**University of Salerno**
Department of Civil Engineering

**Doctor of Philosophy in**
Risk and Sustainability
in Civil, Architectural and
Environmental engineering systems -
XXXIII cycle (2018-2021)

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# TERTIARY TREATMENT OF URBAN WASTEWATER BY ADVANCED OXIDATION PROCESSES

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**Doctoral thesis**

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# ACKNOWLEDGEMENTS

I would like to thank, first of all, my supervisor, *Prof. Luigi Rizzo*, who was always available to help and support me, for his enthusiasm and motivation that he has continuously passed on to me, for his ability to explain complex things in simple words, for his kindness and patience.

I would also like to thank my co-tutor, *Dr. Sixto Malato*, for his valuable scientific contributions to my thesis project and experimental work.

I am also very grateful to *Dr. María Inmaculada Polo-López* and *Dr. Isabel Oller* for them precious help and tireless support during my stay in the *Plataforma Solar de Almería*; to *Irene Salmerón* for the time she devoted to teaching me some practical aspects.

I would like to extend my thanks to *Antonino Fiorentino, Maurizio Carotenuto, Giusy Lofrano* and *Vincenzo Vaiano* for the help with the experimental work at the *University of Salerno*.

I would like to warmly thank *Paolo Napodano* for often being the person in the lab I could always rely on for help, be it either logistical or to find creative solutions to practical problems in the lab.

Many thanks to my unique friends who made bright my days during the studies: *Anastasia*, *Leisan*, *Giuseppe*, *Kasia*, *Ilaria* and *Samira*. Each friend listed (and not) played a special role in my time here.

I would like to give a heartfelt thank to *Laysan* and *Margarita* for supporting me always, for help to make the decision to accept the PhD position and move on to Italy. They kept me going on when I wanted to give up, they were always available to listen to me, in difficult moments they were always close despite the distance.

Acknowledgments could not be complete without giving thanks to my family for their daily unwavering emotional and moral support. My parents have made many sacrifices for me, have taught me to be a hard worker and a resolute woman, have accepted my choices always providing their unconditional care and love.

Lastly (but certainly not least), I wish to thank the person that I have met during the implementation of my PhD thesis. He shared my joys and sorrows and took care of my feelings.

# ABSTRACT

The increasing demand of food to satisfy the needs of a growing world population alongside the rate of economic development and high population are putting incomparable pressure on water resources. As a result of this impact, water resources quality is steadily decreasing. Several organic pollutants such as pesticides, pharmaceuticals, hormones, personal care products and their metabolites also known as contaminants of emerging concern (CECs) are continuously released into the environment from urban wastewater treatment plants (UWWTPs) effluents. Although occurring at low concentrations (ng/L−µg/L) they can persist into the environment resulting in significant acute and chronic toxicity, with potential collateral effect on human health and aquatic ecosystems. Unfortunately, conventional UWWTPs are unable to provide an effective removal of several CECs. To overcome this problem, in recent years research has been focused on the investigation of new processes/technologies for tertiary treatment of urban wastewater in the attempt to effectively remove CECs and pathogens before effluent disposal or reuse. Advanced Oxidation Processes (AOPs), which are based on the formation of highly oxidative species (mainly hydroxyl radicals, $HO^{\bullet}$), have found to be effective in CECs removal as well as in bacteria inactivation.

The main aim of this PhD thesis work was to investigate the effects of different AOPs as tertiary treatment methods of urban wastewater under realistic conditions using different endpoints: CECs removal, pathogens inactivation and toxicity. In this study, five pollutants have been selected as model CECs (Caffeine, Carbamazepine, Diclofenac, Sulfamethoxazole and Trimethoprim) and three human bacterial pathogens (*E. coli, Salmonella spp and Enterococcus spp*) as model of microbial contamination because they are typically detected in UWWTP effluent.

Firstly, homogeneous solar driven AOPs, namely sunlight/$H_2O_2$, solar photo-Fenton (SPF) and SPF with EDDS were compared to a new solar driven heterogeneous photocatalytic (namely sunlight/N-$TiO_2$ doped) process with the aim of contributing to fill the gap between lab scale tests and full scale applications as well as to provide a sustainable solution for tertiary treatment in small UWWTPs. Process efficiency was evaluated in terms of effluent toxicity and degradation of a mixture of three CEC (namely Carbamazepine, Diclofenac and Trimethoprim), at initial concentration of 200 µg/L each, in deionized water (DW) and real UWWTP effluent (WW). SPF with EDDS was found to be the most effective process (99% removal of CEC from WW in 15 min, $Q_{uv}$ =1.2 kJ/L). Conventional SPF was drastically and

negatively affected by water matrix, due to the spontaneous neutral pH and iron precipitation in real WW. Although sunlight/N-$TiO_2$ process was not so affected by water matrix, it was found to be less efficient than SPF with EDDS. Toxicity values were found to be lower in WW compared to DW matrix. WW samples showed a toxicity reduction up to the no acute toxicity level for sunlight/N-$TiO_2$ and SPF with EDDS treatments, while sunlight/$H_2O_2$ and SPF increased the final effluent toxicity up to slightly acute levels.

Subsequently, SPF with EDDS at near neutral pH was investigated in the simultaneous removal of a mixture of CECs and bacteria inactivation in simulated urban wastewater treatment plant effluent (SUWW). Process efficiency was evaluated in terms of (i) degradation of five CECs (namely Caffeine, Carbamazepine, Diclofenac, Sulfamethoxazole and Trimethoprim) at the initial concentration of 100 μg/L each and (ii) bacteria inactivation (*E. coli*, *S. enteritidis* and *E. faecalis*), at the initial concentration of $10^3$ CFU/mL each. The aim of 80% removal of total CECs was selected according to Switzerland regulation in UWWTPs, being the only Country that has established a release limitation for such compounds from UWWTPs into the environment. SPF with EDDS was investigated at lab scale in a solar simulator to evaluate the effect of iron concentration (0.1 mM and 0.05 mM) and Fe:EDDS ratio (1:2 and 1:1). 80% removal of total CECs was not achieved in the experiment with Fe (III) at concentration 0.05 mM with 1:2 molar ratio to EDDS. To evaluate the effect of organic matter, SPF with EDDS was investigated even in SUWW without organic compounds (SUWW-woc). Organic matter negatively affected process efficiency. Total inactivation of all bacteria and 80 % removal of CECs was achieved only in the experiment with Fe:EDDS 1:1 in SUWW-woc, accordingly, such operating condition was chosen for the scaling-up to pilot plant with raceway pond reactor (RPR).

Therefore, in the third part of the work, SPF with EDDS at near neutral pH and sunlight/$H_2O_2$ processes operated in RPR were compared with ozonation under different end points (CECs removal, bacteria inactivation and toxicity). Process efficiency was evaluated first in terms of simultaneous inactivation of *E. coli*, *Salmonella spp* and *Enterococcus spp*. The effect of inorganic matter was also evaluated. The highest bacteria inactivation rate was observed for ozonation. The detection limit (DL) (1 CFU/mL) was reached for all pathogens in 45 min treatment in WW. The inactivation of all bacteria in WW by sunlight/$H_2O_2$ (50 mg/L) and SPF with EDDS (at 1:1 molar ratio, 0.1 mM of Fe and 50 mg/L of $H_2O_2$) showed similar behavior. Although the DL was not reached in WW, faster inactivation kinetics and lower bacterial concentration after 180 min of treatment time was observed for sunlight/$H_2O_2$ process. In general, the results showed different bacterial inactivation profiles under the same operational conditions, observing for both treatments the following order of inactivation kinetics: *Salmonella spp*>*E. coli*>*Enterococcus spp. Enterococcus spp* showed high resistance to solar driven investigated processes. Quantitative microbial risk assessment (QMRA) for the ingestion of a raw-vegetable (lettuce) irrigated with untreated and treated WW was also estimated. Disinfection by ozonation and sunlight/$H_2O_2$ processes was found to drastically decrease the associated microbiological risk. Then, degradation of five CECs (namely Caffeine, Carbamazepine, Diclofenac, Sulfamethoxazole and Trimethoprim) at the initial concentration of 100 µg/L each and toxicity were investigated. Treatment of CECs by SPF and ozonation at neutral pH was demonstrated in CEC-enriched simulated and real WW. SPF has shown to be strongly affected by water matrix, since in SUWW-woc the 80 % removal of the total CECs was reached in 10 min while in WW after 180 min. 80% removal of total CECs could be achieved in 15 minutes by ozonation in real WW. Toxicity of treated effluents keeps as a hot topic to address. Acute toxicity test was not conclusive and chronic toxicity should be also measured to avoid a dispose/ reuse tertiary treated WW more toxic than secondary treated one.

# LIST OF PUBLICATIONS

## Manuscripts published in indexed journals

**Maniakova, G.,** Salmerón, I., Polo-López, M.I., Oller, I.,Rizzo, L., Malato, S. (2020). Simultaneous removal of contaminants of emerging concern and pathogens from wastewater by homogeneous solar driven advanced oxidation processes. Science of the Total Environmental 766 144320 https://doi.org/10.1016/j.scitotenv.2020.144320 (**IF: 6.551; Q1**).

**Maniakova, G.,** Kowalska, K., Murgolo, S., Mascolo, G., Libralato, G., Lofrano, G., Sacco, O., Guida, M., Rizzo, L. (2019). Comparison between heterogeneous and homogeneous solar driven advanced oxidation processes for urban wastewater treatment: Pharmaceuticals removal and toxicity. Separation and Purification Technology, 116249https://doi.org/10.1016/j.seppur.2019.116249 **(IF: 5.774; Q1)**.

K. Kowalska , **G. Maniakova** , M. Carotenuto , O. Sacco , V. Vaiano , G. Lofrano , L. Rizzo (2020). Removal of carbamazepine, diclofenac and trimethoprim by solar driven advanced oxidation processes in a compound triangular collector based reactor: A comparison between homogeneous and heterogeneous processes. Chemosphere 238 124665 https://doi.org/10.1016/j.chemosphere.2019.124665**(IF: 5.778; Q1).**

## Manuscript accepted for publication in indexed journals

**Maniakova, G.,** Salmerón, I., Nahim-Granados S., Malato S., Oller, I.,Rizzo, L., , Polo-López, M.I. (2021). Solar driven advanced oxidation processes Vs ozonation for wastewater safe reclamation. Sustainable Chemistry and Engineering **(IF: 7.632; Q1)**.

## Manuscript submitted for publication in indexed journals

**Maniakova, G.,** Salmerón, I., Aliste M.,Oller, I., Polo-López, M.I. Malato S., Rizzo L, (2021). Solar photo-Fenton compared to Ozonation for tertiary urban waste water treatment: contaminants of emerging concern removal and toxicity assessment. Journal Hazardous Materials **(IF: 9.038)**

**Last updated: 04.05.2021**

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## LIST OF ABBREVIATIONS

AOPs– Advanced oxidation processes

BATs–Best available technologies

BOD5 – Biochemical Oxygen Demand

CAF– Caffeine

CBZ –Carbamazepine

CEC – Contaminants of emerging concern

CTC – Compound triangular collector plant

DALY – Disability adjusted life year

DCF – Diclofenac

DIC – Dissolved inorganic carbon

DL– Detection limit

EDDS– Ethylenediamine-N,N'-disuccinic acid

GWRC– Global Water Research Coalition

LRV- Log Reduction Value

QMRA–Quantitative microbial risk assessment

Quv– Cumulative UV energy

ROS – Reactive oxygen species

rpm–Revolutions per minute

RPR– Raceway Pond reactor

SMX – Sulfamethoxazole

SPF – Solar photo-Fenton

SUWW–Simulated urban wastewater treatment plant effluent

SUWW-woc–Simulated urban wastewater treatment plant effluent without organic compounds

TMP – Trimethoprim

UPLC –Ultra-performance liquid chromatography

UV– Ultraviolet

UWWTPs– Urban wastewater treatment plant

w/v– Weight/volume

WHO–World Health Organization

WW – Wastewater treatment plant effluent

WW-rc–Wastewater treatment plant effluent with reduced carbonates

OUR- oxygen uptake rate

# CHAPTER I

# Introduction

# 1 Introduction

## 1.1 Water scarcity and demand

The increasing demand of food to satisfy the needs of a growing world population alongside the rate of economic development and high population are putting incomparable pressure on water resources. Water is crucial element for the life. Nowadays, it has been estimated that around one more than 3 billion people globally experience severe water scarcity for more than 3 months a year, amongst which a sizable population in industrialized nations in Europe, the US and Australia (Fig. 1).

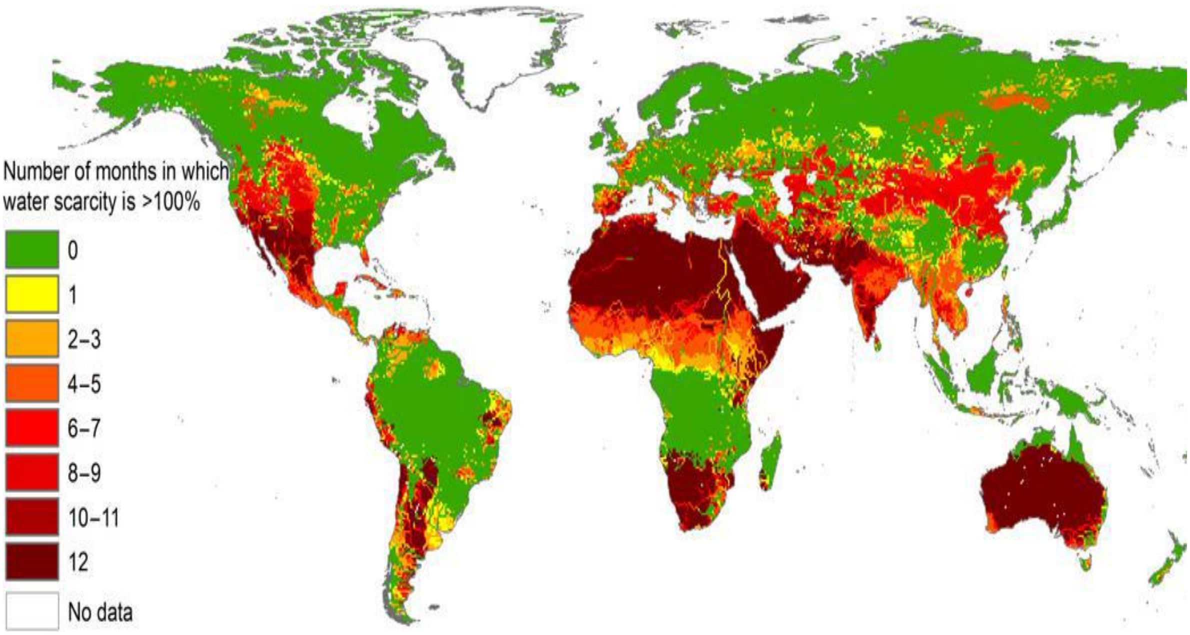


**Figure 1** – Global annual water scarcity (Mekonnen and Hoekstra, 2016)

Kummu et al., (2016), studied the roadmap of water stress in the 20th century, revealed that the population of people facing water shortages had risen from 0.24 billion in the 1900s to 3.8 billion people in the year 2000. These values represent 14% and 58% of the population in their respective years.

In addition, climate change is expected to further compromise the water quality and availability for supply (Sowers et al., 2011). Increases in frequency of abnormal events such as flooding, temperature extremes, temporal drying out of water streams, lower volumes of water recharging aquifers dose climate change cause of major concern (Bates et al., 2008).

Facing demographic growth and economic development, it is increasingly hard to find new sources of water necessary to successfully satisfy growing water demand.

## 1.2 New challenges in urban wastewater effluents disposal and reuse

### 1.2.1 Contaminant of emerging concern

Several pollutants such as pesticides, pharmaceuticals, hormones, personal care products, and their metabolites, also known as contaminants of emerging concern (CECs), are continuously detected in urban wastewater treatment plants (UWWTPs) effluents(Rizzo et al., 2019). Although occurring at low concentrations (ng/L-µg/L) in urban wastewater (WW) and receiving water bodies, they can accumulate into the environment resulting in significant acute and chronic toxicity, with potential collateral effect on human health and aquatic ecosystems (Fent et al., 2006; Hossain and Roy, 2018).

CECs enter the environment through a variety of pathways and move among land, air, and water (SCCWRP, 2012):

- Pharmaceuticals excreted in urine or flushed down the toilet, if not removed by standard WW treatment practices, are discharged with treated effluent into rivers or the ocean.
- Pesticides used on gardens, golf courses, or crops run off into streams, storm drains, and coastal waters when it rains.
- Fire retardants applied to furniture and electronics volatilize into air, leach into water, or bind to particles like house dust and soil.

Concern about CECs stems from the rapid pace of new chemical production, along with an increased focus on CEC detection in the environment and drinking water sources. More than 100,000 chemicals are currently in use, but fewer than 130 constituents are regulated as priority water pollutants.

### 1.2.2 Pathogens in WW

Effluents released from UWWTPs may contain a wide range of waterborne pathogens, including bacteria, viruses, and parasites included *E. coli, Salmonella spp and Enterococcus spp* which are considered the most severe foodborne pathogens found, even at a low infective dose, in raw vegetables and fruits irrigated with contaminated water (Ayaz et al., 2014). *E.coli* can produce Shiga toxins which are one of the most potent bacterial toxins. The related symptoms can vary from asymptomatic and normal diarrhea to more severe symptoms like bloody diarrhea know as hemorrhagic colitis and hemolytic uremic syndrome (EFSA, 2011). The ingestion of food contaminated by *Salmonella spp.* usually presents an incubation period of 18-72 hours known their infection as Salmonellosis which symptoms are chills, abdominal pain, fever, nausea, diarrhea and vomiting (Harris et al., 2003). Infections caused by *Enterococcus* such as endocarditis and bacteremia, can clearly cause serious and often life-threatening disease (Christie et al., 1994)

The occurrence of antibiotics may promote the selection and the diffusion of antibiotic resistance patterns into the environment (Davies and Davies, 2010), such as the development of antibiotic resistant bacteria (ARB) which reduce the therapeutic potential against animal and human pathogens (Rizzo et al., 2013).

### *1.2.3 WW reuse and quality regulation*

The problem of water scarcity has put humans in search of alternative water sources to supplement their daily needs. Treated WW reuse is a suitable alternative to supplement freshwater resources (Scheierling et al., 2011). Several studies have outlined the importance of WW reuse in the areas of agriculture, industry, urban development, domestic reuse, potable water supply, and others. In the United States, for example, treated waste water provides horticultural and urban irrigation system, industrial processes (Schwabe et al., 2020). They reported that WW reuse has expanded from 4 million $m^3$ per day in 1995 to almost 8.5 million $m^3$ per day in 2015.

The increasing reuse of WW promote the spread of contaminants and pathogens in the environment. CECs such as Caffeine, carbamazepine, DEET, dilantin, meprobamate, naproxen, primidone and triclosan can be easly taken up by plants irrigated with treated WW(Wu et al., 2014). Moreover, in tissue lettuce plant can be accumulated Clofibric acid, ibuprofen, naproxen, tonalide and triclosan (Calderón-Preciado et al., 2013). In this light CECs release into the environment should be restricted or at least minimized in order to limit risks to the environment and public health that are difficult to assess and manage.

Directive 2008/105/EC has established a list of 33 Priority Substances for surface water and their associated Environmental Quality Standards, but no CEC were included. This directive modified the WFD (Water Framework Directive 2000/60/EC) and the EQSD (Environmental Quality Standards Directive) in terms of priority substances in the field of water policy, and has extended the list to 45 substances priority, of which 21 are identified as dangerous. The last update was in June 2018 in Decision 2018/840/EU. This UE Watch list highlighted 15 compounds to be monitored: three estrogens, (estrone, 17-β-estradiol and 17-α-ethinylestradiol), five antibiotics (azithromycin, clarithromycin, amoxicillin, ciprofloxacin and erythromycin), and seven pesticides (metaflumizone, methiocarb, imidacloprid, thiacloprid, thiamethoxam, clothianidinand acetamiprid). on March 2019, the

European Commission approved a Communication (COM/2019/128 final) concerning the problem regarding pharmaceutical pollutants. This Communication proposes a strategic approach to this kind of pollutants in order to reach an integral vision. By the way, this Communication does not affect the continuation, development or application of legislation, normative or actions regarding those pollutants by EU members. However, until now, there were no strict regulations established to control micropollutant concentration levels in the EU. At national level, only Switzerland has regulated the removal of these priority substances. This rule reflects that UWWTPs must be updated to achieve 80% micropollutant removal with regard to untreated WW(Bourgin et al., 2018).

The reuse of WW for irrigation raises public health concerns, especially when vegetables are eaten raw or undercooked such as leafy greens, because pathogens still occur in the treated WW(Krzeminski et al., 2019). The contribution of the irrigation water to vegetables contamination and the subsequent foodborne infection outbreaks, mainly in raw-eaten vegetables, represent an important global concern (Beuchat, 2002). For this reason, the number of studies about the microbiological risk associated to irrigation has increased in the last years (Deepnarain et al., 2020; Rasheduzzaman et al., 2019).

Under these circumstances, member states of European Union have proposed minimum quality requirements for water reuse in agriculture irrigation(Regulation (EU) 2020/741).In particular, *E. coli* minimum values were set at ≤ 0.1 or below detection limit, ≤10, ≤100 and ≤1000 CFU/100 mL for class A waters obtained after a secondary treatment, filtration and disinfection by advanced water treatments, class B and C waters from a biological treatment followed disinfection path, and class D water treated by secondary treatment and storage for stabilization ponds or wetlands (Rizzo et al., 2018).

## 1.3 Role of the tertiary treatment in UWWTPs

Ideally, UWWTP facilities include preliminary, primary, secondary and tertiary treatment. There are two end products from the treatment process; sludge solids and liquid effluent.

- Preliminary treatment takes large solids and floating debris from the raw WW.
- Primary treatment separates the smaller solids.
- Secondary treatment uses micro-organisms to remove the biodegradable or organic waste.
- Tertiary treatment includes removal of resistant contaminants and pathogens

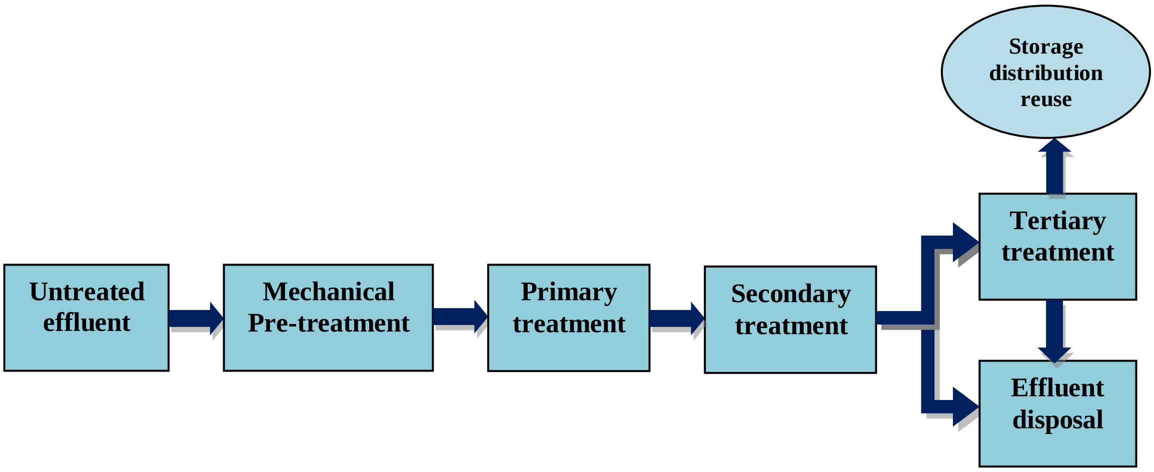


**Figure 2** – Simplified schematic UWWTP

Conventional UWWTPs are not or poor effective in controlling the release of CECs and antibiotic resistant pathogens into the environment(Rizzo et al., 2019b). To minimize the risk associated to CEC and pathogens as well as to be in compliance with the corresponding limits for WW reuse, conventional UWWTPs should be upgraded with an effective advanced tertiary treatment.

Tertiary treatment is an additional water polishing step that may be needed prior to discharge and that is virtually always applied prior to WW reuse, in order to satisfy discharge/reuse regulation in its relevant jurisdiction. The more commonly used tertiary treatments in UWWTPs are chlorination, UVC irradiation and filtration.

Chlorination is the most widely used process for the WW disinfection. Among the factors that affect the efficiency of disinfection with chlorine are the type and concentration of microorganisms, the nature and concentration of the disinfectant, contact time, temperature, pH and organic matter content. However, disinfection by-products formed in the chlorinated WW may have a toxic effect on aquatic organisms that are exposed to them. These products cannot be removed even by dechlorinating UWWTPs effluents before spilling them into the environment (Watson et al., 2012). Moreover, chlorination is poorly effective in CECs removal (Cerreta et al., 2019).

UVC irradiation (200–280 nm) has been extensively used for water disinfection as an alternative to conventional disinfection procedures for killing pathogens. At particular wavelengths such as 254 nm, UV-C light is able to destroy the molecular bonds and, causing death to a variety of environmental microorganisms (Cutler and Zimmerman, 2011). However, serious drawback such as microbial regrowth (due mainly to the lack of residual effect) and mechanisms of self-repair of microorganisms DNA have been observed (Guo and Kong, 2019; Lee et al., 2015). In addition, UVC treatment did not show to be effective in CEC removal (Yu et al., 2019).

