Università degli Studi di Torino
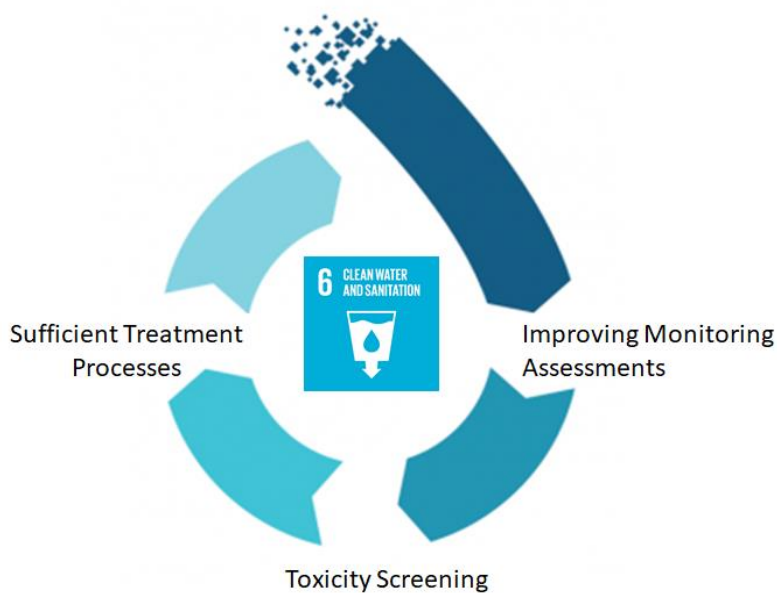
Doctoral School of Sciences and Innovative Technologies

PhD Programme in Innovation for the Circular Economy XXXIII Cycle

Development of analytical methods using LC-MS/MS technique for CECs detection and assessment of CECs in surface and drinking water samples including toxicological screening

Dimitra Papagiannaki

Supervisors:

Dr. Rita Binetti

Prof. Paola Calza

Development of analytical methods using LC-MS/MS technique for CECs detection and assessment of CECs in surface and drinking water samples including toxicological screening

Candidate: **Dimitra Papagiannaki**

Supervisors: Dr. **Rita Binetti**

                  Prof. **Paola Calza**

Jury Members: Prof. **Debora Fabbri**

                  University of Turin

                  Department of Chemistry

                  Prof. **Vasilios Sakkas**

                  University of Ioannina

                  Department of Chemistry

                  Prof. **Stéphane Bouchonnet**

                  Ecole Polytechnique

                  Department of Chemistry

Head of the Doctoral School: Prof. Alberto Rizzuti

PhD Programme Coordinator: Prof. Francesco Quatraro

Torino, 2021

To my parents Thanasis and Katerina.

For everything they gave up to make me who I am today.

Στους γονείς μου,
Θανάση και Κατερίνα

Thesis Abstract

Water scarcity is one of the biggest threats society is facing around the globe and has been on the rise worldwide. With the rapidly increasing global population, the demands for clean and safe drinking water are increasing as well. However, the widespread pollution caused from both natural and anthropogenic factors is one of the main challenges that water companies have to face. For this reason, a more sustainable water management, adopting the Circular Economy concept, is necessary in order to improve water bodies quality, prevent waste and pollution and find efficient remediation technologies.

The main objective of this thesis was to develop advanced and accurate analytical tools for quantifying different classes of Contaminants of Emerging Concern (CECs) in different water bodies. For this purpose, two analytical procedures using Ultra High Performance Liquid Chromatography tandem with Mass Spectrometry were developed and fully validated. Both methods were applied successfully to two original explanatory studies that sought to quantify the levels of per- and polyfluoroalkyl substances, and pharmaceuticals and hormones in the water bodies of the Metropolitan Area of Turin, in Italy. Both monitoring assessments followed the principles of Green Analytical Chemistry, using fast and cost-efficient methods, and took into account the risk-approach by identifying the potential pollution sources. However, even if target monitoring assessments can provide fundamental information about the levels of pollution in an area, useful also for making treatment decisions, they are not sufficient in evaluating the holistic quality status of water bodies. This thesis is highlighting the importance of combining effect-based tools and non-target screening with conventional screening methods in order to better assess the water quality and better manage water. Hence, a non-target screening assessment using High Resolution Mass Spectrometry was done in order to reveal pollution patterns in the aquatic environment and identify potential novel contaminants.

Finally, taking into account the CECs' occurrence results after the monitoring assessments, the removal efficiency of different degradation methods was studied. More specifically, cost-efficient and environmental friendly techniques employing Advanced Oxidation Processes (AOPs) - followed by identification of byproducts in order to understand the degradation pathways and toxicological screening - as well as conventional treatment methods used in the Drinking Water Treatment Plant (DWTP) of Società Metropolitana Acque Torino (SMAT), were examined.

TABLE OF CONTENTS

List of Publications

P1. R. Binetti, P. Calza, G. Costantino, S. Morgillo, **D. Papagiannaki**[*] "Perfluoroalkyl Substance Assessment in Turin Metropolitan Area and Correlation with Potential Sources of Pollution According to the Water Safety Plan Risk Management Approach" *Separations* 2019, *6*, 17.

P2. D. Papagiannaki, C. Medana, R. Binetti, P. Calza, P. Roslev "Effect of UV-A, UV-B and UV-C irradiation of glyphosate on photolysis and mitigation of aquatic toxicity" *Scientific Reports* 2020, 10, 20247.

P3. D. Palma, **D. Papagiannaki**, M. Lai, R. Binetti, M. Sleiman, M. Minella, C. Richard "PFAS degradation in ultrapure and groundwater using non-thermal plasma" *Molecules* 2021, 26, 924.

P4. D. Papagiannaki[*], S. Morgillo, G. Bocina, P. Calza, R. Binetti "Occurrence and human health risk assessment of pharmaceuticals and hormones in drinking water sources in the Metropolitan Area of Turin in Italy" *Toxics* 2021, 9, 88.

P5. D. Papagiannaki, D. Palma, A. Cedrino, M. Lai, M. Minella, C. Richard, R. Binetti "Identification of novel by-products after PFAS degradation with non-thermal plasma" *Manuscript.*

P6. I. Sciscenko, **D. Papagiannaki**, R. Binetti, C. Escudero-Oñate, I. Oller, A. Arques "Dissolved organic matter monitoring along with Turin drinking water plants employing EEM-PARAFAC", Submitted to *Water.*

Abbreviations

ADI	Acceptable Daily Intake
AOPs	Advanced Oxidation Processes
ASTM	American Society for Testing and Material
ARGs	Antibiotic Resistance Genes
AFFF	Aqueous Film-Forming Foams
MEC	Average Detected Concentration
BW	Body Weight
CE	Circular Economy
CA	Concentration Addition
CECs	Contaminants of Emerging Concern
DDA	Data Dependent Acquisition
DIA	Data Independent Acquisition
DWI	Drinking Water Intake
DWTP	Drinking Water Treatment Plant
ESI	Electrospray Ionization
EPs	Emerging Pollutants
EDCs	Endocrine Disrupting Compounds
EPA	Environmental Protection Agency
EQSs	Environmental Quality Standards
EEA	European Environmental Agency
GCPs	Green Chemistry Principles
HCA	Hierarchical Cluster Analysis
HPLC-MS/MS	High Performance Liquid Chromatography coupled to Mass Spectrometry
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
HV	High Voltage
IS	Internal Standard
ICH	International Conference on Harmonisation
LOD	Limit of Detection
LOQ	Limit of Quantification
EC50	Median Effective Concentration
LC50	Median Lethal Concentration
MeOH	Methanol
MDGs	Millennium Development Goals
MVDA	Multivariate Data Analysis

N(L)OAEL	No (Low) Observed Adverse Effect Level
log Kow	n-octanol-water partition coefficient
OLS	Ordinary Least Squares
PLS-DA	Partial Least Squared Discriminant Analysis
ppb	Parts per billion
ppm	Parts per million
ppt	Parts per trillion
PFCs	Perfluorinated Compounds
PFSAs	Perfluoroalkane Sulfonates
PFCAs	Perfluoroalkyl Carboxylates
PFASs	Per- and polyfluoroalkyl Substances
POPs	Persistent Organic Pollutants
PhACs	Pharmaceuticals' and Hormones' Active Compounds
PDBEs	Polybrominated Diphenyl Ethers
PP	Polypropylene
PNEC	Predicted No-Effect Concentration
PC	Principal Component
PCA	Principal Component Analysis
PSs	Priority Substances
pGLV	Provisional Guideline Value
PG	Pyrex glass
Q1	First Quadrupole
Q3	Third Quadrupole
QC	Quality Control
QF	Quantification Frequency
RT	Retention Time
RQ	Risk Quotient
SWATH-MS	Sequential Window Acquisition of all Theoretical Mass Spectra
SMAT	Società Metropolitana Acque Torino
SPE	Solid Phase Extraction
SDG	Sustainable Development Goal
TOF	Time-of-flight
TN	Total Nitrogen
TOC	Total Organic Carbon
VIP	Variables Importance in Projection
WWTP	Waste Water Treatment Plant
WEI+	Water Exploitation Index plus

WFD   Water Framework Directive
WM   Wide Mouth
WHO   World Health Organization

Section I

Introduction

Chapter 1 Water at the heart of circular economy

Transition towards a Circular Economy (CE) has become a popular and important issue for environmental management in the last years [1]. CE is a sustainable development strategy that aims to boost resource efficiency and minimize waste production, while increasing economic and social benefits [2]. Actually CE is a rethinking of the current linear economic systems of "take, use, dispose", by promoting the employment of reuse, recycle, redesign, remanufacture, reduce and recover (the Rs approach) in a way to create a closed-looped system. Valuable materials from one product, at the end of its life, could be recovered and reused as source materials for the production of another product, minimizing resource inputs and waste [3]. The three main principles of CE include designing out waste and pollution, regenerating natural systems and keeping materials in use [4]. There is no clear evidence for the origin of the Circular Economy concept, with a variety of researchers contributing to it since the 1970s [3]. However, it was not until 2010 that its practices started being applied in different sectors, like waste management, sustainable design, food production, etc [5]. In the European level, the CE concept was adopted after two communications with titles ''Towards a circular economy: a zero waste programme for Europe'' [6], published in 2014, and "Closing the loop - An EU action plan for the Circular Economy" [7] published in 2015. Both communications highlighted the importance of adopting more rational use of resources and sustainable waste management in every branch of industry, and every group of materials and waste, while moving towards the CE [1]. As water is essential for human and ecosystems survival, and plays a significant role for industries as a carrier of both materials and energy, implementation of the CE principles in the water sector is necessary in order to improve the management of the already under pressure water bodies.

1.1 Water Scarcity and Sustainable Development Goals *SDGs*

Water is a finite resource - as its amount on Earth remains always the same and circulates through its natural cycle - of vital importance for living organisms, and indispensable for economic development, food production, and energy supplies [1,8]. Due to its high value for the society it should be used in a very sustainable way. However, the water crisis is already a reality in regions around the world, with studies reporting that 1 billion people lack access to clean water, and around one-third of the world population has already experienced severe water stress conditions [9,10]. It is estimated that by 2030 close to 3.9 billion people (almost half of the world's population) will be

living under conditions of severe water scarcity [11]. In the European level, the Water Exploitation Index plus (WEI+) showed that 33% of the population and 20% of the area has already faced severe water stress conditions (Figure 1), a percentage that is estimated to increase to 50% by 2030 [12].

New Chart — Population and area exposed to water scarcity conditions in Europe (summers 1990-2015)

Area affected by water scarcity conditions (WEI+ 20 and greater)
Population exposed to water stress conditions
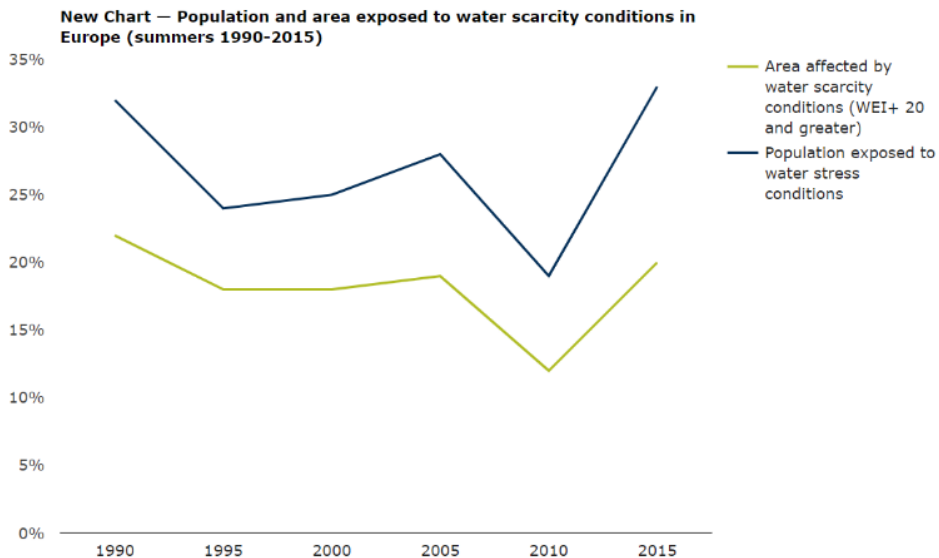
Figure 1: Water Scarcity Conditions in Europe (WEI+).

The continuously increasing world's population, that provokes increased human needs for water and requests for food, is responsible for the lack of sufficient available water resources on the demand side. Agricultural practices are increasingly growing in order to provide higher food yields, achieving a high water consumption, estimated as the 70% of the total one [13]. Their intensified land use with the overexploitation of water sources for irrigation purposes are adding in the risk of water scarcity, posing also challenges to the sustainable management of water. On the supply side, water scarcity is considered as a consequence of climate change. Nowadays, extreme floods or drought periods, alongside with warming temperatures affected significantly the hydrological cycle adding stress on water bodies [5]. Droughts can also have a negative impact on the biodiversity of an area. Different plant and animal species may go entirely extinct in extreme climate change conditions [14]. Furthermore, humanity's increasing water consumption is adding to the decrease of a region's biodiversity, which subsequently can affect humanity. The imbalance between demand and supply can be tremendous for the world population, since lack of clean water and sanitation can result in hunger,

poverty, diseases, and migration [15]. Therefore, protection of water resources for present and future generations is one of the most crucial environmental issues nowadays. A variety of solutions within the context of CE - such as integrated water resources management and wastewater reuse – have been proposed for addressing it. However, apart from quantity, maintaining the high quality of water bodies, is also vital for the population's water demands satisfaction. Extensive use of chemicals in agriculture alongside with uncontrolled discharge of big volumes of untreated industrial and domestic wastewater, undermine water quality posing threats to human health and aquatic ecosystems [16].

Humanity is called to take actions and make collective efforts in order to address these problems. In this context, the Sustainable Development Goals (SDGs) were adopted in 2015 by all the United Nations Member States, as a universal call for action to manage the social, economic, and environmental aspects of sustainable development by 2030 [17]. After the completion of the Millennium Development Goals (MDGs), the 17 SDGs and their 169 global targets were adopted in order to improve life's quality and achieve a better and more sustainable future for everyone (Figure 2) [18]. Water is directly and indirectly linked to all of the 17 SDGs, with SDG 6 being entirely devoted to clean water and sanitation. More specifically, SDG 6 aims to achieve access to safe drinking water and sanitation for all by 2030, improve water quality by reducing pollution and release of hazardous chemicals in water bodies for safe reuse, protection and restoration of water-related ecosystems. Finally, SDG 6 aims to address water scarcity by sustainable withdrawals and better water resources management, highlighting the dependance of social and economic development on the sustainable water management [19,20].

Figure 2: Water centric 17 SDGs for each sector.

1.2 Sustainable water management

In order to face water scarcity and meet the SDGs, different strategies have been published. In December 2019, EU published the European Green Deal [21], which aims to provide better solutions and conditions for achieving a more sustainable climate neutrality and circular economy by 2050. The strategy highlights the need to take actions for protecting and restoring ecosystems, use resources in a more sustainable way, reassure a toxic-free environment and improve human health [21,22]. In order to achieve these goals, EU published in March 2020, the new Circular Economy Action Plan [7], which points the importance of reducing waste externalities, maintain sources in use and regenerate the natural capital. For achieving a more sustainable management, which means to meet the current water supply needs without endangering the ability of future generations to do the same [23], these can be translated as [4,24]:

- optimization of the amount of water, energy and chemicals used in water systems' operation,
- maintain water, minerals, energy and chemicals in use and maximize their reuse,
- preserve the natural capital by river restoration, pollution prevention, and quality of water reassurance,

- ensure minimum disruption of natural water cycle from human actions.

In practice, different methods have translated these principles in the so-called Rs approach, including the concepts of:

- **reduce** water use and pollution at source,
- **reclamation** which is associated with the efficient removal of pollutants from wastewater using highly effective techniques,
- **reuse** of treated water for non-potable needs, as irrigation,
- **recycle** which is associated to reclaimed water from treated wastewater for direct potable use,
- **recovery** of materials, minerals and chemicals as potential resources,
- **rethink** of how to use natural resources in a more sustainable way.

Adoption of these concepts will eventually achieve reduction of the gap between availability and water demand by eliminating the adverse effects of pollution, identification of potential pollution sources, reduction of excessive water abstraction and waste production, better management of wastewater, and finally assurance of sufficient amount of good quality water to meet the needs of human and ecosystem [1,25].

1.3 Green Chemistry to enable circularity and sustainability

As already discussed, adopting a circular economy approach within a sustainable water management system is essential for increasing water resilience and preventing a global water crisis [23]. A key concept in order to enable sustainable development and circular economy is the implementation of Green Chemistry Principles (GCPs).

In general, green chemistry focuses on designing innovative products, with less consumption of natural sources and minimized waste disposal [26]. The twelve GCPs (Table 1) were created by Paul Anastas and John Warner, who tried to explain a more environmental-friendly process or product [27,28]. According to them, the pollution's prevention can be achieved before occurring by using new techniques and products that reduce or eliminate the use and generation of hazardous substances to human health and the environment [29].

Table 1. The twelve principles of Green Chemistry.

The twelve principles of Green Chemistry (GCPs)	
1. Prevent waste	7. Use renewable feedstocks/materials
2. Maximize atom economy in syntheses	8. Avoid chemical derivatives
3. Design less hazardous chemical syntheses	9. Minimize waste by using catalytic reactions and not stoichiometric reagents
4. Design safer chemicals and products	10. Design chemicals and products to degrade after use
5. Use safer solvents and reaction conditions	11. Analyze in real time to prevent pollution
6. Increase energy efficiency	12. Minimize the potential for accidents

Green chemistry focuses on a safe and sustainable design and development of materials and processes, by eliminating waste and improving energy efficiency, which are main components towards a transition to sustainable energy, resources and processes and circular economy [30]. Moreover, since its implementation plays an important role in environment, through pollution prevention and human health through elimination of hazardous compounds - while providing an economic sustainability - it can be a centric piece on addressing the SDGs [28]. Finally, green chemistry could have a significant contribution on the increased demand for development of innovative chemicals and technologies, that are cost-efficient and don't produce waste, in order to address the presence of CECs in the aquatic environment.

Chapter 2 Towards a toxic-free environment

Creating a toxic-free environment is one of the goals set by the European Green Deal towards a more sustainable and circular economy. In order to achieve it more actions for pollution monitoring, preventing its generation and finding better remediation solutions are necessary, alongside with revisions of policies and regulations.

Concerning water management and protection, till now EU has published different directives for achieving good environmental standards. In 2000, the Water Framework Directive (WFD) 2000/60/EC was adopted, aiming to prevent and reduce pollution, promote sustainable water use and protect the aquatic environment [31]. The EU WFD, established a watch list of priority substances (PSs) with high risks to the aquatic environment in order to set their monitoring assessment and establish their Environmental Quality Standards (EQSs). These values represent the maximum concentrations that a pollutant or a group of contaminants can be present in different environment compartments without posing threats to human health or ecosystem. The first list of priority compounds was established in 2008, by the Directive 2008/105/EC [32], including 33 PSs and 8 other pollutants. The choice of these compounds was based on data of their acute and chronic effects to ecosystem and human health. The EU Member States were required to set monitoring campaigns of the PSs and a good status of water bodies was determined if the detected concentrations were not exceeding the established Environmental Quality Standards. In 2013, the list was updated to 45 PSs and 8 other pollutants by the Directive 2013/39/EU [33], grouped as single or classes of substances and containing pesticides, industrial additives, pharmaceuticals, hormones, personal care products, food additives, surfactants, flame retardants and others. Moreover, the 2013/39/EU Directive proposed a first Watch List of 17 unregulated organic pollutants, not regularly included in monitoring programs but able to cause adverse effects on human health and ecosystems, and recommended their occurrence assessments in order to prioritize them and develop innovative treatment technologies for their abatement. This Watch List was later established by the Decision 2015/495/EU [34].

Although, there aren't any Environmental Quality Standards available for these pollutants, an evaluation system based on the frequency and the extent of exceedance of Predicted No-Effect Concentration (PNEC) in order to prioritize the compounds for monitoring and treatment has been proposed from the NORMAN Association [35]. In December 2020 the revised Drinking Water Directive 2020/2184 [36] has been

published, aiming to ensure the quality standards and increase the transparency for the consumer. More specifically, it highlights the need of better understanding, monitoring and evaluating the risks posed from newly detected chemicals in water bodies and their combined effects when present in mixtures. Moreover, it reports that risk assessment and management of the supply systems (Water Safety Plan approach), improvement of treatment techniques and increase of collaboration between stakeholders will allow better reduction of contaminants' release in the aquatic environment and subsequently their impact on drinking water sources.

2.1 Challenges

However, the biggest challenge of these strategies and regulative frameworks, that aim to ensure good quality of water bodies is the pollution originating from the so-called Contaminants of Emerging Concern (CECs).

Currently, water pollution is a serious problem that undermines the already scarce water resources. The majority of European countries rely on surface and groundwater for their drinking water needs, whose quality is affected from natural and anthropogenic factors. Metals, single organic ions and more complex organic molecules, as well as biological components can derive from various sources, such as natural disasters, agricultural run-off, industrial and domestic discharges, increasing population and economic growth, and can affect the quality of water bodies [8,37]. The existence of these factors in the aquatic environment represents a serious threat for human health and ecosystems. In recent years, the availability of robust and sensitive analytical methods and techniques has allowed the identification and detection of a wide variety of pollutants, with those deriving from anthropogenic sources commonly being referred to as micropollutants since their presence in water bodies is usually at trace levels (between few ng/L to some µg/L) [8]. These contaminants may be classified as legacy - whose toxic effects are already known and control measures have been established - or as Contaminants of Emerging Concern (CECs) [37]. This last class concerns compounds that are not currently regulated or included in routine monitoring programs, but are thought to have potential adverse effects to ecosystems and human health, and may serve as candidates for future legislations. CECs are not necessarily chemicals that have been recently introduced in the environment, they might as well include contaminants that have been present for a longer time, but their significance has not been evaluated since now or their occurrence was not known due to lack of adequate analytical techniques [38].

More than 1000 substances are considered as CECs or Emerging Pollutants (EPs) with a variety of sources being responsible for their occurrence in the aquatic environment, such as hospital effluents, landfill leachates, runoff from agriculture, and mainly industrial and domestic wastewater due to insufficient treatment techniques (Figure 3) [39,40]. Different studies have reported the inability of conventional treatment methods used in Waste Water Treatment Plants (WWTPs) to efficiently remove CECs, posing threats to the receiving environment [41,42].
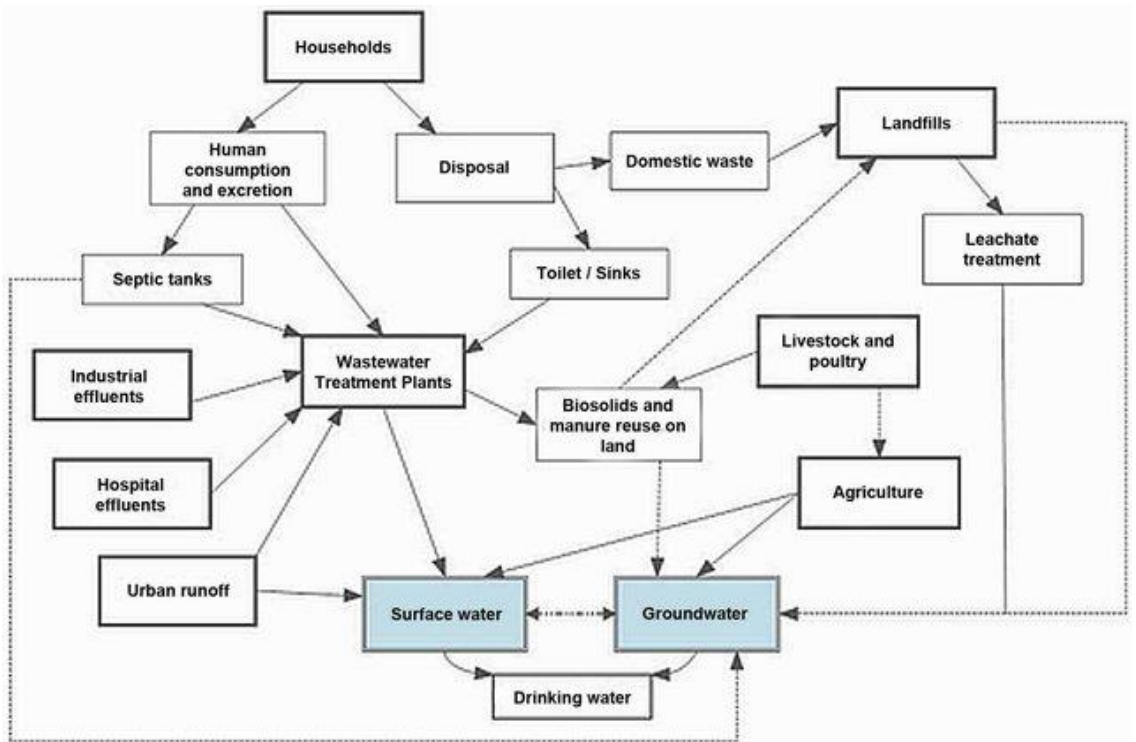
Figure 3: Sources of CECs in the aquatic environment [40].

As CECs are considered compounds included in the following 16 categories [43]: Pharmaceuticals, Personal Care Products, Sunscreens and UV/filters, Veterinary medicines, Pesticides and Herbicides, Persistent Organic Pollutants (POPs), like Perfluoroalkyl Substances (PFASs) and Polybrominated Diphenyl Ethers (PDBEs), Endocrine Disrupting Compounds (EDCs), including estrogens – both naturally occurring and synthetic-, Nanomaterials, Microplastics, Drinking water by-products, Antibiotic Resistance Genes (ARGs) and their transformation products.

Currently, in the European level, there are no existing regulatory limits for the majority of these substances. However, the European Environmental Agency considers that CECs or EPs should be closely monitored by taking into account the risk management approach and the combined exposure assessment, as they are increasingly being detected in the aquatic environment.

2.1.1 Per and polyfluoroalkyl substances (PFASs)

Per and polyfluoroalkyl substances (PFASs) represent a broad class of organic compounds, widely used over the past decades. They belong in the category of fluorosurfactants, since they host a substitution of hydrogen atoms by fluorine, in their carbon chain, building in that way the hydrophobic part of the surfactant [44,45]. PFAS are man-made organic compounds, with two major processes being followed for their production, the electrochemical fluorination and the telomerisation. However, since 2002 mainly the telomerisation method is applied. In general, perfluorinated compounds (PFCs) like perfluoroalkyl sulfonamides and fluorotelomer alcohols can be degraded naturally under aerobic conditions and transformed to perfluoroalkyl carboxylates (PFCAs) and perfluoroalkyl sulfonates (PFSAs) [46]. Based on the length of their perfluorocarbon chain, PFASs (PFCAs and PFSAs) can be classified as short and long chain compounds. In those with long chain are included PFCAs with eight or more atoms of carbon and PFSAs with at least six atoms of carbon [47,48] (Table 2).

Table 2. Categorization of PFASs according to their chain length [46].

Perfluoroalkane Sulfonates (PFSA)		Perfluoroalkyl Carboxylates (PFCA)	
Short Chain $n \leq 5$ e.g. PFBS	Long Chain $n \geq 6$ e.g. PFHxS, PFOS and PFDS	Short Chain $n \leq 7$ e.g. PFBA, PFPeA, PFHxA and PFHpA	Long Chain $n \geq 8$ e.g. PFOA, PFNA, PFDA, PFUdA, PFDoA, PFTrDA, PFTeDA, PFHxDA and PFODA

PFASs represent a vast variety of molecules, with unique physicochemical properties, like extreme hydrophilic and lipophilic character or thermal and chemical stability. Most importantly, these compounds host one of the strongest chemical bonds (C-F) in their

carbon chain, which makes them very stable towards natural degradation or under conditions of heat, acid and oxidation [47,49]. Due to these characteristics, they are essential elements for a wide range of industrial and commercial operations. Some examples include stain- and water- resistant fabrics for clothes and carpeting, cleaning products, food packaging and cookware, paints and fire-fighting foams.

The widespread use of PFASs and their abilities to remain intact in the environment result in continuously increasing contamination levels in the water bodies. As their main sources are considered the industrial and municipal wastewater treatment plants, landfill leachate, dry and wet atmospheric deposition, soil and street surface runoff [48]. Different studies have reported their occurrence in various raw water sources, in concentrations between ng/L and few µg/L, and other environmental matrices, including food [50-55]. Accumulation of certain PFASs in humans and animals has also been reported, with serious concerns being raised about the potential health effects. Due to their stability properties, and the incapability of conventional treatment technologies to efficiently remove them from water, PFASs have also been detected in drinking water, raising significant threats of adverse human health effects [56,57]. Therefore, as mentioned in the EU Chemicals Strategy for Sustainability published in October 2020 [58], it is crucial to find and establish new and more effective degradation techniques, alongside with the prevention of their contamination.

