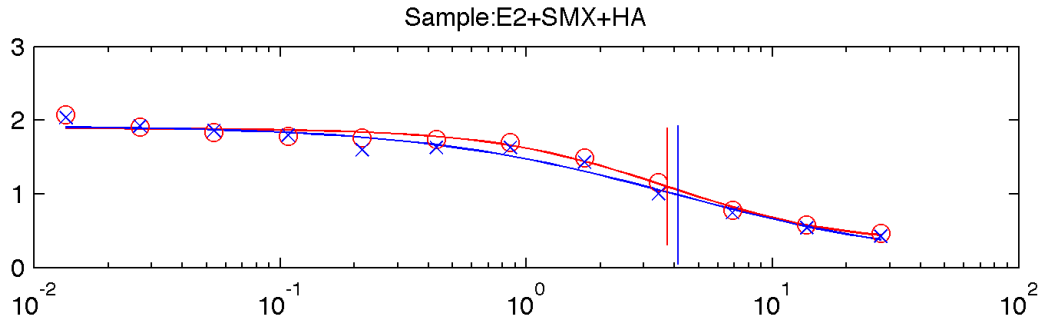
n.d = not detected

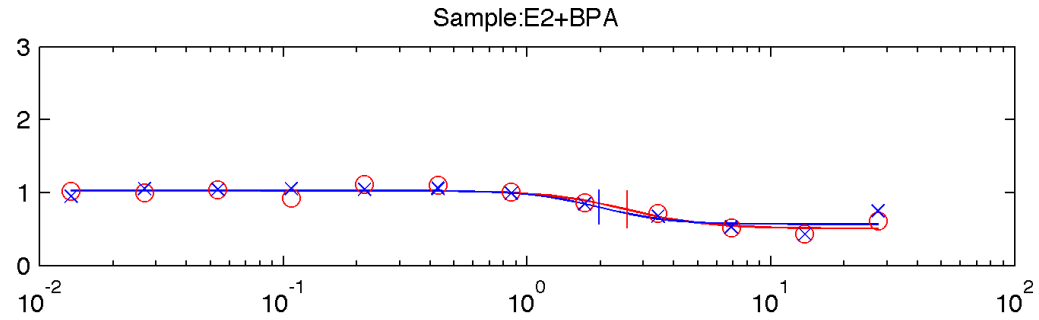
Humic acid can have a masking response of to the estrogenic compound causing low bioavalibility (Tanghe et al. 1999). Membrane transport blockage of gonadotropic hormone, and changes of membrane permeability of E2 can be the reason of antiestrogenic effects (Janosek et al. 2007).