Membrane technologies offer an alternative to the disinfection processes producing a high-quality clarified effluent and avoiding the addition of chemical reagents, thus no harmful by-products are formed. Membrane technologies include reverse osmosis, ultrafiltration, nanofiltration and microfiltration even though the last one is the most widely used for the disinfection of treated WW. Microfiltration efficiently removes particulates, bacteria, algae and protozoans and viruses larger than 0,2 micron. Depending on the chosen membrane, suspended solids, bacteria, viruses and parasites can be separated, along with proteins, pesticides and humic acids, among others, meaning that by applying this technology it is possible to obtain water free of pathogens and chemical compounds (LeChevallier, 2013). The main disadvantage of membrane is clogging, which requires regular cleaning to avoid reducing efficiency and, even periodically, replacing it. The main concern about this treatment is rejection stream management, as it would contain all separated microorganisms and organics.

## 1.4 Advanced oxidation processes

Unfortunately, UWWTPs with or without conventional tertiary treatments (such as sand filtration and or consolidated disinfection processes like chlorination, UV radiation, peracetic acid) are unable to provide an effective removal of CECs (Krzeminski et al., 2019). To overcome this problem, in recent years, research has focused on the investigation of new processes/technologies for tertiary treatment of urban WW in the attempt to effectively remove CECs and pathogens before effluent disposal or reuse. The remarkable characteristic of AOPs is the potential to simultaneously degrade organic contaminants and inactivate microorganisms in water through the promotion of highly reactive oxidative chemical species generation, in particular hydroxyl radicals ($HO^{\bullet}$) (Klamerth et al., 2013; Rizzo et al., 2019b; Soriano-Molina et al., 2019a).They have an oxidation potential of 2.33 V and exhibit fast rates of oxidation reactions.

Although AOPs use different reagents, which result in different treatments such as photochemical processes ($UV/O_3$, $UV/H_2O_2$), photocatalysis ($TiO_2/UV$, photo-Fenton), and chemical oxidation processes ($O_3$, $O_3/H_2O_2$, $H_2O_2/Fe^{2+}$), they all produce $HO^{\bullet}$.AOPs can also be classified as homogeneous or heterogeneous. Homogeneous processes can be further subdivided into processes that use energy and processes that do not use energy (Poyatos et al., 2009).

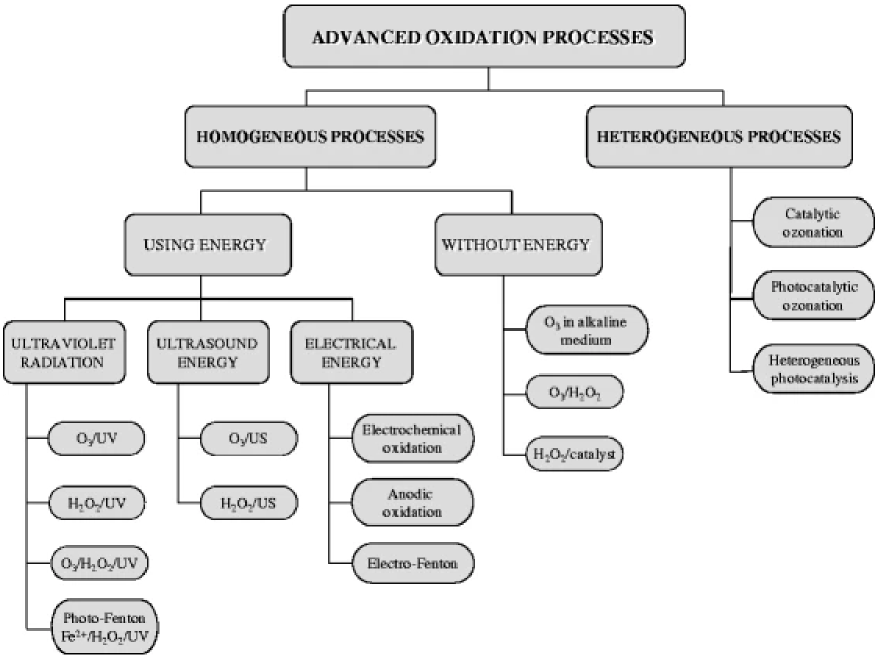


**Figure 3** –AOPs classification (Poyatos et al., 2009)

Depending on the process, a number of drawbacks exist making them a not economically sustainable solution, mainly because of the pH operating conditions (e.g., Fenton and photo-Fenton at acidic pH) and high electricity demand (e.g., ozone and UV based AOPs) (Rizzo et al., 2019b). To save energy cost and make the process more sustainable, several photo-driven AOPs can be operated also with solar radiation (Malato et al., 2009).

## 1.5 Solar driven AOPs

### *1.5.1 Solar driven homogenous AOPs*

Fenton process is one of the AOPs most investigated for water treatment due to its ability to generate a high amount of hydroxyl radicals by a reaction between iron and the oxidant $H_2O_2$ which is decomposed into $H_2O$ and $O_2$ and therefore without generating chemical residues. The reactions involved in the process are shown below (Eq. 1-9).

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

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + H^{+} + HO_2^{\bullet} \quad (2)$$

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe^{3+} + HO^{-} \quad (3)$$

$$H_2O_2 + HO^{\bullet} \rightarrow H_2O + HO_2^{\bullet} \quad (4)$$

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+ \quad (5)$$

$$Fe^{2+} + HO_2^{\bullet} + H^+ \rightarrow Fe^{3+} + H_2O_2 \quad (6)$$

$$2HO^{\bullet} \rightarrow H_2O_2 \quad (7)$$

$$2HO_2^{\bullet} \rightarrow H_2O_2 + O_2 \quad (8)$$

$$HO_2^{\bullet} + HO^{\bullet} \rightarrow H_2O + O_2 \quad (9)$$

In the presence of UV-vis radiation, the rate of hydroxyl radicals production increases, the photo-Fenton process taking place where the ferric ions ($Fe^{3+}$)produced during Fenton reactions (Eq.1-4) are photo-catalytically converted to ferrous ions ($Fe^{2+}$), generating additional hydroxyl radicals (Eq. 10).

$$Fe^{3+} + H_2O + hv \rightarrow Fe^{2+} + H^+ + HO^{\bullet} \quad (10)$$

Decontamination and disinfection of urban WW by solar photo-Fenton (SPF) (sunlight/Fe(II)/$H_2O_2$) and SPF-like (sunlight/Fe(III)/$H_2O_2$) processes have been successfully investigated so far (Fiorentino et al., 2019, 2015; Klamerth et al., 2013; Miralles-Cuevas et al., 2014). Nevertheless, several drawbacks exist making such processes not an economically and environmentally sustainable solution, due to high reagent costs for adjusting pH to 2.8 (optimum pH to avoid iron precipitation),and the subsequent need for a neutralization after treatment, before effluent disposal or reuse (Pignatello et al., 2006) which will also increase water salinity. Therefore the interest of the scientific community for the development of suitable solutions to operate these processes at neutral pH has increased in the last years (Clarizia et al., 2017). In particular, different solutions have been recently proposed and investigated to overcome the pH-related problem, which include heterogeneous photo-Fenton process but also the addition of chelating agents in homogenous phaseto prevent iron precipitation and make the process effective even under neutral pH conditions. The most investigated chelating agents includemono-, poly-, or amino-carboxylic acids, such as Ethylenediamine-N, N′-disuccinic acid (EDDS), and purposely designed metal-organic complexes (Di Cesare et al., 2020; Fiorentino et al., 2018). According to previous works, dosing EDDS in SPF process provides fast CECs degradation(Klamerth et al., 2013; Miralles-Cuevas et al., 2014). The presence of EDDS as an organic ligand produces a soluble ferric and ferrous organo-complex at neutral pH. Degradation of the complex with Fe(III) produces additional radicals in a wide pH range. Under solar irradiation, the complex yields the EDDS radical, promoting the generation of $HO^{\bullet}$ and $O_2^{\bullet}$ (Eq.11-13)(Miralles-Cuevas et al., 2019).

$$\text{Fe (III):EDDS} + h\nu \text{ (290-500 nm)} \rightarrow [\text{Fe (III):EDDS}]^* \rightarrow \text{Fe}^{2+} + \text{EDDS}^{\bullet 3-} \quad (11)$$

$$\text{EDDS}^{\bullet} + O_2 \rightarrow O_2^{\bullet -} + \text{EDDS}^{2-} \quad (12)$$

$$\text{EDDS}^{\bullet} + OH^- \rightarrow \text{EDDS}^{2-} + HO^{\bullet} \quad (13)$$

As WW disinfection is of concern, SPF process implementing EDDS as chelating agent has been investigated too (Bianco et al., 2017; García-Fernández et al., 2019). Nevertheless, the efficiency of this process for the inactivation of bacteria seems to be contradictory and controversy, in particular with regard to the possible effect of photo-Fenton process using Fe:EDDS as complexing agent as it promotes bacteria growth due to its low toxicity and high biodegradability.

### *1.5.2 Solar treatment assisted with $H_2O_2$*

$H_2O_2$ is an oxidant widely used for water treatment due to its oxidation potential of around 1.4 V at near-neutral pH (Giannakis et al., 2016). Moreover, it is cheap, safe, easy to handle and does not generate residues as it easily decomposes to water and oxygen. As a matter of fact, $H_2O_2$ alone (even at non-toxic microbial concentration, lower than 50 mg/L) in combination with natural sunlight can promote very high microbial inactivation rates (Ferro et al., 2015). In addition, better performances of sunlight/$H_2O_2$ for the inactivation of different types of bacteria have been documented (Ferro et al., 2015; Fiorentino et al., 2015; Moreira et al., 2018).

Briefly, the mechanisms of bacteria inactivation, based on the accumulated damages inside cells by internal cellular injure, occur under sunlight and is accelerated in the presence of $H_2O_2$. Internal damages are mainly attributed to the promotion of ROS formation (directly by UVA and UVB photons and indirectly by photo-Fenton-like reactions between $H_2O_2$ that enters the cell and naturally occurring intracellular-iron) that affects different intracellular vital components leading to bacterial death or lack of viability (Giannakis et al., 2016).

### *1.5.3 Solar driven heterogeneous photocatalysis*

Photocatalysis is defined as the change in the rate of a chemical reaction or its initiation under the action of ultraviolet or visible light in the presence of a substance, the photocatalyst, which absorbs light and it is involved in the chemical transformation of the reaction partners (Byrne et al., 2018).

In heterogeneous photocatalysis, the catalyst (a semiconductor), being titania (TiO2) that one with the higher potential compared to other semiconductors such as $ZrO_2$, for example,.

which has a band gap energy wider than that of $TiO_2$ (about 5 eV). $TiO_2$ is one of the most investigated catalyst, generate selectron/hole pairs upon absorption of light. These holes drive to the formation of HO• which degrade the target organic compound near or on the catalyst surface (Chong et al., 2015). However, $TiO_2$ has a band-gap in the range 3.0–3.2 eV, meaning that only less than 5% of the solar spectrum can be used for the activation of the photocatalyst. To use a wider part of the whole solar spectrum, $TiO_2$ should be modified to maximize the absorption of visible light so that more photo excited electrons and holes can be generated (Sa and Mh, 2016) $TiO_2$ doped with non-metal elements, such as nitrogen (Rizzo et al., 2014; Sacco et al., 2018), has been widely investigated because non-metal can significantly extend the visible light absorption of the doped-photo catalysts and considerable decrease photo generated charge recombination (Alshammari et al., 2015). From WW disinfection point of view, heterogeneous photocatalysis with $TiO_2$ has been the most researched process among all AOPs.

Moreover, the photocatalyst used in its powder form would request an additional treatment step to remove it before effluent disposal or reuse, further increasing treatment cost. However, this problem can be addressed by fixing the photocatalyst onto a support (Paredes et al., 2019). These limitations, under both a technological and process viewpoint, make the above described AOPs not ready for full scale applications in tertiary/advanced urban WW treatment (Rizzo et al., 2019b). Several gaps about the efficiency of supported photocatalysts solar driven AOPs in removing CECs at environmentally relevant concentrations from real WW are still present, discouraging the up-scale of this technology. Solar photocatalytic efficiency of $TiO_2$ immobilized on glass spheres (sol being prepared by titanium is opropoxide, commercial P25 and polyethylene glycol) was successful investigated indifferent aqueous matrices spiked with 15 CECs, in a compound parabolic collector (CPC) solar based reactor (Miranda-García et al., 2011).

## 1.6 Ozonation

The first drinking water treatment plant to use ozone for disinfection was built in 1893 at Oudshoorn, Holland. Learning from this one, another one was built in 1906 at Nice, France Ozonation is among the best available technologies (BATs) for advanced treatment of WW, increasingly used for the removal of CECs and water disinfection in several European countries (Rizzo et al., 2020). Due to the high $HO^{•}$ generation potential, ozonation can be considered an AOP (Buffle et al., 2006) and it is highly effective in the inactivation of different microorganism (Alexander et al., 2016; Nahim-Granados et al., 2020).

The application of ozone generally involves contaminants oxidizing by $O_3$ (direct reaction) and/or $HO^\bullet$ (indirect reaction) that are formed during ozone reactions. The issue is that $O_3$ is instable in water. The half-life of ozone depends on the water quality, being the pH especially important. Hydroxide anions, generated by an increase on the pH, decompose ozone ($O_3 + OH^- \rightarrow HO_2^- + O_2$) (von Gunten, 2003). Moreover, $O_3$reacts selectively with compounds containing electron-rich moieties (e.g., unsaturated double bonds, deprotonated amine groups and activated aromatic systems). Hence, CECs with ozone-reactive moieties can usually be completely abated by primarily direct $O_3$oxidation during conventional ozonation (Sonntag et al., 2012).

Several research studies have been performed to evaluate the capability and drawbacks of ozone application for the removal of CECs from WW(Rizzo et al., 2019b). The removal of more than 70 microcontaminants (included CECs) present in WW was investigated at large-pilot scale in a UWWTP of Switzerland by(Margot et al., 2013). In that work it was observed that even at low dose, ozone is able to provide 80% removal of total microcontaminants demonstrating that ozone-based processes are an efficient tool to abate a great variety of microcontaminants (included CECs) (Miklos et al., 2018; von Gunten, 2018).

# CHAPTER II

## Novelty and objectives

## Novelty

Conventional UWWTPs cannot effectively remove most of CECs (Krzeminski et al., 2019) and AOPs, in particular photo driven ones (namely, UV/$H_2O_2$ and photo-Fenton), and ozonation have been widely investigated so far as tertiary treatment methods to remove these contaminants and inactivate bacteria for a safe effluent disposal or reuse (Rizzo et al., 2019b; Rizzo et al., 2020). Noteworthy are the efforts of the scientific community to make these processes more sustainable and certainly an important step in this direction has been taken with the investigation of the SPF process. Important steps forward have also been made in the study of semiconductor-based photocatalysts (such as $TiO_2$) to make them more effective in the presence of solar radiation. However, there is still a lack of information in the scientific literature about the comparative effect of different photo driven AOPs. The progress in the heterogeneous solar driven photocatalytic process should be evaluated with respect to SPF to learn if it can be competitive under realistic conditions as tertiary treatment method of urban WW. More in general, comparisons are needed between ozonation, which is among the BATs for tertiary treatment of urban WW, and photo driven AOPs, under realistic conditions, using different end points (CECs removal, bacteria inactivation, effluent toxicity). The novelty of this thesis work relies on the attempt to fill the gaps explained above thus contributing to advance the knowledge on the new photo driven AOPs as possible alternative solution to the BATs (namely ozonation) in tertiary treatment of urban WW.

## Objectives

To fill the gaps in the knowledge explained in the novelty paragraph above, the main aim of this PhD thesis work was to investigate the effects of different AOPs as tertiary treatment methods of urban WW under realistic conditions using different endpoints: CECs removal, pathogens inactivation and toxicity. In this study, five pollutants have been selected as model CECs (Caffeine, Carbamazepine, Diclofenac, Sulfamethoxazole and Trimethoprim) according to the COST Action ES1403 (New and emerging challenges and opportunities in wastewater reuse (NEREUS)) recommendation due to CECs uptake and accumulation in food crops and consequent diffusion into the food-chain. Three human bacterial pathogens (*E. coli, Salmonella spp and Enterococcus spp*) were chosen as model of microbial contamination because they are typically detected in UWWTP effluent. An heterogeneous set of model CECs and pathogens under realistic conditions and at realistic concentration could help to predict the behave of a mix of hundreds of CECs and several dozens of pathogens.

The specific objectives of this work are:

1) To evaluate the competitiveness of solar driven heterogeneous photocatalysis (namely sunlight/N-$TiO_2$ doped process)with homogeneous ones (SPF, SPF with EDDS, sunlight/$H_2O_2$) in the short-mid-term for tertiary urban WW treatment;
2) To investigate the best operating conditions for SPF process with chelating agent (EDDS)in WW at near neutral pH for the simultaneous removal of a mixture of CECs and bacteria inactivation;
3) To compare SPF with EDDS at near neutral pH and ozonation under different end points (CECs removal, bacteria inactivation and toxicity).

# CHAPTER III

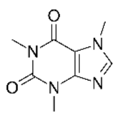
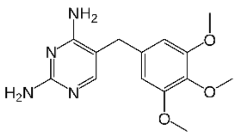
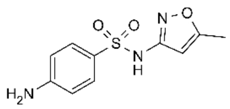
# Materials and methods

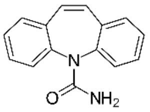
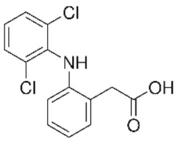
# 3. Materials and methods

## 3.1 Chemicals and reagents

The selected model CECs were all high-purity grade (>99%); Carbamazepine (CBZ), Diclofenac (DCF), Sulfamethoxazole (SMX) and Trimethoprim (TMP)) were purchased from Sigma Aldrich and Caffeine (CAF) was provided by Fluka. This compounds were chosen according to the COST Action ES1403 NEREUS recommendation. Impact of CBZ, DCF, SMX and TMP is of particular relevance to WW disposal and reuse in agricultural settings due to CECs uptake and accumulation in food crops and consequent diffusion into the food-chain(Krzeminski et al., 2019).Moreover, DCF is included to the EU Watch list (Decision 2015/495/EU). CAF is considered an emerging contaminant and can be highlighted due its presence in medicines, beverages, foodstuff and several other products. In addition, it is a compound used worldwide recognized as a marker of anthropogenic activity (Júnior et al., 2019). CBZ is an anticonvulsant, this drug usually is used for the treatment of epilepsy, as well as for various psychotherapy applications (Miao et al., 2005). TMP and SMX have been used in combination in clinical settings for more than 40 years, they usually detected together in UWWTP effluent. The combination of TMP and SMX is used to treat a variety of bacterial infections of the respiratory-, urinary-, and gastrointestinal tracts. Although TMP and SMX are used to treat infections caused by organisms such as *Nocardia, Salmonella, Shigella, staphylococci,* or *streptococci* (Vilcheze and Jacobs, 2012). DCF is a common non-steroidal anti-inflammatory drug that is often detected in UWWTP effluent (Vieno and Sillanpää, 2014). It works by reducing hormones that cause inflammation and pain in the body.

**Table 1**-Chemical structure and physicochemical properties of target CECs

| **Compound** | **Structure** | **Formula** | **Molecular weight, g/mol** | **Dissociation constant (p$K_a$)** | **log($K_{OW}$)** | **Water solubility, mg/L** |
|---|---|---|---|---|---|---|
| Caffeine (CAF) |  | $C_8H_{10}N_4O_2$ | 194.19 | 10.4 | -0.07 | 21600 (at 25°C) |
| Trimethoprim (TMP) |  | $C_{14}H_{18}N_4O_3$ | 290.32 | 7.12 | 0.91 | 400 (at 25°C) |
| Sulfamethoxazole (SMX) |  | $C_{10}H_{11}N_3O_3S$ | 253.28 | 1.6 (p$K_a$1);<br>5.7 (p$K_a$2) | 0.89 | 610 (at 37°C) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Carbamazepine (CBZ) |  | $C_{15}H_{12}N_2O$ | 236.27 | 13.9 | 2.45 | 17.7<br>(at 25°C) |
| Diclofenac (DCF) |  | $C_{14}H_{11}Cl_2NO_2$ | 296.15 | 4.15 | 4.51 | 2.37<br>(at 25°C) |

Data obtained from the PubChem,U.S. National Library of Medicine, National Center for Biotechnology Information, https://pubchem.ncbi.nlm.nih.gov

Sodium thiosulfate (99% w/w), hydrochloric acid (1 N), and glacial acetic acid were obtained from Carlo Erba (France). Hydrochloric acid (37%) was purchased from Titolchimica (Italy).Titanium (IV) oxysulfate, ammonium metavanadate, 1,10-phenanthroline, ammonium acetate, acetic acid used for $H_2O_2$ and dissolved iron measurements were obtained in reagent grade (Sigma-Aldrich). $Fe_2$ $(SO_4)_3 \cdot H_2O$ 75% solution (Panreac) was used as Fe (III) source in photo-Fenton experiments. $H_2O_2$ (35%, w/v), EDDS water solution (35%,w/v), bovine liver catalase, acetonitrile (ACN) (UHPLC ultra-high-performance liquid chromatography)-grade), formic acid (UHPLC-grade), $NaHCO_3$, $MgSO_4$, NaCl, $K_2HPO_4$, beef extract, peptone, humic acid, sodium lignin sulfonate, and sodium lauryl sulphate were purchased from Sigma Aldrich. $CaSO_4 \cdot 2H_2O$, $(NH_4)_2SO_4$, tannic acid, acacia gum powder and KCl were provided by Panreac.

## 3.2 Water matrices

Experiments were carried out in different water matrices: simulated urban wastewater treatment plant effluent (SUWW), simulated urban wastewater treatment plant effluent without organic compounds (SUWW-woc), in real urban wastewater treatment plant effluent (WW) from an UWWTP in Salerno (Italy) and from one in Almeria (Spain) which was also used with reduced carbonates concentration (WW-rc).

### *3.2.1 SUWW samples*

SUWW was prepared according to receipt described in Zhang et al., 2007 and in APHA Standard Methods. The following chemicals were dissolved in dematerialized water: i) $NaHCO_3$(96 mg/L), $MgSO_4$ (60 mg/L), NaCl (580 mg/L), $K_2HPO_4$ (7.0 mg/L), $CaSO_4 \cdot 2H_2O$ (60 mg/L), $(NH_4)_2SO_4$ (23.6 mg/L) and KCl (4 mg/L) to simulate the inorganic content*;* ii) beef extract (1.8 mg/L), peptone (2.7 mg/L), humic acid (4.2 mg/L), sodium lignin sulfonate (2.4 mg/L), sodium lauryl sulphate (0.9 mg/L), tannic acid (4.2 mg/L) and acacia gum powder (4.7 mg/L) to simulate the organic content. Resulting physicochemical properties of SUWW are shown in Table 2.In a second set of experiments, SUWW without organic content (SUWW-woc) was used.

**Table 2** –Physicochemical characterization of SUWW

| **Parameter** | **Value** |
|---|---|
| pH | 7.6±0.3 |
| Conductivity (mS/cm) | 1.4±0.1 |
| Turbidity (NTU) | 3.4±0.2 |
| *DOC (mg/L) | 15.5±0.6 |
| Carbonates (mg/L) | 68.6±6.1 |

*DOC – dissolved organic carbon

### *3.2.2 WW samples*

WW samples in Italy were taken from a large UWWTP (300.000 equivalent inhabitants) located in the province of Salerno (Campania Region, Italy). Samples were taken from the effluent of the biological treatment step. Samples were collected in 25 L tanks. The samples were stored at about 4 °C The average values of the main parameters are given in Table3.

**Table 3** –Physicochemical characterization of the UWWTP effluent from Italy

| **Parameter** | **Value** |
|---|---|
| pH | 7.8±0.2 |
| Conductivity (mS/cm) | 1.1±0.1 |
| Turbidity (NTU) | 4.4±0.2 |
| DOC (mg/L) | 25.2±3.9 |
| *BOD5(mg/L) | 10.0±2.3 |

* BOD5 - Biochemical Oxygen Demand

WW samples in Spain were freshly collected from the UWWTP El Bobar (Almería). The samples from the effluent of the biological treatment step were collected and stored at 4 °C not more than 4 days. The average physicochemical properties of the UWWTP effluent from Spain are shown in Table 4.

**Table 4**–Physicochemical characterization of the UWWTP effluent from Spain

| Parameter | Value | Anions (mg/L) | | Cations(mg/L) | |
|---|---|---|---|---|---|
| pH | 7.8±0.2 | $Cl^-$ | 463.2±33.3 | $NH_4^+$ | 75.9±19.8 |
| Conductivity (mS/cm) | 2.6±0.2 | $Br^-$ | 3.2±0.4 | $Na^+$ | 266.6±6.9 |
| Turbidity (NTU) | 11.0±1.5 | $NO_3^-$ | 2.0±0.4 | $K^+$ | 29.5±1.1 |
| *DOC (mg/L) | 22.1±2.3 | $PO_4^{3-}$ | 6.0±1.3 | $Ca^{2+}$ | 97.5±5.7 |
| **DIC (mg/L) | 97.6±10.6 | $SO_4^{2-}$ | 122.8±7.5 | $Mg^{2+}$ | 52.5±1.6 |
| | | $CO_3^-$ | 488.0±53.0 | | |

*** DIC – dissolved inorganic carbon

Moreover, in an additional set of experiments, the UWWTP effluent from Spain with reduced carbonates concentration (WW-rc) were used. In this case, carbonates were lowered to 75±7.7mg/L (15±1.5 mg/L of DIC) by reaction with sulfuric acid under gentle mixing.