2.1.2 Pharmaceuticals and hormones

Pharmaceuticals and hormones (PhACs) represent another big category of anthropogenic contaminants present in the aquatic environment [43], as a result of increasing population's growth and economic activities. These compounds can have adverse effects both to human health and aquatic ecosystems, like morphological anomalies, endocrine disruption and increasing antimicrobial resistance. In Europe, their use is continuously increasing, with 3000 compounds currently being active in the market [59]. Due to their large consumption, pharmaceuticals and hormones can reach the aquatic environment through different routes mainly including animal and human excretion, improper domestic or industrial discharge and landfill leaching [43], depending upon the substance and its properties. The last decades, a wide variety of studies has reported their presence in water systems of all types, in concentrations ranging from ng/L to few µg/L [60-66], raising concerns about their potential effects on human health, especially after a long term exposure to low level concentrations. However, none of these compounds is regulated in European Union, even though some

of them are included in the Watch List of the WFD, mainly due to the fact that they have insignificant biological effects at their low occurrence concentrations in the environment. Nevertheless, till now none of their assessments considered their effects when they bioaccumulate, or the synergistic interactions of simultaneous contamination with multiple compounds [67].

In order to face these problems, and fill knowledge gaps related to PhACs occurrence concentrations, risk assessments and monitoring of "hotspot" locations, the European Comission published in 2019 the European Union Strategic Approach to Pharmaceuticals in the Environment [6]. This approach proposes that Member States should promote the more careful use of PhACs, develop compounds that are not harmful for the environment, improve their environmental risk assessment and management of waste, broaden their environmental monitoring assessments and finally identify further knowledge gaps to be resolved, such as finding cost-effective remediation methods, towards the transition to a circular economy based environmental management.

2.1.3 Pesticides

Active ingredients from pesticides' formulation are among the most frequently detected organic micropollutants in the aquatic environment, due to their widespread use in agriculture and forestry [68]. They may contaminate the different water bodies types after application through spray drift, surface runoff, and soil leaching [69,70]. Depending on their physicochemical properties and more importantly on their chemical stability, pesticides may undergo natural degradation processes resulting to metabolites equally of even more toxic, considered as contaminants as well. The rapid population growth has increased the needs for food production, enhancing the need for pesticides to protect the yields from undesirable organisms. Pesticides are classified into different categories, based on their toxicity level, target application and chemical properties. Moreover, they can be categorized as organochlorines, organophosphorus, carbamates, pyrethrin and pyrethroids [71]. Advances in analytical techniques have allowed, since 1970s, the multi residual detection of pesticides in different water types and especially in drinking water, raising particular concerns about the greater impacts that can have on human health [72,73]. For this reason, pesticides concern few of the contaminants of emerging concern that -since 1998- are regulated in Europe. The published directives have set a maximum concentration of 0,1 μg/L for individual compounds, their metabolites and transformation products, and 0,5 μg/L for their total amount. Moreover, in a work programme launched in 1992, EU started a review process for all

active ingredients used in pesticides, based on human health, environmental, and ecotoxicity risk assessments and on food residues monitoring assessments and taking into account the requirements of the 91/414/EEC directive [74]. The outcomes are categorizing the active substances as banned, for "essential use" and authorized. Currently, 84 compounds are banned in EU including Atrazine, Alachlor, Dichlorvos, and Diazinon, which however are still detected in water bodies.

Another widely detected class of pesticides, whose approval in agricultural uses is a debated topic, is the glyphosate-based ones. Their active ingredient - N-(phosphonomethyl)glycine (Glyphosate) - affects a broad-spectrum of plants, making it a useful tool for agricultural, public and domestic uses [75-78]. At present, glyphosate has a massive global usage of about 700,000 tons per year, which has caused its ubiquitous occurrence in the aquatic environment, posing threats to humans and ecosystems [75,77-80]. A variety of degradation techniques has been proposed for removing glyphosate from the aquatic environment. However, there is still the need of finding green and cost-efficient techniques, with the potentiality to be used in large scale applications, such as treatment plants, within the circular economy context.

2.2 Improving Monitoring Assessments

In the European level, even if EU Member States achieved significant improvement of water bodies quality after adopting the requirements of the different directives, the current status of many European water bodies is still uncertain, regardless the advanced techniques and the numerous developed methods. According to the latest European Environmental Agency (EEA) Water Europe report [81] the majority of European water bodies faced difficulties in achieving a minimum of good ecological status as set by the different EU directives [1]. This fact highlighted the need of new measurements' and regulations' adoption, focusing mainly on reducing the negative effects of pollution that stress water bodies, and better managing of waste, towards the implementation of CE principles in the water sector [82].

Robust monitoring of water quality is a must for conserving the existing unpolluted resources and restoring the polluted ones within a sustainable water management, and a prerequisite for a safe regulation of chemicals [83]. Monitoring data are fundamental for identifying problems, taking decisions for reducing emissions and evaluating their effectiveness, as well as policy development and registration of chemical substances (ex. under the Regulation for Registration, Evaluation, Authorization and Restriction of Chemicals in Europe –REACH) [84]. Initiatives for new monitoring programmes, like the Watch List strategy published under the WFD, aim to fill the gaps concerning the occurrence and impacts of micropollutants in the European water bodies. However, these approaches, based on target analyses of a specific number of pollutants fail to evaluate the holistic quality status of the different water bodies. Therefore, improving monitoring assessments by combining them with risks evaluation on ecosystem and human health, taking into account also the simultaneous exposure to combined effects of chemical mixtures, is necessary [15].

More specifically, analyses targeting to specific analytes are not able to distinct between the non-existence and the transformation of a compound due to degradation processes. Instead, suspect and non-target screening approaches based on High-Resolution Mass Spectrometry (HRMS), are able to provide a more comprehensive picture of chemicals' presence in water bodies by addressing complex chemical mixtures analytically. Even the valuable information that can be obtained from non-target screening, it can never replace the target analysis but only trigger it after being the first step of the assessment [85]. Finally, bioassays are another available method able to address unknown mixture risks present in the water bodies [86].

2.2.1 Approaches for comprehensive monitoring

A more comprehensive monitoring of chemicals in the aquatic environment will be achieved after the combination of the three previously approaches. In this way, the detection of newly emerging compounds and their transformation products, as well as the identification of their toxicity effects, necessary information for future abatement plans, will be achieved [87]. Target, suspect and non-target screening analyses, based on modern Liquid or Gas Chromatography instruments coupled to HRMS, allow the detection of organic micropollutants in water at trace level concentrations (ng/L). Practically, this means that the combination of these three approaches based on HRMS is able to detect simultaneously complex mixtures of a huge variety of substances at a high level of sensitivity, while identifying unknowns as well [83]. Complementary to chemical analyses, bioassays are increasingly used as bioanalytical tools for water quality assessments in order to measure the combined effects of trace-level mixtures of chemicals [88]. Different biological tests, including cell models, receptors, tissues or small organisms can be used for measuring the effects of chemicals on various biological endpoints. Although bioassay data cannot be used for thorough risk assessments, can identify the presence of one or more compounds that cause effects on biological battery tests relevant to human health and the environment. As each chemical causes different effects, water quality monitoring requires a suitable and adequate set of bioassays, based on chemicals' human health effects [89] or environmental pressures [90]. Consequently, combining chemical and effect-based methods has the potential to improve water quality monitoring, by revealing the cause of an effect and the effect itself.

2.2.2 Sustainability in monitoring assessment

New monitoring approaches need to prove that they are cost-efficient and not time consuming, for being able to be applied in large-scale programmes. For this reason, a massive request for simpler, faster and lower-cost methods, in order to enable more extensive and efficient monitoring of a wide-range CECs in water bodies while minimizing waste, is growing [9]. Currently, the analytical techniques used in monitoring assessments include Liquid and Gas Chromatography coupled to Mass Spectrometry. Usually, a samples' pretreatment step is necessary prior to the analysis, in order to remove potential interferences and preconcentrate the sample. Solid Phase Extraction (SPE) is currently the most used technique, despite its drawbacks of high organic solvents' consumption or high costs [8]. Since improvements in monitoring assessments

are required, the replacement of traditional methods, with more sustainable and energy-efficient alternatives following the principles of Green Chemistry, is necessary and can result to great socioeconomic and environmental impacts. However, many of the opportunities for sustainability and greenness of an analytical method are directly related to the sample preparation process. During this step, the most efficient way to reduce waste is to introduce the sample to the analytical apparatus with no or little pretreatment. However, since in the majority of times, the no pretreatment step is impossible and the analytes must first be extracted from their matrices prior to analyses, sample preparation techniques should be optimized to reduce energy input, solvent use, waste production, and operator exposure as much as possible [91]. This can be accomplished by implementation of the Green Analytical Chemistry Principles, which promote the use of alternative eco-friendlier solvents and avoidance of toxic organic solvents, greener (less polluting) extraction procedures or complete elimination of the pretreatment step, restriction of the size and number of samples and the use of reusable samples extraction devices [91]. Moreover, the importance of methods' development which target to mixtures of multiple analytes and are based on simplified and automated analytical protocols, alongside with reduction of energy consumption and use of mathematical modelling of the data based on chemometrics is encouraged. Consequently, the greenness of the analytical methods promoted for large-scale monitoring assessments applications, is a fundamental step for increasing sustainability in the water management, alongside with the use of modeling tools for identifying contamination hotspots [92]. The combination of all these approaches, can provide the water sector with "smart" tools for more realistic monitoring and risk assessment of mixtures of CECs in water bodies, and help take decisions concerning treatment methods.

2.3 Remediation Methods

Except of the importance of pollution monitoring and preventing its generation at source towards creating a toxic-free environment within a more sustainable and circular economy, it is crucial to find more sufficient remediation solutions [37]. According to EU regulations and laws, treatment of wastewater is obligatory [1] and WWTPs represent the primary barrier of preventing water bodies' contamination. However, it is well-known that WWTPs are one of the most significant CECs pollution sources, since the conventional physical and biological treatment technologies that they rely on are unable to efficiently remove recalcitrant organic compounds, discharging them directly into the environment [93-95]. Drinking water treatment plants (DWTPs) impose another barrier

concerning the prevention of unintended human exposure to CECs. However, studies have shown that utilized conventional treatment techniques such as coagulation, flocculation, filtration and chlorination have low removal efficiencies as well [96,97], while enhanced treatment technologies like ozonation and activated carbon adsorption are able to efficiently remove them [96,98-100]. However, even though these techniques are effective, may require expensive chemicals and equipment. Moreover, as water sector is focusing on alternative water sources for meeting the current potable or non-potable needs while preserving the natural resources, is promoting water reuse. However, a not appropriate water treatment system for the specific reuse needs could lead to undermined and unreliable water quality (e.g. generation of disinfection byproducts in reclaimed water for potable needs) posing risks to human health and ecosystems [101]. For this reason, more efficient, cost-effective treatment techniques dealing with a vast variety of chemicals, while minimizing waste and energy consumption need to be developed. Among the alternatives, Advanced Oxidation Processes (AOPs) represent one viable option [39].

AOPs are eco-friendly chemical methods that rely on chemical and physical processes "in situ" generating highly reactive radical species (such as $HO^{\bullet}$ and $SO_4^{\bullet-}$) for the oxidation of organic compounds [39], without producing waste. The generated radicals are optimal and powerful oxidants, since they do not generate additional waste, they are not corrosive for equipment, they are not toxic and they have a very short lifetime [102]. Their efficiency relies on the fact that the generated radicals are unselective and able to degrade a vast variety of compounds through non-selective reactions into smaller or inorganic molecules (Figure 4) [103]. The degradation of the organic contaminants occurs through hydrogen abstraction, electrophilic attack or electron transfer by the hydroxyl radicals.
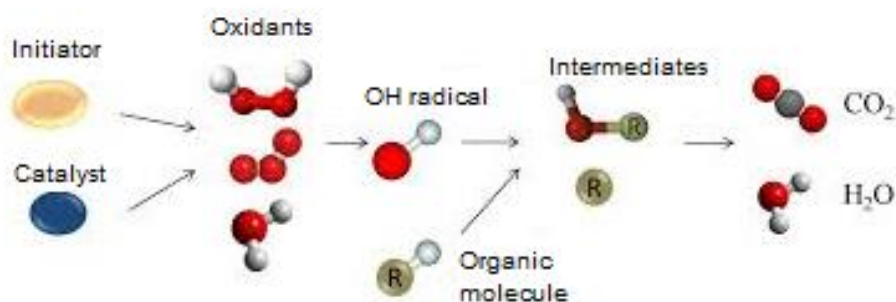

Figure 4: AOPs degradation mechanism when employing a catalyst.

AOPs include a vast variety of processes like Fenton, photo- and electro-Fenton processes, ozonation, UV photolysis, H_2O_2 and heterogeneous photocatalysis (Table 3) [39]. Processes involving treatment with O_3 and UV irradiation are the simplest processes proven to be able to efficiently degrade CECs and are already established in large scales applications, like DWTPs and water reuse facilities. Nonetheless, several AOPs such as electrochemical treatment, microwave, plasma and ultrasound related processes are continuously being studied by the scientific community [104].

Table 3. Different Advanced Oxidation Processes (AOPs) and their sources of radicals.

Advanced Oxidation Processes (AOPs)	Source of Radicals
Photolysis	UV irradiation
O₃-based processes	O_3
	O_3/UV
	O_3/H_2O_2
	$O_3/H_2O_2/UV$
H₂O₂-based processes	H_2O_2/UV
	H_2O_2/Fe^{2+} (Fenton)
	H_2O_2/Fe^{3+} (Fenton-like)
	$H_2O_2/Fe^{2+}/UV$ (Photo-Fenton)
Heterogeneous photocatalysis	TiO_2/UV
	$TiO_2/UV/H_2O_2$
Sonochemical oxidation	Ultrasounds (water sonolysis)
Electrochemical oxidation	Electricity (water electrolysis)

AOPs represent a group of sustainable remediation technologies that mainly follows the principles of Green Chemistry, with rapid reaction rates, mineralization of organics and a great potential to reduce toxicity of organic compounds. Moreover, they do not generate waste that need further treatment (such as the membranes, and activated carbon absorption). However, they have also some drawbacks, such as not being able to treat large volumes of low concentration pollutants (realistic conditions), and their efficiency being affected from the presence of organic matter or inorganic ions, which slow the rates of radicals' reactions. Finally, AOPs can generate several toxic

intermediates which sometimes are even more harmful than the parent compound, proposing the need of additional treatment for their removal [103,105]. For this reason, risk and toxicity of byproducts assessments should be included when planning and designing these processes as well, in order to identify and deal with their potential advantages and disadvantages, and set them in use [101].
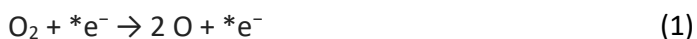
2.3.1 UV photolysis

Photolysis of micropollutants present in water can occur directly via photon absorption in order to reach excited states, or indirectly through chemical reactions mediated by active oxygen species [37,102,106,107]. These can be translated to several potential mechanisms by which UV irradiation could mediate the transformation of CECs in water [106]. Firstly, UV irradiation can cause the direct photochemical dissociation of a compound, through photon absorption, followed by different chemical reactions such as bond cleavage and oxidation-reduction. Moreover, UV irradiation can interact with H_2O and O_2 and form reactive oxygen species including hydroxyl radicals ($^{\bullet}OH$) that interact with the micropollutant and cause degradation. In any process that uses UV irradiation, the above mentioned reactions can take place. The performance of such processes depends on several factors, such as the intensity and wavelength of the UV irradiation, as well as the water matrix. As UV irradiation is considered a group of electromagnetic radiations with different wavelengths (e.g., 10-400 nm) and is classified into different categories such as UV-A (365nm), UV-B (302nm), UV-C (254nm), and Vacuum UV.

One of the main advantages of this AOP is that photolysis represents one sustainable alternative degradation method, since it is a non-invasive and free of added chemicals technique that can destroy the targeted CECs without generating waste or transferring them from one matrix to another [37]. Hence, UV-based AOPs are one of the most widely studied degradation methods for organic micropollutants. Pharmaceuticals, hormones and pesticides are some of the studied compounds classes reported in literature. These processes can promote the degradation of micropollutants via direct or indirect photolysis. However, UV doses used in conventional drinking water treatment lines (e.g., disinfection) are relatively low and not able to ease their removal. For this reason, different additives such as oxidants or catalysts may be added to improve the degradation processes mainly through the formation of radicals. Examples of such water treatment processes are the advanced oxidation process involving the

addition of hydrogen peroxide (e.g., UV-C/H_2O_2) and photocatalysis involving the addition of different catalysts (e.g., UV-C/TiO_2) [108-111].

3.2 Electrochemical Oxidation

Water plasma is another treatment technique utilizing non-selective and reactive radicals in order to degrade organic micropollutants. Even if water plasma is a technology firstly explored in the late 1980s, currently is attracting lots of attention for water treatment applications. Water-plasma technologies include both thermal and non-thermal plasmas used to remove biological or chemical contamination [112,113]. The main advantages of this process, is its ability to activate different reactions in a short time and at lower temperature (room temperature) using a relatively simple equipment [114]. Such reactions include the formation of highly oxidating species such as $H^\bullet$, O and $^\bullet OH$ radicals, free electrons and ozone, as well as oxidants like hydrogen peroxide (Equations 1-7) and ultraviolet light due to the plasma discharge light emission. Therefore, the degradation mechanism of pollutants is complex.

$$O_2 + *e^- \rightarrow 2\ O + *e^- \tag{1}$$

$$H_2O + *e^- \rightarrow HO^\bullet + H^\bullet + *e^- \tag{2}$$

$$O_2 + O \rightarrow O_3 \tag{3}$$

$$H_2O + O \rightarrow 2\ HO^\bullet \tag{4}$$

$$2\ HO^\bullet \rightarrow H_2O_2 \tag{5}$$

$$HO^\bullet + O_3 \rightarrow HOO^\bullet + O_2 \tag{6}$$

$$2\ HOO^\bullet \rightarrow H_2O_2 + O_2 \tag{7}$$

Different electrodes configuration has been explored for water plasma treatment methods, with the most used type being the corona discharge, which is created by applying a high-intensity electric field to sharp pointed electrode tips. Based on the polarity of the electrode, the corona discharge exists in positive and negative forms [114]. Water plasma has proven to be an efficient and cost-effective remediation technique – in short processing times [115]- of many recalcitrant compounds such as pharmaceuticals, pesticides, synthetic dyes and phenols without regenerating waste [116]. However, since its use is still in a lab-scale, further research about its sustainability in industrial-scale applications is needed.

Section II
Aims and structure of this thesis

In the context of the AQUAlity-ETN project, this PhD thesis aimed at the development of analytical methods for CECs' detection and assessment in different water matrices used for drinking water production including a toxicological assessment. The main research activities of this PhD project were carried out at the Research Centre of Società Metropolitana delle Acque Torino S.p.A (SMAT), the company in charge of the water cycle management in the Metropolitan Area of Turin (Piedmont, Italy). Further collaborations and secondment periods in other institutions within the consortium of AQUAlity contributed in the conclusion of this thesis.

The main goal of this work was to evaluate a more sustainable way of water management systems within the context of Circular Economy (CE), by improving water quality monitoring assessments, after combining screening and identification techniques with higher detection sensitivity (even at trace levels) for a broader and more diverse group of Contaminants of Emerging Concern (CECs) with risk assessments, taking as well into account the cocktail effect of their mix. Based on the results of these assessments, the evaluation of conventional and modern degradation techniques to remove them was carried out including toxicological assessments and identification of by-products.

Within this thesis, three monitoring assessments were carried out, including both targeted and non-targeted screening approaches. Two different analytical methods - using High Performance Liquid Chromatography coupled to a Triple Quadrupole Mass Spectrometer (HPLC-MS/MS) - for evaluating the pollution rates, at trace level concentrations, were developed, following the principles of Green Analytical Chemistry, which required them to be fast, cost-effective and more green. Both methods were validated according to the requirements of ISO 17025 and those established by Accredia, the Italian National System for laboratories' accreditation.

More specifically, during the first year of this PhD project an innovative method for the determination of seventeen Perlfuoroalkyl Substances (PFASs) in different water matrices (including surface, ground and drinking water) at really low quantification levels was developed. The target compounds included a mix of seventeen linear perfluoroalkyl substances, with a chain length ranging from four to eighteen atoms of carbon, in order to investigate the variability among their physicochemical properties. The key characteristic of this method is the achievement of the compounds' detection in a maximum analysis time of 10 minutes and in really low concentration levels without using any preconcentration step. In the literature, only three methods using a direct injection analysis [51,117,118] are reported. However, all of them target at less than 17

compounds, and have a longer analysis time than the one reported here. Other reported methods achieved as well low quantitation levels (in the ppt range), but for a smaller number of target compounds and after the use of an extraction/pre-treatment step [46] [119-123]. Furthermore, until now very few standard methods (Table 4) – EU aims to develop standard methods for PFAS until 2024 - exist for the determination of PFASs in water samples, with only one, the American Society for Testing and Material (ASTM) method, not including any pre-treatment step. However, this method is not referring to determination of PFASs in drinking water samples. Similarly, even if both the ISO 25101:2009(E) [124] and the United States Environmental Protection Agency (EPA) 537 [125] methods, concern drinking water matrices, they use a solid phase extraction (SPE) step and focus on a smaller number of compounds than the one reported in the developed method.

The developed method was used in the first monitoring assessment study reported in this thesis, which aimed at estimating the PFASs occurrence levels in the Metropolitan Area of Turin. A correlation study between the obtained results and the potential pollution sources in the area (such as WWTPs, industries and civilian airports) was carried out using spatial multivariate statistical analysis tools, in order to develop a statistical framework that investigates if the presence of PFASs in water bodies is associated with the number of pollution sources within a watershed. In this way, a geographical model that supports "smart" water quality monitoring programs, was developed in order to not only take into account the number of inhabitants or the volume of supplied water when planning quality monitoring programs, as done till now. The results of this study (**P1**) are summarized in **Section III, Chapter 3**.

Table 4. A comparison of standard methods for PFASs analysis [46].

Standard Method	EPA 537	ISO 25101:2009(E)	ASTM D7979-16	ASTM D7868-14
Sample volume	250 mL	500 mL	5 mL	2 g
Sample matrix	Drinking water	Drinking water, groundwater, surface water and seawater	Water; wastewater sludge, influent and effluent	Solid and biosolid
Analytes	PFAS and FOSAAs 14 PFAS	PFOS and PFOA	PFAS, FOSAAs, FTSs, n:2 FTUCAs and FTCAs	PFAS, FOSAAs, FTSs, n:2 FTUCAs and FTCAs
Preservation	Trizma for buffering and removal of free chlorine	Sodium thiosulfate pentahydrate for removal of free chlorine	None	None
Holding time	Before extraction: 14 days refrigerated at ≤6 °C Postextraction: 28 days at room temperature	14 days at 4 ± 2 °C	28 days at 0–6 °C	28 days at 0–6 °C
Extraction Method	SPE-WAX (SPE Weak anion exchange)	SPE	Direct injection	Solvent extraction followed by filtration using polypropylene filters
Analytical instrument	LC-MS/MS (liquid chromatography tandem with mass spectrometry)	LC-MS/MS and LC/MS	LC-MS/MS	LC-MS/MS
Reporting limits	2.9–14 ng/L	2–10,000 ng/L	10–400 ng/L	25–1000 ng/L

The second developed method focused on the quantification of sixteen different pharmaceutical compounds and hormones (PhACs) at trace levels in water. It was developed following the principles of Green Analytical Chemistry and validated according the criteria of ISO/IEC 17025 [126] as well. However, taking into account the variety among the compounds and their properties, in this method a preconcentration step was necessary for achieving low Quantification Levels for all of them. The second monitoring assessment reported in this thesis used this method with the aim to evaluate the concentrations of the sixteen target PhACs in raw drinking water sources (surface and groundwater) of the Metropolitan Area of Turin. However, in order to avoid a blind monitoring, like the first assessment campaign, the geographical tool developed in Chapter 3 was used for choosing the sampling points in higher risk of pollution, according to their geographical position close to potential pollution sources (WWTPS, hospitals and care houses). Finally, a risk assessment was carried in order to evaluate the potential adverse effects that PhACs' occurrence in drinking water can have on human health after a long-term exposure to low doses. Human health risks were evaluated considering average detected concentrations for individual compounds and their mixtures, and provisional guideline values for those that a drinking water regulatory value didn't exist. Since risk assessments are required for establishing priority substances for monitoring and if necessary managing their removal – as reported in the just issued Drinking Water Directive 2020/2184/UE [36]- the overall aim of this study was to fill some knowledge gaps existing in literature for PhACs' risk assessments due to limited datasets and synergistic effects of contaminants mixtures [127,128]. The results of this study (**P4**) are collected in **Section III, Chapter 4.**

However, one fundamental obstacle in evaluating water quality with current monitoring approaches is the fact that they focus on a very small number of chemicals. In order to more comprehensively assess the presence of contaminants in the aquatic bodies and prioritize pollutants within regulatory applications, target, suspect and non-target screening approaches should be combined. Hence, in this thesis a non-target monitoring assessment based on High Resolution Mass Spectrometry (HRMS) was included. Surface water samples from two different countries – Greece and Italy –were analyzed after a conventional SPE step by Liquid Chromatography coupled to a hybrid Quadrupole-Time-of-flight Mass spectrometer (LC-QTOF-MS) using the Sequential Window Acquisition of all Theoretical Mass Spectra mode (SWATH-MS). A suspect list of 100 compounds, with available standards in the laboratory, was developed and a stock solution containing them, was prepared and analyzed as well. After searching the chromatograms for exact

masses of suspects -as an additional help for identifying compounds in the samples- a non-target screening was carried out. However, due to the complexity of environmental samples, thousands of HRMS features were generated by their nontarget screening analyses (in this thesis detected fragments/ions will be referred to as features prior to their identification). For this reason, data reduction efforts for collecting reliable information were pursued, followed by the application of Multivariate chemometric methods - such as Principal Component Analysis (PCA) and Partial Least Squared Discriminant Analysis (PLS-DA) - in order to process the big obtained datasets, reveal pollution patterns and prioritize the features responsible for the discrimination among the samples. The use of a database with more than 4000 entries contributed to high confidence identification of the compounds, which was based on mass accuracy, retention time, isotopic ratio pattern, and MS/MS fragmentation pattern searching. For those that a correspondence to the database was not found, an empirical formula calculation was done by the instrument's software, followed by an *In silico* fragmentation using an online library to recognize the MS/MS fragment ions and successfully identify unknown compounds. The results of this study are reported in **Section III, Chapter 5.**

The importance of monitoring assessments except the fact that they provide valuable information about water bodies' quality status, they can be a helpful tool for treatment decisions. In general, conventional treatment methods used in WWTPs and DWTPs have been found to face difficulties in efficiently removing CECs from water, especially when they occur in trace level concentrations [66,97,129,130]. For this reason, the need of finding new more efficient degradation processes has emerged with great attention being raised towards Advanced Oxidation Processes (AOPs), a green alternative. AOPs rely on the generation of highly reactive species, able to oxidize the emerging pollutants. These techniques have shown great potential in removing efficiently CECs from water bodies, especially when they are combined with the conventional treatment processes. For this reason, after taking into account the results obtained from the monitoring assessments, as well as the general trends of aquatic contamination, the evaluation of the pollutants' removal by conventional and advanced water treatment technologies was carried out, during the second half and third year of this PhD over secondment periods in other Universities. The obtained results are summarized in Section IV.
More specifically, during a secondment period at the University of Aalborg, an attempt of finding environmental sustainable treatments was done by evaluating the degradation efficiency of UV photolysis.

UV photolysis has been reported as a degradation technique of low environmental impact and minimized environmental footprint since it doesn't have a lot of prerequisites and doesn't generate waste [131]. The contaminants' degradation after exposure to UV irradiation can be a result of direct photochemical transformation by photons' absorption, or of interactions with generated reactive oxygen species. In the study (**P2**) reported in **Section IV**, **Chapter 6**, the effects of irradiation on glyphosate - the most detected pesticide in the aquatic environment - were investigated. Samples of the compound were prepared in both MilliQ water and different real water matrices (including drinking and groundwater) from the Municipality of Aalborg, Denmark and exposed to UV-A (365nm), UV-B (302nm) and UV-C (254nm) irradiation under different UV doses. A test battery including aquatic organisms from different trophic levels was used in order to evaluate glyphosate's biological effects and bioactive transformation products before and after treatment. Studies measuring the effectiveness of UV irradiation alone as a remediation treatment for pesticides and more specifically glyphosate, should include an assessment of its potential to alleviate the parent compound's toxicity to non-target organisms as well as the one from the transformation products. For this reason, in this study we combined LC-MS analysis in order to follow glyphosate's degradation and identification of byproducts with bioassays to assess the compound's removal and toxicity to non-target aquatic test organisms before and after treatment was done. Samples taken before and after exposure to different wavelengths of UV irradiation, were analyzed with LC-Orbitrap-MS, and based on a suspect screening, identification of by-products was done.

Finally, in **Section IV, Chapter 7** is presented the High Voltage Pulsed Electric Field process, a promising technology for degrading persistent emerging contaminants that are recurrent in water bodies and can pose a threat to both human health and aquatic environment. One major category of such compounds includes PFASs, which host one of the strongest chemical bonds in their carbon chain (C-F) that makes them highly persistent against conventional treatment methods. Moreover, the fact that these contaminants coexist in mixtures and the variety of physicochemical properties among them, harshens the opportunity to find one efficient degradation technique. Hence, in this study, the performance of non-thermal plasma technique was evaluated. The reactor used for the treatment is a patent of IRIS S.r.l., and the technique basically applies on one or several very high voltage (HV) pulses of very short duration to a reactor containing the contaminated water samples, generating in this way pressure waves, UV light and formation of chemically active species such as $\bullet OH$, $\bullet H$, $\bullet O$, $\bullet O_2^-$, $\bullet HO_2$, $\bullet H_2O_2$,

•O$_3$ [132], that can break the organic molecules. Based on the results from the study reported in Chapter 3, the most abundant compounds in the area were prioritized for treatment. The selected compounds had different chain lengths, included both perfluoroalkane sulfonates and perfluoroalkyl carboxylates, and all of them were linear. Samples of individual compounds as well as their mix were prepared in MilliQ water and real water matrix, and analyzed with LC-QTOF-MS in both HRMS MRM and SWATH acquisition modes. The MRM mode contained the parameters of the method reported in Chapter 3 and was used for the quantification of the compounds before and after treatment, in order to follow the degradation profiles (**P3**). The SWATH mode, based on a full scan acquisition, was used for identifying by-products with both suspect and non-target screening (**P5**).