Sample:E2+HA
3
2
1
0
10-2
10-1
100
101
102


Sample:E2+SMX
3
2
1
0
10-2
10-1
100
101
102


Sample:E2+SMX+HA
3
2
1
0
10-2
10-1
100
101
102


Sample:E2+BPA
3
2
1
0
10-2
10-1
100
101
102

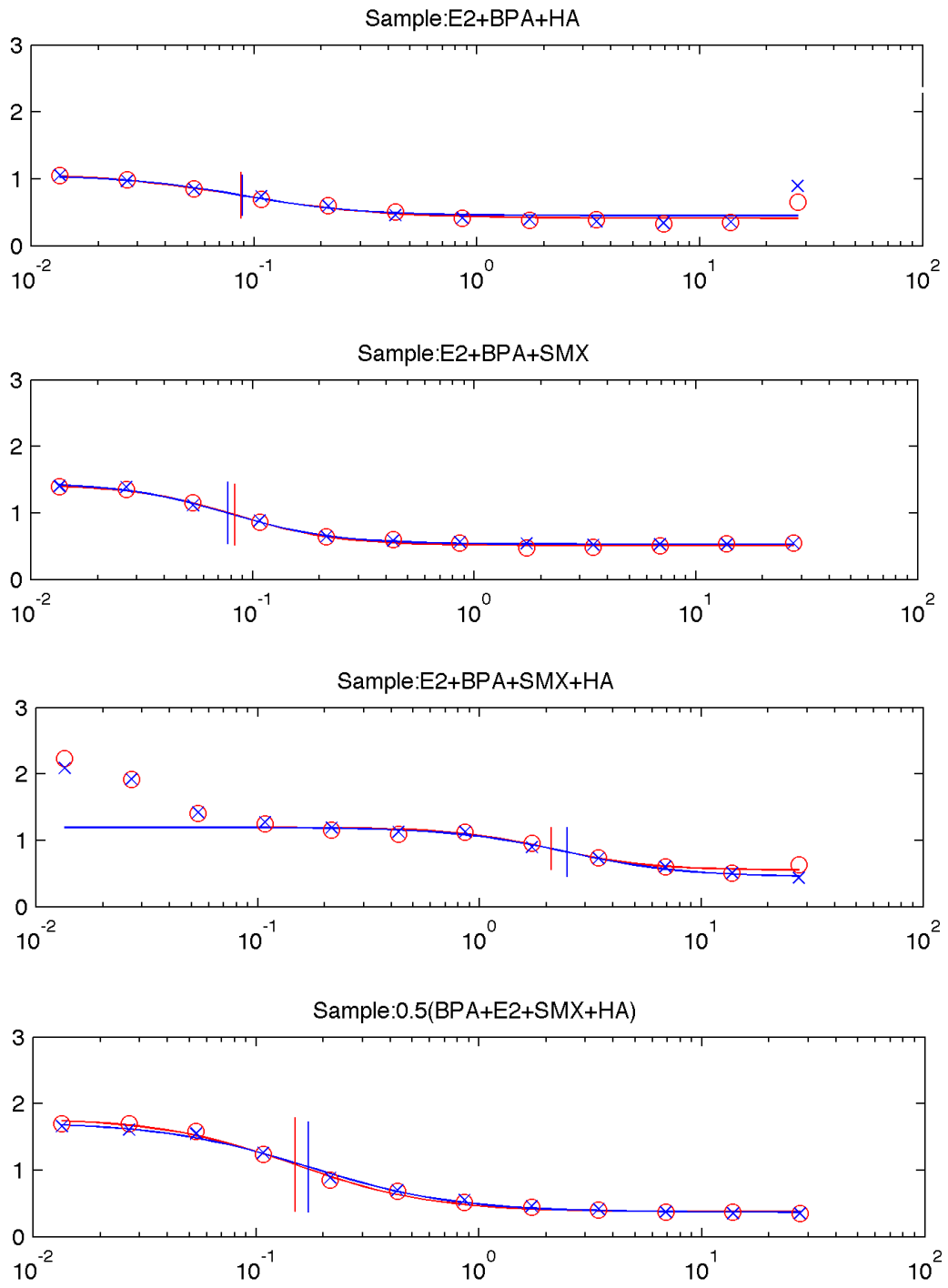


Figure 4.19: The EEQ of different mixture of E2 C_0 = 0.7 mg/L and/ or SMX C_0 = 80 mg/L, and/ or BPA C_0 = 11.6 mg/L and/ or HA C_0 = 1000 mg/L comparing with pure E2, (x) sample number one, (o) sample number two

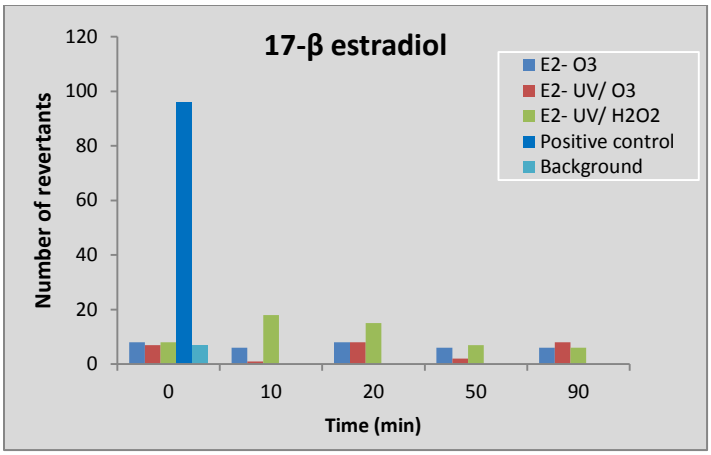
4.3.3 The mutagenicity of 17-β estradiol, Bisphenol A, Sulfamethoxazole, and Humic acid: Effect of different AOPs:

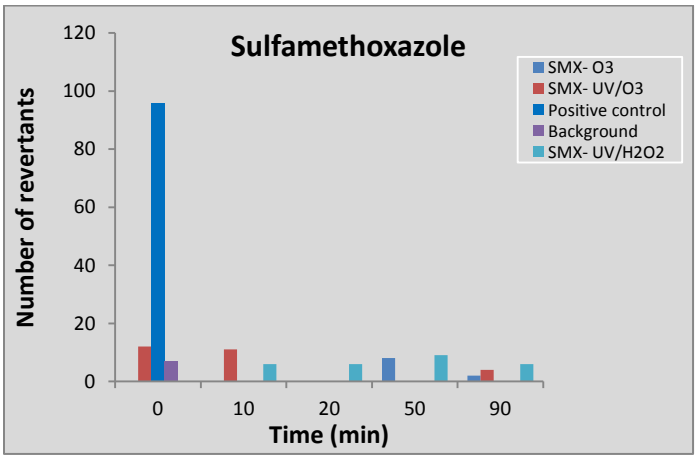
The results of the Ames test for pure 17-β estradiol, bisphenol A, sulfamethoxazole, and humic acid and their mixtures after different exposure times of three advance oxidation treatments (O_3, UV/O_3 and UV/H_2O_2) showed no mutagenicity. Some reverse mutations were observed with *Salmonella typhimurium* TA 98 and TA 100, especially for the UV/H_2O_2 treatment; however, none of them were statistically significant as shown in Figure 4.20 and 4.21.

In a study of the SMX mutagenicity using the Ames spot test with two strains of *Salmonella typhimurium* TA 98 and TA 100, the results were expressed as revertants/µg of antibiotic by analyzing linear regression of the dose–response curves of the samples, which found to be mutagenic (APHA, 1998). In another study done by Dantas et al. (2008), it was found that SMX produced statistically significant increases ($P \leq 0.05$) in mutant frequency.

On the other hand, Nakmuraet al. (1995) performed the Ames spot test; it was found that SMX didn't show mutagenicity to *Sal*. strain TA 98 and TA 100. However, N-acetoxy-SMX showed dose-dependent mutagenicity for *Sal*. TA100. Sulphamethoxazole can form a photodegradation product in aqueous solution by several pathways (Moore, 1998) sometimes forming more harmful byproducts than parent compound (DellaGreca et al., 2003). In addition, it can cause cytotoxic or cytostatic effects in human cells (Abou- Eisha= et al., 2004). However, in these experiments SMX was never mutagenic using any of the advanced oxidation processes.

E2 didn't show mutagenicity in the Ames test, except 16OHE1 is the only estrogen that has been shown to be mutagenic in the Ames test ("Estrogen Metabolism," 2007). On the other hand, Liehr (2000) showed that E2 is a weak carcinogen and a weak mutagen capable of inducing genetic lesions with low frequency. However, the catechol estrogens failed to be mutagenic in the Ames test (Liehr et al., 1986). Humic acid is not mutagenic (Sato et al., 1986): however, the chlorinated humic acid showed a mutagenic response in the Ames test (Coleman et al., 1984) (Coleman et al. 1984& Hemming, J. et al, 1986). BPA is not mutagenic (Ike et al. 2002), and from our previous study Gilmour et al. (2012) was found that BPA is not genotoxic. In this study, it is confirmed that the mixture of BPA, SMX, E2 and HA is also not mutagenic under any of the AOP treatments, however, UV/H_2O_2 treatment produced somewhat higher numbers of mutants in some of the mixtures.