## 3.3 Experimental set-up

Treatment tests were carried out in three pilot plant scale reactors: i) Compound Triangular Collector (CTC), ii) Raceway Pond Reactor (RPR) and iii) ozonation plant. Moreover, a lab-scale reactor under solar simulated radiation (solar simulator) was used to carried out proof of principle experiments under controlled conditions.

### *3.3.1 Compound triangular collector plant*

The photocatalytic tests were carried out under natural solar irradiation outside the Laboratory of Sanitary and Environmental Engineering, University of Salerno (latitude 40°N, longitude 14°E) in a pilot scale CTC solar reactor (Fig. 4). The CTC reactor consists of 10.2 L irradiated volume module, equipped with 8 acrylic glass tubes with an external diameter of 3.3 cm and a length of 150 cm each, housed in the mid of a triangular shaped aluminum collector. The module is mounted on a mobile and inclinable platform, whereby the reactor can be oriented toward the sun. The platform was inclined about 40° during the experiments, according to the latitude of the Laboratory with the aim to recover the maximum UV radiation. A pyrex vessel was connected to a peristaltic pump, which allowed to operate the module in a recirculation mode.

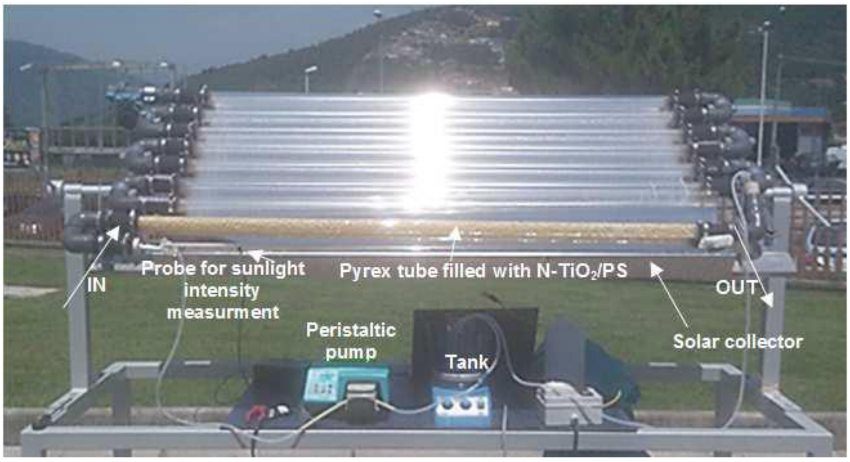


**Figure 4**– Compound triangular collector (CTC) plant

Only one tube of the module was used (1.3 L) for the photocatalytic experiments and it was packed with N-$TiO_2$/PS structured photocatalyst (434 g)(Sacco et al., 2018).

### *3.3.2 Solar lab scale reactor*

Lab-scale experiments under controlled conditions were performed in an open cylindrical reactor (Fig. 5). The working volume (3.9 L) was selected to obtain an optical path length (water depth in the reactor) of 15 cm which is similar to the water depth in RPR, as the objective of the experiments was the selection of the proper operation conditions for RPR tests. The reactor was placed inside a solar simulator SUNTEST XLS+ Benchtop Xenon Tester System (Atlas) (Fig. 5) which is equipped with a Xe lamp (NXE 2201-2500 W, 250–765 W/$m^2$) and a UV glass filter (Suprax) to cut wavelengths<290 nm and provide artificial solar radiation under controlled conditions.

**Figure 5-** Solar simulator

UV irradiance inside the simulator was set at 30 W/m$^2$ on water surface because this value was considered the mean of global UV irradiance under clear skies in sunny countries.

### 3.3.3 *Raceway Pond reactor*

Experiments in the RPR were carried out under natural solar irradiation at Plataforma Solar de Almeria (Spain) in clear sunny days from 11:30 a.m. to 14:30 p.m. local time. RPR is made by polyvinyl chloride(PVC) and its sizes are 0.45 m of width and 0.97 m of length which lead to a 90 L capacity (all the volume are irradiated) and15 cm liquid depth (Fig. 6). The system includes a paddle wheel connected to an engine (with variable frequency to control de paddle wheel speed) for a homogeneous mix of the solution.

**Figure 6-** Raceway Pond reactor

### 3.3.4 *Ozonation pilot plant*

The ozonation pilot plant consists on a column reactor with an inlet ozone diffuser for batch operation with a maximum capacity of 20 L (Anseros PAP-pilot plant, Anseros Klaus Nonnenmacher GmbH, Germany). The reactor (Figure 7) is equipped with an oxygen generator based on molecular sieves (Anseros SEP100), a corona-discharge ozone generator (Anseros COM-AD02), two non-dispersive UV analyzers (BMT 964) to measure inlet and outlet ozone gas concentration, a flow-meter for inlet air regulation and an ozone destroyer to remove residual ozone and avoid its release to the atmosphere.

**Figure 7-**Ozonation pilot plant

The operational conditions of the ozone pilot plant used in this study were 18 L of total volume of water, a constant inlet air flow of 0.06 N $m^3$/h. The inlet ozone flow rate was 1.5 $gO_3$/h, resulting from the ozone generator working at 20% power. Ozone dose used in all experiments was 83 $mgO_3$/L h.

## 3.4 Analytical measurements

### *3.4.1 Water characterization*

Temperature, pH, conductivity, turbidity, dissolved organic carbon (DOC), dissolved inorganic carbon (DIC) and the ionic content of the water samples were measured. pH was measured using a multi parametric sensor GLP22-CRISON (Fig. 8a), temperature using thermometry Hanna (Fig. 8b) and water conductivity using a conductivity meter GLP31-CRISON (Fig. 9).

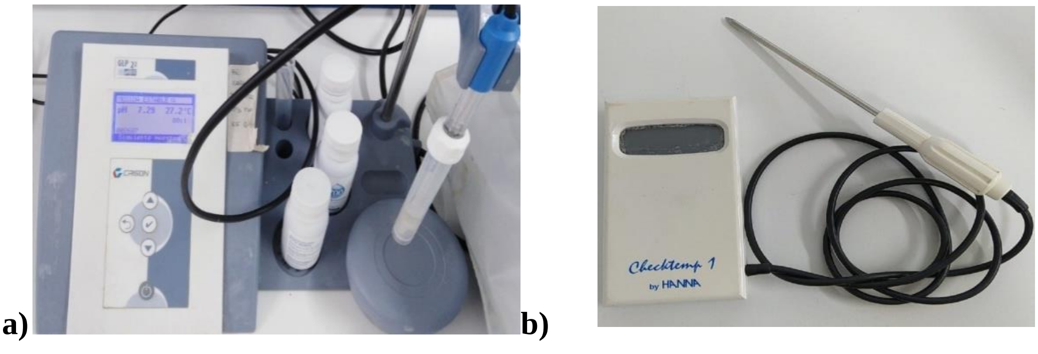


**Figure 8-**a)Multi parametric sensor GLP22 CRISON b) thermometry Hanna 1

**Figure 9 -**GLP 31 Conductivity meter

Turbidity was measured using a 2100AN Turbidimeter from Hach which allow a quantification from 0.1 to 4000 NTU.

**Figure 10 -** Turbidimeter

DOC and DIC defined as the concentration of organic or inorganic carbon contained in a water sample after its filtration through0.45 µm was measured using a Total Organic Carbon Analyser TOC-V-CSN(Figure XX) from Shimadzu, equipped with an ASI-V sampler. DOC is obtained from the difference between total dissolved carbon (TDC) and DIC of the same analyzed sample.

**Figure 11-** TOC-analyzer

The ionic content of the water samples, i.e., anions and cations concentration were analyzed using a Metrohm ion chromatograph Model 850 with a 872 extension module (Fig. 12). Anions determination was performed through a gradient analysis using a Metrosep A Supp 7-150/4.0 column with a 3.6 mM sodium carbonate solution as mobile phase and a flow rate of 0.7 mL/min. Cations were measured by a isocratic analysis using a Metrosep C6-150/4.0 column with an aqueous solution containing pyridine (0.85 %) and nitric acid 2M (0.085 %) as mobile phase with a flow rate of 1.2 mL/min. Samples were filtered through a 0.45 μm syringe-filter before the reinjection into the equipment.

**Figure 12** Ion chromatograph

### *3.4.2 CECs monitoring by UPLC-UV-DAD*

CECs degradation was monitored by ultra-performance liquid chromatography with ultraviolet-diode array detection (UPLC-UV-DAD) and reverse phase. This analytical technique is able to identify and quantify compounds contained in an aqueous mixture based on their separation according to their interaction with the absorbent particles of a stationary phase (chromatography column). A reverse phase system consisting of the use of a non-polar stationary phase (C18 columns) and a polar eluent /mobile phase which usually is a mixture of water and an organic solvent such as acetonitrile or methanol. The mobile phase containing the target compounds is pumped through the stationary phase where depending of the hydrophobic interactions of these compounds they will be retained in the column and detected in a specific order being the less polar or hydrophobic compounds more retained and therefore the last detected.

Two mixtures of CECs were investigated. In the first set of experiments CBZ, DCF and TMP removal was investigated. For this aim, CECs concentration was monitored at different treatment times by an Ultimate 3000 UPLC System (Thermo Fisher Scientific) equipped with an auto sampler, a temperature-controlled column compartment and a UV detector (Fig.13).

**Figure 13**-Ultimate 3000 UPLC System

The chromatographic separation of the organic mixtures was performed by injecting 50 µL and eluting them at 0.20 mL/min through a BEH C18 column (2.1 x 150 mm, 1.7 µm), with a binary gradient consisting of $H_2O$/ACN 95/5, 0.1% formic acid (A) and ACN, 0.1% formic acid (B). The gradient started from 5% B that was held for 4.6 min and then was linearly increased to 80% in 18.5 min and to 100% in further 5.5 min and the final composition was held for 0.5 min. At the end of each run, the system was further rinsed for 6 min using the final eluent composition and then returned to starting conditions and equilibrated for 5 min. The residual concentration of CECs was obtained by MultiQuan 3.0.2 software (AB Sciex).

In the second set of the experiments CAF, CBZ, DCF, SMX and TMP removal was investigated by a UPLC-UV-DAD (Agilent Technologies, Series 1200) using as stationary phase a Poroshell 120 EC-C18 column (Agilent Technologies: 50 mm × 3.0 mm, 2.7 µm particle (Fig. 14) and a gradient elution method.

**Figure 14**- Agilent1200UPLCDAD System

The eluents were 25 mM formic acid ultrapure water solution (A) and ACN (B) at a flow rate of 1 mL/min. The gradient started with 0% B followed by a linearly increase to 50%in 10 min and a final increase to 100% B after additional 2 min which was maintained the next 2 min leading to a total analysis time of 14 min. Injection volume and column temperature were 50 µL and 30°C, respectively. The chromatographic conditions used for CECs quantification are showed in Table 5.

**Table 5 -** Chromatographic conditions used for detection and quantification of each CEC

| Compound | Injection volume: 50 µL | |
|---|---|---|
| | **Detection wavelength(nm)** | **Retention time (min)** |
| Caffeine | 270 | 3.566 |
| Trimethoprim | 270 | 3.938 |
| Sulfamethoxazole | 267 | 5.013 |
| Carbamazepine | 267 | 7.161 |
| Diclofenac | 285 | 10.619 |

Before injection, 9 mL of collected water sample was filtered through 0.22 µm PTFE filter (Millipore) and the filter was subsequent rinsed with 1 mL of ACN to remove any possible adsorbed compound, obtained a final solution 90:10 of water:  ACN.

### 3.4.3 *Spectrophotometric measurements*

The concentration of Fe and $H_2O_2$in water samples was determined by specific spectrophotometric methods using a UV-Vis Evolution 220 spectrophotometer (Thermo scientific) (Fig.15).

**Figure 15**-UV-Vis Evolution 220 spectrophotometer

#### 3.4.3.1 *Hydrogen peroxide quantification*

Hydrogen peroxide concentration in water samples was analyzed according to the DIN 38402H15 protocol, which is based on the determination of the $[Ti(O_2)(OH)(H_2O)_3]^+$ yellow complex at 410 nm. The experimental procedure consisted on the mixture of 0.5 mL of titanium (IV) oxysulfate with 5 mL of water sample previously filtered (0.45 µm nylon filter) and its subsequent Spectrophotometric measurement at 410 nm.

#### 3.4.3.2 *Iron quantification*

Iron quantification in water samples was performed following the method ISO 6332:1998 based on the determination of the $[Fe(1,10\text{-phenanthroline})_3]^{2+}$ complex at 510 nm. The experimental procedure to measure ferrous iron in the sample consisted on the mixture of 4 mL of sample (previously filtered by 0.22 µm for dissolved iron quantification)with 1 mL of 1,10- phenanthroline (0.1 % w/v) and 1 mL of a buffer solution (250 g/L ammonium acetate and 700 mL/L of acetic acid).As in water samples the total iron is a mixture of ferrous and ferric iron, to measure the total iron in the sample, the ferric iron is also reduced by the addition of ascorbic acid (spatula tip).

## 3.5 Irradiance measurement

The solar irradiation (W/m$^2$) was measured by a radiometer (Black Comet, StellarNet Inc.) in the experiments in CTC plant and by UV pyranometer (280-400 nm, Model CUV-5, Kipp&Zonen) mounted on a horizontal platform (as RPR) providing the UV incidence (W/m$^2$) in the experiments in RPR..

Experimental results are plotted in terms of accumulated UV energy per unit of treated volume ($Q_{UV}$, kJ/L) calculated by the following equation (Eq.14)(Malato et al., 2003):

$$Q_{UV,n} = Q_{UV,n-1} + \Delta t_n \cdot \overline{UV}_{G,n} \cdot \frac{A_r}{V_t} \tag{14}$$

Where, $Q_{UV}$ is the accumulated UV energy per treated volume between samples $n$ and $n-1$. $\overline{UV}_{G,n}$ (W/m$^2$) is the average UV radiation measured and $\Delta t_n$ is the experimental time between samples. $A_r$ is the illuminated area (m$^2$) and $V_t$ is the total volume of water(L).

The irradiance value in solar simulator was continuously monitored by a PMA 2100 radiometer (Solar Light Company).

## 3.6 Bacterial quantification and kinetic analysis

### *3.6.1 Fresh cultures preparation*

In the first set of experiments where disinfection was also evaluated, the inactivation of three spiked faecal bacterial strains were investigated. The selected bacteria strains were provided by the Spanish Culture Collection (CECT) as freeze-dried cultures: *E. coli* O157:H7 (CECT 4972), *S. enteritidis* (CECT 4155) and *E. faecalis* (CECT 5143). Briefly, once the stock dishes with the desired isolated bacteria colonies were ready, they were used to prepare the daily fresh liquid cultures needed for bacteria to be spiked in the reactors. To prepare the liquid cultures (also known as inoculum),single colonies of *E. coli*O157:H7, *S. enteritidis,* and *E. faecalis* were inoculated into 14 mL of the specific liquid broth medium for each bacteria (Nutrient-Broth I, Tryptone Soya Broth and Luria-Bertani Broth, respectively)and incubated at 37ºC for 20 h under constant agitation in a rotary shaking incubator (90 rpm) in order to obtain a stationary phase culture (~10$^9$ CFU/mL).After that, the bacterial suspension obtained was centrifugated at 3000 rpm for 15 min (J.P. Selecta), the bacterial pellet obtained was re-suspended in 14 mL of phosphatebuffer saline solution (PBS, Oxoid)and each microbial suspension was then directly diluted in the water intended to be treated to obtain an initial concentration of $10^3$ CFU/mL.

### 3.6.2 *Bacterial enumeration*

In the second set of disinfection experiments *E.coli*, *Salmonella spp*, and *Enterococci spp* were analyzed from freshly collected UWWTP effluent. Enumeration of bacteria was performed by standard plate counting method using selective agar media: Chromocult® (Merck), Salmonella Shigella agar (Scharlau) and Slanetz-Bartley agar (1% TTC, Scharlau), for *E. coli*, *Salmonella spp* and *Enterococci spp*, respectively. Water samples (50-500 µL) were spread onto each corresponding selective agar Petri dishes. Subsequently, plates were incubated for 24 h (*E.coli*) and 48 h (*Salmonella spp* and *Enterococci spp*) at 37 ºC and counted (Fig.16). The detection limit (DL) of this technique is 2 CFU/mL.

In order to quench residual $H_2O_2$ and to avoid any post-effect of oxidation, bovine liver catalase (Sigma-Aldrich) at 0.1 g/L was added to the samples at a ratio catalase: sample of 1:50.

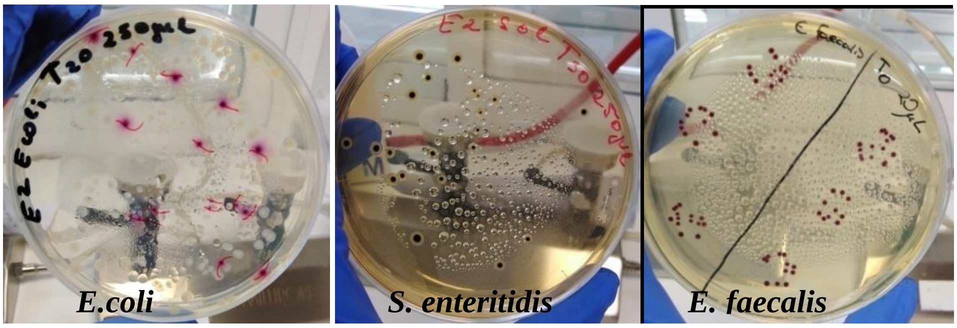


**Figure 16-** *E. coli*, *S. enteritidis* and *E. faecalis* colonies in selective agar Petri dishes

### *3.6.3 Inactivation kinetic models*

Inactivation kinetics constants of each bacterial strain was calculated as function of treatment time in order to compare the efficiency of the different treatments and conditions tested. Inactivation kinetic constants were obtained by fitting experimental data to two commonly mathematic models used for describing bacterial inactivation profiles by AOPs: (i) log-linear decay calculated according to Chick-Watson’s law (Model 1, Eq. 15)

$$Log(\frac{N}{N_0}) = -k \cdot t \qquad (15)$$

and (ii) A constant bacteria concentration or very smooth decay (known as ‘shoulder phase’) followed by a log-linear decay (Chick-Watson’s law) attributed to the accumulation of oxidative damages ending in the loss of cells viability (Model 2, Eq. 16):

$$Log(\frac{N}{N_0}) = -k \cdot t \begin{pmatrix} 0; t_{n+1} \geq t_n \\ -k \cdot t_n; t_{n+1} < t_n \end{pmatrix} \tag{16}$$

Where $N/N_0$ represents bacteria concentration reductions, $k$ is the disinfection kinetic constant and $t$ is the treatment time.

### 3.7 Quantitative microbial risk assessment

Quantitative microbial risk assessment (QMRA) is a probabilistic model that integrates data on pathogen abundance, human exposure, and infection to evaluate the potential health impacts related to a polluted environment (Sampson et al., 2017). QMRA for the ingestion of a raw-vegetable (lettuce) irrigated with secondary treated WW and tertiary treated WW was estimated using the web-based free software FDA-iRISK®. The software analyzes data concerning microbial hazards in food and returns an estimate of the resulting health burden on a population level based on mathematical equations and Monte Carlo simulations.

Based on system and hazards description for the specific scenario under evaluation, QMRA was performed according to four basic elements (FAO/WHO, 2016), briefly described as follows:

*(i) Hazard identification: E. coli* and *Salmonella spp* were selected as microbial hazards because these pathogens are commonly associated with foodborne infections outbreaks in raw-eaten vegetables (Wadamori et al., 2017). Although *Enterococci spp* inactivation was also evaluated, this pathogen was discarded for QMRA analysis because it is investigated only as indicator organism for water quality standards as it is not usually associated with fresh products infections outbreaks (Health Canada, 2011; Wadamori et al., 2017).

*(ii) Hazard characterization:* the disability-adjusted life year (DALYs)health metric was used for this assessment as this parameter is one of the more used health-based risk metrics. This parameter is a measure of overall disease burden, expressed as the number of years lost due to ill-health, disability or early death. The DALY values implemented were 0.143 and 0.049 for *E.coli* and *Salmonella spp*, respectively (Havelaar et al., 2012).

*(iii) Exposure assessment*: it determines the hazards consumers exposure, i.e., the risk scenario based on parameters such as microbial concentration, hazard prevalence and consumption patterns. The concentration and prevalence of *E. coli* and *Salmonella spp* in lettuce was obtained from a previous experimental study, where the microbiological load on harvested lettuces irrigated with secondary treated WW and tertiary treated (by SPF, sunlight/$H_2O_2$) WW were 10 CFU/3 g and 1 CFU/45 g, respectively (Aguas et al., 2019). Hazards prevalence for the crops irrigated with secondary treated WW was 0.33 for *E.coli* and 1 for *Salmonella spp* whereas 0.01 for both pathogens in crops irrigated with tertiary treated WW. The lettuce consumption pattern implemented was selected according to acute exposure data of the European Food Safety Authority (EFSA) (EFSA, 2019): serving sizes of 160 g and 150 eating occasions per person per year (PPPY).

The β-poisson dose-response model was used for both pathogens, where the risk of infection/illness ($R_{ill}$) is estimated based on Eq.17:

$$R_{ill} = 1 - \left(1 + \frac{D}{\beta}\right)^{-a} \qquad (17)$$

Where *D* is the hazard dose and α/β the dose-response parameters. The dose-response parameters used for this assessment were: i) α=0.248 and β=48.8for *E. coli* (Teunis et al., 2008); and ii) α=0.3126 and β=2884 for *Salmonella spp* (Haas, 1999). The probability of illness ($P_{ill}$) with a certain symptom for both pathogens were selected according to previous studies as 69% and 50% for *E.coli* and *Salmonella spp*, respectively (Amha et al., 2015; Seidu et al., 2015).

*(iv) Risk characterization:* in this step, the probability of infection for a population is characterized using all the outputs of the previous defined steps. The output data obtained from the QMRA software were the number of total illnesses per year (i.e., the annual risk of illness), the mean risk of illness per eating occasions ($R_{ill}$), and the DALYs PPPY.

## 3.8 Toxicological analysis

The assessment of acute and chronic toxicity related to the presence of micropollutants in SUWW and UWWTP effluent before and after treatment was carried out. Toxicity results obtained were expressed as a percentage of toxic effect (inhibition and/or immobilization).

### *3.8.1 Acute toxicity analysis*

The acute toxicity assessment of the water samples was performed using three different tests: *Vibrio fischeri (V. fischeri) and Daphnia magna (D. magna)*because they are the two bioassays most commonly used for acute toxicity assessment of urban WW (Rueda-Márquez et al., 2020) and additionally, their toxicity towards activated sludge were also estimated by respirometry.

#### *3.8.1.1 Vibrio fischeri – acute test*

*V. fischeri*, is a marine bacteria with bioluminescent properties, thus the toxicity analysis is based in its bioluminescent response towards an environment influenced by the treated water sample compared with a reference (sample containing only the bacteria in an isotonic solution).The tests were performed using the commercial kit BioFix®Lumi from MACHEREY-NAGEL and according to the manufacturer specifications which follow the standard method ISO 11348-3:1998. Briefly, the water samples previously filtered (0.22 µm) were incubated in contact with the bacteria for 30 min and after that, the luminescence intensity was measured using the BioFix® Lumi-10 luminometer (MACHEREY-NAGEL®).

#### *3.8.1.2 Daphnia magna*

This toxicity analysis consists in the study of the immobilization of the crustacean *D. magna* after its contact with the water sample (previously filtered through 0.45µm) during 24 and 48 h according to the operational procedure of the commercial kit DAPHTOXKIT F (MicroBioTest) and in adherence to standard guidelines (OECD Guidelines for the Testing of Chemicals, Section 2, Test No. 202: Daphnia sp.).

#### *3.8.1.3 Activated sludge – acute test*

Analyses were carried out through the Uina SURCIS BM-Advanced Respirometer. The respirometer was loaded with 1 L of endogenous activated sludge, maintaining it continuously aerated and agitated, corresponding to a LFS (liquid phase principle, flowing gas, static liquid) respirometry. Toxicity was evaluated by the addition of sodium acetate (as highly biodegradable compound) prepared in 30 mL of distilled water with 0.5 g of sodium acetate per g of volatile solids, and once the maximum oxygen uptake rate (OUR) was achieved, 30 mL of treated or untreated sample was added (Salmerón et al., 2020). Changes in OUR gives the percentage of inhibition, or stimulation if the sample is not toxic but contain highly biodegradable organic carbon.

### *3.8.2 Chronic toxicity analysis*

The chronic toxicity assessment of the water samples was performed using activated sludge, *V. fischeri* and the algae *Raphidocelis subcapitata* (*R. subcapitata*) according to the protocols described below.

#### *3.8.2.1 Vibrio fischeri - chronic*

Chronic toxicity was evaluated at the same conditions of acute toxicity test increasing the incubation time till 24 h, following an adapted protocol previously published by other authors (Westlund et al., 2018).