Section III

Freshwater Quality
Monitoring and Assessment

Chapter 3 Per and polyfluoroalkyl Substances (PFASs)

Firstly, an advanced quantitative analytical method for detecting PFASs in different water type samples was developed and validated. The overall aim was to obtain a fast, cost-effective and green method – following the Green Analytical Chemistry principles– in order to be applied in a large scale monitoring assessment of PFASs levels in the Metropolitan Are of Turin. Finally, an estimation study of the potential pollution sources was carried out by correlating the PFASs occurrence results with industrial sites, civilian airports and WWTPs that exist in the area, using spatial and multivariate statistical analysis tools.

1. Materials and Methods

1.1 Reagents and Chemicals

A mix (PFAC-MXB) of seventeen PFASs was examined in this study containing compounds with various carbon chain lengths (between four to eighteen atoms of carbon): thirteen linear perfluoroalkylcarboxylic acids and four perfluoroalkylsulfonates (Table 5). The standard mix solution PFAC-MXB was purchased from Wellington Laboratories (Guelph, ON, Canada) with chemical purities of >98% and a concentration of 2000 ng/mL in Methanol/Water <1% for every individual perfluoroalkylcarboxylic acid and perfluoroalkylsulfonate. Another mix (MPFAC-MXA) containing seven mass-labelled (^{13}C) perfluoroalkylcarboxylic acids and two mass-labelled (^{18}O and ^{13}C) perfluoroalkylsulfonates was used as internal standards (Table 5). The mix solution MPFAC-MXA was purchased from Wellington Laboratories (Guelph, Ontario, Canada) with chemical impurities >98% and a concentration of 2000 ng/mL in Methanol/Water <1% for every individual mass-labelled perfluoroalkylcarboxylic acid and mass-labelled perfluoroaklylsulfonate and with isotopic impurities of 99% per ^{13}C and >94% per ^{18}O. UHPLC-grade Methanol was purchased from Sigma-Aldrich, Co (St. Louis, MO, USA), MilliQ was obtained from MilliPore (MA, USA) and Ammonium acetate for LC-MS LiChropur® was purchased from Merck KGaA (Darmstadt, Germany).

Table 5. Target compounds and their related internal standards.

Target Compounds (PFAC-MXB)		Internal Standard Compounds (MPFAC-MXA)	
Full Name	**Abbreviation**	**Full Name**	**Abbreviation**
Perfluoro-n-butanoic acid	PFBA	Perfluoro-n-[$^{13}C_4$]butanoic acid	MPFBA
Perfluoro-n-pentanoic acid	PFPeA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	MPFHxA
Perfluoro-n-hexanoic acid	PFHxA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	MPFHxA
Perfluoro-n-heptanoic acid	PFHpA	Perfluoro-n-[1,2-$^{13}C_2$]hexanoic acid	MPFHxA
Perfluoro-n-octanoic acid	PFOA	Perfluoro-n-[1,2,3,4-$^{13}C_4$]octanoic acid	MPFOA
Perfluoro-n-nonanoic acid	PFNA	Perfluoro-n-[1,2,3,4,5-$^{13}C_5$]nonanoic acid	MPFNA
Perfluoro-n-decanoic acid	PFDA	Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	MPFDA
Perfluoro-n-undecanoic acid	PFUdA	Perfluoro-n-[1,2-$^{13}C_2$]undecanoic acid	MPFUdA
Perfluoro-n-dodecanoic acid	PFDoA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	MPFDoA
Perfluoro-n-tridecanoic acid	PFTrDA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	MPFDoA
Perfluoro-n-tetradecanoic acid	PFTeDA	Perfluoro-n-[1,2-$^{13}C_2$]dodecanoic acid	MPFDoA
Perfluoro-n-dexadecanoic acid	PFHxDA	Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	MPFDA
Perfluoro-n-octadecanoic acid	PFODA	Perfluoro-n-[1,2-$^{13}C_2$]decanoic acid	MPFDA
Potassium perfluoro-1-butanesulfonate	L-PFBS	Sodium perfluoro-1-hexane [$^{18}O_2$] sulfonate	MPFHxS
Sodium perfluoro-1-hexanesulfonate	L-PFHxS	Sodium perfluoro-1-hexane [$^{18}O_2$]sulfonate	MPFHxS
Sodium perfluoro-1-octanesulfonate	L-PFOS	Sodium perfluoro-1-[1,2,3,4-$^{13}C_4$] octanesulfonate	MPFOS
Sodium-1-decanesulfonate	L-PFDS	Sodium perfluoro-1-hexane [$^{18}O_2$]sulfonate	MPFHxS

1.2 Study area and sampling

As already mentioned SMAT is the company in charge of managing and supplying water to 293 municipalities in Piedmont, Italy (Figure 5, Table A1) and a population of about 2,3 million inhabitants. The sampling campaign was organized by SMAT and carried out between March 2018 and October 2018. In total 930 samples were collected from the whole territory, according to the specifications and requirements of ISO 5667 [133], including 5% of surface, 19% of groundwater and 76% of drinking water. As surface water were considered samples taken from rivers, streams, and at the intake of SMAT DWTP (including river and lagoon water), as groundwater those taken from pumps at each wellhead, and as drinking samples those collected at the end of the DWTP treatment line, fountains, and tanks. The samples were collected in polypropylene bottles WM (wide mouth) with caps (volume 125 mL), purchased from SciLabware Limited (Stoke-on-Trent, Staffordshire, ST4 4RJ, United Kingdom), stored at 4°C prior to their analysis, and analyzed within 15 days from their collection, in order to prevent biodegradation of the matrix and reassure the recovery of the analytes.
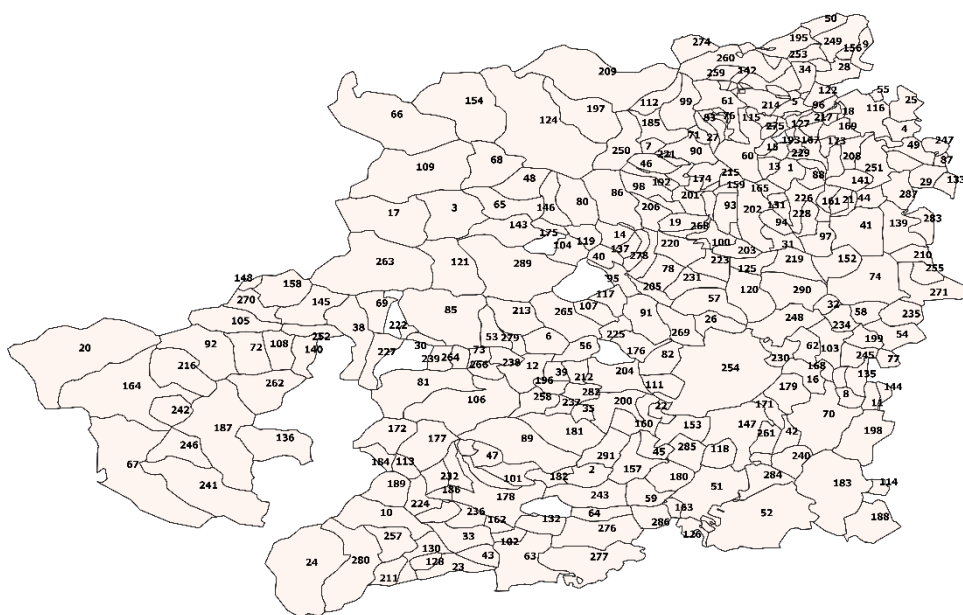

Figure 5: Map of the municipalities in the study area (Metropolitan Area of Turin, Piedmont, Italy), more information available on Table A1.

1.3 Sample preparation

The samples were injected directly into the analytical system without any pretreatment step. A filtration step was not necessary as the samples—mostly drinking water—were not contaminated with soils or suspended organic matter. Two working standard solutions—in 50% MeOH/50% H_2O for the first and in 100% H_2O for the second—were prepared with a dilution from each of the two stock solutions, and used for the calibration. The purchased solutions were stored at 4°C, while the other four were stored at room temperature. A volume of 700 µL of each sample was transferred into 0,7 mL Polypropylene Short Thread Micro-Vials (purchased from CPS Analitica for Chemistry, Milan, Italy), and 1 µL of the Internal Standard mix (50 ng/L) was added.

1.4 Instrumental Analysis

Analyses were carried out using the SCIEX QTRAP® 6500 system (SCIEX, Framingham, MA, USA) with a Thermo Scientific Dionex UltiMate 3000 UHPLC system and a RS-3000 autosampler (Dionex Softron GmbH, Germering, Germany). The UHPLC instrument was equipped with a Luna® C18 (2) HPLC Column (5 µm particle size, 30 mm × 2.0 mm; Phenomenex Inc., Torrance, CA, USA) installed between the eluent mixer and the autosampler, in order to delay the potential contamination originating from the UHPLC system. The chromatographic separation was achieved using a Luna® Omega PS C18 HPLC Column (1,6 µm particle size, 50 mm × 2,1 mm; Phenomenex Inc., Torrance, CA, USA)—heated to 40 °C—by injecting a 50 µL sample volume at a mobile phase consisted of a mixture of 20 mM Ammonium Acetate in Water (A) and Methanol (B), lasting a total time of 12 minutes. The gradient profile, with a flow rate of 0,550 mL/min, started with 98% A and 2% B, increasing to 100% B in 6 minutes, and, after keeping this ratio for 1,5 minutes, reversed into the initial conditions (Table A2). The parameters of the mass spectrometer are summarized in Tables A3, A4.

1.5 Method Validation

In order to reassure the validity of the results, a validation process was necessary and carried out following the ISO/IEC 17025 [126] guidelines and those set by Accredia, the Italian National Accreditation System. Six-point calibration curves of final concentrations 5, 10, 25, 50, 90 and 120 ng/L were built for each target compound, and fifteen replicates of each point were analyzed in order to estimate the uncertainty, trueness, linearity, recovery and limits of Detection (LOD) and Quantification (LOQ). In order to reassure the quality of the method and the best performance of the instrument during the analyses,

blank and control (QC) samples were analyzed after every ten samples. QC samples were prepared by diluting the standard solution in MilliQ water with a final concentration of 50 ng/L and adding 50 ng/L of the internal standard mix. The quantitation was performed using the software MultiQuant^{TM} 3.0.3 software (SCIEX, Framingham, MA, USA).

1.6 Spatial and Statistical Analysis

Spatial analysis was performed in order to develop a framework that investigates the correlation between contamination levels and the number of potential pollution sources within a watershed. For this reason, information about 176 industrial sites (Figure A1) and 800 WWTPs present in the study area were taken from Arpa Piemonte [134,135], and in particular, the geographical data used (coordinates in WGS 84 system and maps of the area) were obtained from the free Diva-Gis platform [136]. The QGIS 3.4 software was used for estimating the correlation between the sampling points with detected concentrations above the LOQs and the industrial sites and WWTPs that exist in the area, within a radius of 5km from the sampling points, and can potentially be characterized as potential pollution sources. However, the lack of available information concerning emerging pollutants employed by the industrial sites, led us to choose them according to their sector activities and manufacturing products known to potentially employ PFASs. QGIS 3.4 was also used for building the thematic maps, which provide a distinct visualization of the spatial distribution of the detected concentrations in the area. The GeoDa 1.12 software was used in order to build the Ordinary Least Squares (OLS) and the Spatial Regression models, following the equation (8):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \lambda \quad (8)$$

where Y is the dependent variable, X_1, X_2 are the explanatory or independent variables, β_0, β_1, β_2 are the coefficients and λ is the random spatial error term. Spatial autocorrelation between the area units was evaluated utilizing the Moran's I statistic, while the Akaike info criterion was used to check the strongest model for the correlation prediction between the potential pollution sources and the contaminated areas [137].

2. Results

2.1. Method development and optimization

During the development of an analytical method aiming to detect trace level contamination, like in nanograms per liter (or parts per trillion; ppt), cross contamination effects can have a large impact on the accuracy and validity of the analytical results. More specifically, since PFASs are compounds with a vast variety of applications,

background contamination can originate even from the laboratory equipment [117]. In order to minimize these effects, online SPE-LC-MS/MS methods for PFASs detection have been proposed and reported in the literature [51]. However, an elimination of the pretreatment step may minimize better these risks, alongside with meticulous and methodical manipulation of the samples. In this study, teflon and glass materials were avoided, as well as a filtration step - even if it was not necessary for drinking water samples - in order to minimize any contamination of the samples. Furthermore, a smaller HPLC column was added between the pump and the injector in order to delay possible contamination originating from the solvents [51,118-120].

However, direct analysis of spiked water was proven unsuccessful, as the results didn't show sufficient recovery levels for the compounds with longer chains. For this reason, taking into account the diversity of chemical properties among the target compounds as well, we chose to add Methanol in the samples following the reports of the EPA 537 method [125]. Optimization of the solvents' percentage ratio in both working standard mixes was done, and satisfying results were achieved after preparing them in 50% MeOH/50% H_2O. Moreover, in order to ensure the best detection results, a mix of isotopically labelled internal standards was added at a concentration of 50 ng/L. For quantifying the molecules, the ratio between the peak areas of the target compounds and that of their related internal standard was used.

Concerning the chromatographic conditions, the best compounds' separation was achieved after using a shorter column as an isolator, placed before the main one, in order to separate the target compounds occurring in the analytical samples, from those that were potentially present in the solvents. Furthermore, the parameters of the gradient elution of the mobile phase were optimized as well before achieving the best for analyte's separation and shape of peaks. Firstly, an elution starting from 40% Methanol and 60% Ammonium Acetate in water (5 mM) increasing to 100% Methanol and returning to the initial conditions within 6 minutes was tried. These conditions provided a satisfactory separation of the longer chain compounds, but some of the shorter chain ones were co-eluting at the beginning of the chromatogram. After different efforts, the best conditions concluded to those finally used (Figure 6, Tables A2, A3).
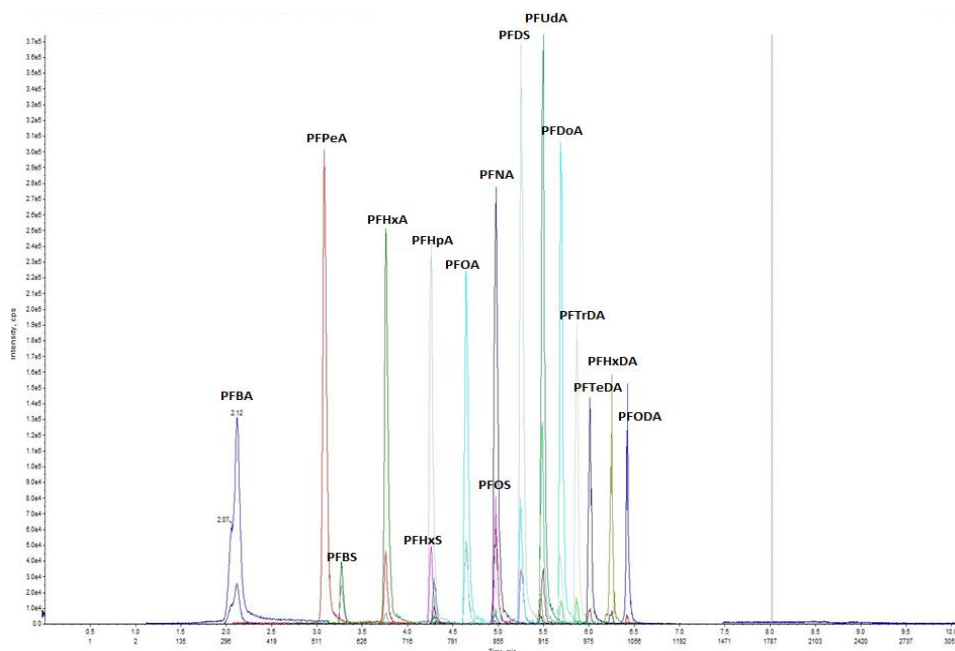
Figure 6: A typical chromatogram.

2.2 Validation and Quality Assurance

Concerning the validation parameters, linearity is based on the linear regression analysis of the obtained quantitative data. The regression coefficients (R^2) of the six-point calibration curves were calculated by the ratio between the peak area of the target compound, the peak area of the relative Internal Standard (IS) and the different concentrations of the calibration curve. A 1/x concentration-weighting factor was used in order to give more emphasis to the lower concentrations and to ensure the best assay performance. Good coefficient results between a range of 0,980 and 0,999 (Table 6) were obtained for all the target PFASs.

Furthermore, the measurement of the systematic and random errors is a crucial step during the validation of a method. According to ISO/IEC 17025 [126], the required values for uncertainty accepted values should be RSD ≤ 20% and for a sufficient recovery within the range of 70%–120%. For this method, the variability and reproducibility of the results were calculated for every point of the calibration curves, offering satisfactory results within the acceptance ranges (Table 6). The recovery of the compounds was also tested in 4 different real water matrices, spiked with the mix of the target compounds at the same concentrations (50 ng/L) offering satisfactory results (Table 7). Similarly, the

45

accuracy of the method, based on the trueness of the results, was calculated for every analyte and found within the range of ≤30% (Table 6).

Table 6. Validation results.

Compounds	Concentration ng/L	Trueness %	Recovery %	Uncertainty %	Linearity	LOQ
PFBA	50	−8,6	91,38	3,13	0,997	5
PFBS	50	−4,2	95,78	2,89	0,997	5
PFPeA	50	1,3	101,28	1,67	0,999	5
PFHxA	50	−5,2	94,84	2,82	0,998	5
PFHxS	50	−3,9	96,12	6,07	0,997	5
PFHpA	50	−4,7	95,28	3,63	0,999	5
PFOA	50	−3,7	96,28	4,36	0,998	5
PFOS	50	−8,0	92,04	1,96	0,992	5
PFNA	50	−9,6	90,38	4,82	0,999	5
PFDA	50	−14,4	85,58	7,59	0,995	5
PFUdA	50	−5,3	94,72	9,64	0,998	5
PFDoA	50	−7,2	92,78	8,72	0,999	5
PFTrDA	50	−26,8	73,22	17,53	0,989	5
PFTeDA	50	−30,0	69,98	13,76	0,987	5
PFHxDA	50	−15,1	84,88	15,97	0,995	5
PFODA	50	−13,7	86,34	5,48	0,980	5
PFDS	50	−51,3	48,68	15,78	0,997	5

Finally, in order to calculate the Limits of Detection and Quantification for each analyte the requirements of the International Conference on Harmonisation (ICH) Method [139] were followed. More specifically, the standard deviation of the y-intercepts and the slope of the calibration curves were obtained from the 15 replicate analyses of the calibration curves. The LOD was calculated following the equation (9) and LOQ following the equation (10)

$$LOD = 3,3\ \sigma\ /\ S\ (9)$$

$$LOQ = 10\ \sigma\ /\ S\ (10)$$

where σ is the standard deviation of y-residuals and S is the slope of the calibration curve. The results for the LOQs of the target PFASs varied between 3 (for shorter chain compounds) to 8 ng/L (for longer chain compounds). However, for practical reasons and

data processing uniformity as a Limit of Quantification for every compound (as stated also in the ASTM D7979-17 method [140]) was considered the lowest point of the calibration curve satisfying the trueness and uncertainty criteria of less than 30% (considering the 15 replicates). In this way, the LOQ for every compound resulted at 5 ng/L (Table 6). However, the validation results for PFDS were not satisfactory (Table 6). Hence, this compound was removed from our method.

Table 7. Recovery results after spiking real water samples.

Compounds	Concentration ng/L	Real Sample 1 Recovery %	Real Sample 2 Recovery %	Real Sample 3 Recovery %	Real Sample 4 Recovery %
PFBA	50	107,52	106,06	90,84	107,05
PFBS	50	106,92	107,22	90,76	103,90
PFPeA	50	108,22	105,85	90,53	109,99
PFHxA	50	104,96	106,89	91,69	107,11
PFHxS	50	104,96	106,73	90,88	105,98
PFHpA	50	102,19	102,93	92,57	105,56
PFOA	50	103,63	114,74	92,09	107,14
PFOS	50	95,92	97,31	86,33	96,11
PFNA	50	106,18	102,78	97,41	104,91
PFDA	50	95,29	90,22	81,93	90,39
PFUdA	50	93,49	83,01	81,57	80,78
PFDoA	50	93,19	100,73	96,98	97,45
PFTrDA	50	97,89	87,62	82,73	97,13
PFTeDA	50	101,03	84,89	94,62	88,16
PFHxDA	50	100,61	101,19	84,50	101,98
PFODA	50	86,63	90,16	87,91	93,71
PFDS	50	72,26	75,31	52,28	74,51

2.4 Monitoring Assessment results

The developed method was used in an estimation study of the PFASs' pollution levels in the Metropolitan Area of Turin. In total, 930 samples were collected from all the steps of a water supply system (from the catchment till the tap), through a sampling campaign organized by SMAT, during March and October 2018. Between the samples, 5% included surface water, 19% groundwater and 76% drinking water.

All the detected concentrations -both for individual compounds as well as their mix- were significantly lower than the drinking water performance values set by the Italian Ministry of Health (30 ng/L for PFOS and 500 ng/L as sum of PFAS), and the parametric limit values reported in the revised Drinking Water European Directive 2020/2184 [36] (100 ng/L as single and 500 ng/L as sum of PFAS). In this study, the highest detected concentration for the sum of the target compounds was 57 ng/L. Only four out of the sixteen compounds were detected in the area in concentrations above their Limits of Quantification (5 ng/L) and they were the perfluoro-n-butanoic acid (PFBA), perfluoro-1-hexane sulfonate (PFHxS), perfluoro-n-octanoic acid (PFOA) and sodium perfluoro-1-octanesulfonate (PFOS). For PFBA the highest detected concentration was 19 ng/L (Figure 7a), while for PFHxS and PFOA, they were 15 ng/L and 9 ng/L, respectively (Figure 7b,c). Concerning PFOS, the highest detected concentration was 23 ng/L (Figure 7d), which was the highest among the four detected compounds. The highest concentrations for these four compounds were detected in raw sources. These results were in contrast with those reported in other studies, where as a general finding the detected concentrations of carboxylates in the aquatic environment are higher than those of sulfonates [137].

In general, PFBA, PFHxS, PFOA and PFOS were present alone or in mixtures of two or three compounds in the analyzed samples. However, only in a groundwater sample of one municipality, which was the one that hosts the civilian airport on the area, were present all together. Even if their sum concentration was low, this was an excepted result since it is known that PFASs constitute some of the main components of the aqueous film-forming foams (AFFF) used in that areas [46]. Detected PFASs concentrations were observed in all the different types of water sources included in this study, with those concerning treated water being significantly lower, but still above the individual LOQ levels. These results alongside with the fact that the highest concentrations were detected close to industrial sites and WWTPs, confirm the studies in literature claiming that conventional treatment methods (both in DWTPs and WWTPs) are not sufficient in efficiently removing PFASs from water. Understanding the correlation between pollution sources and detected concentrations in drinking water, and whether increased concentrations are associated with their number within a watershed is important for water utilities in order to identify the exposure risks. For this reason, in this study multivariate spatial regression models [141] were developed in order to identify correlations between contaminated sampling points and the selected potential pollution sources.
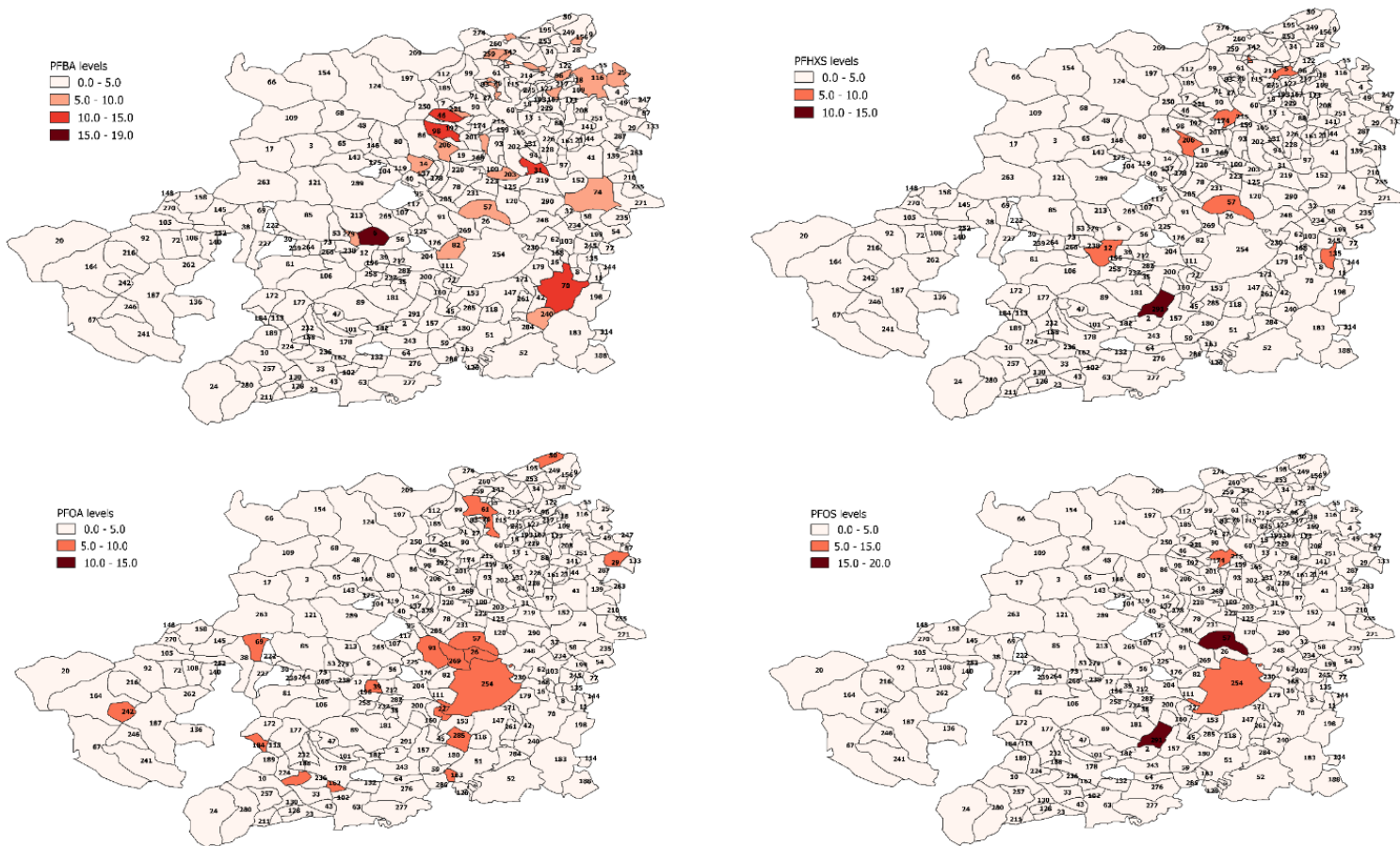
Figure 7: Concentration levels(ng/L) among the different municipalities of the study area for the four detected compounds: (a) PFBA; (b) PFHxS; (c) PFOA; (d) PFOS.

2.5 Spatial and Statistical Analysis

In this study, spatial analysis was performed in order to study the geographic correlation between the "positive" sampling points and the potential contamination sources that are surrounding them. The main principle of spatial statistical analysis is that space is an influence on the observations of a study, meaning that it is very likely for values obtained from nearby points to be more similar to each other than with values obtained from more distant points. Regression analysis is a tool that allows to examine and model these location-oriented relationships, and helps to explain spatial patterns. In this study, the results obtained from the screening assessment were used in order to build three different regression models, the OLS, Spatial Lag and Spatial Error Regression. The model that best explained the relationship between the concentration levels of the compounds (dependent variables) and the number of WWTPs and industrial activities in their surrounding area (independent variables) was chosen, based on the obtained Akaike criterion and R^2 values (Table 8). In order to build the regression models spatial weights -as measures of the influence- were taken into account.

Table 8. Summary of the different regression models.

	Coefficients	OLS	Spatial Error	Spatial Lag
PFBA	Industrial Sites	1,1012	1,1371	1,1233
	WWTPs	0,2790	0,2795	0,2792
	R^2	0,660	0,662	0,662
	Akaike info criterion	1020,04	1021,45	1019,78
PFHxS	Industrial Sites	0,1667	0,1688	0,1678
	WWTPs	-0,0110	-0,0118	-0,0113
	R^2	0,270	0,303	0,296
	Akaike info criterion	848,65	850,48	848,58
PFOS	Industrial Sites	0,2719	0,2859	0,2785
	WWTPs	-0,0119	-0,0123	-0,0121
	R^2	0,078	0,081	0,076
	Akaike info criterion	909,98	911,79	909,97
PFOA	Industrial Sites	0,6166	0,576574	0,59239
	WWTPs	-0,0311	-0,0251	-0,0261
	R^2	0,242	0,225	0,233
	Akaike info criterion	968,21	963,78	965,32

Based on the results obtained from the different regression models, the Spatial Error model was the one that explained better the spatial correlation between the compounds PFBA, PFHxS and PFOS and the potential pollution sources in the area. Concerning PFOA, the spatial regression model that best described the relationship between the contaminated areas and the industrial activities and WWTPs was the OLS. The results of the chosen models are summarized in Table 9. These results explained 8%–66% of the variance in the water samples for the four PFAS compounds that were detected. Increasing PFBA concentrations were positively associated with the number of industrial sites and the WWTPs present in the surrounding area of the sampling point, meaning that each potential pollution source is associated with a 66% increase in PFBA levels. This relationship resulted as statistically significant ($p < 0,001$), and as the strongest statistical association across the compounds and contamination sources.

Table 9. The chosen spatial regression models for PFASs concentrations in drinking water.

Compounds	Industrial Sites	WWTPs	λ^*	R^2
PFBA coefficient p-value	1,1371 0,001	0,2795 0,001	0,092 0,05	0,66
PFHxS coefficient p-value	0,1688 0,002	−0,0118 0,523	0,033 0,07	0,30
PFOS coefficient p-value	0,2859 0,001	−0,0123 0,527	0,082 0,07	0,08
PFOA coefficient p-value	0,6166 0,001	−0,0311 0,151	0,159 0,07	0,24

[*] Spatial error term coefficient showing spatial influence.