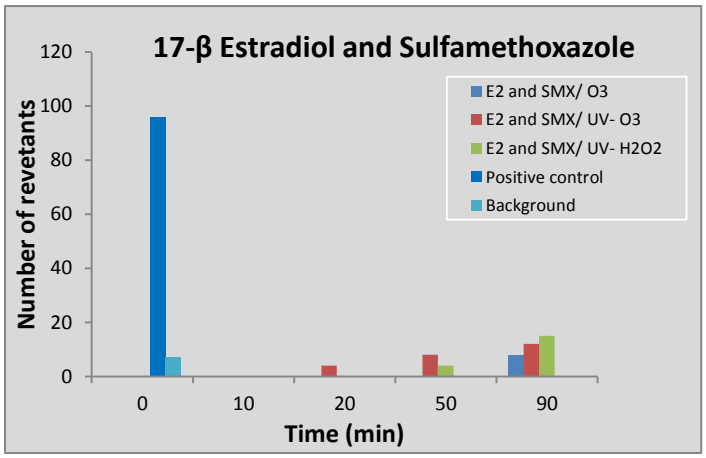
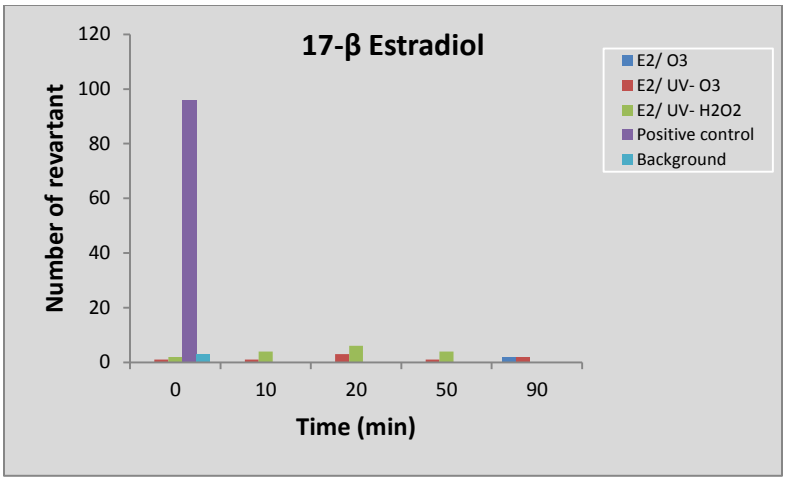
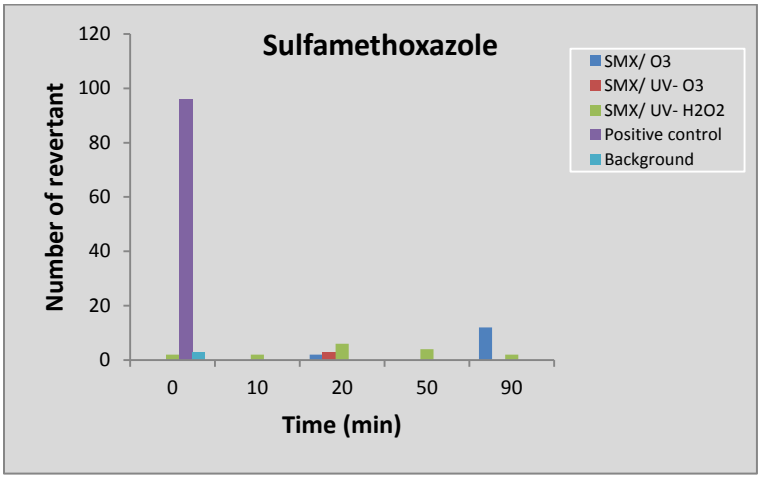