#### 3.8.2.2 *Activated sludge - chronic*

Analysis were performed in a SURCIS BM-Advanced Respirometer by recording the slope of OUR (and so the consumption of dissolved oxygen) when the aeration is stopped, corresponding to liquid phase principle, static gas, static liquid(LSS) respirometry and comparing such values obtained for the fresh activated sludge coming from the UWWTP. Treated and untreated samples after 1, 24, 48 and 72 h of contact with activated sludge were analyzed by OUR measuring. A decrease in the slope of OUR means chronic toxicity (Ponce-Robles et al., 2019).

#### *3.8.2.3 Raphidocelis subcapitata*

A battery of *R. subcapitata* toxicity tests was performed including the 72 h chronic test with *R. Subcapitata* according to the ISO 8692:2012. Cultures were kept in Erlenmeyer flasks. The initial inoculum contained $10^4$ cells/mL. The specific growth inhibition rate was calculated considering 6 replicates exposed at 20±1°C for 72 h under continuous illumination (6000 lx). Effect data were expressed as percentage of growth inhibition.

# CHAPTER IV

# Results and discussion

## 4.1 Comparison between homogeneous and heterogeneous solar driven AOPs: contaminants of emerging concern removal and toxicity

The work detailed in this section was published in the scientific journals *Separation and Purification Technology* and *Chemosphere,* respectively.

**Maniakova, G.,** Kowalska, K., Murgolo, S., Mascolo, G., Libralato, G., Lofrano, G., Sacco, O., Guida, M., Rizzo, L. (2020). Comparison between heterogeneous and homogeneous solar driven advanced oxidation processes for urban wastewater treatment: Pharmaceuticals removal and toxicity. Separation and Purification Technology, 116249 https://doi.org/10.1016/j.seppur.2019.116249 **(IF: 5.774; Q1)**.

K. Kowalska , **G. Maniakova** , M. Carotenuto , O. Sacco , V. Vaiano , G. Lofrano , L. Rizzo (2020). Removal of carbamazepine, diclofenac and trimethoprim by solar driven advanced oxidation processes in a compound triangular collector based reactor: A comparison between homogeneous and heterogeneous processes. Chemosphere238 124665 https://doi.org/10.1016/j.chemosphere.2019.124665 **(IF: 5.778; Q1).**

### *4.1.1 Rationale*

As the occurrence CECs into the environment due to the their release from UWWTPs is of concern, in the last decade increasing attention has been paid to the comparison of different AOPs methods for the tertiary treatment (Sgroi et al., 2020).

Even though, a number of studies on the effect of homogeneous and heterogeneous solar driven AOPs on CECs removal have been carried out, just poor information is available about comparative investigations, and no attempt has been made so far to compare SPF (even with a chelating agent) to solar heterogeneous photocatalysis using new generation photocatalysts (such as N-$TiO_2$) in real WW.

The aim of this study was to comparatively assess the performance of homogeneous and heterogeneous solar driven AOPs in a pilot-scale compound triangular collector (CTC) plant in removing a mixture of CECs (CBZ, DCF, TMP) in real WW. More specifically heterogeneous solar driven AOP using a supported nitrogen-doped $TiO_2$ (N-$TiO_2$) photocatalyst was compared with three different homogeneous solar driven processes at natural aqueous solutions pH: (i) sunlight/$H_2O_2$; (ii) SPF (Fe(II)/$H_2O_2$/sunlight); and (iii) SPF with EDDS (Fe(II)/$H_2O_2$/EDDS/sunlight). The effect of water matrix (deionized water (DW) vs real WW) and toxicity were also evaluated.

### 4.1.2 Experimental set-up

Photo degradation experiments were carried out in a CTC reactor (Fig.4) under natural solar irradiance, outside the Laboratory of Sanitary and Environmental Engineering, University of Salerno (latitude 40°N, longitude 14°E) by spiking mixture of CECs at an initial concentration of 200 µg/L each to DW or to WW (water matrix characterization – paragraph 3.2.2) in a recirculation mode at a flow rate of 260 mL/min.. Only one tube of the CTC reactor was used for experiment. Working volume was 2.7 L. In homogeneous solar driven AOPs, taking into account matrix effect, an initial concentration of 20 mg/L of $H_2O_2$ was used in DW experiments, while it was increased to 50 mg/L in WW experiments due to the higher oxidant demand of the real water matrix and according to the scientific literature (De la Cruz et al., 2013). Fe concentration used in all SPF experiments was 0.1 mM (5.6 mg/L). Fe:EDDS molar ration was 1:2.

In heterogeneous solar driven AOP tests, one tube of the module was packed with N-$TiO_2$ structured photocatalyst (434 g).

The following control tests with DW were performed: i) Sunlight as standalone processes ii) $H_2O_2$dark experiment iii) N-$TiO_2$dark experiment.

A battery of acute (*V. fischeri* and *D. magna)* and chronic *(R. subcapitata)* toxicity tests, was carried out on treated (60 and 300 min), untreated (0 min) WW and DW samples.

### 4.1.3 Control tests

Sunlight effect (photolysis control test) was investigated in DW to discriminate solar contribution to the removal of the CECs target with respect to solar driven AOPs experiments. DCF was effectively degraded by sunlight as standalone process (80% after 120 min treatment (15.3 kJ/L). The obtained results are in agreement with studies available in scientific literature. Moreira et al., (2018) investigated CECs removal in a compound parabolic collector solar reactor (CPC) where complete DCF removal was achieved after 240 min. However, sunlight was poorly effective in the degradation of TMP (only 15% removal after 300 min and 35.0 kJ/L) and not effective in the degradation of CBZ.

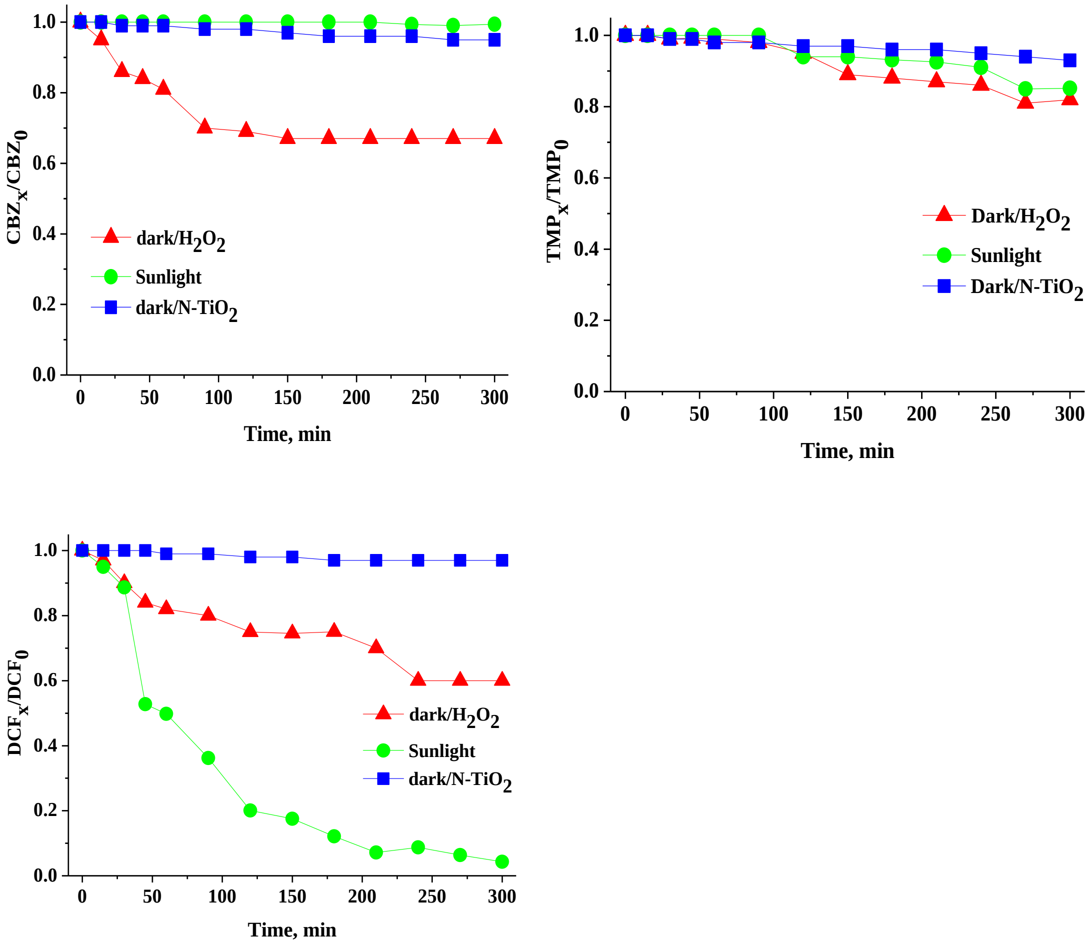


**Figure 17**- Degradation of CECs by control tests: dark/$H_2O_2$ (20 mg/L), dark/N-$TiO_2$ and sunlight as standalone processes in DW

The effect of $H_2O_2$ at 20 mg/Lon the degradation of CBZ, DCF and TMP under dark conditions was investigated. Degradation rate changed depending on the target CEC: from 18% after 300 min for TMP to 33 % after 150 min for CBZ and 40% after 240 min for DCF. The removal of all target CECs by adsorption on N-$TiO_2$under dark condition was very low after 300 min (CBZ 5 %, DCF 3%, TMP 7%).

### *4.1.4 Comparison among solar driven AOPs in DW*

Solar driven homogeneous and heterogeneous AOPs were investigated in DW. CBZ and TMP were effectively oxidized by sunlight/$H_2O_2$ after 150 min (80% removal $Q_{uv}$=19.1 kJ/L) and DCF (99% removal after 120 min, $Q_{uv}$=12.1 kJ/L). $H_2O_2$ consumption was 4 mg/L after 300 min.

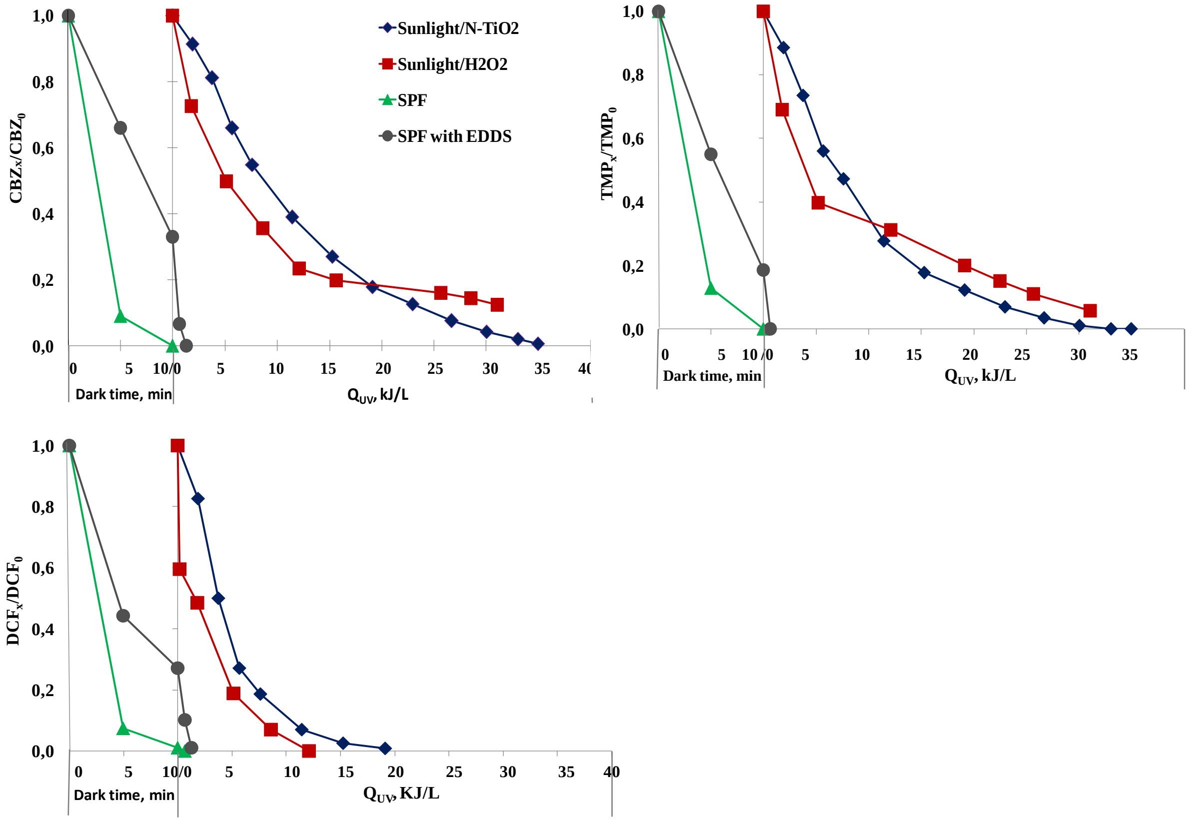


**Figure 18**-Degradation of CBZ, TMP and DCF by Sunlight/N-$TiO_2$, Sunlight/$H_2O_2$ (20 mg/L), SPF (Fe 0.1 mM, $H_2O_2$ 20 mg/L), SPF with EDDS (Fe 0.1 mM, EDDS 0.2 mM, $H_2O_2$ 20 mg/L) processes in DW

When SPF was investigated (spontaneous pH 3.4 in DW), high CECs degradation was observed already under dark conditions (solar reactor covered). As matter of fact, 90 % of all CECs were removed just after Fe and $H_2O_2$ addition (4 min contact time). $H_2O_2$ consumption was 18 mg/L after 300 min. Similar results were observed in the SPF with EDDS. Before uncovering the reactor, CBZ, DCF and TMP were removed at 67%, 70% and 81% respectively, already under dark conditions. In this case the initial spontaneous pH was higher (5.7). $H_2O_2$ consumption was 11 mg/L after 300 min. Fe concentration after 60 min was 0.01 mM.

The results observed under dark conditions are in agreement with the scientific literature. As matter of fact, Miralles-Cuevas et al., (2019) compared degradation of CECs by SPF mediated by EDDS at neutral pH and SPF at pH 3.5 and they also observed a high degradation of CECs in the dark.

Regarding to the heterogeneous AOPs, sunlight/N-$TiO_2$ process resulted in 99% degradation of CBZ after 300 min ($Q_{uv}$=35.0 kJ/L). Total removal was also observed for TMP, at a lower irradiation time (240 min, $Q_{uv}$=30.0 kJ/L). A faster degradation kinetic was observed for DCF and even in this case a total removal was observed after 300 min of solar exposure. The removal efficiency observed for CBZ and DCF is consistent with a previous work where solar photocatalytic efficiency of $TiO_2$ immobilized on glass spheres (sol being prepared by titanium isopropoxide, commercial P25 and polyethylene glycol) was investigated indifferent aqueous matrices spiked with 15 CECs, in a CPC solar based reactor (Miranda-García et al., 2011). In general, high degradation was attained as experiments where performed in DW, where all AOPs behaves so efficiently.

### *4.1.5 Comparison among solar driven AOPs in WW*

Control experiments by sunlight as standalone process in WW were carried out. DCF was completely removed after 300 min ($Q_{uv}$=13.7 kJ/L) while CBZ and TMP showed higher resistance to the process and only 23 and 29 %, respectively, were removed.

Due to the oxidant demand of WW, the initial concentration of $H_2O_2$ was increased to 50 mg/L. Concentration of CBZ, DCF and TMP was decreased up to 45%, 99% and 44%, respectively, in 240 min ($Q_{uv}$=12.1 kJ/L) by sunlight/$H_2O_2$ process. $H_2O_2$ consumption was 5 mg/L after 300 min. The process was less effective in WW matrix than the same process in DW because of the competition with other contaminants and/or radical scavengers occurring in WW. Basically, a similar trend was observed in SPF tests compared to DW experiments. Noteworthy, even dark Fenton resulted in a significant removal of the target CECs, being removed in the following order DCF > TMP > CBZ. The pH of the aqueous solution strongly affected process efficiency. The spontaneous pH of DW was lower than WW, where iron precipitation took place. According to the dissolved iron concentration in the respective water matrices, it decreased faster in WW tests than DW.

SPF with EDDS showed a higher efficiency compared to the other processes. Even in this case the reaction started under dark (Fenton process) and the degradation of the target CECs significantly improved under solar radiation (99% removal of CBZ and TMP was achieved in the early 15 min, $Q_{uv}$ =1.2 kJ/L). The process was affected by the water matrix to a less extent compared to SPF, because the addition of the chelating agent prevented the precipitation of iron at neutral pH.$H_2O_2$ consumption was 32 mg/L after 300 min. Fe concentration after 45 min was 0.01 mM. The observed CECs removal is consistent with a previous work where the degradation of 15 emerging contaminants (including CBZ, and

DCF), at 100 μg/L each was investigated applying SPF process at neutral pH with a CPC based photoreactor(Klamerth at al., 2009).

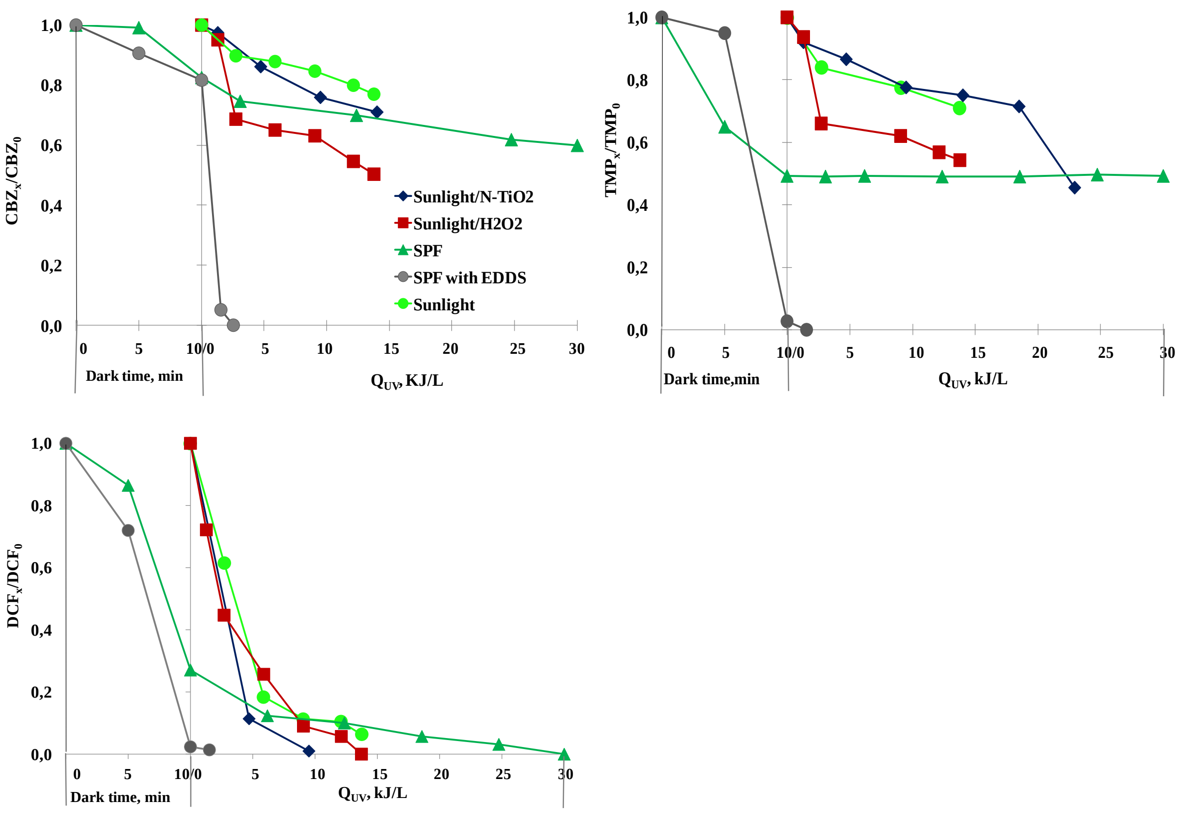


**Figure 19**-Degradation of CBZ, TMP and DCF by sunlight, sunlight/N-TiO$_2$, sunlight/H$_2$O$_2$ (50 mg/L), SPF (Fe 0.1 mM, H$_2$O$_2$ 50 mg/L), SPF with EDDS (Fe 0.1 mM, EDDS 0.2 mM, H$_2$O$_2$ 50 mg/L) processes in WW

Sunlight/N-TiO$_2$ process was less effective in WW compared to DW and less effective than the other investigated processes in the removal of CBZ and TMP (27% and 55% respectively after 300 min, Q$_{uv}$=22.9 kJ/L) in WW. DCF was completely removed after 120 min of the treatment (Q$_{uv}$=9.1 kJ/L).

### 4.1.6 Toxicity effect of solar driven AOPs

Toxicity of treated DW and WW based samples are summarized in Figs. 20 and 21, respectively. Results of spiked DW treated with solar driven AOPs showed species-specific effects with increasing level of toxicity as follows: *V. fischeri*<*R. subcapitata*≈ *D. magna*. The toxicity of untreated samples (not included SPF) varied in the ranges 15-23%, 41-68% and 67-80% for *V. fischeri*, *R. subcapitata*, *D. magna*, respectively. Higher toxicity of the untreated SPF samples can be related to the spontaneous pH (3.4).

Lower toxicity was observed in DW control samples with sunlight as standalone process compared to all others experiments, possibly because the process did not effectively remove CBZ and TMP, thus not forming toxic oxidation intermediates. After 300 min of illumination, toxicity values decreased from 100% up to 53% and from 57% to 38% in *R. subcapitata* in the SPF and SPF with EDDS complex, respectively. *D. magna* test showed high toxicity levels (100%) for both experiments. None of the treatments was able to reduce up to 50% the toxicity, and frequently toxicity increased as treatment time increased from 0 to 300 min for all endpoints. Apparently, CECs toxicity cannot be effectively removed via the considered treatments in DW, suggesting that CECs oxidation intermediates are more toxic than their parental compounds (Rizzo, 2011). Similarly to our findings in DW based treatments, Donner et al., (2013) highlighted that the toxicity of CBZ solution increased as degradation time increased, indicating that the mixture of degradation products formed was more toxic than the parent compound. The three endpoint used were still inhibited more than 60% (compared to negative controls) even after 90 min of UV-treated CBZ solution. Some authors suggested that the increased toxicity after photocatalytic treatment in $TiO_2$ water suspensions, even after 300 min of treatment, could be attributed to the formation of hardly oxidizable organic intermediates (Calza et al., 2006; Rizzo et al., 2009). In any case, toxicity evaluation in DW has interpretation constraints as these are not real conditions. The presence of other organics different to CECs and their oxidation intermediates would drastically change toxicity responses of different microorganisms. Therefore, toxicity evaluation under more realistic conditions is needed.