The other three detected compounds showed positive correlations with the number of the industrial sites, with statistically significant relationships ($p < 0,05$), indicating that each additional industrial site is associated with a 30% increase in PFHxS, 8% increase in PFOS and 24% in PFOA levels. However, the regression models showed negative association between the number of WWTPs and the increasing levels of PFHxS, PFOA and PFOS, with relationships lacking statistical significance ($p > 0,05$) (Table 9). This

indicates that PFAS releases from WWTPs are important but less significant than those from industries, following the findings obtained from Hu et al. [137]. However, the low number of sampling points with concentrations above the LOQ for these three compounds (for PFHxS, only 7 out of the 930 samples were positive, whereas for PFOS, only 6 out of the 930) can explain these results. The Moran's I statistic was used for evaluating the spatial autocorrelation between the area units. Basically this test is used for examining if the attribute values of features (compounds) cluster on not, taking into account locations of other features. A result of -1 would mean a checkered pattern, a results of 0 would mean a random pattern and a result of 1 would mean a clustered pattern. In this study the results of Moran's I statistic for the four regression models built were between 0,131 (for PFOS) and 0,541 (for PFBA), showing a random pattern of spatial autocorrelation among the area units.

The spatial analysis performed in this study was challenging due to the low detection results and the lack of available information, as reported by the λ coefficients that represent the spatial error. Geospatial data for many potentially important PFAS point sources were not present, as well as information about the companies' production processes. Moreover, no data about the employment of this class of substances (as PFAS are not regulated yet) or the airborne emissions were available in order to evaluate the importance of the atmospheric releases. Information about where and if the intake of the water supply was upstream from the point source of pollution was not accessible as well.

3. Conclusions

In this chapter, a new green, fast and validated method with high sensitivity in detecting a mix of sixteen different PFASs in drinking water samples at trace level concentrations is presented. The key characteristic of this method is the absence of an extraction step and a direct injection into the analytical system. Even the numerous difficulties faced in order to achieve its best performance, good recovery results, and really low Quantification Limits (5 ng/L) were achieved for all the compounds. The developed method was applied in the first assessment of PFASs occurrence levels in the Piedmont region of Italy. Despite, the low detected pollution rates, a correlation between the "positive" sampling points and the potential pollution sources in the territory was done in order to understand their influence on the pollution levels and take decisions for reduction of contamination at source. The results showed that the number of point sources within a watershed significantly affects PFASs occurrence levels, providing us

with significant predictors for guiding future choice of sampling points at higher risk. However, the lack of information through the correlation study didn't allow for better assessment, highlighting the need for stronger cooperation and active participation between Regional Health and Environmental Protection Agencies, Water Companies and Stakeholders within policy making.

In conclusion, the results of this study highlighted the fact that chemical analysis alone is not able to evaluate the potential pollution of water bodies sufficiently. In order to take control measures for a safe and sustainable water supply is important to identify the hazardous components, their occurrence areas, and also the points at higher contamination risk. For this reason, and considering the costs, efforts and environmental impact of wide screening assessments, a "smart" monitoring program is better performing thanks to the prioritization of sites at major risks.

Chapter 4 Pharmaceuticals and Hormones

As described in Chapter 3, improvement of monitoring assessments for better evaluating the water bodies' quality is fundamental for better water supply and regulations. Hence, in this chapter is presented the second monitoring assessment done within this PhD thesis, followed a risk-based approach. More specifically, the geographical model developed in Chapter 3 was used in order to identify the points at higher risk of pollution and include them in the evaluation of pharmaceuticals and hormones levels in the Metropolitan Area of Turin. For the selection of the target compounds different analytical protocols' requirements were taken into account and a human health risk assessment for the detected concentrations – both for individual compounds and their mixed effects – was carried out in order to prioritize contaminants for treatment, with the higher aim of managing better a safe drinking water supply.

1. Materials and Methods

1.1 Selection of compounds

For this study, a target list containing different pharmaceutical compounds and hormones, was prepared based on the just revised European Drinking Water Directive (2020/2184/UE) [36], the requirements of the Regional Environmental Protection Agency (ARPA Piemonte) analytical protocol [142] and the NORMAN prioritization framework of emerging substances [35]. In this way, we concluded to sixteen different compounds: Ketoprofen, Atenolol, Trimethoprim, Ofloxacin, Azithromycin, Ciprofloxacin, Cyclophosphamide, Sulfamethoxazole, Erythromycin, Clarithromycin, and Caffeine (Table 10). Caffeine was included in this study as a tracer of anthropogenic pollution.

1.2 Reagents and Chemicals

Stock solutions of the target compounds were prepared in UHPLC-grade MeOH, all purchased from Sigma-Aldrich, Co (St. Louis, MO, USA). MilliQ was obtained from MilliPore (MA, USA), LiChropur Formic Acid 98%-100% and LiChropur Ammonia (NH$_3$) solution 25% for LC-MS were purchased from Merck KGaA (Darmstadt, Germany), Ethylenediaminetetraacetic acid trisodium salt dihydrate (Na$_4$EDTA) and Hydrochloric Acid were obtained from Fluka Analytical (Sigma-Aldrich, MO, USA). Ammonium acetate for LC-MS was purchased from Fisher Chemical Scientific (Geel, Belgium).

Table 10. Selected compounds and their current regulation status in EU.

Compounds	Chemical Group	CAS number	Regulation Status
Atenolol	β-Blockers	29122-68-7	NORMAN framework prioritization
Azithromycin	Macrolide Antibiotic	83905-01-5	EU Watch List/ARPA protocol
Clarithromycin		81103-11-9	EU Watch List/ARPA protocol
Erythromycin		114-07-8	EU Watch List/ARPA protocol
Caffeine	Stimulant	58-05-2	NORMAN framework prioritization
Carbamazepine	Anticonvulsant	298-46-4	NORMAN framework prioritization
Ciprofloxacin	Fluoroquinolones antibiotics	85721-33-1	NORMAN framework prioritization
Ofloxacin		82419-36-1	NORMAN framework prioritization
Cyclophosphamide	Alkylating agent	50-18-0	NORMAN framework prioritization
Diclofenac	Analgesics anti-inflammatory drugs	15307-79-6	EU Watch List/ARPA protocol
Ketoprofen		22071-15-4	NORMAN framework prioritization
Ibuprofen		15687-27-1	NORMAN framework prioritization
Sulfamethoxazole	Antibacterial sulfonamides	723-46-6	EU Watch List/ARPA protocol
Trimethoprim		738-70-5	EU Watch List/ARPA protocol
17-beta Estradiol	Estrogens	50-28-2	EU Watch List/ARPA protocol
Estrone		53-16-7	NORMAN framework prioritization

1.3 Study Area and Sampling

The focus area of this study was the same with Chapter 3 (Figure 5, Table A1). However, in order to avoid a large-scale blind monitoring and all the costs and effort that has, a risk-based approach was followed. More specifically, in order to more sufficiently evaluate the pollution levels of PhACs in the area, a prioritization of the sampling points at higher risk was done based on the geographical model presented in Chapter 3. In this case, as potential pollution sources were considered 44 hospitals and care houses, and 24 major WWTPs, that occur within a radius of 5km from the 683 already existing sampling points in the SMAT network, usually included in regular monitoring campaigns (Figure 8). As a result, 270 sampling points were characterized as "hotspots" and were included in this study.
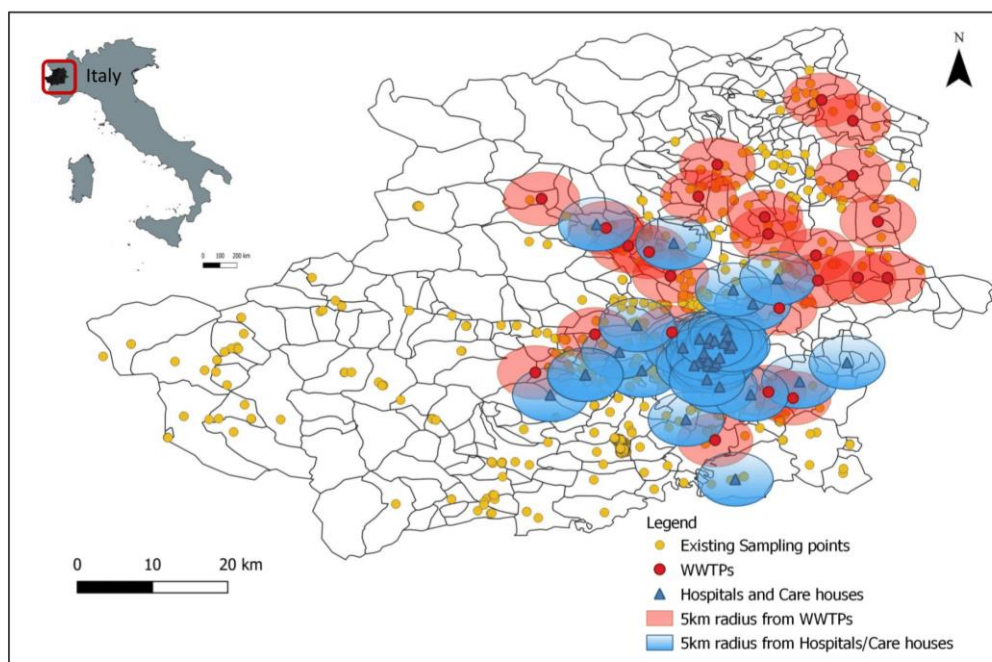

Figure 8: Map of the study area, including all the SMAT existing sampling points in the catchment areas, WWTPS and hospitals/care houses taken into account as potential pollution sources.

The sampling campaign was organized by SMAT, and carried out between October 2019 and October 2020. In total 328 samples including both raw and treated drinking water samples, following the specifications and requirements described in Chapter 3. As raw drinking water sources were considered surface (rivers, streams, and those taken at the

intake of SMAT's DWTP) and groundwater, while as treated were considered samples taken from fountains, tanks and the end of the SMAT's DWTP line. The samples were collected into amber glass bottles (1L) – previously decontaminated and rinsed with MeOH, according to the EPA 1694 method – stored at 4°C prior to their analysis and analyzed within 7 days of their sampling.

1.3.1 SMAT Drinking Water Treatment Plant

In figure 9 -which shows a photo of SMAT's DWTP- and figure 10 –which shows its schematic diagram- are shown the different pre-treatments steps of the plants. They include the catchment (D), where a wire mesh filter removes the majority of coarse contaminants and sediments, and a static horizontal flow pre-settling basin (E) consisting of a circular pool, equipped with a rotary dredge for the mechanical removal of sludge. In this stage additives can also be used in order to promote the sedimentation process. At the outlet of the pre-settling basin, the treatment plant is divided into three different lines. Po1 and Po2 (output 1100L/s), and Po3 (output 1500L/s). For Po3, an ozonation process (F) takes place, then the water is transferred to three CYCLOFLOC clarifiers (capacity of 1,5 m³/sec) with addition of aluminum polychloride. The basic principle of this system is the precipitation of sludge formed by the aluminum polychloride clumps and added microsand. During this clarification-flocculation stage (H), in one CYCLOFLOC basin, a further oxidation treatment is carried out with addition of sodium hypochlorite, for the elimination of ammonia and the nitrogenated compounds (G). Lastly, the water is filtered in two filtration batteries, each of which contains twelve overlapping units. The uppermost and lowermost filters, consist of 0,80 m thick filtering surface of granular activated carbon, trapping any particles left in the water after the settling process. After the filtration, the water is transferred into a tank where a final disinfection treatment with chlorine dioxide takes place in order to avoid the regrowth of bacterial colonies along with the distribution system during the delivery of the water to the users (I). For the two identical treatment lines Po1 and Po2, after the first sedimentation process, a pre-chlorination step takes place by means of chlorine dioxide and sodium hypochlorite, then the water is headed to an "Accelator" type sludge recirculation tank for the clarification step (L). Subsequently, the treated water is filtered on granular activated carbon and accumulated in a tank (M) where the final disinfection step with chlorine dioxide occurs (Figures 9,10).
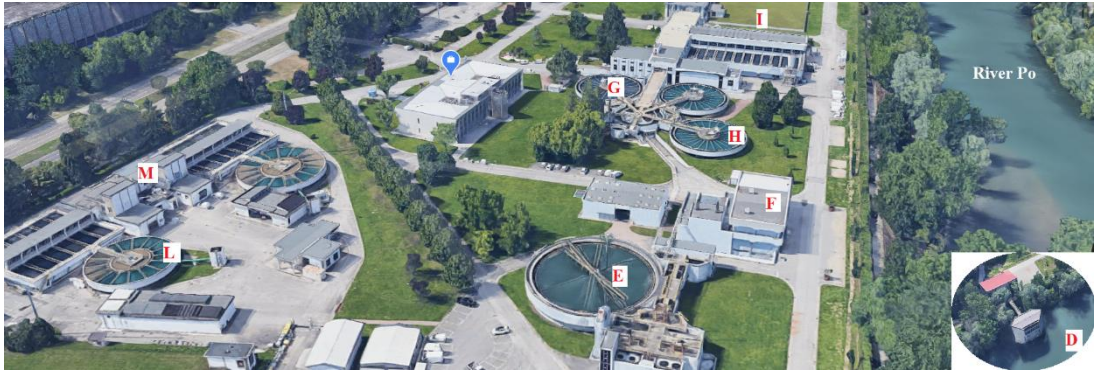
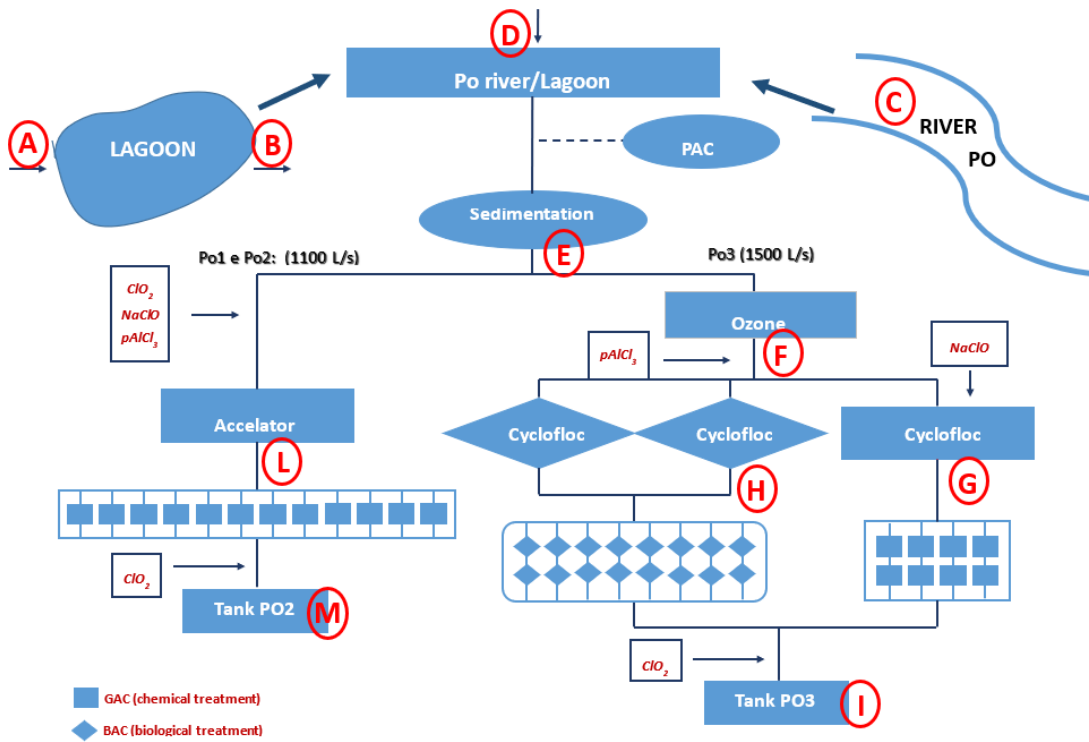**Figure 9:** SMAT Drinking Water Treatment Plant

Figure 10: Schematic diagram of treatment lines in SMAT DWTP.

1.4 Sample preparation and instrumental analysis

In order to achieve adequate extraction of the mainly acidic target analytes, the samples' pH was adjusted to 2,0 using HCl, and after the addition of 500mg of Na_4EDTA to each of them. The 1L samples were loaded on to Oasis-HLB (200mg) solid phase extraction (SPE) cartridges (Waters, Milford, MA, USA) - which were preconditioned with 12 mL MeOH, followed by 6 mL MilliQ and 6 mL MilliQ with pH 2,0 - with a flow rate of 10mL/min. Elution of the analytes was done with 12 ml MeOH and after the solvent's evaporation with a rotary evaporator (BUCHI Rotavapor R-114), they were reconstituted in 1 ml MilliQ water. The chromatographic separation was achieved with a HPLC-triple quadrupole MS system equipped with a C18 HPLC column. The MS system operated in both Positive and Negative ESI using different MRM scan modes targeted to every analyte, while three subsequent HPLC methods were developed, due to the heterogeneity among the compounds. For the substances ionized in the Positive ESI mode (Table A5) a sample volume of 5μL was injected into a mobile phase consisted of a mixture of 0,1% Formic Acid in MilliQ Water (A) and Methanol (B), in a total run time of 10 minutes. The gradient profile, with a flow rate of 0,250 mL/min, started with 98% A and 2% B, increased to 100% B after 6 minutes, and after keeping this ration for 2 minutes, returned to the initial conditions. For the Negative ESI compounds (Table A5) a volume of 10μL of sample was injected into a mobile phase consisted of a mixture of 0,02% Ammonia in MilliQ Water (C) and Methanol (D) with a total run time of 10 minutes, while Ibuprofen was analyzed alone. In this case a sample volume of 10μL was injected into a mobile phase of a mixture of 0,1% Ammonium Acetate and 0,1% Formic Acid in MilliQ Water (C) and Methanol (D) in a total run time of 10 minutes. For both analysis cycles the gradient profile, with a flow rate of 0,400 mL/min, started with 98% C and 2% D, increased to 100% D after 6 minutes, and after keeping this ratio for 2 minutes, returned to the initial conditions.

1.5 Method Validation

For reassuring the applicability of this method a validation study was carried out according to the requirements of ISO/IEC 17025 [126] and the ICH method [139], described in Chapter 3. Six-point calibration curves with final concentrations of 1000, 2000, 4000, 6000, 8000 and 10000 ng/L (preconcentration factor of 1000 after the SPE step) were built for each target compound and used for quantification and calculation of linearity, trueness, uncertainty and recovery as well as LOD and LOQ for each target compound. Blank and quality control samples were analyzed in order to ensure the best performance of the instrument and the repeatability of the results during the whole analysis time. Quality control samples were prepared at a final concentration of 4000 ng/L (in the middle of the calibration curve range) and analyzed after every ten samples. The quantitation was performed using the MultiQuant^{TM} 3.0.3 software (SCIEX, Framingham, MA, USA) and the 1/x concentration-weighting factor was applied.

1.6 Human Health Risk Assessment

A human health risk assessment study was carried out by comparing PhACs' concentrations to guideline values. The risk assessment was carried out for both individual compounds and their mixture, and as PhACs' concentrations were considered their average detected concentrations. In order to avoid wrong estimation, non-detects were considered at a value of ¼ of the individual LOD for each target molecule as proposed by Houtman et al. [62], since removing them or setting their values as zero would have under or overestimated their average concentrations. Moreover, compounds with n-octanol-water partition coefficient (log K_{ow}) higher than 3 were not included in the risk assessment study as in general, there is a smaller possibility that they will pass all the drinking water treatment line steps and end up in the treated water [143]. For this reason, log K_{ow} values for each compound were obtained with the KOWWIIN algorithm of the EPI Suite 4.11 software [144], and only those with higher values than 3 were included in the study.

The Risk Quotient (RQ_i) for individual compounds (equation 11) was obtained by the ratio between the average Detected Concentration (MEC_i) and the corresponding guideline value or, where it didn't exist, the calculated provisional guideline value ((p)GLV) [143]. The pGLVs were calculated using the equation 12,

$$RQ_i = MEC_i/pGLV_i \qquad (11)$$

$$pGLV_i \ (\mu g/L) = [ADI \ x \ BW \ x \ 10\% \ drinking \ water \ allocation]/DWI \qquad (12)$$

where ADI is the Acceptable Daily Intake (μg/kg bw/day); BW is the body weight set at a default value of 70kg, as it is the closest to the average European bodyweight value of 70,8 kg [146]; DWI is the drinking water intake (L/day) set at a default value of 2 L/day as reported by WHO; a 10% of drinking water allocation factor was taken into account as drinking water is not the only exposure way for humans [62,143,147]. ADI values for each target compound were obtained from the literature, and in the case of absence they were calculated after dividing N(L)OAEL values with an uncertainty factor of 100 [148]. For RQ values ≥ 1 there is the possibility of risk, if a lifelong exposure to the compound occurs only after drinking water consumption, while for RQ values ≤ 0,2 the risk for adverse human health effects is considered negligibly low [62,143]. For the calculation of the mixed health Risk Quotient (RQ_{mix}) the Concentration Addition (CA) concept [149] was followed by comparing the sum of individual RQs.

2. Results

2.1 Validation and Quality Assurance

Good coefficient results with R^2 within the range of 0,995-0,999 were obtained for all the molecules, indicating good linear correlation. Concerning the systematic and random errors, satisfying results within the required ranges (as reported in Chapter 3) were obtained for each point of the calibration curve, with those obtained for 4000 ng/L being reported in Table 11. The recovery of the compounds after the SPE treatment was checked in 4 different real water samples at concentrations of 4 ng/L and 10 ng/L, and resulted in a range of 85,5-128% for all the compounds. For the calculation of the limits of Detection and Quantification, equations (9) and (10) were followed and resulted in a range of 0,010-3,492 ng/L for LOD and 0,034-11,369 ng/L for LOQ (Table 11).

Table 11. Validation results for every target compound

Compounds	C (ng/L)	Trueness %	Uncertainty %	Linearity	LOD (ng/L)	LOQ (ng/L)
Atenolol	4000	-3,977	3,047	0,9996	0,196	0,655
Azithromycin	4000	-10,090	7,290	0,9951	0,736	2,454
Caffeine	4000	-1,300	1,912	0,9991	0,322	1,073
Carbamazepine	4000	-14,831	9,761	0,9999	0,066	0,219
Clarithromycin	4000	-3,519	2,786	0,9996	0,031	0,074
Ciprofloxacin	4000	-1,603	0,892	0,9996	0,788	2,625
Cyclophosphamide	4000	-0,161	2,563	0,9996	0,010	0,034
Diclofenac	4000	-7,627	2,531	0,9998	0,376	1,254
Erythromycin	4000	-4,793	3,114	0,9998	0,244	0,814
Ketoprofen	4000	-10,221	4,476	0,9999	0,115	0,385
Ofloxacin	4000	-1,1769	2,735	0,9978	0,493	1,644
Sulfamethoxazole	4000	-4,823	2,202	0,9983	0,110	0,366
Trimethoprim	4000	-7,457	5,497	0,9998	3,492	11,369
17-beta Estradiol	4000	-7,129	6,546	0,9972	0,303	1,010
Estrone	4000	-23,144	3,655	0,9971	0,400	1,333
Ibuprofen	4000	-1,599	1,770	0,9969	0,412	1,375

2.2 Monitoring Assessment results

In total 325 samples were analyzed, of which 287 were groundwater and 24 surface water. For the samples with the highest detected PhACs concentrations, treated samples from the same areas were analyzed in order to reassure the quality of treated water, and take the appropriate countermeasures if necessary.

Concerning the raw drinking water sources, in 40 samples none of the target compounds was detected above their individual LOQs, while in 52 samples only one compound was detected. This result, highlights the fact that in the majority of the samples a mix of PhACs usually is present. The maximum number of coexisting compounds in one sample

in this study was eleven, and it was detected only in one groundwater sample. The point from which the sample was taken, is close to two WWTPs and one care house, confirming the higher risk of pollution when more point sources are closer to one sampling point, as indicated from the geographical model. The average detected concentration as sum of all the target compounds was found to be 28,32 ng/L, with a range between 2,02 and 523,36 ng/L in groundwater samples and 18,54 ng/L (2,02-82,05 ng/L) in surface water.

The range of individual detected concentrations was ranging between 0,08 ng/L and 483,94 ng/L, as it is concluded from the results reported in Table 12 and Figure 11. Only two out of the sixteen target compounds – ofloxacin and erythromycin- were not detected in the study area, in concentrations higher than their individual LOQs (1,64ng/L and 0,81ng/L for ofloxacin and erythromycin subsequently). Human consumption trends in the area, the physicochemical characteristics of the compounds enabling them to be adsorbed on different particles of the soil or biodegradation processes could explain the absence of these compounds. However, these results are in accordance with a study from Verlicchi et.al [64], that doesn't report higher than their individual method detection limits concentrations of Ofloxacin and Erythromycin in surface water from the Po Valley in Italy. On the contrary, the most detected compounds in the study area were Caffeine and Ketoprofen. Caffeine is considered as one of the most abundant compounds in the aquatic environment worldwide, and was present in 176 groundwater samples with an average detected concentration of 4,61 ng/L (1,15-65,92 ng/L), while in 23 surface water samples was detected with an average concentration of 5,34 ng/L within a range of 1,31-61,28 ng/L. However, its occurrence concentrations in the study area are significantly lower than those reported in other studies, whose ranges are in the scale of μg/L. The second most abundant compound in the area, was Ketoprofen as it was present in 143 groundwater samples, with an average concentration of 6,51 ng/L within a range of 0,16-152,98 ng/L, and in 21 surface water samples with an average concentration of 5,84 ng/L (0,43-71,84 ng/L). As shown by the results, Ketoprofen's concentrations varied significantly across the territory, with the areas closer to WWTPs showing higher levels, following trends from other studies [65,150], and could be correlated with socioeconomic aspects and consumption trends.

The next two more abundant compounds in the area were the two target estrogens, raising fears about their negative endocrine disrupting effects on humans and animals. Estrone's average concentration in the 117 groundwater samples that was detected was 4,03 ng/L, with a range between 1,09 and 125,97 ng/L, and in the 12 surface water

samples was 1,003 ng/L (1,30-8,33 ng/L). Concerning, 17-beta estradiol, which is the only compound from those included in this study that is subjected to a guideline (its concentration should not exceed 1 ng/L in drinking water), it's concentration in the 114 raw surface and groundwater that was present, was higher than the guideline level with an average of 1,50 ng/L, with the highest being detected in an area close to a hospital, highlighting the risks originating from discharges of untreated wastewater effluents. A mix containing only the two hormones was present in 24 samples, while in 148 none of the two was detected.

Table 12. Detection results of the target PhACs in the study area.

Compounds	QF* n = 325	C_{min} (ng/L)	C_{max} (ng/L)	$C_{average}$ (ng/L)	C_{median} (ng/L)	Q1 (ng/L)	Q3 (ng/L)
Atenolol	12,54 %	1,07	483,94	18,73	3,96	1,64	7,93
Azithromycin	4,18 %	2,55	82,46	14,84	3,28	2,64	14,63
Caffeine	61,67 %	1,15	65,92	5,69	3,53	2,21	5,51
Carbamazepine	39,37 %	0,23	183,49	6,93	2,44	1,07	5,24
Clarithromycin	21,95 %	0,10	101,30	7,57	1,48	0,40	4,60
Ciprofloxacin	3,83 %	2,86	7,00	4,16	3,25	2,88	5,33
Cyclophosphamide	11,15 %	0,08	1,10	0,31	0,26	0,19	0,34
Diclofenac	11,15 %	1,26	121,46	12,41	3,62	2,22	11,89
Erythromycin	0,00 %	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
Ketoprofen	49,83 %	0,4	152,88	8,28	2,58	1,40	7,31
Ofloxacin	0,00 %	<LOD	<LOQ	<LOD	<LOD	<LOD	<LOD
Sulfamethoxazole	29,27 %	0,41	99,47	4,94	1,91	0,92	3,68
Trimethoprim	2,79 %	12,87	87,16	37,80	31,02	22,07	41,71
17-beta Estradiol	36,59 %	1,08	9,00	1,28	1,18	1,45	2,04
Estrone	40,77 %	1,35	125,97	7,69	3,20	2,12	5,71
Ibuprofen	2,79 %	1,46	10,54	3,77	3,15	1,78	3,73

*QF = quantification frequency

The highest concentrations of PhACs in raw drinking water sources of the study area were detected for Atenolol 483,94 ng/L, Carbamazepine 183,49 ng/L, Ketoprofen 152,88 ng/L, Estrone 125,97 ng/L, and Diclofenac 121,46 ng/L. All of them were detected in groundwater samples taken from points around WWTPs, indicating that conventional treatment techniques are not able to efficiently remove them from wastewater and highlighting the need to find new ones. As a conclusion, the findings of this study are in accordance with occurrence patterns in Italy and other countries, reported in literature [62,63,65,96,97,129,150-154].
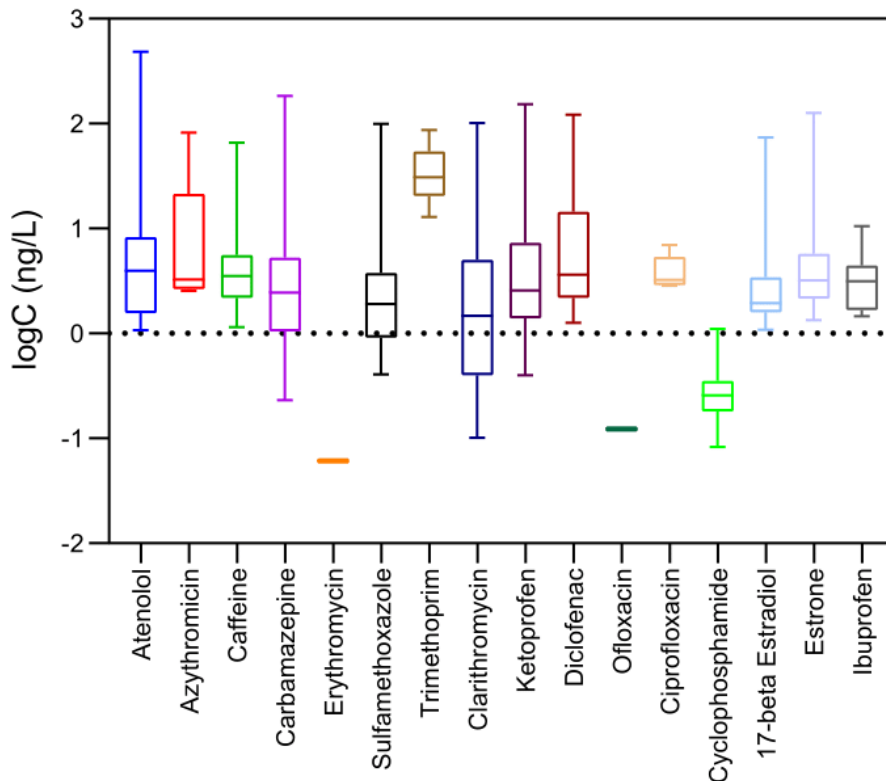

Figure 11: Boxplots showing the differences among the PhACs' detected concentrations in drinking water sources of the study area.