Figure 4.20: The Ames result for *Salmonella typhimurium* TA 97





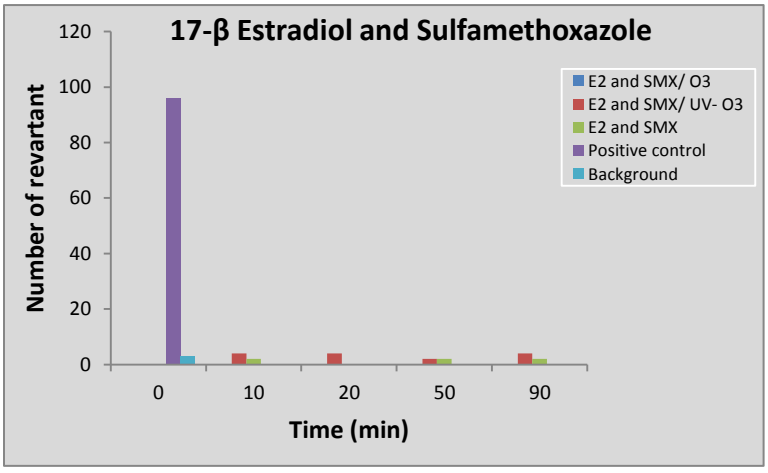


Figure 4.21: The Ames result for *Salmonella typhimurium* TA 100

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Chapter Five

Conclusions and Recommendations

5.1 Conclusions

- SMX showed ~ 100% removal in all the AOPs; whereas E2 and BPA showed much higher degradation in ozonation compared to UV processes.
- The addition of UV with O_3 produced significant increase in degradation rate for SMX; however, it only increased by 18% and 5% for E2 and BPA, respectively.
- The combination of H2O2 with UV produced faster degradation rate for SMX; whereas, it was the lowest for E2, and the rate was reduced by 86% from that of UV/O3 for BPA .
- Humic acid demonstrated the lowest degradation rate of all the compounds tested, and UV/ O3 and UV/ H2O2 demonstrated comparable rates.
- All the mixtures of SMX after ozonation gave a higher degradation rate when they were combined with HA. On the other hand, E2 gave a higher rate when it was alone. While the effect of HA on BPA degradation was mixed.
- There is a significant difference between the degradation of the parent compounds and complete mineralization indicated by low TOC removal.
- The percentage of TOC removal was reduced when HA was added to the mixture.
- HA and SMX are not estrogenic; however, when they were in the mixture with E2 they had a synergistic interaction that led to increase in estrogencity by 2.7- 4.7 times.
- BPA is a weak xenoestrogen that was able to create an impact upon E2 which is a strong estrogen by increasing the estrogencity of E2 by 2.4 times.
- Some mixtures showed an antagonistic interaction that resulted in dropping EEQ. The exact mechanism for this drop in estrogencity needs to be investigated.
- UV/ O3 is the best AOPs in this experimental conditions in terms of parent compound degradation, mineralization and reduction in the estrogencity, followed by ozonation. UV/H2O2 performed poorly in many of the cases.
- No mutagenicity was shown by the Ames test for all pure compounds and mixtures after different exposure times, which means that the intermediates that produced from the parent compound are not mutagenic.

5.2 Recommendations for future study

On the basis of the present study, some areas were revealed to be of significant interest for future research. They are listed as follows:

- Further testing to include assays to monitor in-vivo effect of these micropollutants mutagenicity and estrogencity and environmental ecotoxicity

- Further bioassay analysis for spiked SMX, BPA, E2 and HA into wastewater and drinking water samples to evaluate complex matrix effect on the toxic by-product formation during the degradation in AOPs.

- Studying the impact of pH and other AOPs such as Fenton's reagent, microwave and OH• radical scavengers.

- Quantifying intermediates of 17-β estradiol, bisphenol A, sulfamethoxazole, and humic acid that have been formed which are more estrogenic; in order to determine the effective AOPs to degrade them.