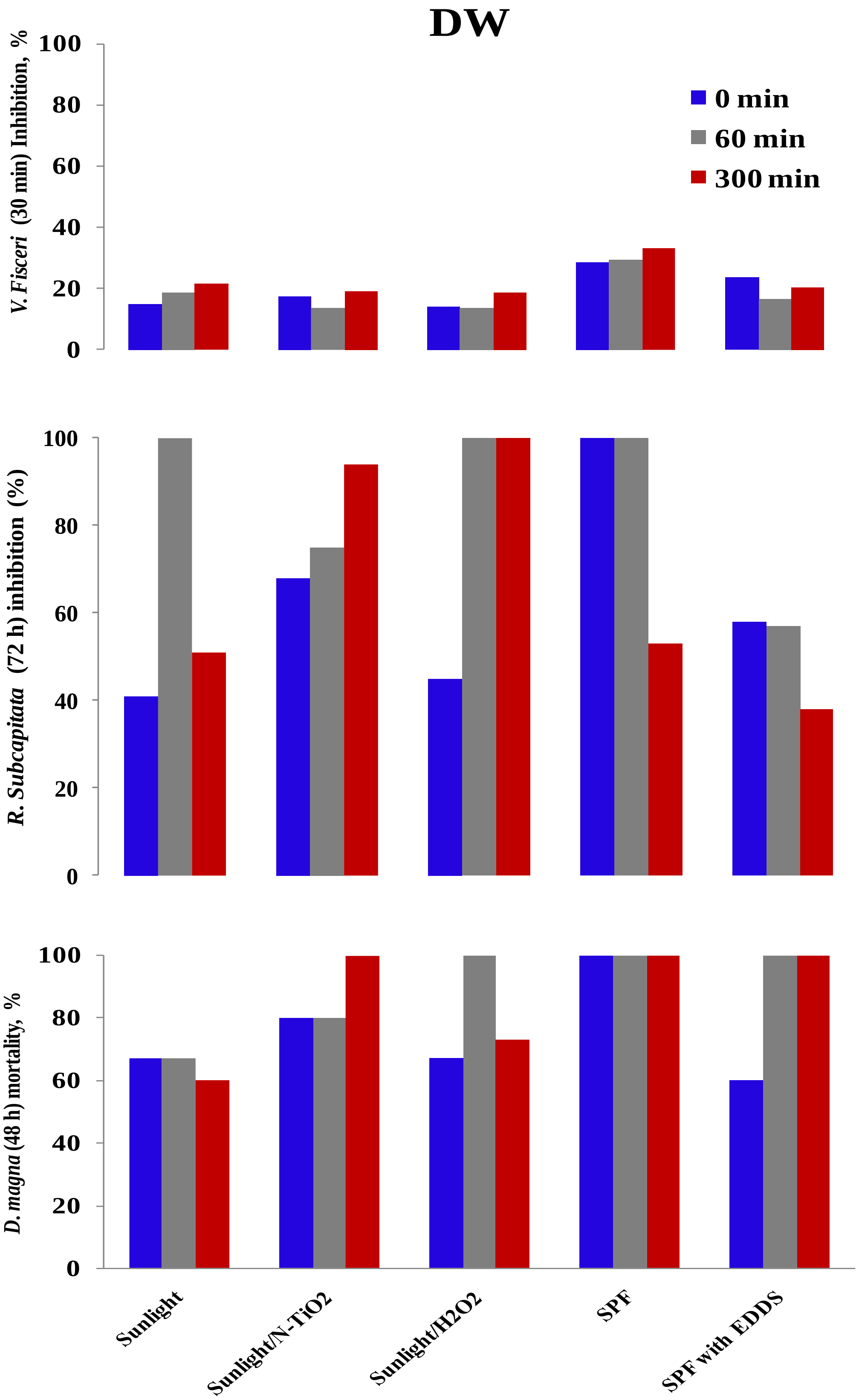


**Figure 20**- Toxicity test untreated and treated samples by sunlight, sunlight/N-$TiO_2$, sunlight/$H_2O_2$, SPF and SPF with EDDS considering *V. fischeri*, *R. subcapitata* and *D. magna* in DW

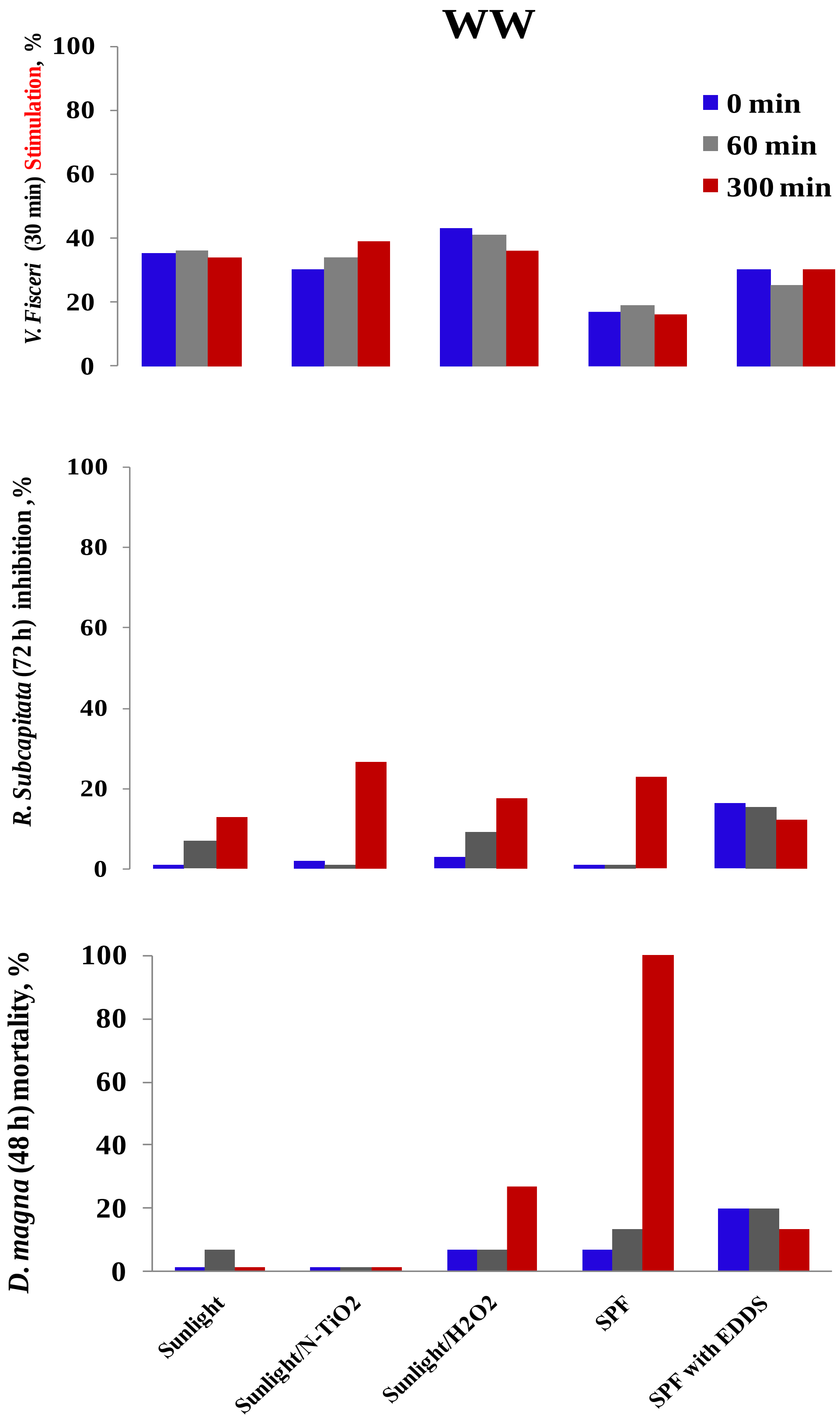


**Figure 21-** Toxicity test untreated and treated samples by sunlight, sunlight/N-$TiO_2$, sunlight/$H_2O_2$, SPF and SPF with EDDS considering *V. fischeri*, *R. subcapitata* and *D. magna* in WW

Lower toxicity was observed in the WW treated samples compared to DW tests. This trend can be possibly explained by i) presence in WW organic matter which could balance the toxic effect of the CEC due to more favorable condition for the microorganism ii) the matrix effect which resulted in a lower efficiency in the removal of the target CECs compared to DW matrix in treated samples, which in turn possibly resulted in a lower formation of oxidation intermediates. *V. fischeri* acute toxicity test showed only stimulation in all analyzed samples, which ranged between 16 and 43%. This result can be explained by the presence of organic matter in WW which supposedly promoted *V. fischeri* growth.

Microalgae and *daphnids* also evidenced that the whole toxicity in WW was lower than in DW. Results basically show that toxicity was not affected by the treatment, confirming that DW results are very often not conclusive and doubtful. Only in the case of SPF toxicity substantially increased. Paying attention to CECs degradation, it could be concluded that SPF was able to degrade DCF but only partially CBZ and TMP. Only SPF with EDDS was able to degrade parent compounds and possibly even oxidation intermediates, thus resulting in a decreased toxicity trend.

### *4.1.7 Conclusions*

SPF with chelating agent EDDS was found to be the most effective process of those tested in the removal of the target CECs from the investigated water matrices. Noteworthy, sunlight/N-$TiO_2$ was less effective than the other investigated processes in the removal of CBZ and TMP in WW. Toxicity values were confirmed to be substantially different in WW compared to DW matrix, possibly due to both a lower formation of oxidation intermediates (as consequence of a lower removal rate of the target CECs) and a positive effect of water matrix natural constituents on microorganism metabolism. These results also confirm that monitoring residual concentration of the target CECs in the effluent is not sufficient and toxicity should be also measured to avoid a dispose/reuse tertiary treated WW more toxic than secondary treated one. Although progresses to improve heterogeneous solar driven AOPs have been made by synthesizing new photocatalysts active under sunlight, this process (namely sunlight/N-$TiO_2$) is not yet competitive with SPF with EDDS in the removal of CECs from WW. According to the results achieved, SPF with EDDS process is a promising sustainable solution for tertiary treatment in UWWTPs.

## 4.2 Simultaneous removal of contaminants of emerging concern and pathogens from wastewater by photo-Fenton at neutral pH under simulated solar radiation

The work detailed in this section was published in scientific Journal *Science of the Total Environment*

**Maniakova, G.,** Salmerón, I., Polo-López, M.I., Oller, I.,Rizzo, L., Malato, S. (2020). Simultaneous removal of contaminants of emerging concern and pathogens from wastewater by homogeneous solar driven advanced oxidation processes. Science of the Total Environmental 766 144320 https://doi.org/10.1016/j.scitotenv.2020.144320(**IF: 6.551;Q1**).

### *4.2.1 Rationale*

SPF with EDDS at neutral pH has been successfully investigated for CECs removal (Costa et al., 2020; Miralles-Cuevas et al., 2014). Nevertheless, the efficiency of this process for the inactivation of bacteria seems to be contradictory and controversy, in particular with regard to the possible effect of photo-Fenton process using EDDS. Simultaneous removal of CECs and bacteria inactivation has received only poor attention so far (Soriano-Molina et al., 2019a).

Therefore, the main goal of this work was to investigate the capability of photo-Fenton process with EDDS at near neutral pH to simultaneously remove a mixture of CECs (CAF, CBZ, DCF, SMX, TMP) and inactivate bacteria (*E. coli*, *S. enteritidis*, and *E. faecalis)* in a SUWW. In this work, photo-Fenton using different iron concentrations and the influence of Fe:EDDS ratio were investigated in a solar simulator in order to find an optimal condition for investigation at pilot plant scale. The effect of dissolved organic carbon (DOC) was also evaluated.

### *4.2.2 Experimental set-up*

Experiments were carried out at neutral pH in solar simulator (Fig.5) by spiking CECs at an initial concentration of 100 μg/L each and bacteria mixtures at an initial concentration of $10^3$ CFU/mL each to SUWW or to SUWW-woc (water matrix characterization - 3.2.1) in the dark and keeping stirring. After 5 min of homogenization, Fe:EDDS was added and mixed for further 5 min. Finally, $H_2O_2$ was also added (50 mg/L) and the lamp was turned on to start photo-Fenton experiment.

The following set of experiments were carried out:

i) to evaluate Fe concentrations effect on bacteria inactivation and CEC removal by SPF two Fe(III) concentrations (0.05 mM and 0.1 mM) with Fe:EDDS molar ratio 1:2 were investigated in SUWW

ii) to evaluate DOC effect on bacteria inactivation and CEC removal by SPF, Fe:EDDS molar ratio 1:1 and 1:2 (Fe(III) 0.1 mM) was investigated in SUWW-woc

### *4.2.3 Effect of Fe concentration*

Results of CECs degradation and bacteria inactivation under simulated solar light by photo-Fenton with 0.1 mM Fe(III) and 0.2 mM EDDS in SUWW are shown in Fig.22. The aim of getting 80% removal of total CECs was achieved after 45 min. The target of 80% removal was selected according to Switzerland regulation in UWWTPs, being the only country that has established a release limitation for such compounds from UWWTPs into the environment (Bourgin et al., 2018). The highest removal rate of the target CECs was observed in the early 30 minutes of the experiment, then reaction rates slowed down, and the concentration of the target contaminants did not significantly change till the end of the experiment.

$H_2O_2$ consumption in the early 60 min was high (46 mg/L), which contributed to the fast degradation of CECs. Concentration of dissolved Fe was stable within the early 30 min. DCF was completely degraded by the process in 45 min. The degradation of the target CECs ranged from 70 to 99% according to the following order TMP<CAF<SMX<CBZ.

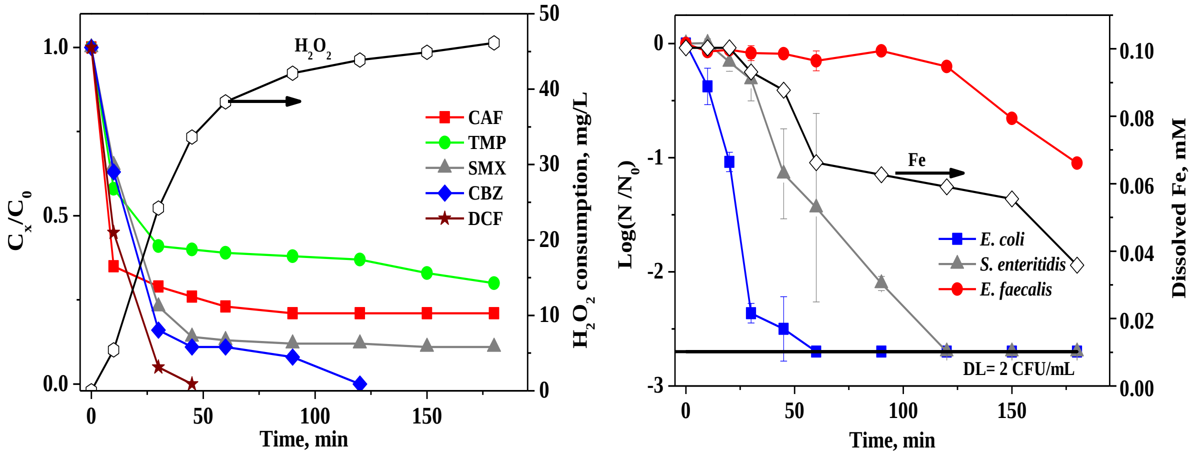


**Figure 22**– CEC degradation and bacteria inactivation by photo-Fenton with EDDS (Fe(III)= 0.1 mM, EDDS=0.2 mM, $H_2O_2$=50 mg/L) in SUWW at neutral pH

Total inactivation of *E. coli* and *S. enteritidis* (below the DL, 2 CFU/mL) was achieved after 60 min and 120 min, respectively, while *E. faecalis* showed a higher resistance to the disinfection process (76 CFU/mL after 180 min). Such different inactivation rates of the pathogens attributed to the different architectures of the cytoplasmic membranes. As a matter of fact, Gram-negative bacteria (*E. coli*) have a cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane containing lipopolysaccharides, while Gram-positive bacteria (*E. faecalis*) have only a cytoplasmic lipidmembrane with a thicker peptidoglycan layer, which confers more resistance to extracellular oxidative stress. Moreover, higher resistance to AOPs of Gram-positive bacteria compared to Gram-negative was observed in previous studies, as they need a higher number of oxidative attacks by $HO^{\bullet}$ to complete bacterial inactivation (Chung et al., 2009; van Grieken et al., 2010).

The second commonly used Fe (III) concentrations 0.05 mM with Fe:EDDS molar ratio 1:2 was also investigated in SUWW (Fig. 23 ). The aim of getting 80% removal of total CECs was not achieved. $H_2O_2$ consumption was 20 mg/L after 180 min. In the end of the treatment dissolved Fe concentration was 0.03 mM.

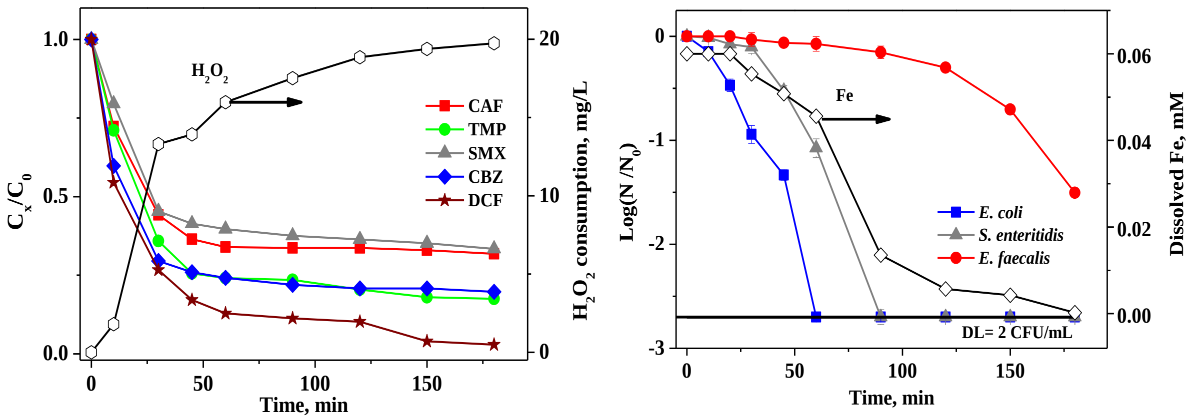


**Figure 23**– CECs degradation and bacteria inactivation by photo-Fenton with EDDS (Fe(III)= 0.05 mM, EDDS=0.1 mM, $H_2O_2$=50 mg/L) in SUWW at neutral pH

The inactivation rate of *E. faecalis* was faster (24 CFU/mL residual density after180 min) with Fe 0.05 mM than with higher Fe concentration, 0.1mM.Complete inactivation of *E. coli* and *S. enteritidis* (below the DL, 2 CFU/mL) was reached after 60 min and 90 min, respectively. Higher efficiency of the treatment using lower reagents concentration for the disinfection can be related to the lower DOC concentration as EDDS concentration was decreased.

### *4.2.4 Effect of organic matter*

The effect of organic matter on the simultaneous degradation of CEC and disinfection by photo-Fenton at neutral pH with EDDS was also evaluated by using two different water matrices, SUWW and SUWW-woc. Photo-Fenton treatment in SUWW-woc were investigated using Fe (0.1 mM) with EDDS at molar ratio 1:2 as 80 % CECs removal was not achieved with lower Fe concentration (0.05 mM). CECs removal was faster in SUWW-woc (Fig. 24) compared to SUWW (Fig. 22). 80% removal of total CECs was achieved before 10 min of the treatment. *E. faecalis* and *E. coli* showed a lower resistance to the disinfection process in SUWW-woc than in SUWW. *S. enteritidis* had a similar inactivation in both SUWW-woc and SUWW. $H_2O_2$ consumption was 48 mg/L after 180 min. In the end of the treatment, dissolved Fe concentration was 0.05 mM. Therefore, degradation of CECs was quicker in SUWW-woc than in SUWW, but disinfection results were quite similar.

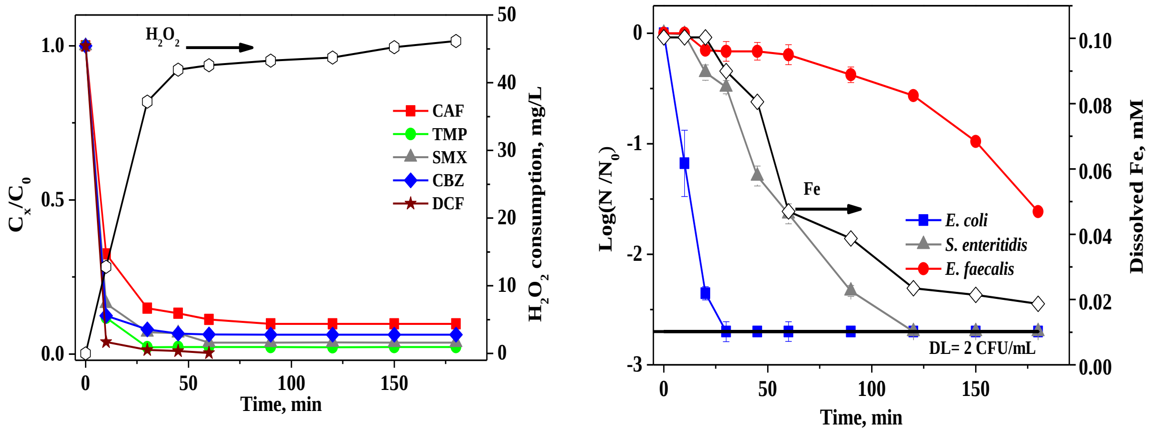


**Figure24**– CECs degradation and bacteria inactivation by photo-Fenton with EDDS (Fe(III)= 0.1 mM, EDDS=0.2 mM, $H_2O_2$=50 mg/L) in SUWW-woc at neutral pH

Table 6 shows treatment time, $H_2O_2$ consumption and Fe concentration when 80% removal of total CECs was achieved using Fe:EDDS at different concentration and at circumneutral pH along all the tests. A much faster CECs degradation rate was achieved with SUWW-woc, >80% degradation being observed in less than 10 min.

**Table 6** - CEC degradation by photo-Fenton at neutral pH with EDDS at neutral pH

| | | 80% removal of total CECs | | | |
|---|---|---|---|---|---|
| **Water matrix** | **Fe:EDDS mM** | **Time, min** | **$H_2O_2$ consum., mg/L** | **Fe mM** | **DOC initial, mg/L** |
| SUWW | 0.1:0.2 | 45 | 33.6 | 0.88 | 57 |
| SUWW | 0.05:0.1 | <60 | 16.0 | 0.04 | 41 |
| SUWW-woc | 0.1:0.2 | <10 | 12.8 | 0.1 | 48 |
| SUWW-woc | 0.1:0.1 | 10 | 15.8 | 0.1 | 33 |

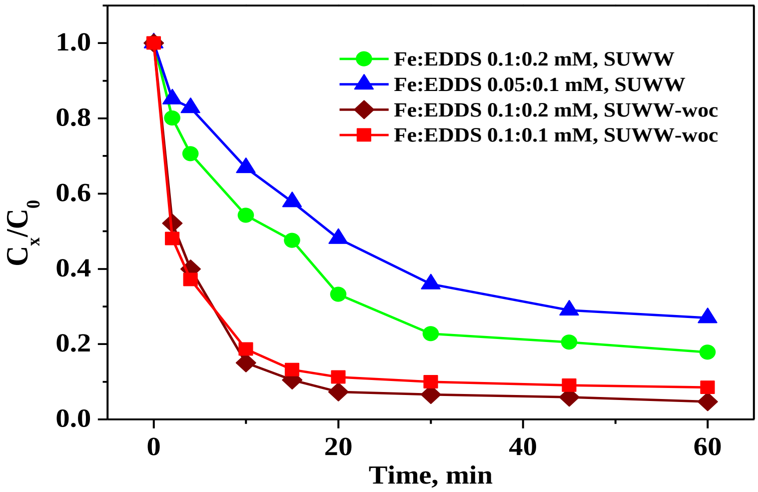


**Figure 25** –CEC degradation by photo-Fenton with EDDS at different Fe(III) and EDDS ratios and $H_2O_2$=50 mg/Lin SUWW and SUWW-woc at neutral pH

Table 7 shows pseudo-first order kinetic constants (*k*) for inactivation of all bacteria under simulated solar radiation, which were calculated according to Model 1 and 2, stated in Eqs.15 and 16 (Chapter 3.6.3)

**Table 7** –Pseudo-first order kinetic constants (*k*) for inactivation of all bacteria

| | | ***E.coli***[1] | | ***S. enteritidis***[1] | | ****E. faecalis***[2] | | |
|---|---|---|---|---|---|---|---|---|
| Water matrix | Fe:EDDS, Mm | k, $min^{-1}$ | DL, min | k, $min^{-1}$ | DL, min | k, $min^{-1}$ | CFU/ mL | SL, min |
| SUWW | 0.1:0.2 | 0.049 | 60 | 0.025 | 120 | 0.011 | 76 | 90 |
| SUWW | 0.05:0.1 | 0.043 | 60 | 0.030 | 90 | 0.015 | 24 | 90 |
| SUWW-woc | 0.1:0.2 | 0.093 | 30 | 0.025 | 120 | 0.011 | 26 | 60 |
| SUWW-woc | 0.1:0.1 | 0.061 | 45 | 0.023 | 120 | 0.020 | <DL | 60 |

*Since total inactivation of *E. faecalis* was not achieved, residual concentration at 180 minis provided.
[1] Log-Linear kinetic model (Model 1, Eq. 12)); [2] Shoulder phase + Log-linear kinetic model (Model 2Eq. 13); DL: Detection limit; SL: Shoulder length;

In order to achieve complete *E. faecalis* inactivation by photo-Fenton, a lower ratio EDDS:Fe at Fe 0.1 mM was tested. Results with molar ratio1:1 of Fe:EDDS, in SUWW-woc are shown in Fig. 26. As far as the concentration of EDDS was decreased in SUWW-woc, DOC decreased as well. In such a case, bacteria have less favorable conditions due to the lower concentration of organic matter in the water matrix, which may act as a carbon source for their metabolism, but also as scavenger of $HO^{\bullet}$ (Rincón and Pulgarin, 2004).

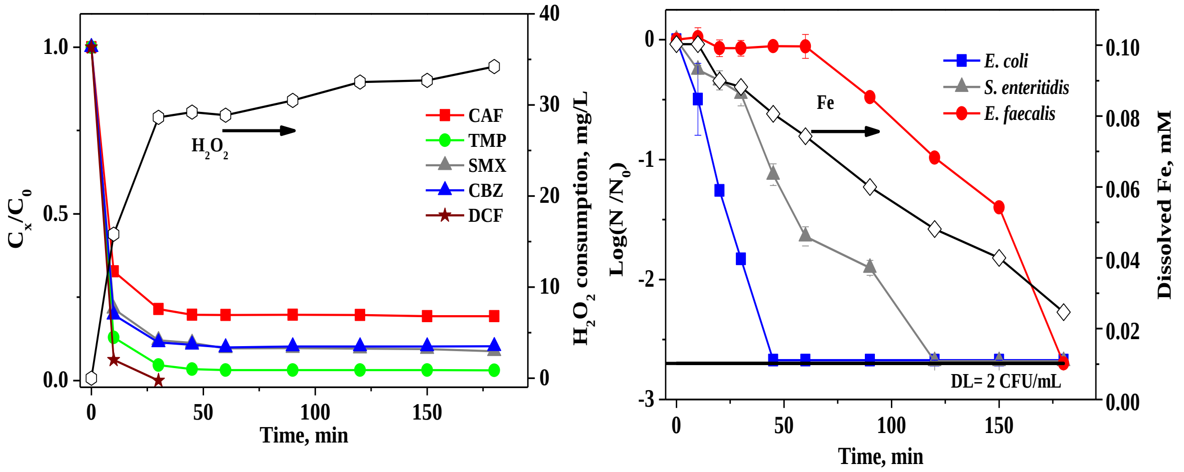


**Figure 26**– CECs degradation and bacteria inactivation by photo-Fenton with EDDS (Fe(III)= 0.1 mM, EDDS=0.1 mM, $H_2O_2$=50 mg/L) in SUWW-woc at neutral pH

High efficient removal of CECs was observed in the early 10 min (>80 % removal) of photo-Fenton with 0.1 mM of Fe (III) at 1:1 molar ratio with EDDS in SUWW-woc. A complete inactivation of *E. faecalis* (<DL) was observed after 180 min (Fig. 26). *E.coli* was quickly inactivated under all the investigated conditions (Table 7). Kinetic constant for *E. faecalis* with Fe:EDDS at molar ratio 1:1 in SUWW-woc was 2 times higher compared to the experiment with Fe:EDDS at molar ratio 1:2 in the same water matrix. A similar inactivation rate was observed for *S. enteritidis* under all tested conditions. Total inactivation was achieved after 90-120 min of treatment.