In literature the occurrence of PhACs in tap water around the world has been reported, as well, mainly due to insufficient treatment in DWTPs [97,148,151,155-157]. They claim that conventional treatment techniques like flocculation and sedimentation are not sufficient in removing PhACs and hormones from water completely, especially when

their concentrations are in trace levels (in the order of ng/L) [18]. However, addition of steps like ozonation and activated carbon filters, have be proved as a sufficient improvement of the treatment lines. Hence, the best solution for water companies in order to reassure a good and safe water quality supply is to combine these techniques. This concept has been incorporated in the study DWTP -as it is presented in Figures 9 and 10.

In order to evaluate the efficiency of the treatment lines in removing PhACs from water, treated samples were included in this study. The results showed that Atenolol, Azithromycin, Clarithromycin, Ciprofloxacin, Cyclophosphamide, Diclofenac, Erythromycin, Estrone, Ofloxacin, Sulfamethoxazole, Trimethoprim, and 17-beta-estradiol were not present in concentrations higher than their individual LOQs, in contrast with Carbamazepine, Caffeine, Ibuprofen and Ketoprofen. The reasons of these occurrence trends could vary among different phenomena, such as biodegradation, sufficient removal through adsorption on carbon filters and chlorination for those absent, and consumption trends and hydrophilic behavior (log K_{ow} < 3) for those present. Nevertheless, the detected concentrations of PhACs after the treatment line were significantly lower than those found in raw water sources, as shown in Figure 12.
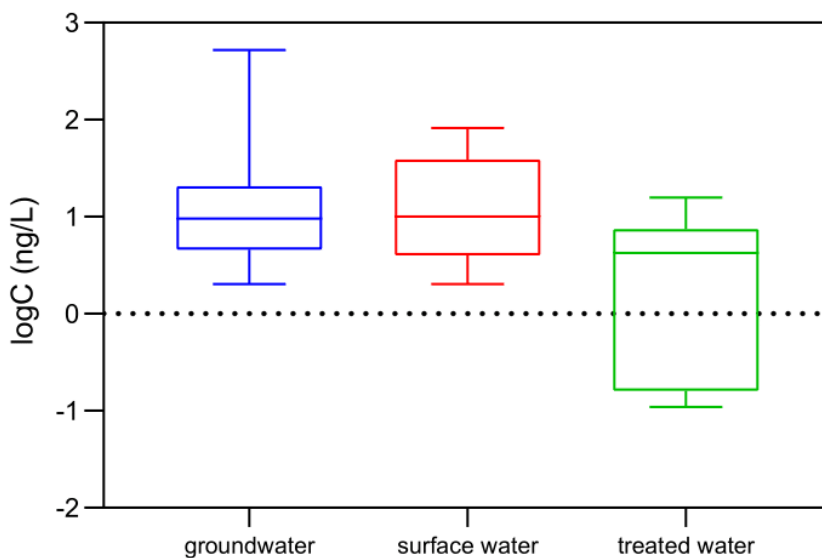

Figure 12: Average of sum detected concentrations ± standard deviation for groundwater, surface and drinking water.

2.3 Human Health Risk Assessment

Based on the log K_{ow} values obtained, only ten out of the sixteen target compounds – Atenolol, Caffeine, Carbamazepine, Ciprofloxacin, Cyclophosphamide, Diclofenac, Erythromycin, Ofloxacin, Sulfamethoxazole, Trimethoprim - were resulted as potentially able to be present in drinking water. Even if they were not detected in the treated water samples, they were included in the risk assessment study. On the other hand, log K_{ow} values for Ibuprofen and Ketoprofen resulted higher than 3, which means that their hydrophobic character probably would not allow them to pass from all the treatment steps. However, since they were detected in treated samples they were included in the assessment as well.

Risk Quotients were calculated for both individual compounds and their mixtures, as the ratio between the average detected concentrations and the pGLV values. However, pGLV values could not be derived from toxicological data in literature – confirming the existence of knowledge gaps in PhACs risk assessment estimation. Hence, ADI values - and where not available N(L)OAEL values- were used for their calculation. All the ADI and N(L)OAEL values were obtained from literature, with the most restrictive value found, being selected (Table 13). The obtained pGLVs ranged from 0,07 µg/L for Ofloxacin to 5285 µg/L for Caffeine. The derived $RQis_{average}$ were much lower than 0,2 (Table 13) ranging between 9,80 x 10^{-7} for Cyclophosphamide and 2,21 x 10^{-3} for Carbamazepine, indicating that none of the target compounds could potentially pose a risk of adverse health effects to humans, even after a lifelong exposure. In accordance with other studies, these outcomes show that even most of the compounds were detected in raw drinking water sources, they do not pose threats to human health individually [148, 158-160], mainly because of their low quantification frequency. However, in order to have a more holistic view of the risks and examine the need of strict measurements to guard pollution on specific points, the RQs were calculated also using the highest detected concentrations for each compound. The results obtained were again much lower than 0,2 ranging between 9,52 x 10^{-6} for Cyclophosphamide and 1,54 x 10^{-1} for Carbamazepine indicating low human health risks (Table 13).

In this context, since a calculation of the risks posed only from individual compounds would result in a total risk underestimation [148,161], the mix Risk Quotient (RQ_{mix}) was calculated as a sum of individual RQs. Since, toxicological data for mixtures of compounds are rare in literature, the Concentration Addition (CA) [161] concept was followed. This concept assumes that no interactions among the different compounds of

the mixture will occur, because of their same action mechanism and toxicity targets [149,162]. Moreover, it is expected that all the different components of a mixture will contribute to the total toxicity depending however on their concentration, resulting to the expectation that even if the individual compounds do not pose a risk, their mixture could potentially do, due to the addition effect [162]. In fact, the obtained results confirmed this assumption with the combined risk of exposure being higher than the individual one, but still it was negligibly low (lower than 0,2 for the sum of $RQi_{average}$, and 0,2 for the sum of RQi_{max}).

Table 13. Human health risk assessment parameters for target PhACs.

Compounds	Log K_{ow}	ADI (µg/kg bw/day)	Source	pGLV (µg/L)	MEC (ng/L)	$RQi_{average}$	RQi_{max}
Atenolol	-0,03	2	[163]	7	2,29	$3,27 \times 10^{-4}$	$6,91 \times 10^{-2}$
Azithromycin	3,24	N/A	N/A	N/A	N/A	N/A	N/A
Caffeine	0,16	1510	[164]	5285	3,52	$6,65 \times 10^{-7}$	$1,25 \times 10^{-5}$
Carbamazepine	2,25	0,34	[163]	1,19	2,63	$2,21 \times 10^{-3}$	$1,54 \times 10^{-1}$
Clarithromycin	3,18	N/A	N/A	N/A	N/A	N/A	N/A
Ciprofloxacin	-0,001	12	[163]	42	0,37	$8,75 \times 10^{-6}$	$1,67 \times 10^{-4}$
Cyclophosphamide	0,97	33	[166]	115,5	0,11	$9,80 \times 10^{-7}$	$9,52 \times 10^{-6}$
Diclofenac	0,57	200	[166]	700	1,5	$2,14 \times 10^{-6}$	$1,74 \times 10^{-4}$
Erythromycin	2,48	0,7	[163]	2,45	0,06	0	0
Ketoprofen	3,00	20	[163]	70	4,07	$5,81 \times 10^{-5}$	$2,18 \times 10^{-3}$
Ofloxacin	-0,20	0,02	[166]	0,07	0,12	0	0
Sulfamethoxazole	0,48	510	[166]	1785	1,39	$7,78 \times 10^{-7}$	$5,57 \times 10^{-5}$
Trimethoprim	0,73	190	[163]	665	1,78	$2,68 \times 10^{-6}$	$1,31 \times 10^{-4}$
17-beta Estradiol	3,94	N/A	N/A	N/A	N/A	N/A	N/A
Estrone	3,43	N/A	N/A	N/A	N/A	N/A	N/A
Ibuprofen	3,79	400	[166]	1400	0,2	$1,52 \times 10^{-7}$	$7,53 \times 10^{-6}$

3. Conclusions

Given the production, consumption and disposal trends of CECs in the aquatic environment, a successful implementation of the just issued Drinking Water Directive requirements is challenging. Finding more adequate strategies in order to protect the quality status of water bodies is necessary. Within this context and following the conclusions derived from the study presented in Chapter 3, a prediction of pollution risk approach prior to the monitoring assessment was followed. Spatial and statistical tools identified the areas at higher risk, based on the number of point sources present within the watershed. Raw water samples from these areas were included in a more comprehensive assessment aiming to quantify the occurrence levels of PhACs in drinking water sources, which was the first in the Piedmont region in Italy. A fast and green method using advanced analytical technique was developed and used for this study. The results confirmed the presence of the target compounds in the area, in concentrations of the ng/L scale, with those geographically closer to the considered pollution sources showing higher detection rates. A risk assessment study was then followed in order to evaluate the potential effects on human health, considering the mixture occurrence as well. Concluding, this study provided us with important information that will contribute to decisions making for a safer and more sustainable water management and supply system, by identifying point sources and potential health risks.

Chapter 5 Non-target screening monitoring assessment

The increasing number of chemicals released in the aquatic environment makes it practically impossible to evaluate the quality of water bodies, after following only target analyses dependent on individual standards. Therefore, suspect and non-target screening methods can be used, as they are able to reveal the full spectrum of occurring compounds, and provide valuable information. High-resolution mass spectrometry is a fundamental tool in order to facilitate these analyses.

In this study, a non-target screening of surface water samples was carried out in order to evaluate the current status of different European bodies. SWATH-HRMS acquisition mode – a technique relatively new and not widely studied in the field of environmental monitoring assessments - was used in order to detect as many analytes as possible, without losing those of lower intensity. Finally, multivariate statistical tools were used in order to identify pollution patterns among the samples, and find the compounds that are responsible for their discrimination.

1. Materials and Methods

1.1 Sample collection

In this study 17 surface water samples from different points in two different European countries were included. The samples concerned river water – taken from the Po river, in Turin, Italy – and lake water – collected from 2 different lakes in Italy, Orta and Comabbio and the lake Pamvotis in Ioannina, Greece. All sampling points were selected to be close to known pollution sources. Information about the sampling points are summarized in table A6, while their maps in Figure A2. The sampling campaign was executed between July and September 2020. For each sampling location, 1L of water was collected into amber glass bottles with stoppers of Teflon lined screw caps. The samples were filtered with 0,7μm GF-F fiberglass filters (Whatman, UK) and stored in dark at 4°C prior to their extraction and analysis.

1.2 Reagents and Chemicals

MilliQ was obtained from MilliPore (MA, USA), UHPLC-grade Methanol was purchased from Sigma-Aldrich (Saint Louis, MO, USA), LC-MS grade Water LiChrosolv® and Ammonium acetate for LC-MS LiChropur® were purchased from Merck KGaA (Darmstadt, Germany). Analytical standards of 100 compounds were included in this

study, and their information are summarized in Table A7. As an internal standard (IS) the Flunixin-d3 was used.

1.3 Sample preparation

For the extraction of the analytes two protocols using different SPE materials were followed. The Oasis HLB (200mg/6 mL) SPE cartridges (Waters, Milford, MA, USA) were used for the extraction of non-polar and slightly polar compounds, while the ENVI-Carb Plus (0,4 g/1 mL) reversible tubes (Sigma-Aldrich, Co, St. Louis, MO, USA) were used for high polarity compounds. Each sample was divided into two 500 mL portions and each portion was spiked with 1 µg/L Internal Standard (IS) solution. The first 500 mL portion of each sample was loaded on to the Oasis-HLB cartridges, previously preconditioned with 5 mL MeOH, followed by 5 mL HPLC-grade water at a flow-rate of 5mL/min. Elution of the analytes was done with 2x5 mL MeOH. Extracts were evaporated until dry under a gentle nitrogen stream and reconstituted in 500µL of 10:90 (v:v) MeOH:H_2O. The second 500 mL portion of each sample was loaded on to the ENVI-Carb cartridges, after conditioning them with 1 mL Methylene Chloride, followed by 2x3 mL MeOH and 3 mL Deionized water at a flow rate of 5mL/min. The elution was conducted with 2x5 mL of 50:50 MeOH: Methylene Chloride (v/v) after arranging them in the forward direction. The obtained aliquot was evaporated until dry, under a gentle nitrogen stream and reconstituted to 500µL of 10:90 (v:v) MeOH:H_2O. Three replicates of control samples for each extraction protocol were prepared from MilliQ water.

1.4 Instrumental analysis

Analyses were carried out using a Quadrupole-time-of-flight mass spectrometer (X500R QTOF, SCIEX, Framingham, MA, USA) coupled to an ultra-performance liquid chromatography (UHPLC) system (ExionLC, Shimadzu, Japan). The chromatographic separation was achieved using a Luna® Omega Polar C18 100 LC Column (3µm particle size, 100 x 2,1mm) heated at 40°C, by injecting a 50µL sample volume into the mobile phase at a flow of 0,350 mL/min. The mobile phase used for the positive ionization mode consisted of a mixture of 5mM Ammonium Formate in H_2O (A) and 5mM Ammonium Formate in MeOH (B), and the elution followed a gradient profile starting from 95% A and 5% B, keeping the ratio for 1 minute and then gradually changing to 100% B within 14 minutes. This profile was kept for 2 minutes and then gradually reversed into the initial conditions until 20 minutes of elution, accordingly also for the negative ionization. For the negative ionization mode, the mobile phase consisted of a mixture of 5mM Ammonium Acetate in H_2O (C) and 5mM Ammonium Acetate in MeOH (D). Also in this

case the elution followed a gradient profile, starting from 95% C and 5% D, holding this ratio for 1 minute and then gradually changed to 100% B within 14 minutes, which was kept for 2 minutes and then gradually reversed into the initial conditions until 20 minutes of elution. During the whole analysis period, the samples were cooled at 4°C inside the autosampler.

The X500R QTOF source parameters for the positive polarity were as follows: ion source gas 1: 45 psi, ion source gas 2: 55 psi, curtain gas: 30 psi, collision gas (CAD): 7 psi, temperature: 350°C; spray voltage: 5500V. The parameters used in full-scan MS mode were as follows: accumulation time: 0,05 sec; declustering potential: 50 V; TOF start mass: 100 Da; TOF stop mass: 1000 Da. A generic collision energy spread of 35 ± 15 was used. For the Q1 isolation strategy (MS/MS) the parameters were TOF start mass: 50 Da; TOF stop mass: 1000 Da; total number of windows: 24; window accumulation time: 0,035 s. An external calibration was performed daily, using a mixture of 10 compounds with a mass range between *m/z* 132,90 and *m/z* 2034,63. This mixture was also automatically injected every 5 samples in order to maintain the mass accuracy below 2 ppm. Similarly, the source parameters for the negative polarity were as follows: ion source gas 1: 45 psi, ion source gas 2: 55 psi, curtain gas: 30 psi, collision gas (CAD): 7 psi, temperature: 350°C; spray voltage: -4500V. The parameters used in full-scan MS mode were as follows: accumulation time: 0,05 s; declustering potential: -80 V; TOF start mass: 100 Da; TOF stop mass: 1000 Da. A generic collision energy spread of (-35) ± 15 was used. For the Q1 isolation strategy (MS/MS) the parameters were: TOF start mass: 50 Da; TOF stop mass: 1000 Da; total number of windows: 24; window accumulation time: 0,035 sec. An external calibration was performed daily, using a mixture of 10 compounds with a mass range between *m/z* 68,99 and *m/z* 2233,91. This mixture was also automatically injected every 5 samples in order to maintain the mass accuracy below 2 ppm. SCIEX OS 1.7 software (SCIEX, Massachusetts, USA) was used for data acquisition and elaboration.

1.5 Data elaboration

In general, the SCIEX OS software accepted as features the detected fragments that had at least 10 data points across each peak, a minimum intensity of 2000 counts/s, were within ± 0,01 Da and had a peak area 10 times greater than the blank sample. Moreover, it performed peak alignment across samples if features were detected within ± 0,2 minutes from each other, and assigned adducts. Due to the large volume of the obtained data, some reduction steps - after visual inspection of the detected features - were

followed, based on low peak intensity, bad integration quality, isotopes and adducts from the analytical apparatus removal. The last step in the reduction process was to remove features that were present in the blank, due to the fact that even if the software subtracted the blank prior to the peak list formation, it gave the possibility to use only one sample.

Moreover, elaboration of the data was done also with the software MZmine 2.52 [167] in order to prepare the tables for statistical analysis. Firstly the raw data obtained from the instrument were converted into .mzXML format (compatible with MZmine) using the software ProteoWizard [168]. Then they were loaded in MZmine and were elaborated following the steps of peak picking, peak deconvolution, peak alignment, and isotope removal. The parameters used are summarized in Table A8. The result was a table which as rows had the observations (samples) and as columns the variables (detected features-ions).

1.5.1 Suspect Screening workflow

For the suspect screening, 100 analytes (Table A7) were taken into account. Their selection was done according to the in house availability of chemical standards. A mix of them was prepared (100 ng/L) in 10:90 (v:v) MeOH:H_2O and analyzed at the end of the sequence in order to obtain MS spectra and retention time data and compare them with those found in the samples for facilitating and enhancing reliability in the identification.

1.5.2 Non-target screening workflow

After data reduction, the instrument's software prepared peak lists including information about the exact *m/z* value, retention time RT (min), and peak area for each detected feature in each sample. All the peaks were visually inspected for shapes and intensities, and those not showing satisfactory results not being included in the further identification process. Moreover, peaks were inspected for isotopic patterns, with those that in their MS1 (precursor ions) spectra had peaks with differences of ^{1}H, ^{37}Cl, or ^{81}Br, from the major ion peak, being further processed. Moreover, the SCIEX OS software provided molecular formulas for each peak, based on the fragmentation in MS1 (precursor ion) and MS2 or MS/MS (product ion) spectra, following specific parameters. More specifically, atoms up to $C_{49}H_{75}Br_2Cl_5F_3I_3N_{10}O_{16}P_1S_3$ were considered, the mass error of the parent ion had to be ± 5ppm and the MS/MS fragments had to support the proposed formula within an error of ± 10ppm.

For the identification of the compounds, libraries provided from the SCIEX OS software were used. They included entries for 4656 components, including pesticides, pharmaceuticals, hormones, personal care products, perfluoroalkyl substances and different toxins with high resolution data. SCIEX OS evaluated the matching grade between the measured MS spectra and those registered in the libraries, and scored each match on a scale of 0 to 100. Only those with a compatibility score of more than 65 were considered, and were manually inspected in order to discard false matches, like those for whom parent ion and molecular formulas didn't match. For those features that a match in the instrument's libraries was not found, searches on the online library ChemSpider, to which SCIEX OS is directly connected, were done. ChemSpider also offers the possibility of *In silico* fragmentation, for spectral predictions that could enhance the identification process.

1.5.3 Statistical analysis

The reduced peak lists, containing information about accurate mass, retention time, and peak area were exported from MZmine and further processed using Microsoft Excel in order to transform it into a table compatible with the SIMCA 14.1 software, which was used for the multivariate analysis of the results. Principal Components Analysis (PCA) and Partial Least Square Discriminant Analysis (PLS-DA) were done in order to identify patterns among the samples, while the Variables Importance in Projection (VIP) technique was used in order to categorize the features that are responsible for the discrimination among the samples. Unit Variance (UV) and Pareto scaling were used as weighting methods, in order for every variable to give equal relevance to the model.

2. Results

2.1 SWATH-MS acquisition

When performing a non-target LC-MS analysis of water samples, many analytes are eluting from the column and are entering the mass spectrometer. Using a Data Dependent Acquisition (DDA) mode, a full scan MS - as Q1 isolation- on a broad range of *m/z* values is acquired and the peaks of the analytes are categorized based on their intensity. However, MS/MS fragmentation is captured only for the most abundant analytes of the sample, risking to lose information about compounds at a lower concentration level. For this reason, in this study we used a Data Independent Acquisition (DIA) technique – the so-called SWATH Acquisition. In that mode, the mass spectrometer uses a wider Q1 isolation size -with the possibility to create smaller Q1 windows in the *m/z* regions where the most compounds are expected to occur- and

collects MS/MS spectra for every detected analyte, by reducing the risk to lose low abundance analytes in complex samples.

However, this type of acquisition in non-target screening analyses generates massive data, that are difficult to handle and require a proper preprocessing workflow in order to obtain reliable information. In this study, 38631 features were detected in total, from which 20796 in the positive and 17835 in the negative ESI mode. Therefore, elaboration of the data prior to identification was done, including the following steps: (a) peak picking, including the removal of chromatographic peaks, based on peak intensities and shapes, (b) blank subtraction, including removal of features present in the matrix, (c) peak alignment, where detected features' peaks are linked among different samples, (d) adducts and isotopes grouping and removal. In this way, we resulted to peak lists containing information for 2741 features detected in Positive and 2526 detected in Negative ESI mode. The high percentage of decrease on the data volume is reported and confirmed also by other studies, that follow this procedure [169,170].

2.2 Multivariate statistical analysis

Due to the complexity and the big volume of data obtained from HRMS non-target screening assessments, statistical analysis is a fundamental processing step that allows better results' visualization. Thus, the reduced peak lists need to undergo further elaboration in order to prioritize features for identification. Multivariate Data Analysis (MVDA) is a useful approach for handling big data and achieving maximum recovery of information [171]. Explanatory analysis of these data can be done based on unsupervised methods such as PCA and Hierarchical Clustering Analysis (HCA), or supervised methods like PLS-DA.

PCA is a multivariate technique that aims to convert a set of observations, possibly related to each other, into a new set of small variables, which have the property of being linear combinations of their initial observations, without being related to each other. Basically, PCA is used for data visualization by reducing their dimensionality into orthogonal principal components (PC) that explain the largest amount of variance among the observations. The first PC (PC_1) defines the highest variance among the samples, the second PC (PC_2) is a vector of 90° to PC_1 that defines the highest variance that can't be explained from PC_1, and finally PC_n is a vector of 90° to PC_{n-1} that defines the highest variance that can't be explained from PC_{n-1}. PLS-DA is another multivariate method, which aims to investigate the features that are more statistically important for the discrimination among the observations. More specifically, in the PLS-DA model the X-matrix -which contains information about the variables and observations- is transformed by adding a Y parameter, which contains the classification of the

observations. Basically, PLS-DA is a linear classification model between the X-matrix and the Y-observations that enables the selection of the most predictive or discriminant features in the data which help to classify the observations [171].

In this study, PCA was performed on the data for examining the relationships between different samples and study the possibility of different locations or sample types being responsible for their variance. The PCA models were constructed after transformation of the data with two types of scaling, the UV and the Pareto, in order for every variable to give equal relevance to the model. In UV scaling every variable is multiplied with the ratio $1/sd_j$, where sd_j is the variable's standard deviation, while in Pareto is multiplied with the ratio $1/\sqrt{sd_j}$. The coefficients R^2 and Q^2 were used for evaluating the goodness of fit and prediction of every model to the dataset used. R^2 explains how well the model fits the data, with high values (close to 1) indicating the best conditions, while Q^2 explains how well the model predicts the data, with values > 0,5 indicating good predictivity. These two coefficients tend to have a completely different behavior as the number of PCs increases. More PCs result to an increasing R^2 value (going closer to 1), showing a better fitting of the data. On the contrary, the Q^2 value reaches a maximum, after of which the addition of PCs is decreasing the predictivity of the model. Concerning the PLS-DA models, were constructed taking into account the two different scaling types, and were validated using the Permutation test. This test uses random tests in order to assess the risk that a difference between the observations is random and not statistically significant. More specifically, its idea is to compare the goodness of fit (R^2 and Q^2) of the original PLS-DA model with the goodness of fit of several random tests, based on the alternation of the Y-observations while the X-matrix are kept intact. A model is valid when the R^2 and Q^2 values of random tests are lower than those of the initial model, and when the regression line between the Q^2 values of the initial test and of the random tests intersects the vertical axis at, or below zero (Figure A3). Finally, the VIP plot was used in order to rank the variables (detected features). The VIP values are calculated as the sum of PLS squares, weighted by the sum of squares explained in each model. VIP values > 1 are considered as important and responsible for the discrimination among the samples variables, while VIP values < 0,5 are considered as not important [169-171].

Figure 13 shows the obtained PCA models for the features detected in ESI (+). In the UV scaling-PCA the variability explained by PC_1 was 22%, and 11% by PC_2, with R^2 equal to 0,221 and Q^2 equal to 0,108. In the Pareto scaling-PCA model, PC_1 explained 25% of the variability and PC_2 15%, with R^2 equal to 0,250 and Q^2 equal to 0,131. These information show that the Pareto scaling-PCA model fits and predicts better the data of this study. Nevertheless, in both models samples S2 and S10 cluster closely together in the score plot and far from the rest of the samples, indication similarities in the chemical composition. Both samples are collected from the two different lakes in Italy (Table A6).

Moreover, in the score plot the sample S9 is plotted far away from the rest of the samples, indicating the occurrence of different compounds. All these three samples, which were collected from the two Italian lakes, were the most chemical dissimilar, highlighting the influence of different locations on the variance. Samples S12, S5, S7 were clustered closely to the other samples, with S12 being alone and the S5, S7 being closer. This result is reasonable, since S5 and S7 are samples from the same lake while S12 concerns a river sample. Finally, the rest of the samples, even if originating from different lakes in different countries are plotted together, without highlighting variance among them.

Figure 14 shows the obtained PLS-DA models (in both scaling types) for the features detected in ESI (+). In both models is obvious the classification of the samples between countries (green-Greece, blue-Italy), while S2 and S10 are clustered together and far from the rest of the samples. In the UV scaling-PLS-DA the variability explained by PC_1 was 83%, and 55% by PC_2, with R^2 equal to 0,831 and Q^2 equal to 0,398. In the Pareto scaling-PCA model, PC_1 explained 81% of the variability and PC_2 49%, with R^2 equal to 0,810 and Q^2 equal to 0,331. These information show that the model obtained after the transformation of the data with the UV scaling can predict better the features responsible for the discrimination among the observations. However, the validation of the two models showed that it was not valid (Figure A3), since it was not fitting satisfactorily the data (R^2 value was very close to 1, while the intersection of the Q^2 regression line with the vertical axis was higher than 0, indicating the overestimation of the model). On the other hand the Pareto-scaling PLS-DA model provided satisfactory results. Hence, it was used in order to build the VIP plot, and select the significant features for the discrimination among the samples, that would be identified (Table 16).

The models for the data detected in ESI (-) are not reported, since they resulted in overestimation of the variance among the samples, due to the high number of zero values.
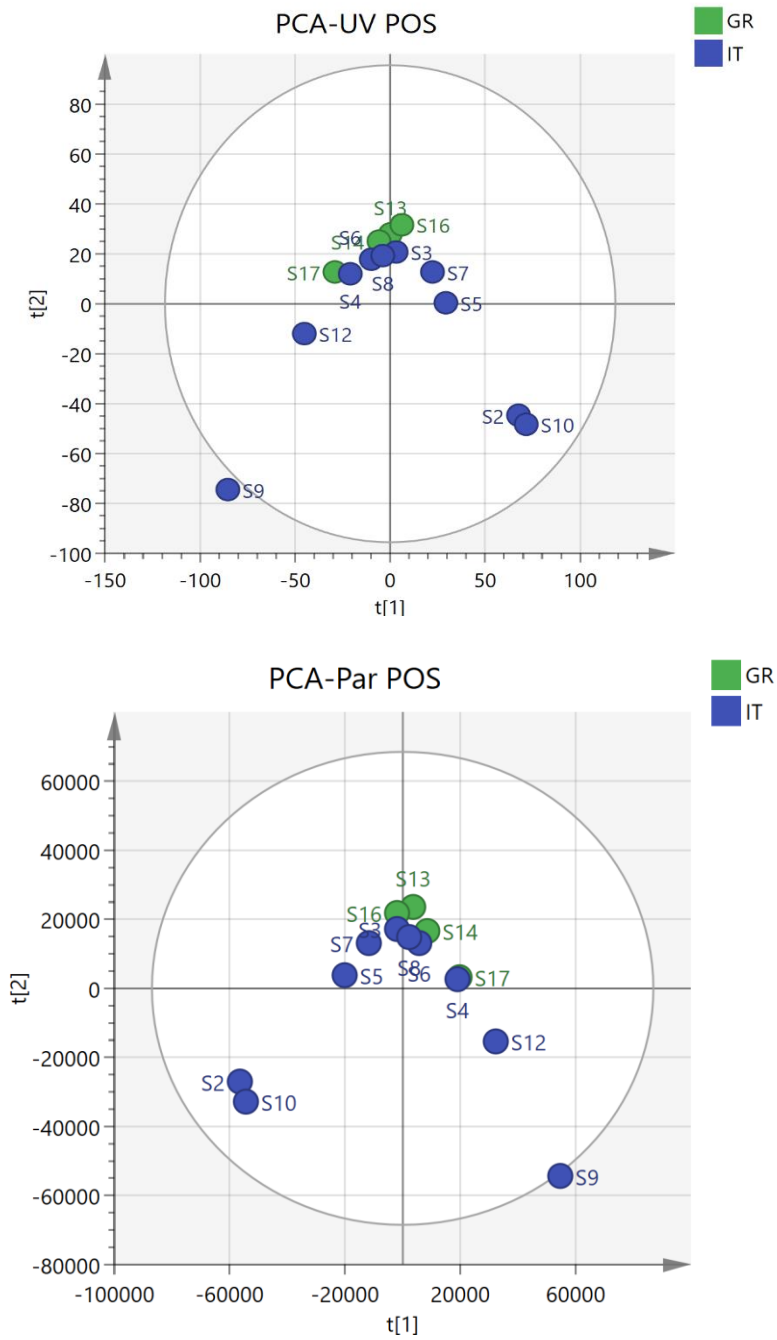
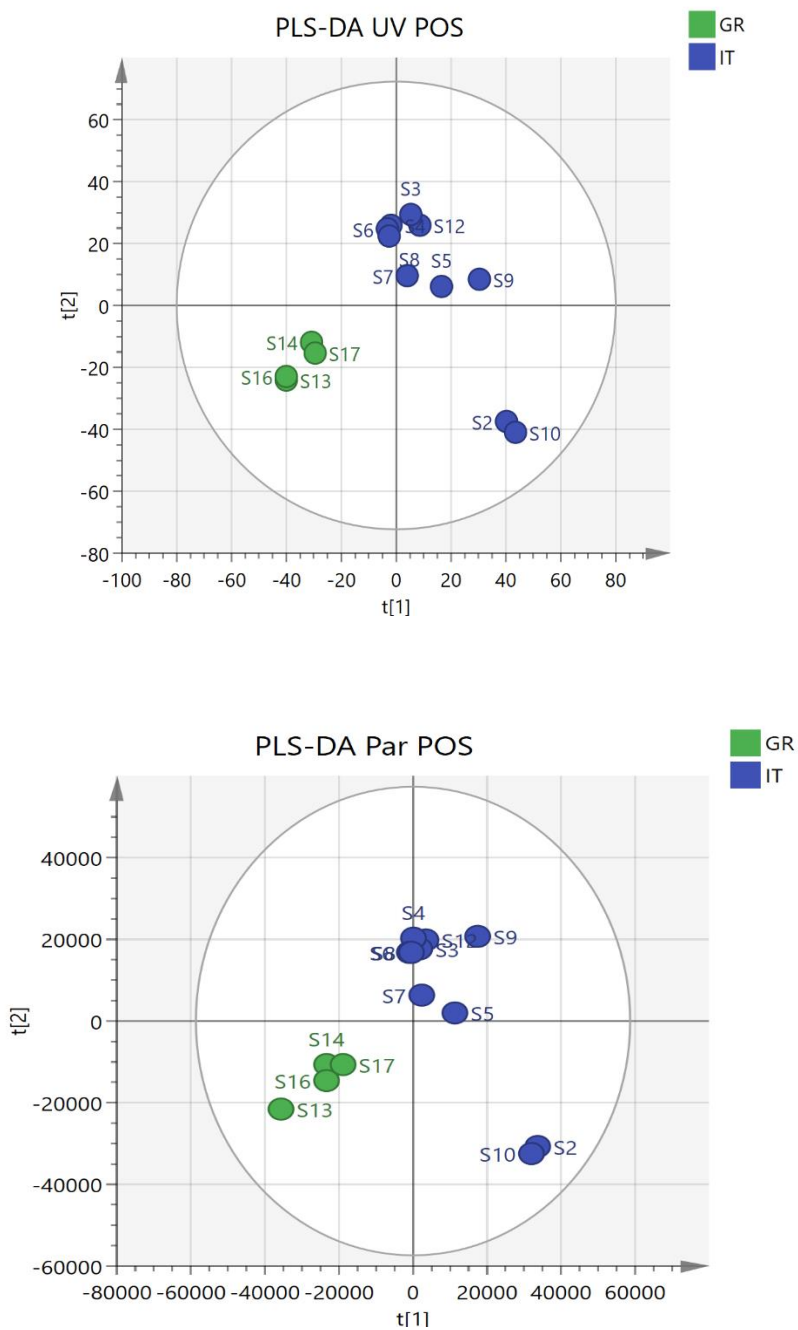**Figure 13**: PCA models with UV and Pareto Scaling, for the features detected in ESI (+).