Appendix

Appendix 6.1: the MAT Lab code for the EEQ calculation of the YES assay.

```
[data,text,raw]=xlsread('All_Data.xlsx','','F5:Q536');
%blank=xlsread('All-Data.xlsx','','F6:Q7');
%standsample=xlsread('All-Data.xlsx','','F3:Q5');
[times,text, raw]=xlsread('All_Data.xlsx','','A5:C536');
times=num2str(times);
AOP_Titles=strcat('Sample:  ', raw(:,1), '; AOP: ', raw(:,3), '; Time: ',times);

[ndata,mdata]=size(data);


%examin data
highvalues=max(data')';
stdev_high=std(highvalues)/mean(highvalues)*100;
highvalue=mean(highvalues);
%=> fix upper fitting parameter to highvalue and lower parameter to zero



%create vector with dilition factor assuming first well was diluted 10 in
%200 uL
DF=0.05;
for i=2:12
   DF(i)=DF(i-1)/2;
end

c=logspace(log10(min(DF)),log10(max(DF)));


% %Fitting all four parameters for data

for g=1:ndata
   Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
   p0=[0.001,2,0.7,1.8];
   options = fitoptions(Hilleq2);
   options.Lower = [0,0,0,0];
   options.StartPoint = p0;
   curve = fit( DF', data(g,:)', Hilleq2, options );
   newcoeffcientsdata(g,:)=coeffvalues(curve);

end


% fixing outliers

   %fixing set 29 and 30 by fixing b
for g=29:30;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
   p0=[0.001,2,0.7,1.8];
```

```
    options = fitoptions(Hilleq2);
    options.Lower = [0,0,0,1.6];
    options.Upper = [10,10,10,1.601];
    options.StartPoint = p0;
    curve = fit( DF', data(g,:)', Hilleq2, options );
    newcoeffcientsdata(g,:)=coeffvalues(curve);
end
    %fixing set 39 by fixing b
g=39;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
    p0=[0.001,2,0.7,1.8];
    options = fitoptions(Hilleq2);
    options.Lower = [0,0,0,1.65];
    options.Upper = [10,10,10,1.651];
    options.StartPoint = p0;
    curve = fit( DF', data(g,:)', Hilleq2, options );
    newcoeffcientsdata(g,:)=coeffvalues(curve);

    %fixing set 49 by fixing b
g=49;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
    p0=[0.001,2,0.7,1.8];
    options = fitoptions(Hilleq2);
    options.Lower = [0,0,0,2.25];
    options.Upper = [10,10,10,2.251];
    options.StartPoint = p0;
    curve = fit( DF', data(g,:)', Hilleq2, options );
    newcoeffcientsdata(g,:)=coeffvalues(curve);

    %fixing set 331 and 340 by fixing b
for g=331:340;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
    p0=[0.001,2,0.7,1.8];
    options = fitoptions(Hilleq2);
    options.Lower = [0,0,0,1.2];
    options.Upper = [10,10,10,1.201];
    options.StartPoint = p0;
    curve = fit( DF', data(g,:)', Hilleq2, options );
    newcoeffcientsdata(g,:)=coeffvalues(curve);
end

    %fixing set 341 and 350 by fixing b
for g=341:350;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
    p0=[0.001,2,0.7,1.8];
    options = fitoptions(Hilleq2);
    options.Lower = [0,0,0,1.34];
    options.Upper = [10,10,10,1.341];
    options.StartPoint = p0;
    curve = fit( DF', data(g,:)', Hilleq2, options );
    newcoeffcientsdata(g,:)=coeffvalues(curve);
end
```

```
   %fixing set 351 and 360 by fixing b
for g=351:360;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
   p0=[0.001,2,0.7,1.8];
   options = fitoptions(Hilleq2);
   options.Lower = [0,0,0,1.1];
   options.Upper = [10,10,10,1.11];
   options.StartPoint = p0;
   curve = fit( DF', data(g,:)', Hilleq2, options );
   newcoeffcientsdata(g,:)=coeffvalues(curve);
end

   %fixing set 443 and 446 by fixing b
for g=443:446;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
   p0=[0.001,2,0.7,1.8];
   options = fitoptions(Hilleq2);
   options.Lower = [0,0,0,2.8];
   options.Upper = [10,10,10,2.81];
   options.StartPoint = p0;
   curve = fit( DF', data(g,:)', Hilleq2, options );
   newcoeffcientsdata(g,:)=coeffvalues(curve);
end

  %fixing set 465 by fixing b
g=465;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
   p0=[0.001,2,0.7,1.8];
   options = fitoptions(Hilleq2);
   options.Lower = [0,0,0,3.6];
   options.Upper = [10,10,10,3.61];
   options.StartPoint = p0;