### *4.2.5 Conclusions*

Photo-Fenton with EDDS at circumneutral pH is an effective solution for CECs removal. Regarding to WW disinfection, *E.coli*, *S. enteritidis* and *E. faecalis* showed different resistance to the process being *E. faecalis* the most resistant pathogen among those investigated. Noteworthy, Fe (III) at concentration 0.05 mM was less effective than higher Fe concentration, 0.1m M with 1:2 molar ratio with EDDS. The results also confirm that DOC strongly affects to the efficiency of CECs degradation and WW disinfection. Total inactivation of all bacteria and 80 % removal of CECs was achieved only with Fe:EDDS 1:1 in SUWW-woc. Such operating condition were chosen for the scaling-up to RPR to evaluate the potential of SPF to be competitive with BATs (namely ozonation) in real WW matrix, as shown in  Chapters 4.3 and 4.4.

# 4.3 Ozonation Vs solar driven AOPs – Part I: bacteria inactivation

The work detailed in this section was published in scientific Journal *Science of the Total Environment* and also submitted to *Sustainable Chemistry and Engineering*

**Maniakova, G.,** Salmerón, I., Polo-López, M.I., Oller, I.,Rizzo, L., Malato, S. (2020). Simultaneous removal of contaminants of emerging concern and pathogens from wastewater by homogeneous solar driven advanced oxidation processes. Science of the Total Environmental 766 144320 https://doi.org/10.1016/j.scitotenv.2020.144320 (**IF: 6.551;Q1**).

**Maniakova, G.,** Salmerón, I., Nahim-Granados S., Malato S., Oller, I.,Rizzo, L., , Polo-López, M.I. (2021). Solar driven advanced oxidation processes Vs ozonation for wastewater safe reclamation. *Sustainable Chemistry and Engineering***(IF: 7.632; Q1)**.***Submitted***

### *4.3.1 Rationale*

The contribution of vegetables contamination and the subsequent foodborne infection outbreaks, mainly in raw-eaten vegetables, due to irrigation of crops by treated WW, represent an important global concern. The most widely used methodology for the estimation of microbiological risk associated with eating vegetables irrigated by WW is the quantitative microbial risk assessment (QMRA).

UWWTP upgraded with SPF with EDDS or sunlight/$H_2O_2$ can be an sustainable solution to minimize the risk associated to pathogens as well as to be in compliance with the corresponding limits for treated WW reuse (Rizzo et al., 2020).

Among the BATs for advanced treatment of WW, ozonation is increasingly used for the removal of CECs and water disinfection in several European countries (Rizzo et al., 2020). However, there is lack of data available about the comparison under realistic conditions between consolidated technologies (namely, ozonation) and photo driven AOPs as tertiary treatment for WW disinfection (Rizzo et al., 2019b).

Therefore, the main goal of this part of the thesis project was to investigate the efficiency of solar driven process (namely, sunlight/$H_2O_2$), AOPs (namely, SPF with EDDS at near neutral pH), operated in a raceway pond reactor (RPR), and ozonation at pilot scale for the simultaneous inactivation of *E. coli, S. enteritidis and E. faecalis* in simulated urban WW treatment plant (SUWW) effluent. The effect of carbonates on disinfection performance was also evaluated to clarify the main role of this inorganic specie on WW disinfection. Microbial risk of the reclaimed WW was carried out by QMRA analysis, adjusting the results obtained by the herein investigated treatment technologies to WW with different chemical composition taken from the scientific literature.

### *4.3.2 Experimental set-up*

Experiments were carried out at circumneutral pH by bacteria mixtures at an initial concentration of $10^3$ CFU/mL each in SUWW-woc. In the case of real WW disinfection. naturally occurred bacteria were investigated.

Experiments with SUWW-woc and WW (water matrix characterization – paragraph 3.2) in the RPR (Fig. 6) were carried out under natural solar irradiation in clear sunny days, from 11:30 a.m. to 14:30 p.m. local time. Additionally, in a set of experiments to evaluate the effect of $CO_3^{2-}$ and $HCO_3^-$ on disinfection performance, WW with lower carbonates concentration (WW-rc) were used. In this case, carbonates were lowered to 75±7.7mg/L (15±1.5 mg/L of DIC) by reaction with sulfuric acid under gentle mixing.

SPF was operated with EDDS and Fe at 0.1 mM and 50 mg/L of $H_2O_2$ sunlight/$H_2O_2$ treatment with 50 mg/L of $H_2O_2$ was also investigated. Working volume was 90 L.

Ozonation experiments were performed through the ozone pilot plant explained above (Fig. 7) with SUWW-woc and WW, a constant inlet air flow of 0.06 N $m^3$/h and ozone flow rate of 1.5 $gO_3$/h (ozone generator operated at 20% power). Therefore, ozone generation in all experiments was 83 $mgO_3$/L h. Working volume was 18 L.

QMRA for the ingestion of a raw-vegetable (lettuce) irrigated with secondary and tertiary treated WW was estimated using the web-based free software FDA-iRISK®. The software analyzes data concerning microbial and chemical hazards in food and returns an estimate of the resulting health burden on a population level based on mathematical equations and Monte Carlo simulations (paragraph 3.7).

### *4.3.3 Simulated WW disinfection by ozonation*

The inactivation profiles of *E. coli, S. enteritidis* and *E. faecalis* by ozonation in SUWW-woc are shown in Fig.27.DL from an averaged initial concentration of $10^3$ CFU/mL was reached for all pathogens in 6 min of treatment ($O_3$ consumption 7.5 $mgO_3$/L).

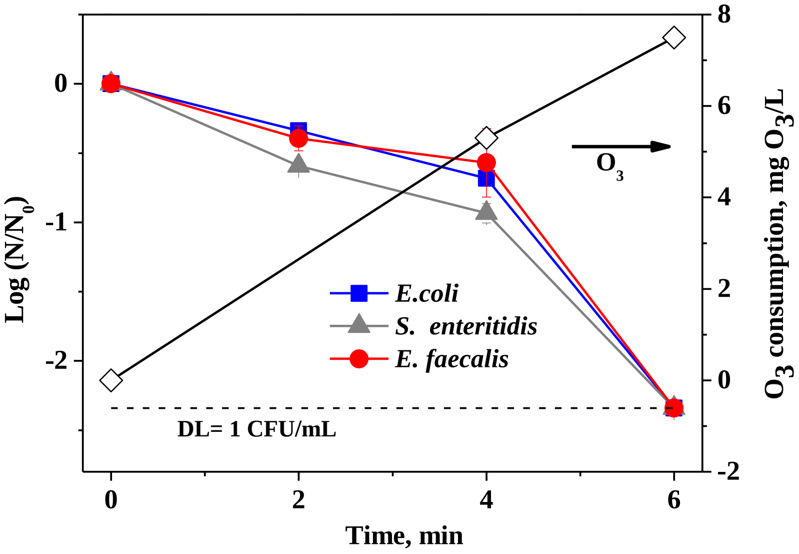


**Figure 27**–Bacteria inactivation by Ozonation at pilot plant scale in SUWW-woc

Having a high oxidation potential, ozone reacts with microorganisms fast, resulting in high lethality (Kim et al., 1999).Murray et al. (1965) proposed that lipoprotein and lipopolysaccharide layers of bacteria would be subjected first to be attacked by ozone resulting in changing cell permeability, eventually leading to lysis.

### *4.3.4 Real WW disinfection by ozonation*

The inactivation profiles of *E. coli, Salmonella spp* and *Enterococcus spp* by ozonation in WW are shown in Fig.28. The DL from an averaged initial concentration of $10^3$ CFU/mL was reached for all pathogens in 45 min of treatment ($O_3$ consumption 42.5 mg$O_3$/L). SUWW-woc (22.1 mg DOC/L) needed <30 mg$O_3$/L for 2 Log (1.36 g $O_3$/g DOC) inactivation and <42 mg$O_3$/L for reaching the DL in all the cases. DOC, carbonates and pH remained constant during the treatment. Turbidity decreased from 10 to 5 NTU since macromolecular organic compounds decompose into smaller molecules by ozonation (Setareh et al., 2020).

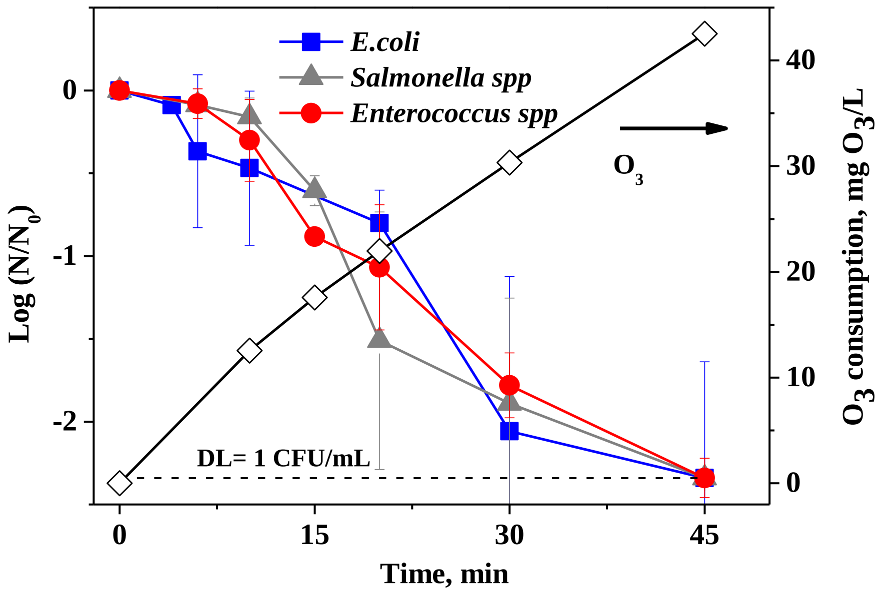


**Figure 28**–Bacteria inactivation by Ozonation at pilot plant scale in WW

The observed results are consistent with previous works in literature reporting bacterial inactivation by ozonation in actual WW. Iakovides et al., (2009) achieved total inactivation of *E. coli (*4- LRV) after 40 min the treatment. In other study, 3.8 and 3.9 Log Reduction Value (LRV) of *E.coli* and *Enterococci*, respectively, after 20 min of the treatment by ozonation was observed (Lüddeke et al., 2015).

### *4.3.5 Simulated WW disinfection by SPF*

Bacteria inactivation in RPR by SPF at neutral pH using Fe:EDDS 1:1 with Fe 0.1 mM in SUWW-woc are shown in Fig.29. A complete *E.coli* inactivation (below DL) was observed in 60 min of treatment (0.45 kJ/L) by SPF with EDDS, followed by *S. enteritidis* (8 CFU/mL after 180 min, 1.38 kJ/L), while *E. faecalis* showed the higher resistance. The dissolved Fe concentration decreased during the treatment; after 180 min the concentration was ca. 0.03 mM. The residual concentration of $H_2O_2$ decreased to 20 mg/L after 180 min of the treatment (initial concentration 50 mg/L).

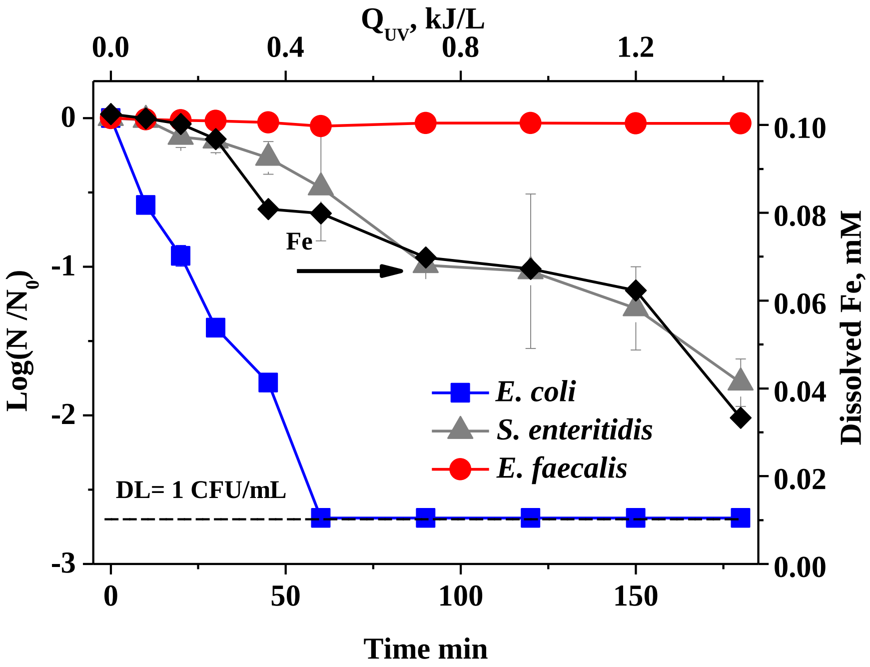


**Figure 29**–Bacteriain activation by SPF (Fe(III)= 0.1 mM, EDDS=0.1 mM, $H_2O_2$ =50 mg/L in SUWW-woc

However, SPF with EDDS was not sufficiently effective to totally inactivate microorganisms usually detected in UWWTP effluents. It has been concluded by García-Fernández et al., (2019) that the main reason would be the beneficial effect on bacteria metabolisms of highly biodegradable EDDS. Therefore, in the attempt to achieve a complete bacterial inactivation, sunlight/$H_2O_2$ was also investigated, as no organic addition would be needed for this process. As a matter of fact, this process is highly effective for microbial inactivation (Ferro et al., 2015; Fiorentino et al., 2015), and it will take advantage compared to SPF with EDDS of a lower DOC. Bacteria inactivation in RPR by sunlight/$H_2O_2$ (50 mg/L) at circumneutral pH in SUWW-woc are shown in Fig. 30.

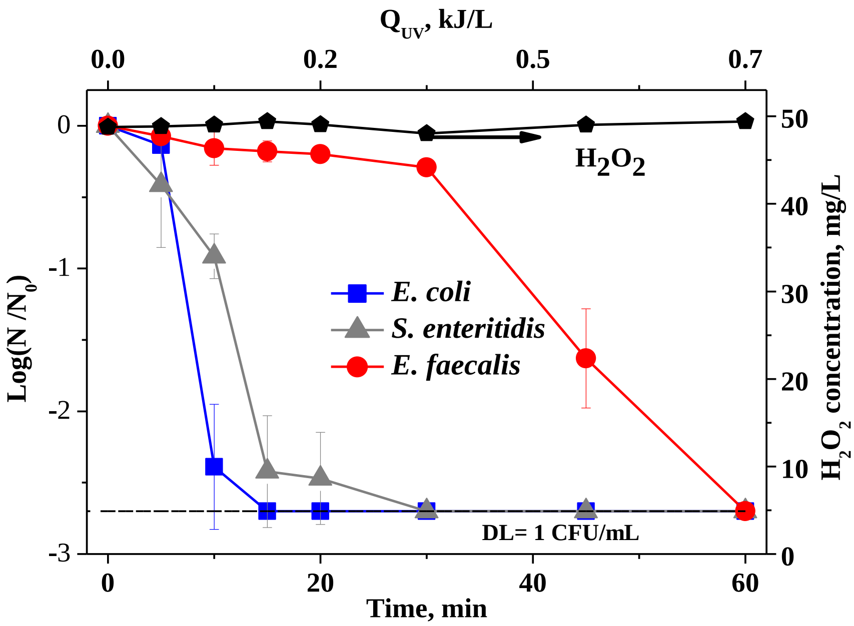


**Figure 30**–Bacteria inactivation by sunlight/$H_2O_2$ ($H_2O_2$ =50 mg/L) in SUWW-woc

Total inactivation for all the target bacteria was observed during sunlight/$H_2O_2$ process in RPR for *E. coli* (15 min and 0.16 kJ/L), *S. enteritidis* (30 min and0.33 kJ/L) and even *E. faecalis (*60 min and 0.69 kJ/L). Regarding *E. faecalis*, results are consistent with those observed by Formisano et al., (2016) who investigated inactivation efficiency in actual WW by CPC reactor with 50 mg/L of $H_2O_2$. The higher disinfection efficiency by sunlight/$H_2O_2$ compared to SPF-EDDS at circumneutral pH obtained in this study is in agreement with a previous work. García-Fernández et al., (2019) carried out disinfection experiments at laboratory scale (200 mL of total volume) and observed total inactivation of *E.coli* and *E. faecalis* (DL reached with 10.4 kJ/L and 20.3 kJ/L of $Q_{UV}$, respectively) for sunlight/$H_2O_2$ (0.3 mM) process.SPF with EDDS (0.1:0.2:0.3 mM Fe:EDDS:$H_2O_2$) process resulted in a slower inactivation rate (*E.coli* and *E. faecalis*) and DL was reached with 12.5 kJ/L and 29 kJ/L, respectively).

The higher disinfection efficiency of sunlight/$H_2O_2$ compared with SPF with EDDS can be explained by the mechanism that describe the inactivation of microorganisms by exposure to sunlight/$H_2O_2$. It is based on the accumulated damages inside cells by internal cellular injures occurring under sunlight and accelerated in the presence of $H_2O_2$ (Cadenas and Davies, 2000; Giannakis et al., 2016; Polo-López et al., 2011). Internal damages are mainly attributed to the promotion of ROS formation by chromophores direct photon-absorption (mainly solar UVA wavelengths) and indirectly by photo-Fenton-like reactions between

$H_2O_2$ and naturally occurring intracellular-iron. The increased ROS generation affects different intracellular vital components leading to bacterial death or lack of viability. During solar exposure, the intracellular ROS formation, mainly superoxide and $H_2O_2$ have been found critical in the facilitation of the internal photo-Fenton reaction, in both direct damage to bio-molecules and indirect intensification of ROS production. The addition of $H_2O_2$and its freely diffusion into the cell, can offer conditions for effective internal photo-Fenton reaction. Briefly, bacteria inactivation prevailing mechanism by sunlight/$H_2O_2$ occurs as followed:

- Light affects the DNA and the enzymes responsible for its reparation as well as ROS-scavenging enzymes into the cells such as catalase, superoxide dismutase, peroxidases;
- $H_2O_2$ penetrates the cell, causing imbalance of ROS, that jointly with light facilitate the release of iron into the cytoplasm, with reacts with $H_2O_2$ to create $HO^•$. Light reduces ferric iron to ferrous iron directly, through ligand-to-metal charge transfer or indirectly, through the reactive intermediates available by the light-induced malfunctioning into the cell, initiating a photo-catalytic cycle.
- Added $H_2O_2$may damage bacterial membrane, initiating its auto-oxidation.

The mechanisms of pathogens inactivation through SPF are produced by the simultaneous action of $HO^•$ attacking pathogen membranes and the damage generated by ROS formed due to the action of solar UVA and the diffusion of iron and $H_2O_2$ into the cell (internal photo-Fenton reactions). When EDDS is used for iron complexation, the same Fenton-like reactions explain the $HO^•$ radicals formation, being analogous to those occurred from free Fe(III) ion with $H_2O_2$ (García-Fernández et al., 2019).

The slower inactivation rate in the case of SPF was mainly attributed to the increased DOC content due to the presence of EDDS as it may act as a carbon source for bacteria metabolism as well as a scavenger of $HO^•$(Rincón and Pulgarin, 2004).

### *4.3.6 Real WW disinfection by SPF*

The inactivation of the three bacterial strains by SPF with EDDS at circumneutral pH and sunlight/$H_2O_2$ in real WW are shown in Figure 31 and 32, respectively. In general, the results showed different bacterial inactivation profiles under the same operational conditions, observing for both treatments the following order of inactivation kinetics: *Salmonella spp*>*E. coli*>*Enterococcus spp.*

Regarding SPF, DL after 180 min of treatment time (1.9 kJ/L of $Q_{UV}$) was not reached for all microorganisms. The dissolved Fe concentration decreased during the treatment (Fig.31) reaching 0.01 mM after 90 min. The concentration of $H_2O_2$ decreased to 10 mg/L after 180 min of the treatment (initial concentration 50 mg/L). Inactivation of pathogens by SPF (50 mg/L of $H_2O_2$ and 0.1 mM Fe:EDDS) in RPR was also investigated by Soriano-Molina et al., (2019a) and *E. coli* concentration below the DL was achieved in 80 min.

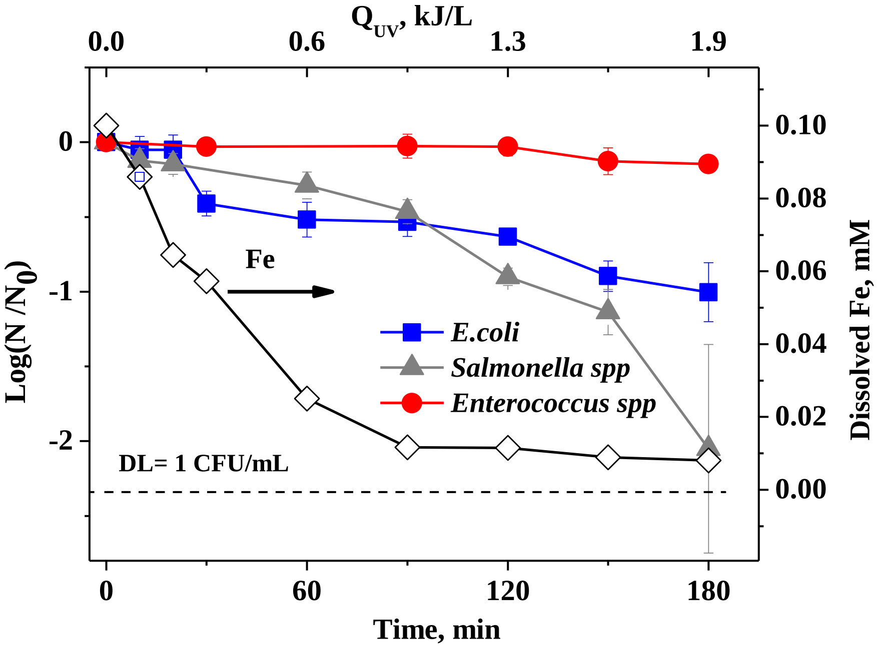


**Figure 31**–Bacteria inactivation by SPF (Fe(III)= 0.1 mM, EDDS=0.1 mM, $H_2O_2$ =50 mg/L) in WW

The inactivation of all bacteria by sunlight/$H_2O_2$ in WW (Fig. 32) showed similar behavior than the previously described for SPF. Although the DL was not reached even in this case, a lower bacterial concentration after 180 min of treatment time was observed. 22 mg/L of $H_2O_2$ were consumed.

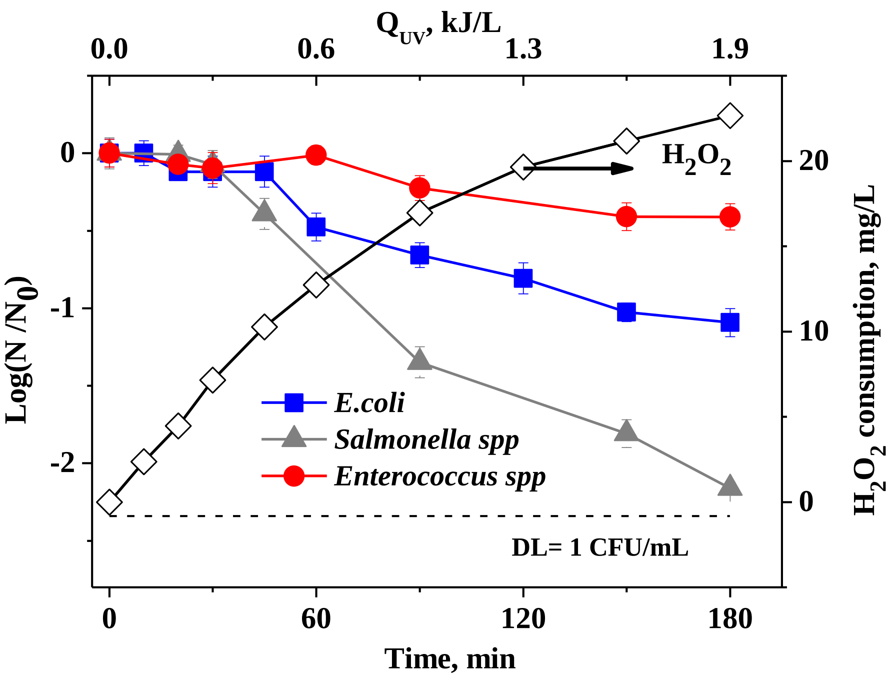


**Figure 32**–Bacteria inactivation by sunlight/$H_2O_2$ ($H_2O_2$ =50 mg/L) in WW

Moreover, a remarkable difference on the resistance to disinfection process was observed among the pathogens investigated. Such behavior can be explained by the different architectures of the cell wall. Gram-positive bacteria (*e.g.*, *Enterococcus spp*) have a cytoplasmic lipid membrane with a thicker peptidoglycan layer, which confers more resistance to extracellular oxidative stress. In contrast, Gram-negative bacteria (*e.g.*, *E. coli, Salmonella spp*) have a cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane containing lipopolysaccharides (van Grieken et al., 2010). As Gram-positive bacteria need a larger number of oxidative attacks by $HO^{\bullet}$ to complete bacterial inactivation compared to Gram-negative, they show higher resistance to AOPs (García-Fernández et al., 2019).