Figure 14: PLS-DA models with UV and Pareto Scaling, for the features detected in ESI (+).

2.3 Suspect and non-target screening

The suspect screening compounds detected in the samples and their responses are summarized in Table 14. All the suspect screening detections are confirmed identifications; as analytical standards were used for these analyses. Pesticides, like Terbuthylazine, Atrazine, Metolachlor and Primicarb were the most detected compounds in the samples, followed by the pharmaceuticals Carbamazepine, Ibuprofen, Clarithromycin and Sulfamethoxazole. PFAS was the next most frequently detected class of compounds, with PFOA and PFOS being detected in 7 out of the 17 samples.

Table 14. Suspect screening analytes' detection information.

Compound	Observed *m/z*	RT (min)	Compound	Observed *m/z*	RT (min)
17-beta estradiol	271,3814	5,16	Epoxiconazole	330,0897	6,27
Acetamiprid	223,0743	7,89	Molinate	188,1109	10,86
Acetochlor	270,1262	11,12	Ofloxacin	362,1582	5,19
Alachlor	270,1143	12,54	Oxadiazon	362,0819	3,52
Ametryn	228,1271	10,40	Penconazole	284,0719	11,35
Atenolol	267,1707	2,90	Pendimethalin	282,1156	15,23
Atrazine	216,1007	9,95	Pethoxamid	296,1414	11,13
Atrazine-desethyl	188,0701	8,00	PFHxA	312,9730	9,05
Azythromycin	749,5164	8,95	PFOA	412,9662	10,31
Boscalid	343,0411	10,6	PFOS	498,9321	12,54
Caffeine	195,0877	6,89	Phosalone	368,0218	7,26
Carbamazepine	237,1019	9,64	Pirimicarb	239,1498	9,67
Chlorfenvinphos	358,9777	11,5	Prochloraz	376,0388	11,66
Chloridazon	222,0429	7,76	Procymidone	301,0241	8,91
Chlorotoluron	213,0791	9,75	Prometryn	242,1419	5,37
Ciprofloxacin	332,1457	4,09	Pronamide	257,1238	11,03
Clarithromycin	748,4841	10,70	Propachlor	212,0837	10,08
Cyanazine	241,0966	9,07	Propazine	230,1166	10,52
Cyclophosphamide	262,0792	8,19	Propiconazole	342,0874	9,34
Dichlorvos	220,9541	13,23	Pyraclostrobin	388,1058	11,56
Diazinon	305,1078	11,50	Pyrimethanil	200,1182	10,48
Diclofenac	296,1163	2,45	Parathion (ethyl)	292,1762	3,29
Dimethenamide	276,0822	10,70	Simazine	202,0855	9,27
Ethofumesate	287,2694	1,48	Spirotetramat	374,197	11,10
Erythromycin	734,4693	10,20	Spiroxamine	298,2745	10,69
Estrone	269,1529	5,27	Sulfamethoxazole	254,0598	7,13

Compound	Observed m/z	RT (min)	Compound	Observed m/z	RT (min)
Fenamidone	312,1176	10,53	Tebufenozide	353,1672	14,92
Fenbuconazole	337,1211	11,15	Terbuthylazine	230,1165	16,69
Fenhexamid	302,071	10,91	Terbuthylazine-desethyl	202,0855	15,06
Hexazinone	253,1659	9,38	Tetraconazole	371,9982	7,93
Ibuprofen	204,9879	1,56	Thiacloprid	253,0310	8,39
Indoxacarb	528,0856	14,20	Thiamethoxam	292,0012	4,29
Kresoxim-methyl	314,1616	10,82	Thiobencarb	258,0719	11,6
Ketoprofen	255,1026	10,36	Triticonazole	318,1342	5,18
Lenacil	235,1448	10,13	Tolylfluanid	364,0021	9,35
Linuron	249,0193	10,46	Trimethoprim	291,1446	6,73
Metazachlor	278,106	10,09	Vinclozolin	287,1192	4,49
Methyl parathion	264,1987	3,54	Zoxamide	336,0391	6,38
Metolachlor	284,1412	11,26			

Concerning the non-target screening, the identification of the compounds was done based on libraries using the accurate mass, retention time, isotopic pattern and MS/MS spectra of the detected ions. In this way, 28 compounds were identified in the negative ionization mode, and 327 in the positive (with VIP values >1). This big difference between the numbers of detected compounds in ESI (+) and ESI (-) can be explained by the lack of available information on the libraries, as well as the ionization type of the majority of the compounds. Among the results, a variety of chemicals was detected including pharmaceuticals - 39% in ESI (+) and 21% in ESI (-), pesticides - 34% in ESI (+), PFAS - 17% in ESI (-), toxins, food additives, drugs and other. These results are summarized in Tables 15 and 16.

Table 15. Compounds detected in ESI (-).

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples
15-Acetyldeoxynivalenol	337,166	337,1636	1,39	1,2,3,5,6,7,8,10,11,12, 13,14,15,16,17
Agistatin E	227,093	227,0925	1,39	1,3,5,6,7,10
Altersolanol A	334,961	334,9592	2,15	11,3
Asperlactone	183,067	183,0668	1,39	14
Aspinonene	187,098	187,0981	1,37	14,15,16,3,6,8
Aspyrone	183,067	183,0661	1,41	13,15,2,4,5,6,7,9,10
Chanoclavine	255,102	255,1022	14,75	17,12
Closantel-$^{13}C_6$	667,060	667,0618	18,40	11
Deoxynivalenol	295,228	295,2284	1,44	13,14,15,16,17,11,12, 1,2,3,5,6,7,8,10
Fatty acid C20:4 Garbage	303,234	303,2335	18,95	2,3,4,5,7,8,9,10
Heptelidic acid	279,124	279,1239	1,42	8
Ibuprofen	204,991	204,9879	1,56	11
Isofusidienol A	299,260	299,2592	17,75	11
Mycophenolic acid	319,142	319,1398	1,45	12
Nivalenol	311,114	311,1134	1,42	1,2,3,4,5,6,7,8,9,10,11 ,12,13,14,15,16,17
Norsolorinic acid	369,244	369,2434	18,54	17
Palitantin	253,144	253,1436	1,64	14
Patulin	153,093	153,0916	1,49	3
Penicillic acid	169,088	169,0861	1,38	13
Pentobarbital Negative	225,074	225,0736	13,69	17
PFBA	213,056	213,0563	15,74	11,12
PFHpA	362,970	362,9688	15,21	1,16,12,11,3,4
PFHxA	312,973	312,9728	12,82	1,2
PFOA	412,966	412,9659	16,02	12,1,16,3,11,
PFPeA	263,165	263,1642	11,24	2
Radicicol	363,182	363,1806	1,63	11,13,16,17,14,12,2,1 5
Secobarbital	236,917	236,9155	1,05	15
Vedaprofen	281,177	281,1766	15,15	16

Table 16. Compounds detected in ESI (+).

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
25I-NBOMe	428,255	428,2550	14,43	12	1,97464
2-Benzyltetronic Acid	190,912	190,9090	1,21	11,1,6,7,10	1,71775
2C-E	210,112	210,1115	1,51	12,6,8,7,10	1,50137
2C-P	241,154	241,1559	1,48	11,16,17,15	1,34957
3,4-Dimethoxyphenethylamine	182,082	182,0819	1,46	16,15,13	1,77064
4-Beta-hydroxystanozolol	182,082	182,0817	1,38	15,16,17,11,1,8,3,6,7,10,	1,07988
4-Beta-hydroxystanozolol	345,231	345,2308	1,40	14	1,93558
4-EEC Ethylethcathinone	206,103	206,1024	1,60	14	1,93883
4-Fluoroamphetamine	154,142	154,1424	12,72	2	1,92382
4-Fluoromethamphetamine	168,066	168,0628	1,39	14,16,13,6	1,90835
4-methylnitrosamino-1-3-pyridyl-1-butanol	210,135	210,1357	1,41	6	1,99311
4-MTA	182,080	182,0818	1,52	3,6	1,82334
5-Hydroxythiabendazole	218,211	218,2113	14,23	7	1,90379
5-Methyl-mellein	207,138	207,1382	1,43	6	1,91168
7-Aminodesmethylflunitrazepam	270,280	270,2797	18,96	12,4,9	1,61323
8-Hydroxyquinoline	146,060	146,0598	10,34	1,10	1,80248
9-Hydroxyrisperidone	427,340	427,3401	19,31	15,16,13,11,3,10,8	1,23423
Acecarbromal	279,233	279,2328	1,80	14	1,90548
Aceclidine	170,154	170,1539	13,32	4,9	1,86929
Acetamiprid	223,064	223,0742	7,90	4	1,96346

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Acetochlor	270,126	270,1262	11,1	12,4,9	1,62789
Actinoquinol	254,154	254,1542	18,03	15	1,93761
Aflatoxin B2	315,196	315,1986	17,52	16,9	1,97832
Ajmaline	327,079	327,0794	17,82	13,15,16	1,80012
Albendazole-D3	269,248	269,2494	1,83	2,3,5,7,8,10	1,45079
Albendazolsulfonamin	240,139	240,1397	1,89	15	1,92363
Aldicarb-sulfoxide	207,159	207,1591	14,24	10	1,95348
Alpha-PPP	204,087	204,0812	1,89	8	1,93077
Alpha-Pyrrolidinopentiophenone	232,170	232,1743	1,56	12,4,9,10	1,53898
Alverine	282,093	282,0956	16,13	12,3	1,81898
Aminoflubendazol	256,263	256,2632	1,79	16	1,96888
Amisulpride	370,217	370,2169	18,70	5	1,95816
AMT	174,992	174,9924	1,42	13,16,14,17,11,12,1,6,8,10,2,3,9	1,10455
a-Nortestosteron	275,149	275,1436	9,13	8	1,92023
Apophedrin	170,081	170,0821	1,51	15,16	1,80837
Aramite	352,307	352,3063	1,78	17,8	1,86342
Aspinolide B	285,222	285,2217	19,87	12	1,99593
Atenolol	267,171	267,1707	2,90	17	1,91923
Atratone	212,201	212,2009	17,11	12	1,91676
Atrazine	216,086	216,1007	9,95	4,9,12	1,65839
Atrazine-2-hydroxy	198,185	198,1845	16,60	12	1,99523
Atrazine-desethyl	188,070	188,0698	13,02	12	1,97218
Atrazine-desisopropyl	174,055	174,0550	6,75	8,6,5,7	1,62605

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Austdiol	237,112	237,1122	1,52	13,15	1,81243
Axeen	227,128	227,1281	1,50	11	1,99334
Azaperol	330,210	330,2105	1,69	9,12,4	1,76984
Beflubutamid	356,196	356,1954	13,29	9,12,9,4	1,68854
Benazolin	244,090	244,0907	14,37	15,1	1,84029
Benzoctamine	250,087	250,0874	1,87	12	1,95955
Benzoximate	364,325	364,3136	18,40	12	1,92986
Benzylpiperazine BZP	177,054	177,0541	15,99	3,6,7	1,6353
Betamethasone-21-phosphate	473,320	473,3265	1,72	4	1,91477
Bifenazate	323,146	323,1347	1,42	7,10	1,86396
Bioallethrin	303,231	303,2312	1,80	17,8	1,82974
Biotin	245,227	245,2267	1,80	17,3	1,9933
Buprenorphine	468,325	468,3256	13,54	10,8,1,10,3,5,4,2	1,00012
Butylate	218,211	218,2113	14,27	14,17,4	1,76627
Butylone	222,113	222,1186	13,39	17,13,14,11,12,4,9	1,20488
Caffeine	195,087	195,0870	11,39	12	1,97385
Cannabinol	311,164	311,1641	17,82	2	1,9719
Capsaicin	306,291	306,299	17,84	12,4,9	1,63193
Carbamazepine	237,103	237,1025	15,22	13,12	1,86959
Carboxin	236,107	236,1070	16,35	13,14,16	1,75915
Cathine	169,097	169,0986	1,49	14,3,5,9	1,74871
Cathinone	150,027	150,0262	19,77	6	1,94434
CBD	315,195	315,1954	1,45	1,14,17,11,4,9	1,56184
Cerulenin	224,118	224,1271	10,92	16,4	1,84696

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Chanoclavine	279,249	279,2491	19,73	1	1,96412
Chloroquine	320,288	320,2886	17,67	1	1,91292
Chlorphenethiazine	305,175	305,1788	1,45	1	1,93923
Cimetidine	253,216	253,2163	1,57	1	1,99649
Citreoviridin A	403,234	403,2343	1,68	17,11,3	1,76125
Citrinin	251,164	251,1642	14,98	12	1,95085
Clemastine	344,196	344,1950	11,96	12	1,94401
Clibucaine	337,179	337,1814	11,69	12	1,9190
Climbazol	293,106	293,1058	17,02	12	1,93284
Clobazam	301,142	301,1419	1,60	12	1,91014
Clobendazole	330,137	330,1373	14,67	9	1,90776
Clodinafop-propargyl	350,248	350,2482	19,41	4	1,97762
Clofentezine	303,163	303,1561	1,56	17	1,92601
Clomipramine	315,161	315,1614	16,54	13,16,11	1,7209
Clonidine	230,154	230,1541	17,90	15,12,8	1,73321
Corticosterone	347,184	347,1845	1,41	17,10,12,4,9	1,42151
Cotinine	177,102	177,1022	10,73	10	1,91386
Crotetamide	227,175	227,1753	13,74	14,17,15,11,1,2,4,5,6,7	1,22091
Curvularin	291,197	291,1963	1,37	12	1,91708
Cyclizine	267,174	267,1742	17,30	17	1,9363
Cyclobenzaprine	276,145	276,1443	1,51	2	1,90127
Cyclovalone	367,320	367,3207	1,26	3	1,91846
Cyhalofop-butyl	392,374	392,3741	19,73	2,4,9	1,81147
Cyprodinil	226,217	226,2173	1,75	4,5	1,89571

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Cytochalasin E	496,449	496,4436	19,92	17,11,12,4,9	1,55721
DEET	192,139	192,1384	15,93	13	1,91091
Delorazepam	337,237	337,2371	17,06	6	1,94854
Demeton-O	291,196	291,1963	18,74	1,2,10,8	1,70727
Deoxynivalenol-3-glucoside	459,306	459,3062	1,51	12	1,94334
Desmethylcitalopram	311,146	311,1548	14,47	5	1,94378
Desmethyl-formamido-pirimicarb	253,159	253,1621	18,56	12	1,92795
Detomidine	187,096	187,0962	8,41	13,14,15,16,17,11,1,2,3,4,5,6,7,8,9,10	1,03726
Dexamethasone	393,226	393,2246	16,93	11	1,96466
Diacetylmorphine	370,368	370,3687	18,77	7	1,92043
Dibutyl phthalate sodiated	301,141	301,1415	18,26	13,17,14,12,1,2,6,7,8	1,55739
Dienestrol-D2	269,175	269,1746	14,15	5,7,4	1,71973
Dihydrolysergol	257,138	257,1414	1,50	12	1,90485
Dilazep	605,145	605,1452	11,31	12	1,90725
Dimethomorph	388,132	388,1316	16,81	17	1,97567
Dioxacarb	224,118	224,1129	11,18	17,15,16,11,12	1,55673
Dipyridamole	505,334	505,3340	1,40	12	1,90718
Diuron	233,025	233,0245	9,98	9	1,90512
DMT	189,128	189,1276	15,63	15	1,93161
D-Norpseudoephedrine	169,097	169,0985	1,50	14	1,90875
Dodemorph	282,205	282,2076	10,76	16	1,91828
DOEt	224,128	224,1282	1,50	15	1,96997
DOM	210,135	210,1355	1,46	11,10	1,92882

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Doramectin	921,690	921,6905	19,24	7	1,96182
Doxepin	280,199	280,1988	1,40	7	1,99683
Emamectin B1b	872,704	872,7008	1,45	11,1,3	1,79217
EMDP	264,196	264,2006	1,64	2	1,94601
Ephedrine	166,086	166,0866	1,38	13	1,95787
Epioxandrolone	329,175	329,1757	1,36	13,14,15,16,17,1,6,8,2,3,7,10	1,2253
Eprinomectin B1a	936,686	936,6846	19,80	12,9	1,89869
Eprosartan	425,215	425,2162	18,76	13	1,97473
Erginine	268,263	268,2652	19,58	1,4	1,91029
Ethambutol	237,149	237,1489	11,31	17	1,96684
Ethiofencarb-sulfone	258,182	258,1849	1,53	16	1,9927
Ethofumesate	287,270	287,2694	1,48	2	1,98802
Ethylone	222,149	222,1489	1,47	16,1	2,00484
Etilefrine	182,082	182,0812	1,33	13,1	1,94503
Etorphine	412,349	412,3488	13,61	2	1,93064
fenoxycarb	302,246	302,2322	17,42	17,10	1,96871
Fenproporex	189,075	189,0743	1,43	2,12,5	1,85652
Flecainide	415,145	415,1483	14,62	9	1,97004
Flubendazole	314,269	314,2696	1,52	2,7	1,94074
Flumethasone	411,215	411,2221	11,72	12,4,11,16	1,62781
Foramsulfuron	453,344	453,3445	2,12	15	1,95191
Fumonisin B1	722,507	722,5071	1,87	8,3,11	1,73928
Furosemide	331,001	331,0024	1,40	8	1,92939
Fusaproliferin	445,284	445,2837	1,50	1,2,3,4,5,6,7,8,10,13,16,14,15	0,90517

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Gabapentin	172,133	172,1332	1,58	9,6,1,16,5,7,12,3,8	1,09125
Gallopamil	485,382	485,3818	19,55	3	1,9310
Glufosinate	182,080	182,0814	1,68	7	1,99065
Glutethimide	218,211	218,2110	1,48	8,5,10,1	1,74446
Glycopyrrolate	318,240	318,2405	1,33	8	1,94281
Haloxyfop-etotyl	434,267	434,2493	18,77	2	1,96252
Helvolic acid	569,435	569,4349	1,68	8	1,91681
Hexaconazole	314,083	314,0828	17,70	12	1,92966
Hexazinone	253,166	253,1659	9,38	11	1,90187
Histamine	112,050	112,0502	1,51	9	1,98965
Histidine	156,102	156,1019	1,50	3,7	1,94454
Hordenine	166,123	166,1226	1,47	3,14,7	1,80238
HT-2 Toxin	447,294	447,2944	1,41	3,7,8,2,15,6,11,10,17,5,16,1	1,11109
Hydralazine	161,071	161,0712	11,45	4,9	1,95659
Hydroxychloroquine	336,252	336,2515	1,42	8	1,98085
Hydroxymethylpyridine	110,060	110,0600	1,91	9	1,92264
Inabenfide	356,315	356,3158	18,73	12	1,97593
Infectopyrone	265,144	265,1443	1,47	16,15,10,13,17,6,3,11,14,2,5,7,8	1,0226
Ipconazole	334,204	334,2039	18,84	12	1,95642
Irbesartan	429,241	429,2414	16,50	12	1,97948
isoprocarb	211,133	211,1331	11,31	14,11,9,4,3	1,55031
Isoproturon	207,149	207,1490	10,02	4,9	1,92176
Isothipendyl	286,238	286,2373	18,12	12	1,9925
Josamycin	828,677	828,6765	1,40	6	1,9629

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Ketorolac	256,133	256,1333	15,01	9,4	1,8889
Ketotifen	310,311	310,3112	19,65	15,14,8,10	1,5185
Kresoxim-methyl	314,159	314,1616	10,82	4	1,9958
Lenacil	235,144	235,1448	10,09	14,10	1,9853
Levamisole	205,105	205,1051	1,65	5,12,6,13,15,14,16	1,0289
Levocabastine	438,379	438,3792	18,09	5	1,9085
Levodopa	198,185	198,1852	1,75	10,12,13,16,15,6,8	1,1777
Lidocaine	235,181	235,1809	11,54	12	1,9303
Lincomycin	407,222	407,2221	10,68	12	1,9016
Lonazolac	313,274	313,2750	12,98	14	1,9122
Lorazepam	321,169	321,1684	14,88	12,15,17	1,6405
Lysergol	255,175	255,1770	17,55	3,17	1,8006
Malaoxon	315,301	315,3013	18,88	5	1,9014
Maleic hydrazide	113,107	113,1069	2,08	11,7,12,3	1,7003
Maprotiline	278,190	278,1902	1,40	7,8,4,3,1,5,2	1,3077
Mazindol	285,076	285,0763	17,26	9,12,13	1,7039
Mebeverine	430,151	430,1512	1,83	1	1,9667
Medetomidine	201,107	201,1131	11,71	1	1,9453
Medroxyprogesterone acetate	387,285	387,2842	1,55	8,1,3,6,16,13,15,7,10,8,11	1,2471
Mefenamic acid	242,144	242,1535	17,70	3	1,9852
Mepenzolate	340,246	340,2472	18,86	2,11	1,9140
Mesoridazine	387,199	387,1989	14,61	5,2,12	1,8480
Metaxalone	222,149	222,1488	1,45	11,8,5,7,3,17,15,1,10,6,16,2,12	1,3399
Metformin	130,109	130,1083	1,39	4,9	1,9707

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Methacrifos	241,217	241,2168	1,35	4,9	1,9025
Methamphetamine	150,128	150,1372	19,89	9	1,9186
Methaqualone	251,126	251,1263	11,34	4	1,9057
Methazolamide	237,148	237,1481	14,30	9,14,11	1,8009
Methfuroxam	230,175	230,1750	14,28	13,15	1,8273
Methocarbamol	242,154	242,1441	1,45	1,2,6,7,8,10,11,12,13,14,15,16	1,2046
Methoprotryne	272,259	272,2590	1,76	1	1,9636
Methyl Prednisolone	375,217	375,2167	1,39	15	1,9348
Methylprednisolone	375,347	375,3471	19,81	10,13,9,12,16,14	1,5102
Methyltestosterone	303,180	303,1795	1,51	8,5	1,9098
Metolachlor	284,141	284,1412	11,20	11,12,9,4	1,7763
Metolcarb	166,123	166,1226	1,51	7,2,15,1,5,13,12,11	1,1001
Mexacarbate	245,079	245,0790	16,09	2	1,9079
Minoxidil	210,135	210,1349	12,70	14	1,9373
Mitragynine	399,309	399,3088	12,84	5	1,9800
Molinate	188,111	188,1109	10,86	8	1,9878
Molsidomine	243,122	243,1224	11,50	14	1,9952
Monocerin	309,134	309,1341	15,91	12	1,9435
Monuron	199,169	199,1699	12,10	10,3	1,8895
Moxisylyte	280,164	280,1636	8,40	17,10,14	1,7851
Moxonidine	242,248	242,2482	19,41	9,10,8	1,7069
Nandrolon	275,166	275,1661	1,53	6,15,4,7,13,16,5,3,2,8,14	1,1203
Nandrolone	275,201	275,2014	1,45	5,10,16,11,3,8,1,10	1,3110
Naphazoline	211,149	211,1479	18,04	9,12	1,8014

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Naproxen	230,891	230,8904	2,28	5	1,9993
Neburon	275,147	275,1465	10,88	4,9	1,9349
N-Ethyl Hexedrone Hexen	220,096	220,0969	1,49	7,3,1	1,7474
N-Ethyl Pentylone	250,146	250,1486	1,34	13,16	1,8112
Nicotinamide	123,055	123,0546	3,03	4,9,17	1,8109
Nicotine	163,133	163,1337	11,56	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17	1,0086
Niflumic acid	283,153	283,1528	15,54	17	1,9976
N-Isopropylsalicylamide	180,102	180,1020	2,01	11,17,1,2,8,3,4,9,7	1,0084
N-methyltryptamine NMT	175,123	175,1228	9,79	17	1,9254
Nonivamide	294,207	294,2068	1,47	10	1,9154
Norbuprenorphine	414,270	414,2724	19,79	11	1,9048
Norcotinine	163,039	163,0392	14,33	14,17	1,9547
Norephedrine	194,098	194,0918	10,88	13	1,9705
Norethisterone	299,220	299,2199	1,39	15,11	1,9498
Norethisterone acetate	358,241	358,2413	1,53	5,8,7,16,6,3,10,17,11,3,12	1,0076
Norgesterel	313,236	313,2384	12,07	13,12,10,15,9	1,5191
Norhydrocodone	286,311	286,3088	18,21	9	1,9062
Norsertraline	292,227	292,2203	19,63	12	1,9105
Noscapine	414,155	414,1547	1,48	2,3,7,1,4,5,12	1,1552
N-propylamphetamine	178,058	178,0581	16,07	9,12	1,9081
Obidoxime	144,066	144,0731	1,49	3	1,9035
Ochratoxin alpha	295,095	295,0948	12,39	17,12,9,4	1,6196
Omethoate	214,125	214,1256	1,53	11	1,9685
Ophiobolin A	401,123	401,1214	14,21	17,3,11	1,7438

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Oxadixyl	279,194	279,1944	15,96	9,7	1,8347
Oxamyl	237,149	237,1486	14,92	17	1,9087
Oxitriptan	221,118	221,1177	1,42	16,13,15,2	1,8403
Oxydemeton-methyl	247,133	247,1334	14,27	17	1,9534
Oxymorphone	305,175	305,1751	14,90	14	1,9030
Oxypendyl	371,102	371,103	7,45	6	1,9543
Oxyphencyclimine	345,337	345,3364	2,23	11	1,9727
Paclobutrazol	294,243	294,2428	12,53	17	1,9268
Palitantin	287,147	287,1477	11,20	2,3,5,6,7,8,10,11,12,13,14,15,16,17	1,0079
Papaverine	340,264	340,2645	18,86	3,5	1,9169
Para-Methoxyamphetamine	166,086	166,0851	1,40	14,7	1,8214
Paraoxon-methyl	248,149	248,1483	14,14	12	1,9198
Penconazole	284,072	284,0719	11,35	3,1	1,9382
Penicillic acid	171,101	171,1014	12,66	9,7,10,1,2,5,6,3,12,8,11	1,1704
Pentedrone	209,165	209,1648	1,47	17,16,11,10,7,15	1,0552
Pentylenetetrazole	156,102	156,1015	1,51	5,6,15,3,16,8,10,17,2,7	1,1770
Perazine	340,264	340,2641	18,95	7,13	1,9225
Pestalotin	215,093	215,0931	1,85	1,2,4,5,6,7,8,10,11,12,13,14,15,16,17,	1,0487
Phenelzine	137,096	137,0961	1,51	1,2,3,5,6,7,8,10,11,12,15,16,17	1,0238
Pheniramine	241,144	241,1439	14,71	17	1,9983
Phenmedipham	301,141	301,1427	12,30	12	1,9379
Phentermine	150,027	150,0269	1,61	13,15,6,11,7,16,8,5,3,10,12,2	1,0836
Phenylephrine	168,063	168,0645	1,59	16,8,12,6,5,7,1,11,2,4,3,17,6	1,1054
Picoxystrobin	368,241	368,2415	17,34	12,4,9	1,0757