   curve = fit( DF', data(g,:)', Hilleq2, options );
   newcoeffcientsdata(g,:)=coeffvalues(curve);

     %fixing set 467 by fixing b
g=467;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
   p0=[0.001,2,0.7,1.8];
   options = fitoptions(Hilleq2);
   options.Lower = [0,0,0,2.6];
   options.Upper = [10,10,10,2.61];
   options.StartPoint = p0;
   curve = fit( DF', data(g,:)', Hilleq2, options );
   newcoeffcientsdata(g,:)=coeffvalues(curve);

   %fixing set 473 and 480 by fixing b
for g=443:446;
Hilleq2=fittype(@(EC50, m, a, b, x) a+(b-a)./(1+(x./EC50).^(-m)));
   p0=[0.001,2,0.7,1.8];
   options = fitoptions(Hilleq2);
```

```
    options.Lower = [0,0,0,2.6];
    options.Upper = [10,10,10,2.61];
    options.StartPoint = p0;
    curve = fit( DF', data(g,:)', Hilleq2, options );
    newcoeffcientsdata(g,:)=coeffvalues(curve);
end



% fixing irrelevent EC50 vlaues
newcoeffcientsdata(1:10,1)=100;
for fixer=44:2:70
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=67:2:69
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=85:1:90
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=105:1:110
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=125:1:130
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=147:1:150
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=161:1:290
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=297:1:300
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=307:1:310
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=317:1:320
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=377:1:380
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=397:1:400
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=401:1:430
   newcoeffcientsdata(fixer,1)=100;
end
for fixer=433:1:440
   newcoeffcientsdata(fixer,1)=100;
end
```

```
for fixer=447:1:450
  newcoeffcientsdata(fixer,1)=100;
end
for fixer=491:1:532
  newcoeffcientsdata(fixer,1)=100;
end

%plotting newly fitted data
for g=2:2:528
  AOP=ceil(g/10);
  figure(AOP)

  fitteddatanewdata(g-1,:)= newcoeffcientsdata(g-1,3)+(newcoeffcientsdata(g-1,4)-newcoeffcientsdata(g-
1,3))./(1+(c./newcoeffcientsdata(g-1,1)).^(-newcoeffcientsdata(g-1,2)));
  fitteddatanewdata(g,:)= newcoeffcientsdata(g,3)+(newcoeffcientsdata(g,4)-
newcoeffcientsdata(g,3))./(1+(c./newcoeffcientsdata(g,1)).^(-newcoeffcientsdata(g,2)));
  subplot(5,1,(g-10*(AOP-1))/2)
  semilogx(c, fitteddatanewdata(g-1,:),'r')
  hold on
  semilogx(c, fitteddatanewdata(g,:))
  semilogx(DF, data(g-1,:), 'ro')
  semilogx(DF, data(g,:), 'x')
  title(AOP_Titles(g-1))
  semilogx([newcoeffcientsdata(g-1,1), newcoeffcientsdata(g-1,1)],[newcoeffcientsdata(g-1,3),
newcoeffcientsdata(g-1,4)], 'r', [newcoeffcientsdata(g,1), newcoeffcientsdata(g,1)],[newcoeffcientsdata(g,3),
newcoeffcientsdata(g,4)])
  axis([10^-5 10^-1 0 3])

end

for g=530:2:532
  AOP=ceil((g+2)/10);
  figure(AOP)

  fitteddatanewdata(g-1,:)= newcoeffcientsdata(g-1,3)+(newcoeffcientsdata(g-1,4)-newcoeffcientsdata(g-
1,3))./(1+(c./newcoeffcientsdata(g-1,1)).^(-newcoeffcientsdata(g-1,2)));
  fitteddatanewdata(g,:)= newcoeffcientsdata(g,3)+(newcoeffcientsdata(g,4)-
newcoeffcientsdata(g,3))./(1+(c./newcoeffcientsdata(g,1)).^(-newcoeffcientsdata(g,2)));
  subplot(5,1,(g+2-10*(AOP-1))/2)
  semilogx(c, fitteddatanewdata(g-1,:),'r')
  hold on
  semilogx(c, fitteddatanewdata(g,:))
  semilogx(DF, data(g-1,:), 'ro')
  semilogx(DF, data(g,:), 'x')
  title(AOP_Titles(g-1))
  semilogx([newcoeffcientsdata(g-1,1), newcoeffcientsdata(g-1,1)],[newcoeffcientsdata(g-1,3),
newcoeffcientsdata(g-1,4)], 'r', [newcoeffcientsdata(g,1), newcoeffcientsdata(g,1)],[newcoeffcientsdata(g,3),
newcoeffcientsdata(g,4)])
  axis([10^-5 10^-1 0 3])

end

  %exporting tif files
```

```
  for AOP=1:ceil(g/10) +1
    fnam=strcat('Fig_',num2str(AOP),'.tif');
    snam='sura';
    s=hgexport('readstyle',snam);
    s.Format='tiff';
    hgexport(AOP,fnam,s);
  end


EC50=newcoeffcientsdata(:,1);
xlswrite('EC50.xlsx', EC50);
```