### *4.3.7 Effect of carbonates on the bacteria inactivation by SPF*

The effect of carbonates on the efficiency of SPF and sunlight/$H_2O_2$ processes was assessed under the same operational conditions (90L-RPR and the same reagents concentration used in experiments discussed in section 3.2.2), but using WW-rc for comparison purposes. Carbonates in WW were lowered to ca. 75±7.7mg/L (15±1.5 mg/L of DIC) by reaction with sulfuric acid under gentle mixing. Inactivation profiles of *E. coli, Salmonella spp* and *Enterococcus spp* by SPF and sunlight/$H_2O_2$ are shown in Fig. 33 and 34, respectively. In the case of WW-rc, similar results compared to those observed with WW were obtained in terms of the different bacteria resistance but both SPF and sunlight/$H_2O_2$ processes were more effective. Namely, sunlight/$H_2O_2$ has shown to be more effective than SPF.

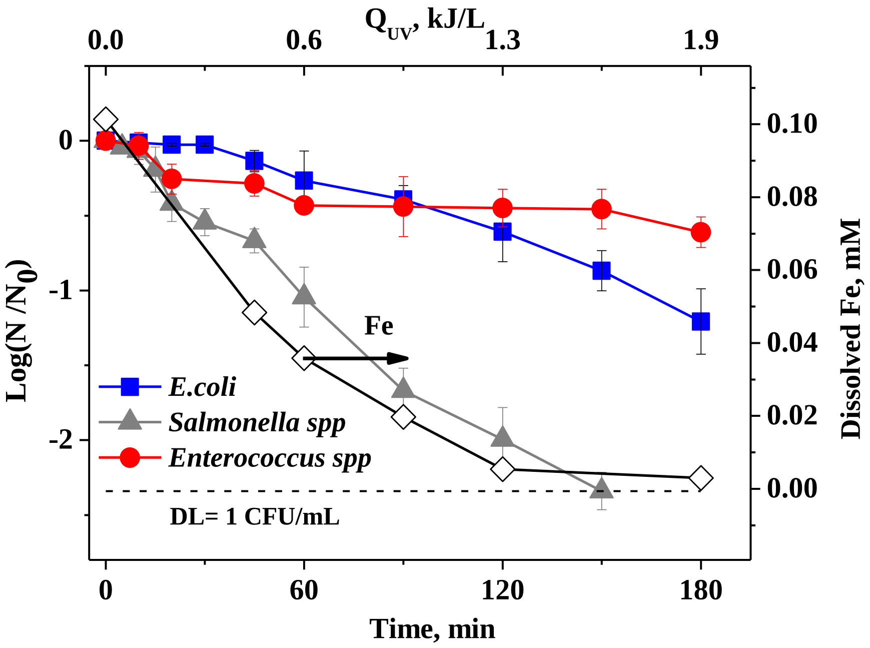


**Figure 33**–Bacteria inactivation by SPF (Fe(III)= 0.1 mM, EDDS=0.1 mM, $H_2O_2$ =50 mg/L) in WW-rc

In WW-rc, total inactivation of *E.coli* (below DL) was achieved in 150 min of sunlight/$H_2O_2$ treatment (2.2 kJ/L), while only 1-LRV was observed for SPF. Similar inactivation kinetics constants were obtained for *Enterococcus spp* and *Salmonella spp* in both solar processes but better disinfection performance was achieved in sunlight/$H_2O_2$ treatment (Table 2). Dissolved iron concentration decreased to 0.01 mM after 120 min and 45 mg/L of $H_2O_2$ were consumed after the SPF treatment. As discussed in detail by Soriano-Molina et al., (2018), iron precipitation begins with the Fe:EDDS decomposition into EDDS radical species and ferrous iron (Eq.18), which is later oxidized in the Fenton reaction (Eq.19) forming ferriciron, that could be complexed again if EDDS is still available, or ferrichydroxide, which would immediately precipitate:

$$\text{Fe (III):EDDS} + h\nu\ (290\text{-}500\ \text{nm}) \rightarrow \text{Fe}^{2+} + \text{EDDS}^{\bullet 3-} \quad (18)$$

$$\text{Fe}^{2+} + H_2O_2 \rightarrow \text{Fe}^{3-} + \text{HO}^{\bullet} + \text{OH}^{-} \quad (19)$$

DOC, carbonates concentration and pH remained constant during the treatment. Regarding to the sunlight/$H_2O_2$ process, a lower concentration of $H_2O_2$ (10 mg/L) was consumed after 180 min of the treatment and the other measured parameters remained unchanged.

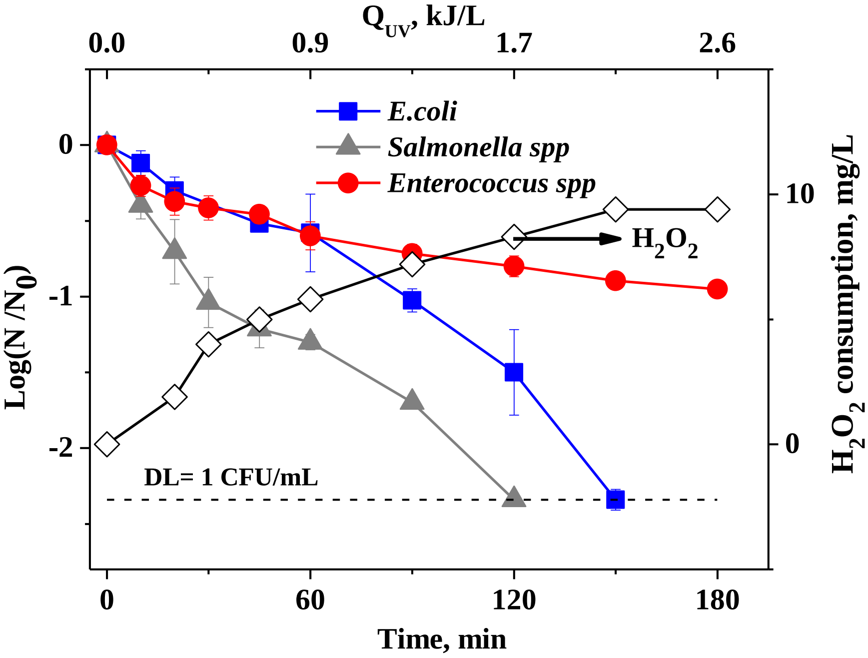


**Figure 34**–Bacteria inactivation by sunlight/$H_2O_2$ ($H_2O_2$ =50 mg/L) in WW-rc

Focusing on the influence of the carbonates concentration, it is clear that bacteria inactivation rates were faster in WW-rc compared to WW, where the concentration of carbonates was 488.0±53.0 mg/L, 6.5 times higher than in WW-rc (75 ±7.7mg/L). In general, bacteria inactivation was higher in WW-rc than in WW. These differences can be mainly attributed to the well-known reactivity of carbonates/bicarbonates against $HO^•$(Eq. 20-21). They act as a strong $HO^•$ radical scavenger and limit therefore the oxidative efficacy of any AOPs, including photo-Fenton process (Buxton and Elliot, 1986).

$$HO^• + CO_3^{2-} \rightarrow CO_3^• + HO^- \quad (20)$$

$$HO^• + HCO_3^- \rightarrow CO_3^• + H_2O \quad (21)$$

Regarding to sunlight/$H_2O_2$ process, the inactivation rate of all pathogens were higher in WW-rc compared to WW. Besides, a lower $H_2O_2$ consumption (10 mg/L) in WW-rc compared to WW (23 mg/L) was observed. The different $H_2O_2$ decomposition rate could be also attributed, among other factors, to the presence of carbonates in water. The alkalinity ($CaCO_3$) of the solution affects the $H_2O_2$ ionization in $H^+$ and $OOH^-$ because it is a weak acid (pKa = 11.62) (Phibbs and Giguère, 1951), accelerating therefore the $H_2O_2$

decomposition in WW compared to WW-rc. This difference may explain therefore the lower microbial inactivation kinetics observed in WW by the lower availability of $H_2O_2$ (Agulló-Barceló et al., 2013).

Effluents characteristics from UWWTP, in particular carbonates concentration, mainly depend on the characteristics of their geological source. There are many WW all over EU (and the world) with variable carbonates content ranging from around 50 to 650 mg/L (Table 8).

**Table 8** - Effluents characteristics from UWWTP in different areas of the world

| № | country | City/region | Carbonates, mg/L | Conductivity, mS/cm | pH | reference |
|---|---|---|---|---|---|---|
| 1. | Spain | El Ejido | 625.0 | 2.2 | 7.4 | (Soriano-Molina et al., 2019b) |
| 2. | Spain | Girona | 238.5 | 0.9 | 7.2 | (Soriano-Molina et al., 2019b) |
| 3. | Spain | Alcoy | 283.5 | 1.2 | 7.6 | (Soriano-Molina et al., 2019b) |
| 4. | Colombia | Florencia | 61.1 | 0.2 | 6.6 | Manrique Losada , 2019 |
| 5. | Poland | UpperSilesia | 214.8 | 1.0 | 7.4 | DudziakandKudlek., 2019 |
| 6. | USA | Minnesota | 402.5 | 2.2 | 8.0 | Damschen et al., 2008 |
| 7. | USA | North Dakota | 281.4 | 2.3 | 8.3 | Damschen et al., 2008 |
| 8. | USA | Ohio | 81.0 | 1.14 | 8.0 | (Hermosilla et al., 2020) |
| 9. | Russia | Orenburg | 528.7 | 2.1 | 7.2 | Shabanova et al., 2015 |

The choice of the best option for tertiary/advanced treatment of urban WW should also take into account UWWTP effluent characteristics. When UWWTP effluent is characterized by high concentration of carbonates (such as in the case of El Ejido, Spain or Orenburg, Russia), solar driven AOPs are expected to be relatively poorly effective and ozonation process may be a valid alternative rather than to remove carbonates before solar driven AOP application. However, in such cases, like those shown in Table 8, where WW are characterized by a low carbonates concentration, SPF or sunlight/$H_2O_2$ are expected to be a good disinfection option. In other cases reducing carbonates by a slight dose of acid would be also a feasible solution for applying AOPs instead of direct ozonation. Therefore, it would be needed a case by case study, as it is so usual in any WW treatment design.

### *4.3.8 Quantitative microbiological risk assessment*

QMRA was carried out only for those treatments were DL was reached, namely ozonation and sunlight/$H_2O_2$. The DL reached in both treatment, 1 CFU/mL, fits the minimum value for *E. coli* concentration detected in reclaimed WW reuse for agriculture according to Spanish RD1620/2007 (all categories) and the new EU 2020/741 regulations (Categories B to C). The results obtained from the quantitative analysis performed considering a scenario of reusing tertiary treated WW for lettuces irrigation are shown in Table 9.

**Table 9** - QMRA results obtained for the consumption of lettuces irrigated by secondary treated and tertiary treated WW

| Risk | ***E.coli*** | | ***Salmonella spp*** | |
|---|---|---|---|---|
| | Secondary treated WW | Tertiary treated WW | Secondary treated WW | Tertiary treated WW |
| Total illnesses | 15.7 | $2.02 \cdot 10^{-2}$ | 3.85 | $3.31 \cdot 10^{-4}$ |
| Mean risk of illness | 0.10 | $1.35 \cdot 10^{-4}$ | 0.03 | $2.21 \cdot 10^{-6}$ |
| Total DALYs PPPY | 2.20 | $2.89 \cdot 10^{-3}$ | 0.19 | $1.62 \cdot 10^{-5}$ |

In general, the data obtained for both pathogens from the QMRA analysis indicated a very high risk associated with the consumption of the crops irrigated with secondary treated WW, as the number of annual illnesses (per 150 eating occasions) is ca. 16 for crops contaminated by *E. coli* and almost four times less (ca. 4) for crops contaminated by *Salmonella spp.*

However, if crops are irrigated with tertiary treated WW, the microbiological risks associated to their consumption would be drastically reduced for both pathogens (more than three and four orders of magnitude for *E.coli* and *Salmonella spp.*, respectively). The results obtained for *Salmonella spp* are in agreement with the acceptable value of the annual risk of illness ($\leq 10^{-4}$) but the value of the DALYs PPPY is higher than the acceptable value ($\leq 10^{-6}$). Nevertheless, the risk values observed for *E.coli* (ca. $10^{-2}$ and $10^{-3}$) are higher than the acceptable values. The higher risk observed for *E. coli* can be explained based on the low infectious dose needed by this pathogen to cause illness, i.e., it may be related to the higher values of $P_{ill}$ (69 %) and to the health metrics (0.143DALYs) implemented for this pathogen with respect to *Salmonella spp.* The QMRA results obtained in this study for *Salmonella spp* are consistent with previous reported studies, such as the study performed by Deepnarain et al., (2020) whereat least 6 consumers out of 100 would be infected due to the intake of vegetables irrigated with WW containing *Salmonella.* On the other hand, the non-tolerable health risk obtained for *E.coli* is not in agreement with previous QMRA studies (Beaudequin et al., 2016) and it can be explained based on the conservative conditions implemented. Namely, some scenarios which could reduce the pathogenic effects were not implemented in this study, such as, lettuce washing prior to sending to market, withholding irrigation before harvesting, pathogen die-off rate during elapse time between harvesting and consumption (Beaudequin et al., 2016).

### *4.3.9 Conclusions*

Ozonation process was more effective for WW disinfection compared to the SPF with EDDS and sunlight/$H_2O_2$. *Enterococcus spp* did show a higher resistance to disinfection process than *E.coli* and *Salmonella spp.* Carbonates strongly affected SPF and sunlight/$H_2O_2$ disinfection efficiency. Accordingly, the selection of the most suitable disinfection process should take initial concentration of carbonates in WW into account. If the target WW is characterized by a high carbonates concentration, its removal before solar driven processes would be a suitable/sustainable option to improve the efficiency of the treatment. Although efficiency of the solar driven AOPs improves in WW with low carbonates concentration, SPF with EDDS cannot be used as alternative solution to the BATs (namely ozonation) in WW which content high resistant bacteria as *Enterococcus spp*. Sunlight/$H_2O_2$ being more effective than SPF with EDDS for bacteria inactivation can be recommended for WW disinfection but the treatment time should be increased. The process can be driven without additional reagent due to high residual concentration of $H_2O_2$ (39 mg/L) in WW after 180 min of the treatment. QMRA analysis indicated a very high risk associated with the consumption of the crops irrigated with untreated WW. On the opposite, the microbiological risk associated to *E.coli* and *Salmonella spp* was drastically reduced for crops irrigated with ozonation and sunlight/$H_2O_2$ treated WW.

# 4.4 Ozonation Vs solar driven AOPs – Part II: CECs removal and toxicity

The work detailed in this section is in preparation for submission to a high IF (Q1) scientific Journal

**Maniakova G.,** Salmerón I., Aliste M., Polo-López M.I., Oller I., Malato S., Rizzo L.Comparison between solar photo-Fenton at neutral pH and ozonation for tertiary treatment of urban wastewater: CEC removal and toxicity assessment.

### *4.4.1 Rationale*

The detection CECs, in the UWWTP effluents arouses concern for human health, in particular with regard to reuse for crops irrigation. Even though, a number of studies on the effect of SPF and ozonation on CECs removal have been carried out, just poor information is available about comparative investigations, and no attempt has been made so far, to our knowledge, to compare SPF (even with a chelating agent (EDDS)) to ozonation in WW using both endpoints: CECs removal and toxicity. Sunlight/$H_2O_2$ process was added to the study due to its interest after demonstrating the substantial efficacy for pathogens removal in UWWTP effluents.

Therefore, the main goal of this work was to compare SPF with EDDS at near neutral pH, operated in RPR, with ozonation to remove a mixture of CECs (CAF, CBZ, DCF, SMX and TMP) in simulated UWWTP effluent (SUWW-woc) and in real UWWTP effluent (WW). Acute and chronic toxicity was also evaluated.

### *4.4.2 Experimental set-up*

Experiments in the RPR (Fig. 6) and at ozonation pilot plant (Fig.7) were performed according to the chapter 4.3.2.

Acute toxicity evaluation of the samples was performed using activated sludge *V. fischeri* and *D. magna* Assessment of chronic toxicity of the samples was performed using activated sludge and *V. fischeri* according to the protocols described in chapter 3.8.

### *4.4.3 Simulated WW decontamination by ozonation*

Degradation of target CECs by ozonation was investigated in SUWW-woc first.

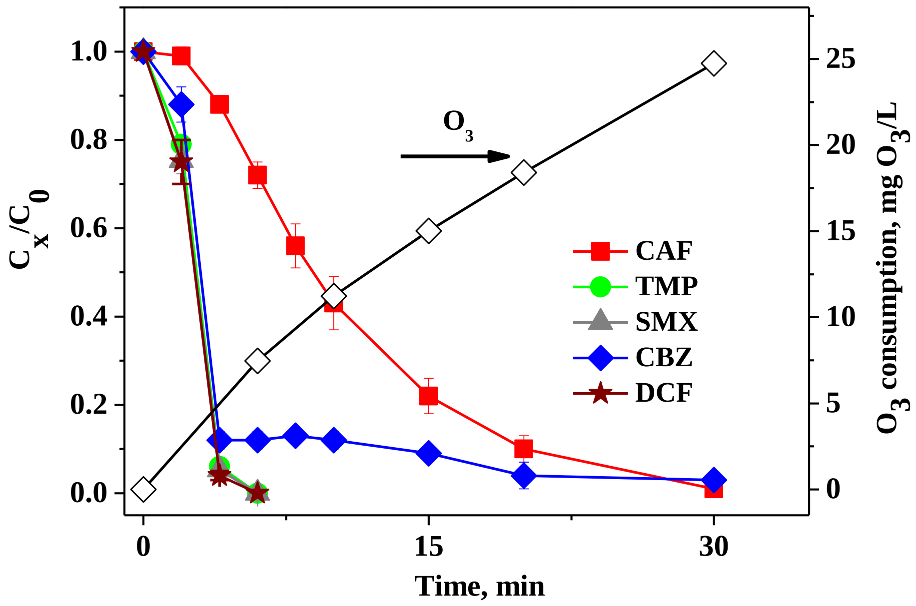


**Figure 35**–CEC degradation by Ozonation at pilot plant scale in SUWW-woc

The 80% removal threshold of the total sum of CECs by ozonation was reached in 5 minutes of the treatment where $O_3$ consumption was 6.4 mg$O_3$/L. DCF, TMP and SMX were remove at a rate >99% in 6 minutes ($O_3$ consumption 7.5 mg$O_3$/L). Abatement of CBZ followed the same trend of the above mentioned CECs till the early 6 minutes, but then the removal rate did not change significantly until the end of the experiment (97 % of removal). CAF was the most recalcitrant CEC, attaining the 80 % of degradation after 15 minutes treatment ($O_3$ consumption 15 mg$O_3$/L). Commonly applied specific ozone doses are in the range of 0.4–0.6 g $O_3$/g DOC to achieve CECs removals >80% (Rizzo et al., 2019b). SUWW-woc (15.5 mg DOC/L) needed <6.4 mg$O_3$/L for the 80% abatement, making the applied specific ozone dose (0.41 g $O_3$/g DOC) consistent with the range quoted above**.** Ozonation generates quite different kinetics in the case of CBZ and CAF. Rizzo et al. (2019) classified CECs in three groups according to their reactivity with ozone. Group A included CECs which have a $k_{O3} > 10^3$ $M^{-1}s^{-1}$ (high reactivity); those in group B have a $k_{O3} = 10^2$-$10^3$ $M^{-1}s^{-1}$ (intermediate reactivity); and CECs in group C have a $k_{O3} < 10^2$ $M^{-1}s^{-1}$ (low reactivity). The application of ozone generally involves contaminants oxidation by $O_3$ (direct reaction) and/or $HO^{\bullet}$ (indirect reaction) that are formed during ozone reactions. $O_3$ is instable in water and half-life time of ozone depends on the water quality, being the pH especially the most important factor affecting ozone decomposition and $HO^{\bullet}$ formation.

Hydroxide anions, generated by an increase on the pH, decompose ozone ($O_3 + OH^- \rightarrow HO_2^- + O_2$) (von Gunten, 2003). Moreover, $O_3$reacts selectively with compounds containing electron-rich moieties (e.g., unsaturated double bonds, deprotonated amine groups and activated aromatic systems)but $HO^\bullet$ exhibits a low selectivity and fast reaction with a wide range of organic and inorganic compounds. TMP, SMX, CBZ and DCF have a high reactivity with ozone, while CAF has a very low $k_{O3}$(Table 10). Ratios $k_{O3}/k_{HO^\bullet} < 10^{-5}$ indicate that indirect reaction are predominant over direct reaction with ozone (Yang et al.,2011), as it happens for CAF in this work. CAF is considered an ozone refractory compound and its abatement is influenced by indirect reactions with $HO^\cdot$. Hence, CECs with ozone-reactive moieties can usually be completely abated by primarily direct $O_3$ oxidation during conventional ozonation (Sonntag et al., 2012).

**Table 10.** Kinetic rate constants of studied CECs with $O_3$ ($k_{O3}$, direct reaction) and $HO^\cdot$ ($k_{HO^\bullet}$, indirect reaction) ($M^{-1}s^{-1}$)(Luo et al., 2012; Quiñones et al., 2015; Rizzo et al., 2019b; Yang et al., 2011)

| **CECs** | $\mathbf{k_{O3}}$ | $\mathbf{k_{HO^\bullet}}$ | $\mathbf{k_{O3}/k_{HO^\bullet}}$ |
|---|---|---|---|
| CAF | 0.80 | $5.9x10^9$ | $1.4x10^{-10}$ |
| TMP | $2.7x10^5$ | $8.7x10^9$ | $3.1x10^{-5}$ |
| SMX | $5.5x10^5$ | $5.5x10^9$ | $1.0x10^{-4}$ |
| CBZ | $3.0x10^5$ | $8.5x10^9$ | $3.5x10^{-5}$ |
| DCF | $1.0x10^6$ | $7.5x10^9$ | $1.3x10^{-4}$ |

### *4.4.4 Real WW decontamination by ozonation*

The 80% removal threshold of the total sum of CECs by ozonation in real WW was reached within the first 15 min ($O_3$ consumption 17.6 mg$O_3$/L). The results are consistent with those observed for SUWW-woc, TMP and DCF showed similar behavior, being removed at a rate >99% after 20 min treatment ($O_3$ consumption 22.0 mg$O_3$/L). On the contrary, CAF showed the highest refractoriness to the process, because more than 30 min were necessary to get the 80% threshold ($O_3$ consumption 30.3 mg$O_3$/L). At the end of the treatment (60 min), $O_3$ consumption was 54.6 mg$O_3$/L.

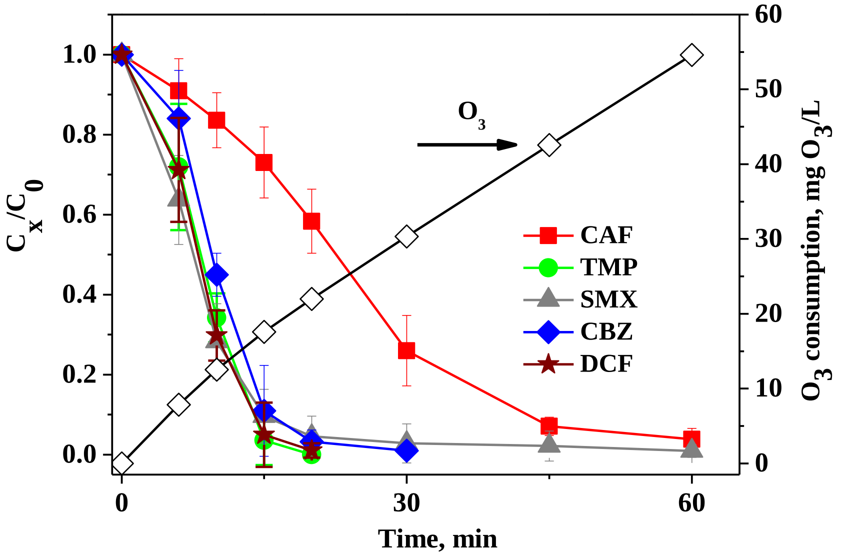


**Figure 36**–CEC degradation by Ozonation at pilot plant scale in WW

Our results are in agreement with previous studies. Lee et al.(2013) investigated the degradation of 16 CECs (included TMP, CBZ, SMX and DCF) in 10 effluents from different UWWTPs in Australia, Switzerland and the U.S. and fast abate (80%) of CECs were observed. Hollender et al., (2009) and Bourgin et al., (2018) investigated the abatement of 220–550 micropollutants at two full-scale UWWTPs upgraded with ozonation (followed by sand filtration). CECs such as DCF, or CBZ were abated by 80%.Lower efficiency of ozonation in real WW compared to SUWW-woc was observed due to the higher DOC, being considered one of the most important parameters affecting ozonation process efficiency (Nöthe et al., 2009).