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Piperacetazine	411,346	411,3453	11,20	3,8,6,5,2,10,7	1,0091
Piperonyl-butoxide	356,244	356,2437	18,68	12	1,9291
Pirimiphos-methyl	306,243	306,2429	14,63	17	1,9104
Pizotifen	296,295	296,2954	19,58	9,17	1,8254
PMMA	180,16	180,1603	18,21	14,17,16,15,12,1	1,3604
Prenylamine	330,338	330,3373	1,82	1,8,7,6,10,5	1,1116
Progesterone	347,298	347,2933	19,15	7,1,3	1,6392
Prohexadione	213,112	213,1123	1,49	13,12,8,7,10,15,5,6,1,11	1,4528
Prometon	226,142	226,1433	1,44	15	1,9330
Propoxycarbazone	438,379	438,3794	18,02	8	1,9121
Prosulfocarb	252,046	252,0459	1,26	13,14,15,17,11,12,10,1,2,3,4,5,6,7,8	1,0698
Prothioconazole Desthiometabolite	312,327	312,3263	5,39	14	1,9709
Pymetrozine	218,211	218,2111	14,18	9	1,9036
Pyrenocine A	209,129	209,1289	1,50	3	1,9492
Pyridoxine	170,117	170,1173	1,50	14	1,9967
Pyrifenox	295,153	295,1606	11,98	14	1,9094
Pyrilamine	286,238	286,2368	18,25	1	1,9001
Pyrimethamine	249,185	249,1854	1,76	16,17	1,8831
Pyrimethanil	200,118	200,1182	10,48	7	1,9449
Pyrvinium	383,314	383,3137	19,44	6	1,9043
RCS-8	376,260	376,2602	1,37	14	1,9549
Selegiline	188,128	188,1282	1,79	14	1,9540
Serotonin	177,092	177,0919	1,51	11	1,9288

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Simazine-2-hydroxy	184,169	184,1697	14,68	13,14,16,6,2,11,10,5,8,3,12,9,17	1,0447
Spinetoram A	748,542	748,5414	18,72	12,6	1,8430
Spiromesifen	371,102	371,1023	7,28	4,12	1,8459
Stanozolol	329,157	329,1572	12,58	2	1,9408
Strychnine	335,222	335,2179	17,44	8	1,9466
Sulfadiazine	251,060	251,0603	9,18	12,2	1,9018
T-2 triol	383,208	383,2076	1,49	12,4	1,8392
Tapentadol	222,186	222,1856	12,77	12	1,9980
Tebuconazole	308,153	308,1527	17,60	16,17,15,11	1,7711
Tebuthiuron	229,108	229,1083	16,30	12	1,9056
Telmisartan	515,244	515,2436	17,60	9,12	1,9416
Temazepam	301,217	301,2168	19,06	12,9,4	1,8437
Terbumeton	225,936	225,9364	2,27	12,4,9,10,2,5,11,7,6	1,1006
Terbuthylazine	230,117	230,1165	16,69	4,6	1,9204
Terbuthylazine-2-hydroxy	212,151	212,1508	14,07	4,9,13	1,8557
Terbuthylazine-desethyl	202,086	202,0855	15,06	12,9	1,9765
Terbutryn	242,144	242,1439	17,27	12	1,9442
Theobromine	181,072	181,0720	8,67	12,9,4	1,8813
Theophylline	181,072	181,0717	9,77	12,9,17,9,14,4	1,7538
Thiabendazole	202,181	202,1806	1,49	12,7	1,8655
Thiacloprid	253,031	253,0310	8,39	12,9	1,7944
Thionazin	248,872	248,8716	1,24	9	1,9379
Thioproperazine	447,294	447,2932	1,43	17,2	1,9158
Thioridazine	371,290	371,2912	17,98	1	1,9759

Library Detection	Theoretical m/z	Detected m/z	RT (min)	Samples	VIP
Thymopentin	680,516	680,5163	1,52	5,16,15,14,13,17,6	1,4462
Tiocarlide	401,287	401,2877	1,78	9,5,16	1,7633
Tocainide	193,072	193,0723	9,44	5	1,9626
Tramadol	264,196	264,1964	12,09	1,2,6,3,11,8,7,16,11,15,9,12	1,0015
Tranexamic acid	158,027	158,0269	1,56	9	1,9701
Triamcinolone	412,218	412,2193	12,47	12	1,9960
Triamterene	254,248	254,2478	18,12	8	1,9043
Triazoxide	248,237	248,2377	18,94	9,15,2,13,3,8,10,17	1,0539
Triflupromazine	353,304	353,3033	19,81	1,10,9,5,7,2,3,12,6,8	1,0236
Trihexyphenidyl	302,246	302,2455	5,79	2,3,5,7,8,10	1,0914
Tritoqualine	501,377	501,3772	18,69	2,13,3,10,5	1,0211
Tryptamine	161,096	161,0962	1,39	2	1,9944
Tryptophol	162,076	162,0763	1,47	11,2,7,13,14,15,16,17	1,0001
Tylosin A	916,733	916,7327	3,41	13,14,16	1,7227
Valsartan	436,234	436,2347	16,25	3	1,9809
Vardenafil	489,359	489,3590	1,76	3	1,9972
Verapamil	455,372	455,3719	3,11	8	1,9150
Vincamine	355,012	355,0129	1,13	12	1,9717
Xylazine	221,154	221,1538	17,34	13,14,15,16,17,11	1,3044
Xylometazoline	245,247	245,2268	19,50	1,7,12	1,7033
Zinniol	267,123	267,1211	16,77	10	1,9218
Ziprasidone	413,267	413,2670	19,87	12	1,9806

3. Conclusions

In this chapter, suspect and non-target screening analyses were combined in order to obtain a more holistic view of the quality of European water bodies. Analyses were carried out with HRMS due to its high sensitivity especially at low concentration levels. Pollution patterns among samples originating from different surface water sources were identified using multivariate statistical analysis tools. PCA models were used for their identification among the samples while PLS-DA models were used in order to show which features (micropollutants) were more important for the discrimination between the water samples. Identification of the compounds showed the wide range of compounds present in water sources, indicating the high occurrence of pharmaceuticals, pesticides and PFAS. The results of this study will be implemented in the NORMAN database, and can make an important contribution in decision making for more target monitoring assessments – in order to evaluate the pollution levels and plan the appropriate treatment activities, as well as for prioritization of compounds for policy making.

Section IV

Degradation Processes

Chapter 6 Degradation of glyphosate

Firstly, the degradation rate of glyphosate after direct photolysis with UV irradiation was examined. The major aim of this study was to determine the effectiveness of UV irradiation in decreasing the toxicity of glyphosate and identify the relevant UV exposure regimes (UV wavelength, UV dose). A test battery including different aquatic organisms was used in order to observe changes in activity and growth of the target organisms before and after UV irradiation. The test organisms were selected from different trophic levels for better assessing the biological effects of all the bioactive compounds in the samples after UV treatment including glyphosate's degradation byproducts. Suspect-screening analyses of the samples before and after treatment with LC-HRMS provided valuable information for understanding the degradation mechanism of glyphosate.

1. Materials and Methods

1.1 Chemicals and materials

Stock standards of *N*-(phosphonomethyl)glycine, monoisopropylamine salt solution (CAS 38641-94-0), and *N*-(phosphonomethyl)glycine (CAS 1071-83-6) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). For the irradiation a 4W UVP UVGL-25 lamp equipped with separate tubes for UV-A (365nm) and UV-C (254nm) (Analytic Jena US), and an 8W UVP 3UV™ lamp equipped with separate tubes for UV-A (365nm), UV-B (302 nm) and UV-C (254nm) were used. Some initial experiments with the two UV lamps gave comparable results for comparable UV doses and in order to shorten exposure times the 8W lamp with greater intensity was selected to continue with. The irradiation intensity at 15 cm distance from the UVP 3UV™ lamp was 970 $\mu W/cm^2/sec$ for UV-A, 1900 $\mu W/cm^2/sec$ for UV-B, and 327 $\mu W/cm^2/sec$ for UV-C, and it was measured with an Extech SDL470 Light meter equipped with UV-AB and UV-C sensors.

1.2 Sample preparation and treatment

Stock solutions of glyphosate were prepared in both autoclaved distilled deionized water and real water matrices, using the purchased stock standards and the commercial herbicide solutions, at a final concentration of 1 g/L. All stock solutions were stored in the dark at 5°C. As real water matrices, drinking water samples from Aalborg, Aarhus, Skagen, and Sønderborg municipalities in Denmark were used. Moreover, samples from the influent (raw water) and effluent (treated water) of a drinking water treatment plant in Elsted, Denmark were included in this study as well. All real water samples originated from groundwater sources, and regional differences in organic and inorganic

constituents among them were observed, based on the information found at the national Danish well database for water quality https://eng.geus.dk/products-services-facilities/data-and-maps/national-well-database-jupiter:

The following parameters were tested during the experiments in order to examine the photodegradation and biotoxicity of glyphosate under UV irradiation:

a) effect of UV wavelength (UV-A, UV-B, UV-C);

b) effect of UV dose (range between 2,3 and 70 J/cm^2);

c) effect of glyphosate's concentrations (range between 0,18 and 100 mg/L);

d) importance of water matrices.

More specifically, solutions of glyphosate and glyphosate-based herbicides with concentrations of 50 and 100 mg/L were prepared in different water matrices. These samples were exposed to different doses of UV-A, UV-B and UV-C irradiation (aim a) using quartz cuvettes (10 mm 3,5 mL, Science Outlet Optical Quartz QS10 and Hellma Precision Quartz SUPRASIL® QS10) at room temperature (22°C). Moreover, control samples were included in the study in order to evaluate any toxicity caused from active oxygen species generated during the irradiation experiments and apart from glyphosate.

Different UV doses (J/cm^2) were calculated from the measured UV irradiation intensity (μW/cm^2/sec) and the exposure time (sec) and were achieved by alternating the exposure times and the distance of the solution form the UV lamp (aim b). For example, a UV dose of 20 J/cm^2 was achieved by using the same exposure time but different distances from the UV lamp for the different wavelengths (20 cm for UV-A, 35 cm for UV-B, and 5 cm for UV-C).

Moreover, diluted glyphosate concentrations were prepared in UV bottom - transparent 96 well microplates (Nunc 96-well UV microplates, Thermo Scientific) and exposed to UV irradiation in order to examine the effects of glyphosate's concentrations on the outcome (aim c). The exposure was done from the top or bottom of the 96-well UV microplates using similar UV doses as the quartz cuvettes, while they were placed on a cooling plate in order to maintain the temperature at around 22°C and avoid evaporation of the small samples' volumes used (100 μL) due to overheating. Control samples without any UV exposure were covered with aluminum foil and stored in the dark. Finally, solutions of glyphosate at a concentration of 100mg/L were prepared in drinking water and exposed to UV irradiation (aim d).

The addition of different oxygen radical probes, helped us to identify the presence of active oxygen species in the aqueous samples after the treatment. Superoxide radicals ($^\bullet O_2^-$), were detected by measuring chemiluminescence after post-treatment addition of 1 mM luminol. Similarly, hydroxyl radicals ($^\bullet OH$) were detected by measuring fluorescence after pretreatment addition of 1 mM coumarin, terephthalic acid, and benzoic acid. A Victor X2 Multilabel Plate Reader (Perkin Elmer) was used in order to measure chemiluminescence and fluorescence originating from oxygen radical probes after reactions with active oxygen species.

1.3 Toxicity tests

1.3.1 *Aliivibrio fischeri*

Toxicity screening of glyphosate samples was examined with a standard inhibition test using the luminescent bacterium *Aliivibrio fischeri* (ISO 11348-1, 2009) [172]. *A. fischeri* DSM 7151 was incubated in white 96-well plates (CulturPlates, Perkin Elmer), and exposed to different glyphosate concentrations (0,098, 0,195, 0,390, 0,780, 1,560, 3,130, 6,250, 12,5, 25, 50 mg/L) before and after UV irradiation. Changes in bioluminescence were quantified after 30 minutes of bacteria's exposure to glyphosate, using a Victor X2 Multilabel Plate Reader (Perkin Elmer).

1.3.2 *Bacillus subtilis*

A newly developed inhibition test with *Bacillus subtilis* was used for glyphosate's toxicity on bacteria screening. The endpoint was inhibition of growth and hydrolase activity after 18 h. *Bacillus subtilis* DSM 10 (German Collection of Microorganisms and Cell Cultures) was grown at 30 °C in Davis Minimal Broth (Sigma-Aldrich) supplemented with: 25 µM $FeSO_4$, 0,5 µM $ZnCl_2$, 0,5 µM Na_2MoO_4, 0,5 µM $MnCl_2$, 0,5 µM H_3BO_3, 0,5 µM $CoCl_2$, 0,5 µM $NiCl_2$, and 2,0 µM $CuSO_4$. Serial dilutions of glyphosate were made in 96-well clear microplates (Nunclon, Thermo Scientific) starting from 100 µL glyphosate stock solutions of 100 mg/L and serially diluted in 100 µL autoclaved distilled water resulting in different glyphosate concentrations. After the dilution, 50 µL of 4 x strength Davis Minimal Broth was added to each well, followed by the addition of 50 µL of diluted *B. subtilis* culture (1:1000 dilution in 0,9% NaCl). This resulted in a final sample volume of 200 µL in each well and 10 different concentrations of glyphosate: 0,098, 0,195, 0,390, 0,780, 1,560, 3,130, 6,250, 12,5, 25, 50 mg/L. Four replicates were included for blanks (medium only), controls (no glyphosate), and each glyphosate concentration. Sealed plates were left for 18 h incubation at 30 °C while shaking at 250 rpm (PST-60HL-4 Plate Shaker Thermostat,

Biosan). The absorbance at 620 nm was then measured for each well (Thermo Multiskan Plate Reader). Finally, hydrolase activity in *B. subtilis* was measured by adding 20 μL fluorescein diacetate stock solution (5 mM) to each well to obtain a final concentration of 5 μM. After 60 minutes incubation at 30 °C and shaking at 250 rpm, fluorescence was quantified in each well using a Victor™ X2 Multilabel Plate Reader with a 485 nm excitation and 535 nm emission filter (Perkin Elmer).

1.3.3 *Raphidocelis subcapitata*

Toxicity of glyphosate to phytoplankton was examined by the unicellular green microalgae *Raphidocelis subcapitata* (ISO 8692, 2012) [173] inhibition tests. The toxicological endpoint was inhibition of growth measured after 72 h of incubation (ISO 8692, 2012) [173]. *R. subcapitata* (MicroBioTests Inc) was cultivated in a test medium at 23 ± 2°C and under continuous illumination at 6500 lux (ISO 8692, 2012) [173]. Diluted culture was exposed in 96-well clear microplates (Nunclon, Thermo Scientific) to the following concentrations of glyphosate with and without prior UV irradiation: 0,098, 0,195, 0,390, 0,780, 1,560, 3,130, 6,250, 12,5, 25, 50 mg/L. Eight replicates were included for blanks (medium), controls (no glyphosate), and each glyphosate concentration. Plates were incubated at 23 °C shaking at 70 rpm under continuous illumination (6500 lux) for 72 h. Growth was measured after 0, 24 h, 48h, and 72h as absorbance at 450 nm using a Thermo Multiskan Plate Reader. Growth measurements for selected samples were done by measuring cell sizes (μm) and cell abundance (cells/mL) using a Multisizer 4e Coulter Counter (Beckman Coulter).

1.3.4 *Daphnia magna*

Glyphosate's toxicity to zooplankton was examined with inhibition tests of the crustacean *D. magna* (ISO 6341, 2012) [174]. The toxicological endpoint was inhibition of mobility and was determined by visual inspection of the animals (ISO 6341, 2012) [174]. *D. magna* STRAUS was cultivated from a laboratory clone originating from pure-culture ephippia [175]. Each treatment consisted of 20 juvenile animals distributed among 4 glass vials containing 5 animals and 10 mL freshwater medium in each. The mobility of each animal was determined after 24 h and 48 h (ISO 6341, 2012) [174].

1.4 Chromatographic Analysis

Analyses of the samples before and after UV treatment were done using an Ultimate 3000 High-Pressure Liquid Chromatography coupled to a high resolution LTQ-Orbitrap mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with an ESI source.

Chromatographic separation was achieved with a reversed-phase C18 column (Phenomenex Luna, 150 × 2 mm, 3 μm, 110 Å; Phenomenex, Castel Maggiore, BO, Italy) by injecting a sample volume of 10μL at a mixture of 0,1 mM Formic Acid (A) and Acetonitrile (B) used as the mobile phase. The elution followed a gradient profile which started from 5% B, increased up to 100% B in 40 minutes and arrived to 100% A after 10 minutes. Mass spectra were acquired in both Positive and Negative ESI modes. Nitrogen was used as sheath and auxiliary gas in order to deliver the LC effluent to the ESI ion source with the following parameters: auxiliary gas 15 arb, sheath gas 34 arbitrary unit (arb), capillary voltage 4.48 kV, and capillary temperature 270°C. The elaboration of the data followed a suspect screening technique and was carried out using the MZmine 2.52 [167] software in order to achieve peak alignment, peak grouping, chromatogram deconvolution and isotope removal (Table A8). The online databases ChemSpider and METLIN were used in order to identify the transformation byproducts [176-178].

1.5 Data analysis and statistics

The toxic response measured for all the endpoints was expressed as inhibition (I) relative to control samples:

$$I = 1 - (R_i / R_c),$$

where R_i is the response measured for inhibited samples and R_c is the one measured for control samples.

The concentration-response curves were fitted to a log-logistic model using iterative non-linear regression [179]:

$$\text{Response} = 1/ (1+10^{\wedge}(\log EC50 - \log C) * \text{Slope} \quad (13)$$

where C is the concentration of the toxicant (mg/L), EC50 is the median effective concentration (mg/L), and Slope is the parameter that represents the slope of the curve. Non-linear regression models and calculation of 95% confidence limits for EC50 values were performed using the GraphPad Prism 8.0.1 (Graphpad Software).

Relative Effect Potency (REP) [179] was used in order to estimate the toxicity of a sample before and after UV treatment:

$$REP = EC50_{(before)}/EC50_{(after)} \quad (14)$$

where $EC50_{(before)}$ is the median effective concentration (mg/L) before UV irradiation and $EC50_{(after)}$ is the median effective concentration (mg/L) after treatment.

The nonparametric Mann-Whitney U test (Wilcoxon rank-sum test) was used to perform statistical analyses of the results and evaluate differences between treatments, that have a significance level of p <0,05 (KaleidaGraph 4.5.4; Synergy Software).

2. Results

2.1 Test organisms

Initial experiments were conducted in order to identify which test organisms were responsive to glyphosate exposure (Figure 15). A battery of non-target organisms including *Bacillus subtilis, Aliivibrio fischeri*, *Raphidocelis subcapitata,* and *Daphnia magna* was tested. The results showed that the traditional toxicity screening organism *A. fischeri* was the least responsive with a median effective concentration (EC50) value of 25,0 mg/L. On the other hand, the crustacean *D. magna*, the bacterium *B. subtilis,* and the green microalgae *R. subcapitata* responded at the exposure of much lower glyphosate's concentrations with EC50 values of 0,990 mg/L, 3,670 mg/L, and 1,130 mg/L, respectively (Figure 15).

Subsequently, we decided to focus on *D. magna, B. subtilis* and *R. subcapitata* for our experiments, examining the changes in toxicity before and after UV irradiation of aqueous glyphosate.
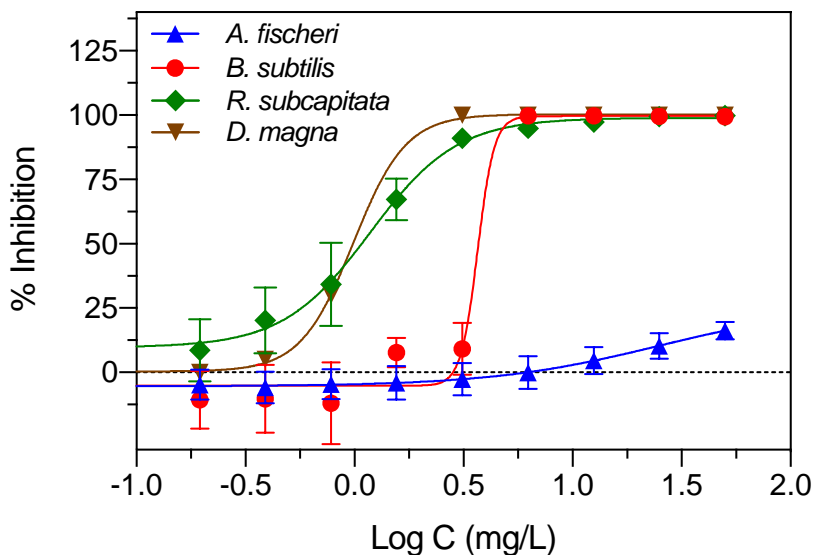

Figure 15: Concentration-response curves showing the toxicity of glyphosate to different aquatic test organisms. Data points represent means ± standard deviation.

2.2 Effect of UV irradiation on glyphosate toxicity

The toxicity of glyphosate before and after exposure to UV irradiation under three different wavelengths - UV-A (365 nm), UV-B (302 nm), UV-C (254 nm) - was evaluated using *B. subtilis*, *R. subcapitata* and *D. magna* as test organisms (Figure 16, Figure 17, Table 17).

Exposure of glyphosate to UV-A and UV-B at a UV dose of 20 J/cm^2 did not have any noticeable effect on the toxicity to *B. subtilis* and *R. subcapitata* (Figure 16).
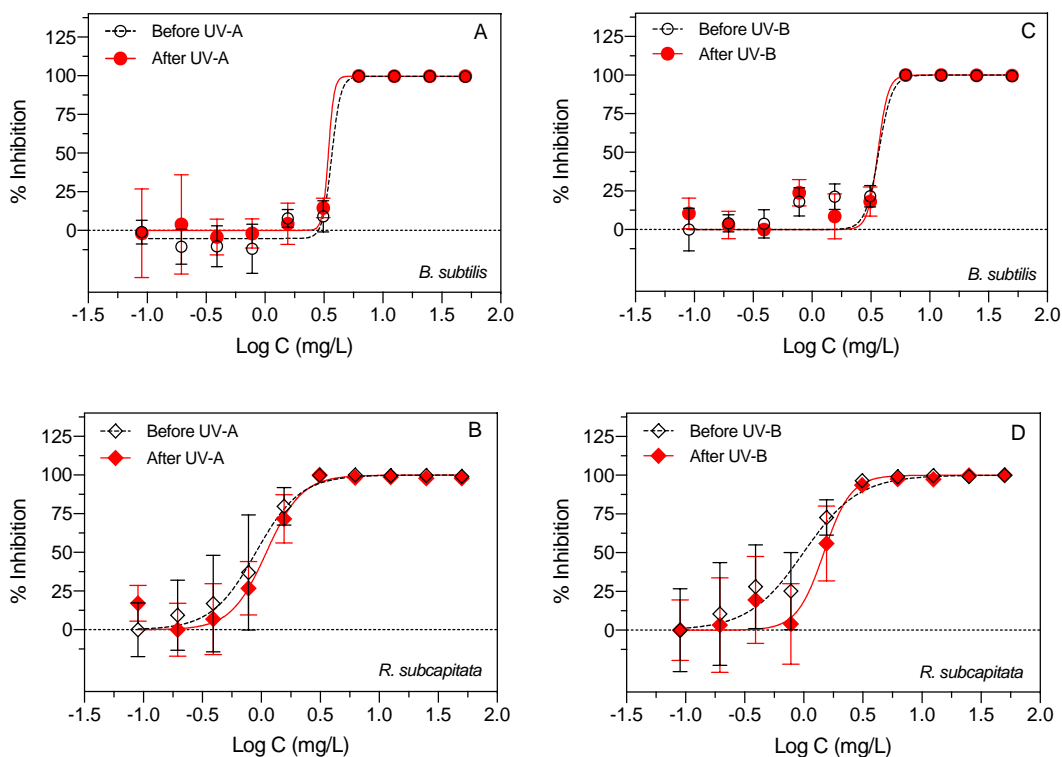

Figure 16: Effect of UV-A irradiation (panel A and B) and UV-B irradiation (panel C and D) at a UV dose of 20 J/cm^2 of aqueous glyphosate on toxicity to *B. subtilis* (panel A and C) and *R. subcapitata* (panel B and D). Data points represent means ± standard deviation.

The same result was achieved also after increasing the UV-A dose to 70 J/cm^2 (Figure 17D). However, exposure of glyphosate to UV-B at a dose of 70 J/cm^2 resulted to a significant toxicity decrease comparing the dose of 20 J/cm^2 (Mann-Whitney test; p=0,028). On the other hand, UV-C exposure at UV dose of 20 J/cm^2 clearly decreased the toxicity of aqueous glyphosate to *B. subtilis* and *R. subcapitata* (Figure 17A-C). Furthermore, when the UV-C dose was increased from 20 J/cm^2 to 70 J/cm^2 showed a significant decrease of the toxicity of glyphosate (Mann-Whitney; p=0,029). This suggests that UV-C and also UV-B irradiation are able to decrease the ecotoxicity of glyphosate if the UV dose is sufficiently high.
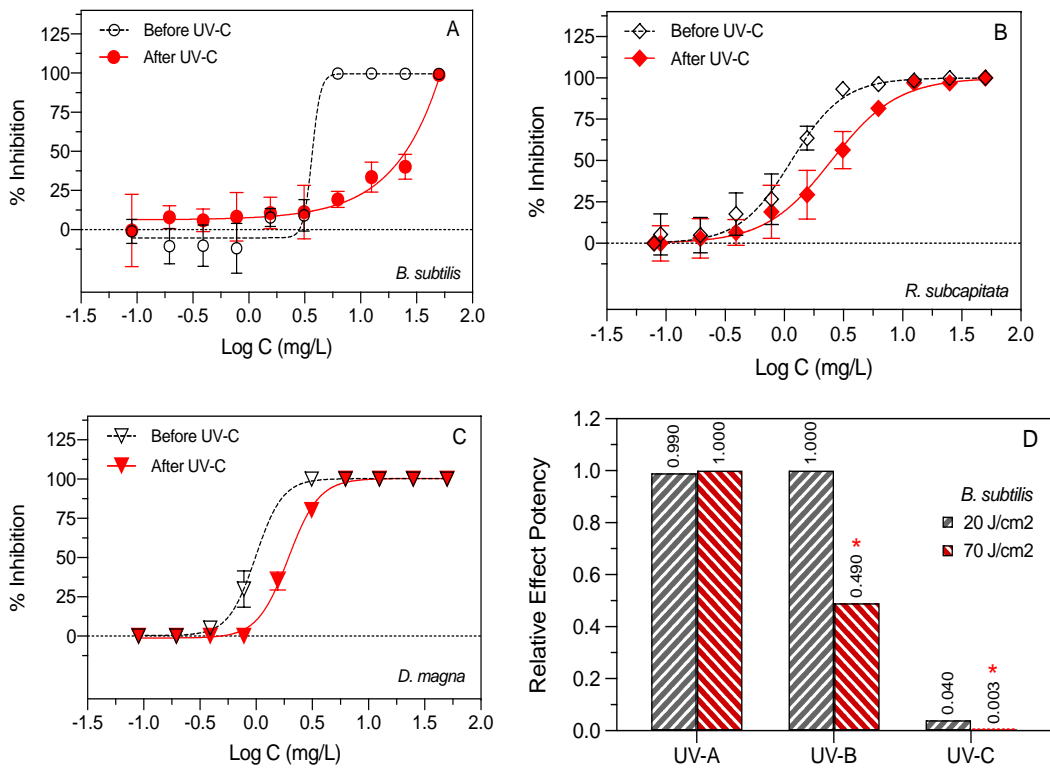

Figure 17: Effect of UV-C irradiation (A, B, and C) of aqueous glyphosate at a UV dose of 20 J/cm^2 on toxicity to *B. subtilis* (A), *R. subcapitata* (B), and *D. magna* (C). Data points represent means ± standard deviation. D shows the effect of an increased UV irradiation dose of 70 J/cm^2 on the relative effect potency of glyphosate to *B. subtililis*. The asterisk (*) indicates the significant difference between 20 J/cm^2 and 70 J/cm^2 (Mann-Whitney test, p<0.05).

Changes in the growth of *R. subcapitata* after UV-C treatment of glyphosate were measured as changes in the absorbance as described in international standards (ISO 8692, 2012)[173]. The results were also confirmed by counting and sizing individual algae cells using a Multisizer Coulter Counter (Figure 18). Important changes in the cell numbers of *R. subcapitata* were observed after its 72 h exposure and growth in solutions which had and had not undergone UV-C irradiation (Figure 18). The results of the Mann-Whitney U test, which was done for each of the four glyphosate concentrations, showed that the difference between non-irradiated and UV-C irradiated solutions was significant (p=0,026; p<0,001; p<0,001; p<0,001, respectively).
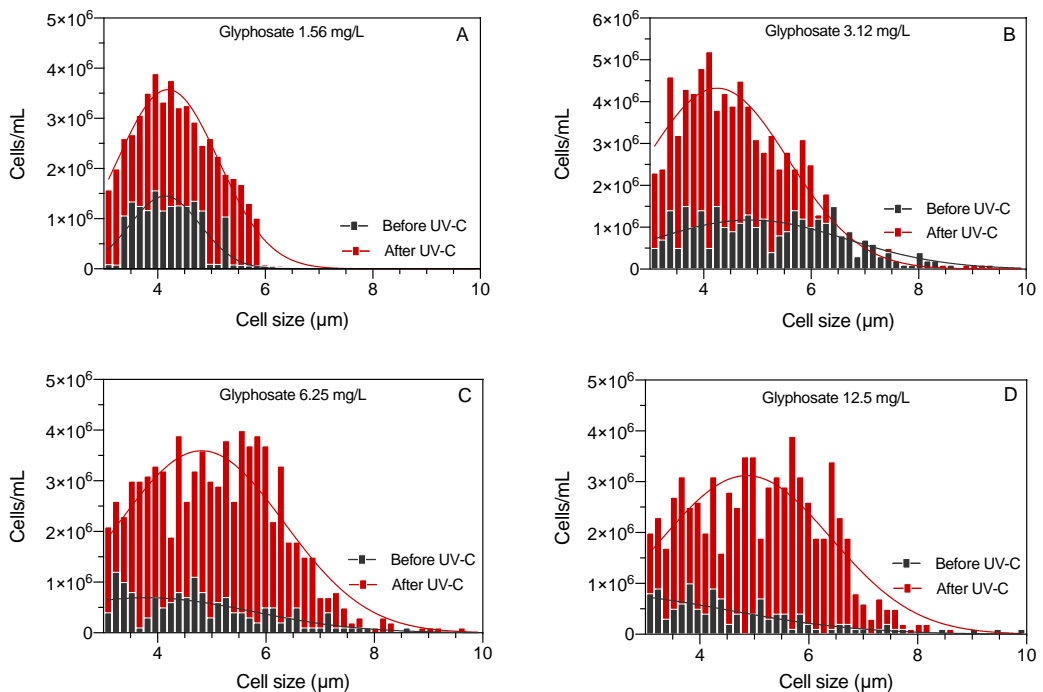

Figure 18: Effect of UV-C irradiation (20 J/cm^2) of aqueous glyphosate on toxicity to *R. subcapitata* measured as differences in cell numbers and cell sizes after 72 h of growth in the presence of glyphosate before and after UV treatment.