Appendix 6.2:

The rest of the EEQ figures for the YES assay using Mat Lab is attached in appendix 6.2.

Curriculum Vitae

Name: Sura Ali

Post-Secondary Education and Degrees:

Master Degree of Science in Veterinary Medicine/ Microbiology 1999
University of Baghdad

Bachelor of Veterinary Medicine and Surgery 1996
University of Baghdad

Awards and Scholarships

Western Graduate Research Scholarship- WGRS.

Related work experiences

Microbiologist R&D/ Germiphene Corporation/ Canada	May 2014- Current
Teaching assistant/ Western University/ Canada	Sep 2012- Dec 2013
Research associate / The University of Western Ontario/ Canada	May- Nov, 2011
Microbiologist/ EMC Scientific Incorporated/ Canada	Dec 2006- Mar 2011
University Faculty Member/ Faculty of Science-	Jan 2000- Aug 2006
-The 7th of April University/ Libya	
Laboratory Technician/ Al-Canal Medical Laboratory /Iraq	Sep 1996- Dec 2000

Selected publications:

Sarkar, S, **Ali, S.,** Nakhla G., Rehmann, L. and M. Ray. 2014. Degradation of Estrone in Water and Wastewater by Various Advanced Oxidation Processes. Journal of Hazardous Materials 278, 16–24.

Chawla, C. Sarkar, S**, Ali, S**., Nakhla G., Rehmann, L. and M. Ray. 2014. Anaerobic Digestibility of Estrogens in Wastewater Sludge: Effect of Ultrasonic Pretreatment. Journal of Environmental Management 145, 307-313.

Sarkar, S, **Ali, S**., Nakhla G., Rehmann, L. and M. Ray. 2013. Advanced Oxidation of Estrone in Water and Wastewater. American Institute of Chemical Engineers (AICHE) Conference, San Francisco, California, USA. Aug 2013.

Referred Conference Proceedings:

Ali, S., Rehmann, L., Ray, M.. 2013, Genotoxicity and estrogencity of sulfamethoxazole and 17-estradiol: effect of advance oxidation treatments. 63rd Canadian Chemical Engineering Conference, October 23, 2013, Fredericton, NB, Canada..

Ali, S., Sarkar, S, Rehmann, L., Ray, M. 2013, Bioassay for Estrogencity of Micropollutants in Wastewater after Ultrasonication as a pre-treatment, 15th CSChE Quebec-Ontario Biotechnology meeting, May 30- 31, 2013, Quebec, Canada.

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