### *4.4.5 Simulated WW decontamination by SPF*

CECs degradation in RPR by SPF at neutral pH using Fe:EDDS 1:1 with Fe 0.1 mM in SUWW-woc is shown in Fig. 37. 80 % removal of the total sum of CECs after 10 min of the treatment was observed (0.10 kJ/ L). Specifically, TMP, CBZ and DCF were removed at 85%, 82 %, and 95%, respectively, after 15 min (0.15 kJ/L). This result is consistent with a previous work where 90% pharmaceuticals removal was achieved with an accumulated UV radiation of around 1 kJ/L by SPF with EDDS at a 1:2 molar ratio in a RPR (De la Obra et al., 2017).

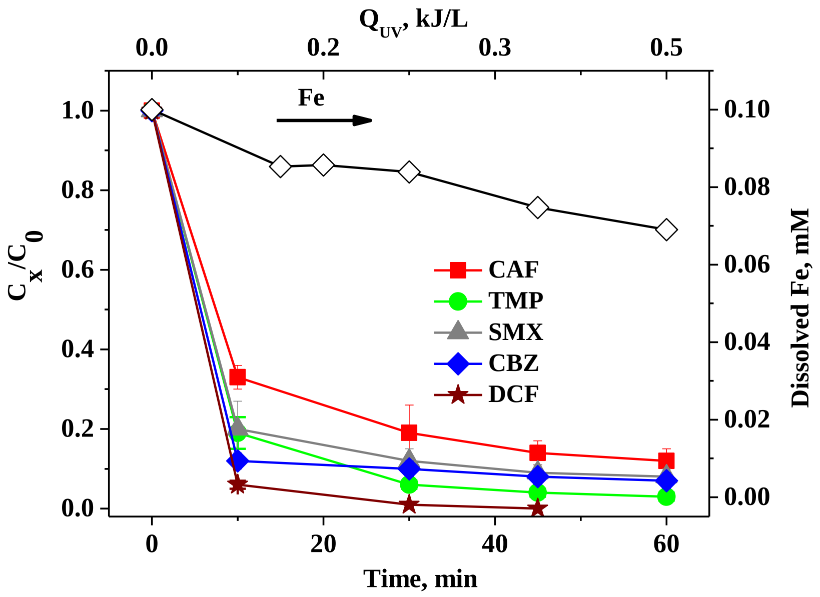


**Figure 37**–CEC degradation by SPF (Fe(III)= 0.1 mM, EDDS=0.1 mM, $H_2O_2$ =50 mg/L) in SUWW-woc

SPF with EDDS performance is related mainly to the synergistic effect of the EDDS radical, $H_2O_2$, $HO^{\bullet}$ and $O_2^{\bullet -}$.The most common and strongest oxidizing species are $HO^{\bullet}$ radicals (E° = 2.86 V). Under natural solar radiation, the complex yields the EDDS radical (Eq. 22) promoting generation of $HO^{\bullet}$ and$O_2^{\bullet -}$ radicals (Eqs 23-24):

$$\text{Fe (III):EDDS} + h\nu \text{ (290-500 nm)} \rightarrow [\text{Fe (III):EDDS}]^* \rightarrow \text{Fe (II)} + \text{EDDS}^{\bullet 3-} \quad (22)$$

$$\text{EDDS}^{\bullet} + O_2 \rightarrow O_2^{\bullet -} + \text{EDDS}^{2-} \quad (23)$$

$$\text{EDDS}^{\bullet} + OH^{-} \rightarrow \text{EDDS}^{2-} + HO^{\bullet} \quad (24)$$

When iron is released as the complex breaks up, most of it precipitates, but a small amount could react in the photo-Fenton cycle, or the complex could be regenerated if there is still EDDS available in the solution (Miralles-Cuevas et al., 2019). After 60 min of the treatment dissolved Fe concentration was 0.07 mM.

CECs degradation by sunlight/$H_2O_2$ (50 mg/L) at neutral pH in SUWW-woc did show different behavior (Fig. 38).

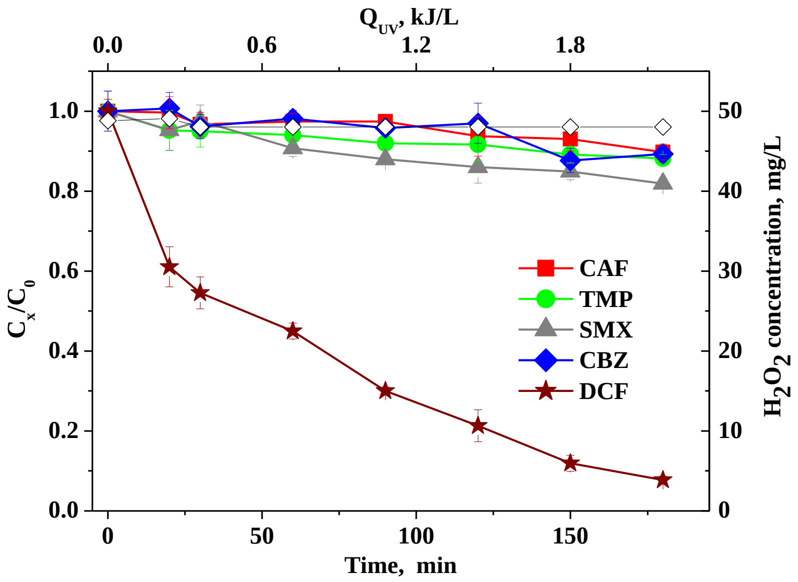


**Figure 38** – CEC degradation by sunlight/$H_2O_2$ in RPR ($H_2O_2$ =50 mg/L) in SUWW-woc

The process was not effective in CECs degradation because the final removal efficiency of the total sum of CECs was as low as 27 % after 180 min of treatment (2.12 kJ/L). CAF, CBZ, TMP and SMX were only removed at 10%, 11%, 12%, and 18%, respectively. However, 98 % removal of DCF was observed at the end of the treatment, mainly provoked by direct photolysis. These results are consistent with previous work (Moreira et al, 2018). In general, $H_2O_2$ absorption of solar radiation is so low, not permitting photolysis of it and therefore formation of $HO^{\bullet}$. $H_2O_2$ photolysis and efficient conversion in $HO^{\bullet}$ could be attained using UVC lamps (< 280 nm).

### *4.4.6 Real WW decontamination by SPF*

The highest removal rate by SPF with EDDS in WW-rc of the target CECs was observed in the early 60 minutes of the experiment (0.9 kJ/L), then reaction rates slowed down, and the concentration of the target contaminants did not significantly change till the end of the experiment(Fig. 39). The target of 80% removal of the total sum of CECs was almost achieved after 180 min of treatment (2.6 kJ/L). DCF was completely degradedwithin150 min (2.1 kJ/L), while the most refractory CECs were SMX and CAF (64% and 68% removal at the end of the process, respectively). A significant Fe precipitation was observed during the treatment till to 90 min. Such high Fe precipitation means that Fe:EDDS was not sufficiently stable as Fe was released as the complex broke up (Miralles-Cuevas et al., 2019).45 mg/L of $H_2O_2$ was consumed after SPF treatment in WW-rc.

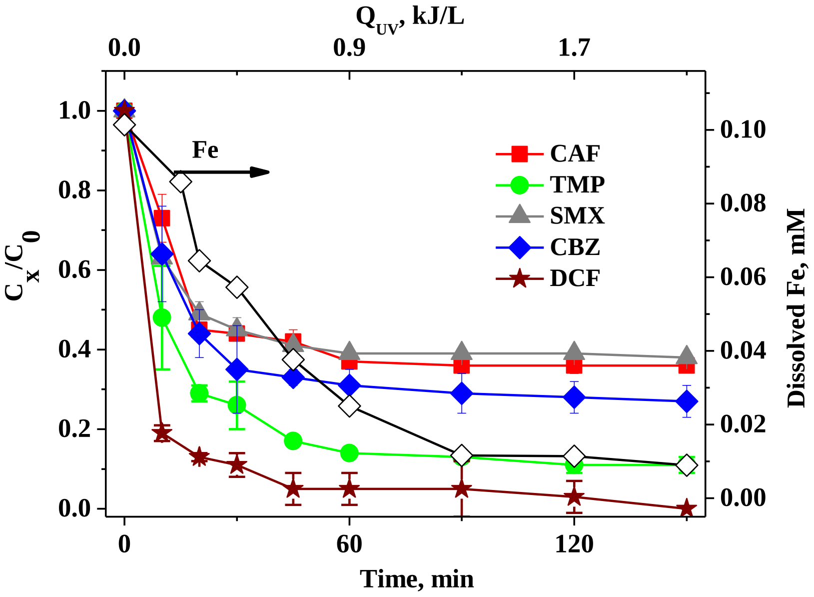


**Figure 39**–CEC degradation by SPF (Fe(III)= 0.1 mM, EDDS=0.1 mM, $H_2O_2$ =50 mg/L) in WW-rc

SPF process has shown to be strongly affected by the water matrix, since in SUWW-woc the 80 % removal of the total CECs was reached in 10 min while in WW-rc after 180 min. This behavior can be explained by the fact that WW-rc presented higher amount and variety of organic compounds, which can compete for the oxidative radicals generated. It must be remarked that in any photo-process the increase in turbidity due to iron precipitation could hamper the light pathway as well as the UV light can be adsorbed by organic matter (Arrigo and Brown, 1996), increasing the negative effect of DOC on the removal of the target CECs.

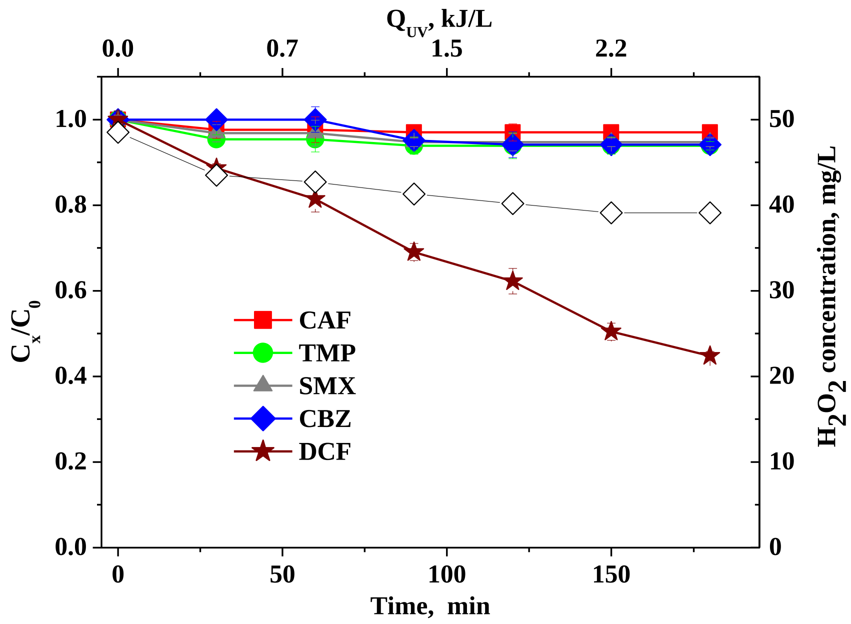


**Figure 40** – CEC degradation by sunlight/$H_2O_2$ in RPR ($H_2O_2$ =50 mg/L) in WW-rc

Sunlight/$H_2O_2$was not effective in CECs degradation in WW-rc because the final removal efficiency was as low as 15 % after 180 min of treatment (2.6 kJ/L)(Fig. 40). CAF, CBZ, TMP and SMX were not significantly affected by the process (3-6%). However, 55 % removal of DCF was observed at the end of the treatment. The same reasons stated in the discussion of Fig 38 applies herein.

### *4.4.7 Toxicity results*

Toxicity tests in complex samples can be affected by synergistic, additive and antagonistic interactions, this is why it is highly important to evaluate toxic effects in organisms representing different trophic levels(Ponce-Robles et al., 2019).

Acute and chronic toxicity tests with different techniques were carried out in untreated (secondary WW spiked with CECs (100 µg/L of each)) and treated (tertiarytreated WW by SPF and ozonation) samples in which almost complete or around 80 % removal of total CECs was achieved. Samples were analyzed for acute and chronic toxicity without any dilution and the results are plotted as % of toxic effect.

Different sensitivity was observed in terms of acute toxicity for the different organisms tested. No acute toxic effect was detected in activate sludge (data not shown) when exposed to samples treated by either ozonation or SPF in both analyzed water matrices. This can be explained by the fact that the sludge is integrated by a huge variety of different microorganisms, which can assimilate the organic matter on the samples and therefore it has less sensitivity to toxicity.

Acute toxicity tests with *V. fischeri* (30 min contact) on samples before tertiary treatment did not show any toxic effect in both SUWW-woc and WW matrices (Fig. 41 and 42).41 % inhibition of samples treated by ozonation in SUWW-woc was observed; on the opposite SUWW-woc sample treated by SPF showed a slight stimulation (5%). The increased toxicity observed for ozonation after 30 min treatment may be due to the formation of oxidation or transformation by-products generated during the process. Regarding to the *V. fischeri* toxicity test with WW samples treated by ozonation, stimulation increased from 44% in untreated sample to 58 % in the treated sample. Slight increase in stimulation maybe explained by the transformation of biorefractory organics by ozonation in more biodegradable compounds(Chen et al., 2019).

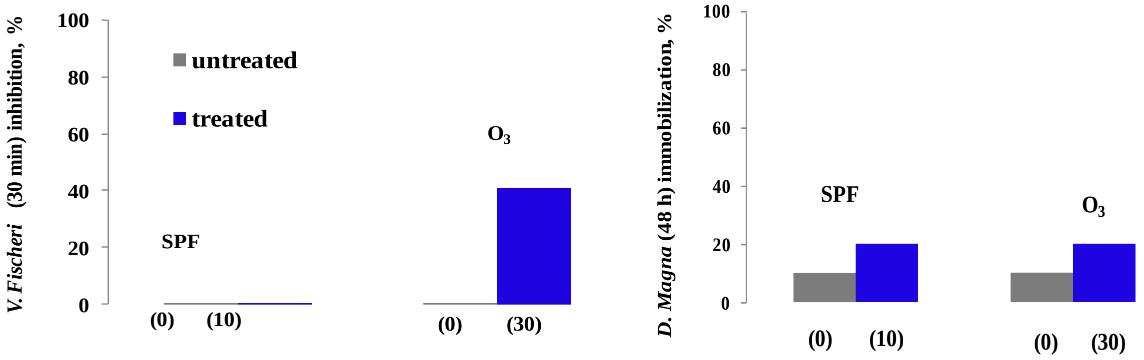


**Figure 41**– Acute toxicity tests on untreated and treated SUWW-woc samples by SPF and ozonation considering *V. fischeri* (30 min) and *D. magna* (48 h). Numbers in brackets indicate treatment time of each process to reach 80 % or more than 80% removal of total CECs, in minutes.

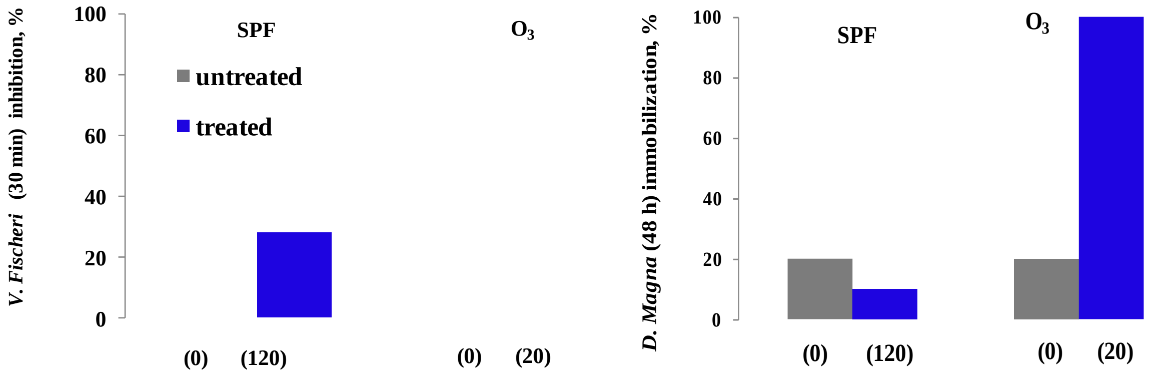


**Figure 42**– Acute toxicity tests on untreated and treated WW samples by SPF and ozonation considering *V. fischeri* (30 min) and *D. magna* (48 h). Numbers in brackets indicate treatment time of each process to reach 80 % or almost 80% removal of total CECs, in minutes.

*V. fischeri* gave less sensitive results compared to *D. magna*. In SUWW-woc low toxicity (20 %) was observed in both treated samples (Fig.41).WW samples treated by ozonation were more toxic compared to SPF (Fig.42). Highest acute toxicity was observed for *D. magna* in WW treated by ozonation where immobilization increased during the treatment from 20 to 100%. These results are in agreement with Miralles-Cuevas et al., (2017) previous work, where 100 % immobilization of *D. magna* was observed in all WW samples treated by ozonation where 90% of CEC removal was achieved.

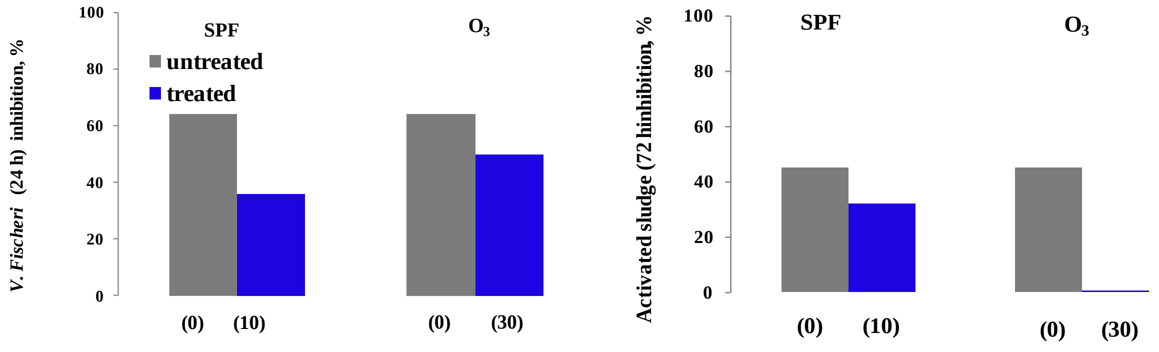


**Figure 43**– Chronic toxicity tests on untreated samples and treated SUWW-woc samples by SPF and ozonation considering *V. fischeri* (24 h) and activated sludge (72 h). Numbers in brackets indicate treatment time of each process to reach 80 % removal of total CECs, in minutes.

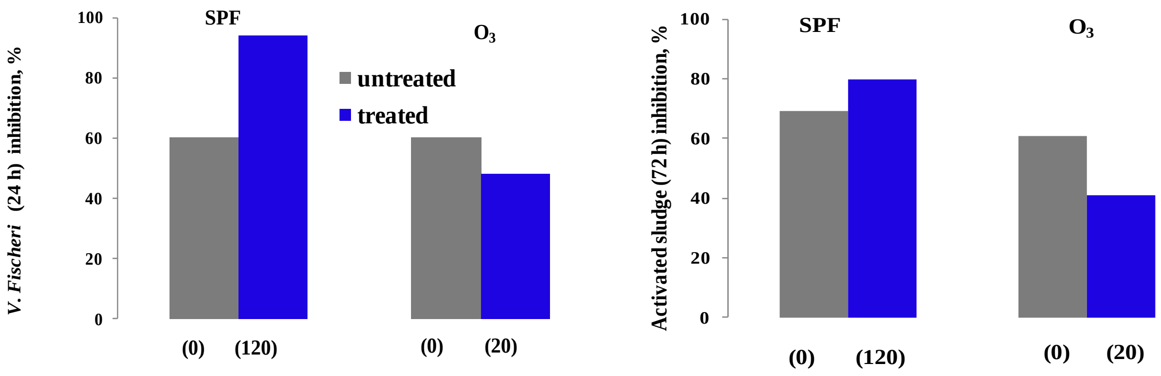


**Figure 44**– Chronic toxicity tests on untreated samples and treated WW samples by SPF and ozonation considering *V. fischeri* (24 h) and activated sludge (72 h). Numbers in brackets indicate treatment time of each process to reach 80 % or almost 80% removal of total CECs, in minutes.

Chronic toxicity assessed by *V. fischeri* (after 24 h of sample contact) and activated sludge (after 72 h of sample contact) was observed in almost all analyzed samples despite 80% of the CECs were eliminated (Fig. 43 and 44). Regarding to the *V. fischeri* chronic toxicity test, in the case of SPF in SUWW-woc inhibition decreased from 64 % to 36 % after the treatment due to CECs removal (Fig.43). The highest inhibition (94%) was observed in the WW sample treated by SPF; a 34% increase was observed after treatment (Fig.44). Regarding to the ozonation, toxicity in all samples did not vary significantly (48-64 %) and inhibition decreased by 14 and 12 % in SUWW and WW, respectively.

The chronic toxicity test results by activated sludge also showed higher toxicity of SPF samples compared to the ozonation in both water matrices (Fig 43 and 44).The highest inhibition (80%) was observed in the WW sample treated by SPF (Fig. 44). It can be explained by the generation of toxic by-products. Treatment by ozonation decreased inhibition from 45% to 0% and from 61% to 41% in SUWW-woc and WW, respectively. According to these results, and taking into account that the parent compounds (CECs) disappeared quite fast, it is possible to speculate that ozonation was able to either substantially degrade oxidation intermediates in SUWW-woc or the resulting intermediates are not toxic.

#### *4.4.8 Conclusions*

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Ozonation and SPF with EDDS were found to be effective in the removal of the target CECs from the investigated water matrices.SPF with EDDS is a possible alternative solution to the BATs (namely ozonation) for CECs decontamination in tertiary treatment of urban WW.

The results confirmed that 80% removal of total CECs was achieved by SPF with EDDS even in real WW. Toxicity of treated effluents keeps as a hot topic to address. The results basically show that acute toxicity test are not conclusive and chronic toxicity should be also measured to avoid to dispose/reuse tertiary treated WW more toxic than secondary treated one. Toxicity values were confirmed to be higher in WW compared to DW matrix, possibly due to formation of oxidation intermediates. Chronic toxicity was observed in analyzed WW samples despite 80% of the CECs were eliminated. Thus, further research is needed to optimize the treatment process scheme to improve the final ecotoxicological quality of the effluent.

# CHAPTER V

# Conclusions

In this PhD thesis work the effects of different AOPs as tertiary treatment methods of urban WW under realistic conditions using different endpoints (CECs removal, pathogens inactivation and toxicity) have been investigated.

1. Heterogeneous solar driven photocatalytic process has been compared with different homogeneous photo driven AOPs. Although, important steps forward have been made in the study of semiconductor-based photocatalysts (such as $TiO_2$) to make them more effective in the presence of solar radiation, this process (namely sunlight/N-$TiO_2$) is not yet competitive with SPF with EDDS in the removal of CECs from WW. SPF with EDDS was able to remove CECs and possibly even oxidation intermediates, thus resulting in a decreased toxicity trend in WW. SPF with EDDS process is a promising sustainable solution for tertiary treatment in UWWTPs.
2. Photo driven AOPs (including SPF with EDDS) were compared with ozonation (which is considered among the BATs for tertiary treatment of urban WW) under realistic conditions and using different end points (CECs removal, bacteria inactivation, effluent toxicity). Carbonates strongly affected SPF and sunlight/$H_2O_2$ disinfection efficiency. Accordingly, the selection of the most suitable disinfection process should take initial concentration of carbonates in WW into account. If the target WW is characterized by a high carbonates concentration, its removal before solar driven processes would increase operating costs and water salinity, making them possibly not competitive with ozonation for disinfection of WW. Partial removal until around 50 mg/L of carbonates could be a choice in some cases. QMRA analysis indicated a very high risk associated with the consumption of the crops irrigated with WW after secondary treatment. Tertiary WW treatment can drastically reduce microbiological risk associated to *E.coli* and *Salmonella spp* for irrigated crops.
3. Ozonation and SPF with EDDS were found to be effective in the removal of the target CECs from the investigated water matrices while sunlight/$H_2O_2$ was not effective. Although, ozonation is able to provide faster CECs removal in WW, SPF with EDDS in low-cost RPR reactor may be a feasible and sustainable solution under particular conditions. Monitoring residual concentration of the target CECs in the effluent is not sufficient and toxicity should be also measured to avoid a dispose/reuse tertiary treated WW more toxic than secondary treated one.

The choice of the most suitable tertiary treatment depends on different factors, including WW characteristics, UWWTPs size, geographical location, land and electricity cost, country income level. For example, in small UWWTPs in a mid income country in sunny areas SPF with EDDS operated in low-cost RPR reactor may be more suitable option that ozonation.

However, further investigation needs to holistically assess the optimization of the processes and facilitate its application at a large scale taking into account the variety and complexity of factors related to WW treatment. Ozonation and SPF with EDDS should be evaluated in environmental terms, through life cycle assessment (LCA) to comprehensively assess the potential environmental impacts using different end points (bacteria inactivation, CECs removal, toxicity, cost assessment etc.).

# CHAPTER VI

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