In the present study, the EC50 value for *D. magna* exposed to glyphosate was found to be 0,99 mg/L, a value comparable to those reported in literature [175,180,181], while for *R. subcapitata* was 1,13 mg/L and for *B. subtilis* 3,67 mg/L (Table 17) -values also

within the range reported in other studies [182-186]. The median effective concentration (EC50) values of glyphosate samples were increased after UV-C irradiation at a UV dose of 20 J/cm^2 for all the three test organisms, a result that suggests lower toxicity (Table 17). A 2-fold decrease in toxicity of glyphosate to *D. magna*, a 5-fold decrease for *R. subcapitata* and a 23-fold decrease *B. subtilis* were observed*.*

Table 17. Median effective concentrations (EC50) values for three aquatic test organisms before and after exposure of glyphosate to UV-A, UV-B, or UV-C at comparable UV doses (20 J/cm^2). *ND: not determined.*

Test organism	EC50 values (mg/L)			
	Before UV	After UV-A	After UV-B	After UV-C
B. subtilis	3,67	3,45	3,54	85,41
R. subcapitata	1,13	1,19	1,53	5,70
D. magna	0,99	*ND*	*ND*	1,93

3.4 Effect of different UV-C exposure conditions on glyphosate toxicity

Increase of irradiation time and UV dose resulted to an exponential decrease of the toxicity of glyphosate to *B. subtilis* and *R. subcapitata* (Figure 19). Increasing UV doses and decreasing toxicity, a relationship calculated as log(1/EC50) suggests that a loss of 90% of the initial glyphosate to the bacterium after UV-C irradiation of 23,4 J/cm^2 and of 23,7 J/cm^2 for the green algae occurs. Therefore, toxicity was mitigated for both organisms in a dose-dependent ratio and at comparable grades, suggesting that the test organisms responded equally to attenuation of UV-C glyphosate inhibition (Figure 19).

Mainly, the exposure of aqueous solutions of glyphosate to UV irradiation was carried out in sealed quartz samples. Examining though the different conditions that could affect the degradation, a different exposure method using UV-C plastic transparent microplates was considered as well. However, for reasons of eliminating sample loss due to the small amount of sample treated in the microplates (100µL) and based on the results that lower of 10 J/cm^2 UV doses may affect the toxicity, we focused on a comparison between the two exposure techniques at a UV-C dose of 5,4 J/cm^2. The results obtained did not show great differences in median effective concentrations for

B. subtilis and *R. subcapitata* for the different exposure regimes (quartz cuvettes vs. transparent plastic microplates) (Table 18).
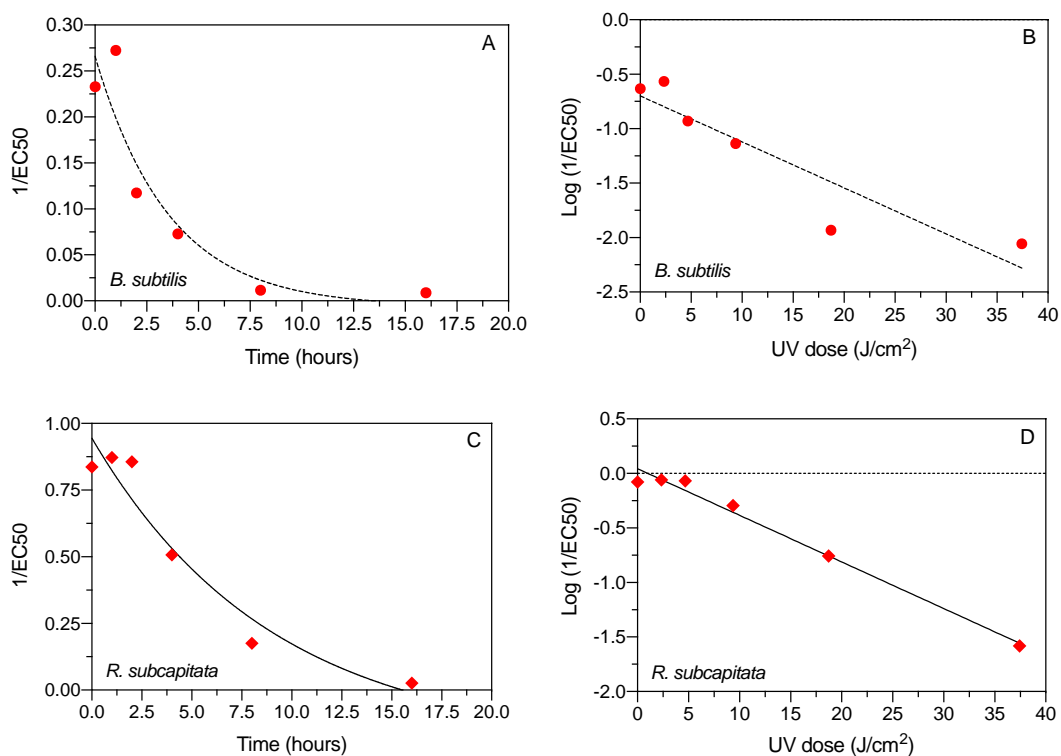

Figure 19: Effect of UV-C irradiation time (h) and UV dose (J/cm^2) on the toxicity of glyphosate to *B. subtilis* (A and B) and *R. subcapitata* (C and D).

Table 18. Median effective concentrations (EC50) for *B. subtilis* and *R. subcapitata* before and after exposure of glyphosate to 5,4 J/cm^2 UV-C, in two different UV exposure techniques

Exposure technique	*B. subtilis* EC50 values (mg/L)		*R. subcapitata* EC50 values (mg/L)	
	Before UV-C	After UV-C	Before UV-C	After UV-C
Quartz Glass	3,67	7,92	1,13	4,18
UV plate	2,34	7,12	1,47	3,65

In this study, control samples for all the exposure experiments using blank solutions without glyphosate were included, in order to assess potential toxicity originating from the generated reactive radicals. The results showed no apparent inhibition of test organisms, an outcome that can be explained by the fact that these products are short-lived.

3.5 Effect of UV irradiation on glyphosate's toxicity in real water samples

The effect of UV irradiation on mitigating glyphosate's toxicity was also studied in real water matrices, in order to simulate more realistic conditions, and examine the effects from unknown water constituents, such as organic and inorganic contaminants, on degradation. Initially, the tests were conducted in a test matrix prepared with deionized distilled water and artificial freshwater. After some promising initial results, natural drinking water samples spiked with glyphosate concentrations were used for UV-C irradiation experiments. The obtained results (Figure 20) showed clear differences in toxicity before and after UV-C irradiation of 20 J/cm^2. In some cases, the decrease in toxicity due to UV-C treatment of aqueous glyphosate was slightly larger for the natural drinking water samples compared to parallel experiments conducted in distilled water (Figure 17 vs. Figure 20). The UV effect was also greater for glyphosate added to drinking water compared to groundwater (raw water) (Figure 20F). The raw water was slightly colored and contained elevated concentrations of natural elements such as iron, manganese, and ammonia because it was sampled before filtration at the Drinking Water Treatment Plant. For glyphosate irradiated in drinking water, the EC50 values before UV-C varied between 2,03 mg/L and 7,30 mg/L whereas the EC values after UV-C varied between 17,57 mg/L and >100 mg/L. The differences in EC50 before and after UV-C irradiation were significantly different (Mann-Whitney; p=0,002). The Relative Effect Potency after UV-C treatment was 0,02-0,40 corresponding to a 3 to 44-fold reduction in toxicity to the test organism *B. subtilis*. Hence, the effect of UV on glyphosate was not inhibited by constituents in the drinking water samples and may even be stronger in some water matrices than in deionized water suggesting that natural drinking water may even facilitate the process.
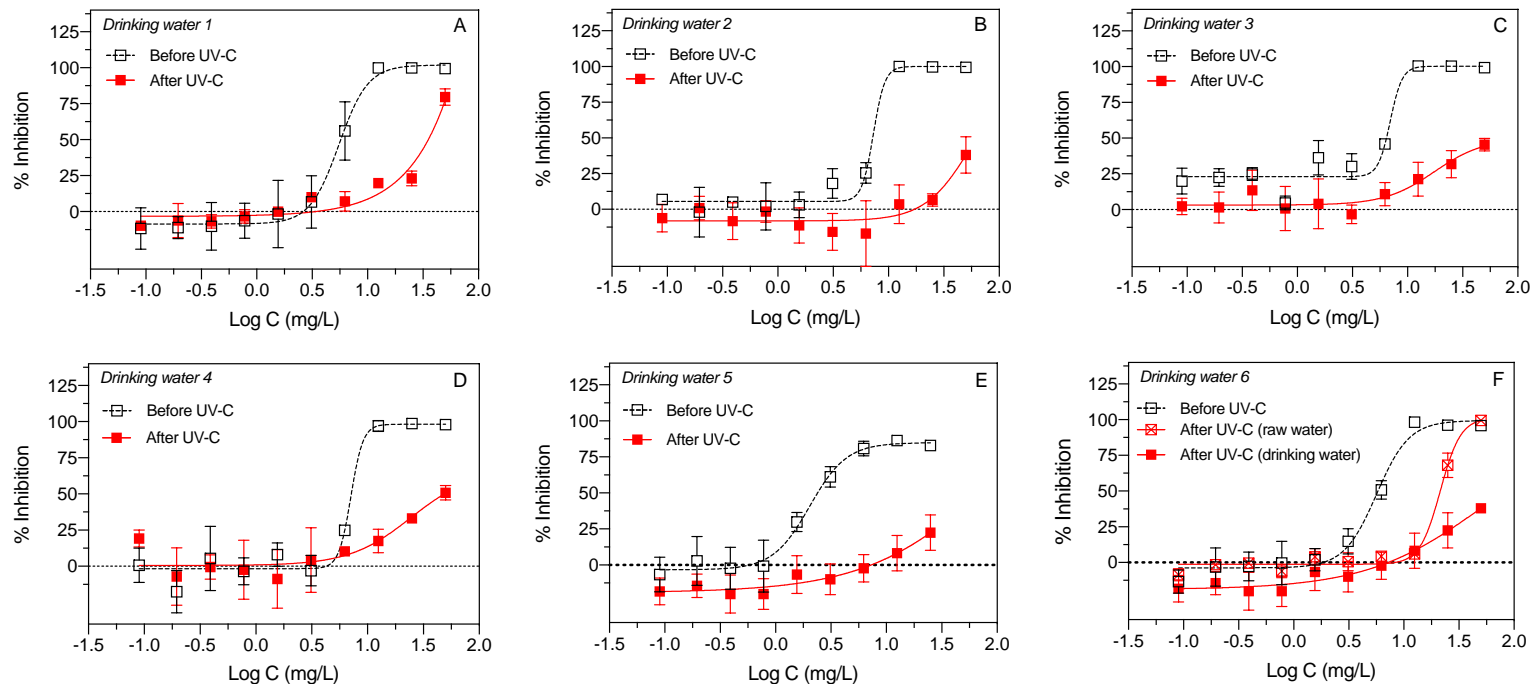
Figure 20: Effect of UV-C irradiation (20 J/cm^2) of glyphosate in municipal drinking water on toxicity to *B. subtilis*. Drinking water produced from groundwater was collected at six locations in three Danish municipalities: Aalborg Municipality (A – Aalborg East; B - Aalborg Center; C – Aalborg West) Sønderborg Municipality (D); Aarhus Municipality (E), and Elsted drinking water treatment plant in Aarhus Municipality sampled before and after water treatment (F). Data points represent means ± standard deviation.

3.6 Byproducts' identification

Liquid Chromatography tandem with High Resolution Mass Spectrometry (LC-HRMS) was used in order to analyze the aqueous solutions of glyphosate before and after UV exposure in order to identify the transformation products and understand the degradation mechanism. Samples after UV-C irradiation at doses of 20 J/cm^2 and 70 J/cm^2 were used. The identification followed a suspect screening approach focusing on transformation products likely to be generated from glyphosate, and was done using the ChemSpider and METLIN databases (http://metlin.scripps.edu/) [176-178]. However, it was a challenging procedure due to lack of analytical standards. More than 20 byproducts were detected (Table 19) after UV-C exposure at 20 J/cm^2 and 70 J/cm^2. Among them, there were Sarcosine ($C_3H_7NO_2$), Glycine ($C_2H_5NO_2$), Glyoxylic acid ($C_2H_2O_3$), Aminomethylphosphonic acid (CH_6NO_3P; AMPA), Acetic acid ($C_2H_4O_2$) and Phosphoric acid (H_3PO_4), transformation byproducts already mentioned in other studies employing different Advanced Oxidation Processes for glyphosate's remediation, as well as other potentially intermediates.

Table 19. Detected transformation products after UV-C irradiation in negative ESI (-) and positive ESI (+).

Compound	m/z	RT (min)	ESI	Compound	m/z	RT (min)	ESI
H_3O_4P	96,968	25,94	-	$C_5H_9NO_2$	116,069	15,66	+
CH_6NO_3P	110,001	1,75	-	$C_3H_3NO_2$	84,009	27,82	-
$C_2H_5NO_2$	74,020	1,86	-	$C_3H_4O_4$	103,003	1,69	-
$C_2H_2O_3$	72,908	16,64	-	$C_3H_6O_4$	105,017	26,14	-
$C_3H_7NO_2$	90,054	29,62	+	C_3H_6O	58,080	11,80	+
$C_2H_7NO_2$	77,084	1,77	-	$C_3H_9NO_2$	92,069	1,66	+
C_2H_5NO	59,070	35,32	-	$C_4H_{10}O_2$	91,074	28,37	+
$C_2H_6N_2O_4$	91,016	28,99	-	C_4H_8O	73,028	4,43	+
$C_2H_4O_2$	59,015	8,52	-	$C_4H_8O_4$	119,033	6,85	-
$C_3H_8N_2O$	89,070	30,13	+	$C_4H_{10}O_3$	106,120	29,42	-
$C_6H_{11}NO_2$	130,085	28,58	+	C_5H_5N	80,048	32,84	+

The proposed degradation mechanisms of Glyphosate after UV photolysis are shown in Figure 21. Both pathways are following those proposed from previous studies and are resulting in less toxic transformation products confirming the toxicity test results [109]. The identified byproducts, show that in UV photolysis treatment, degradation of Glyphosate follows the two mechanisms related to the C-P and C-N bonds, known as the "C-P pathway" and the "C-N pathway". In the first one, Glyphosate's (A) molecule is attacked by the generated hydroxyl radicals resulting to the cleavage of the C-P bond, the formation Sarcosine (B) and the release of Phosphate which after hydrogenation results to Phosphoric acid (C) production. Sarcosine after further treatment is subsequently transformed to Glycine (F), which can be further degrade to Glyoxylic acid (G) and Acetic acid (E). The second pathway involves the breakdown of the electrophilic C-N bond, or directly by UV irradiation, or after attack by the generated hydroxyl radicals, and results to AMPA (D) and Acetic acid (E), or directly to Glyoxylic acid (G). These two mechanisms can exist alone or together during Glyphosate's oxidation process, and are the same as those occurring through biodegradation processes. Hence, the results of this study suggest a potential combination of UV treatment with bioremediation to increase the remediation of Glyphosate further. Detection of other compounds was done as well, for which a

Moreover, LC-HRMS analyses showed that concentrations of glyphosate after UV-C irradiation at 20 J/cm^2 were no longer detected after treatment at a dose of 70 J/cm^2. Subsequently, the concentrations of AMPA and glycine were increasing after 70 J/cm^2, while sarcosine's increased with the treatment at 20 J/cm^2 and later decreased. Phosphoric acid was detected at both high and low UV-C doses while glyoxylic and acetic acid were only observed after UV-C irradiation at 70 J/cm^2. Concentrations were not quantified, since the analyses followed a full scan acquisition for identifying transformation products. However, differences in peak areas were considered for evaluating the differences in the samples before and after treatment.
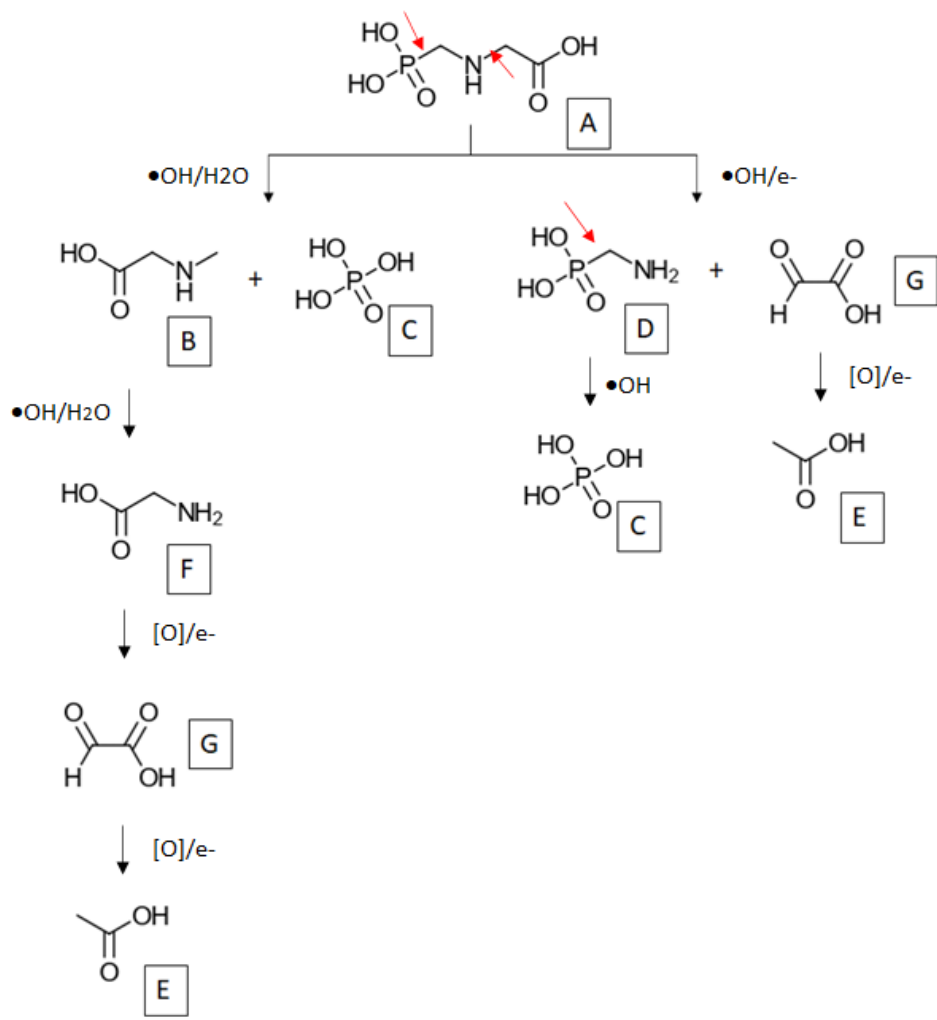
Figure 21: Potential pathways for UV mediated photolysis of Glyphosate (A) through two main routes resulting in Sarcosine (B), and Phosphoric acid (C), and AMPA (D), Acetic acid (E) and Glyoxylic acid (G). Sarcosine (B) may subsequently be transformed into Glycine (F).

3. Conclusions

Glyphosate is the most frequently detected herbicide in the aquatic environment, posing threats to the ecosystem and human health. Thus, efficient remediation techniques for its removal are fundamental. A vast variety of studies employing different methods have reported satisfactory results. However, towards a sustainable and toxic-free environment the implementation of fast, cost-efficient and environmental friendly techniques that don't generate waste or more toxic byproducts are necessary. In this way, the aim of this study was to investigate the degradation of glyphosate after UV-A, UV-B, and UV-C irradiation. Different parameters that could affect the process were taken into account and their effects on toxicity to aquatic organisms from different trophic levels were studied. The effect-based monitoring approach for the evaluation of the method's efficiency was combined with chemical analyses in order to identify transformation byproducts. Toxicity assays are an important supplement to chemical analyses in order to assess water quality, as bioassays can integrate changes in water chemistry and bioactivity before and after water treatment.

The results suggest that UV-C and to some extend UV-B photolysis of glyphosate in water could decrease concentrations of this pesticide and reduce overall ecotoxicity by generating less toxic transformation products. Even if, UV doses used in treatment plants are mainly used for disinfection processes, and they are not adequate to degrade organic contaminants, UV photolysis represents one cost-efficient, and green remediation process that doesn't produce waste and should be considered for industrial scale applications.

Chapter 7 Degradation of PFAS

Per and polyfluoroalkyl substances (PFASs) represent one of the most problematic classes of compounds - whose occurrence in different water sources has been reported worldwide - due to their ubiquitous physicochemical properties, that make them extremely difficult to be degraded and subsequently very persistent in the environment. At present, activated carbon and ion exchange are mainly used as removal technologies of PFASs from water, facing efficiency problems with shorter chain compounds [187-189] and generating waste that need to undergo further treatment, producing additional costs. For this reason, search of new remediation technologies is necessary. AOPs have been reported of being able to efficiently degrade PFASs, with promising defluorination of the compounds being reported after following electrochemical methods [190]. However, further study in order to identify the produced byproducts for understanding the degradation mechanisms and the toxicity of these compounds is still required. For this reason, in this work we evaluated the degradation mechanism of PFASs after non-thermal plasma treatment and identified the produced byproducts in individual compounds' solutions and in a mixture. The effect of different matrices was also examined, by preparing solutions in both MilliQ water and real groundwater matrices.

1. Materials and Methods

1.1 Selection of compounds

The compounds included in this study were selected based on the study reported in Chapter 3 with the highest abundance in the environment and after following the trends in literature. In this way, 3 substances were chosen, two perfluoroalkyl acids, Perfluorooctanoic acid (PFOA) and Perfluorohexanoic acid (PFHxA), and one perfluorosulfonic acid, Perfluorooctanesulfonic acid (PFOS). Two of the compounds had a carbon chain with 8 atoms while a shorter chain compound, including 6 atoms of carbon was included in order to test the efficiency of the thermal plasma treatment to degrade also shorter chain compounds.

1.2 Reagents and Chemicals

Stock compounds of PFOA (Perfluorooctanoic acid, CAS 307-24-4), PFOS (Perfluorooctanesulfonic acid, CAS 1763-23-1) and PFHxA (Perfluorohexanoic acid, CAS 335-67-1) (linear chain only) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). MilliQ was obtained from MilliPore (MA, USA), UHPLC-grade Methanol was purchased from Sigma-Aldrich (Saint Louis, MO, USA), LC-MS grade Water LiChrosolv® and

Ammonium acetate for LC-MS LiChropur® were purchased from Merck KGaA (Darmstadt, Germany).

1.3 Sample preparation and treatment

Solutions of the three selected compounds, PFOA, PFOS and PFHxA, were prepared in MilliQ water and a real water matrix (groundwater) at a concentration of 1 µg/L. Treatment of the different solutions was done using a custom-built Marx generator powered with 220V AC equipped with a pulse-width modulation circuit, a high-voltage transformer and four 990 pF capacitors. A small continuous flow of compressed air with relative humidity around 14% was fed to the spark-gaps' chamber to stabilize the generator's internal atmosphere. The discharge's peak voltage was typically 100-130 kV with peak current values of 20-40 A. The pulse duration was approximately 250 ns and the frequency of discharge could be manually adjusted between 5 and 17 Hz. Electrical measures have been performed using a BK Precision 2190D oscilloscope. Total absorbed power of the generator laid between 299 and 322 W. Water was treated using two different reactors: a 20 mL cylindrical polypropylene (PP) reactor was used in the first part of the work aimed at the optimization of working parameters while a 50 mL Pyrex glass (PG) reactor was used for all other experiments. The PP reactor on one site limited the risk of damage of the reactor wall as a consequence of the generated shock waves, whilst on the other side caused Toral Organic Carbon (TOC) and Total Nitrogen (TN) potential contamination from plastics and rubber parts. For both reactors, the same electrodes have been used consisting in one stainless steel bar having 10 mm diameter and a tungsten sintered electrode with a diameter of 3 mm on the other side. In the PG reactor the distance between the electrodes determined whether the discharge was forming underwater (distance < 2 mm) or at the water surface (distance > 2 mm). For both reactors, the headspace was in connection with the room air. Schematic representation of the experimental setup is shown in Figure 22. Both the reactors and the plasma generator were placed inside a Faraday cage. The optimization of its functional parameters was done in another study from our research group [191] using experimental design.
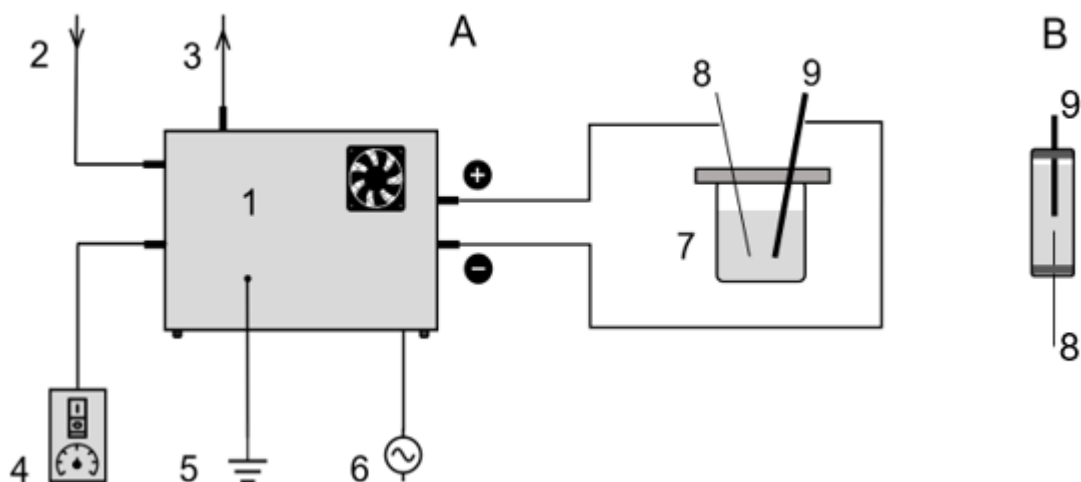
Figure 22: Schematic representation of the plasma generator connected to the PG reactor (A): 1) plasma generator, 2) air inlet, 3) air outlet, 4) plasma switch and frequency control, 5) ground, 6) 220V power supply, 7) PG reactor, 8) sintered tungsten electrode, 9) stainless steel electrode. PP reactor (B).

At the beginning of the experiments the reactor was filled with 50ml of each solution for treatment. The treatment time was 30 minutes and samples to be analyzed were taken at different time intervals (0min, 1min, 2,5min, 5min, 7,5min, 10min, 15min, 20min, 30min) in order to evaluate the degradation profiles and understand the generation of by-products. A mix of the compounds was prepared as well, in the same matrices and with a final concentration of 1 µg/L for each compound. The treatment time was extended to 60 minutes with samples taken at different time intervals (0min, 1min, 2,5min, 5min, 7,5min, 10min, 15min, 20min, 30min, 40min, 50min, 60min) in order to observe the differences in the degradation mechanisms, understand the potential cocktail effects and overall simulate a more realistic situation, since these compounds usually exist in the environment as mixes. Caution throughout the experiments – as reported in Chapter 3- was taken in order to minimize as much as possible the background contamination of the samples, that could potentially interfere with the results. In this way, Teflon materials were avoided throughout the whole treatment and analysis time.

1.4 Instrumental Analysis

Samples before and after treatment were analyzed with a SCIEX X500R QTOF system coupled to a Shimadzu ExionLC UHPLC system. The chromatographic separation was achieved using a Luna® Omega Polar C18 100 LC Column (3µm particle size, 100 x 2.1mm) –heated at 40°C- by injecting a 50µL sample volume into a mixture of 5mM Ammonium Acetate in H_2O (A) and 5mM Ammonium Acetate in MeOH (B) as mobile phase at a flow of 0,350 mL/min. The elution followed a gradient profile starting from 95% A and 5% B, keeping this ratio for 1 minute and then gradually changing to 100% B within 10 minutes, and keeping it for the next 2 minutes. Finally, the conditions returned to the initial within 15 minutes of elution. The samples were kept at a temperature of 4°C throughout the whole analysis time. The X500R QTOF system operated in Negative Electrospray Ionization Mode (ESI) with the following parameters: Ion source gas 1: 45 psi, ion source gas 2: 55psi, curtain gas: 30 psi, gas temperature: 500°C, ionspray voltage: -4500V, in both MRM and SWATH acquisition modes.

The samples were analyzed with the MRM acquisition mode in order to follow a target screening, which allowed the estimation of the degradation rate of the molecules. Quantification was achieved with six points calibration curves of final concentrations of 10, 50, 100, 500, 1000 and 5000 ng/L for each compound. Good coefficient results with $R^2 > 0,990$ were obtained for all the compounds.

On the other hand, SWATH was used for a qualitative screening, in order to identify degradation byproducts and it consisted of a full scan acquisition, followed by a Q1 isolation. The parameters used in full-scan MS mode were as follows: accumulation time: 0,05 sec; declustering potential: -80 V; TOF start mass: 100 Da; TOF stop mass: 1000 Da. A generic collision energy spread of (-35) ± 15 was used. For the Q1 isolation strategy (MS/MS) the parameters were: TOF start mass: 50 Da; TOF stop mass: 1500 Da; total number of windows: 24; window accumulation time: 0,035 sec. An external calibration of the instrument was performed, using a mixture of 10 compounds with a mass range between *m/z* 68,99 and *m/z* 2233,91. This mixture was automatically injected every 5 samples in order to maintain the mass accuracy below 2 ppm. SCIEX OS 1.7 software (SCIEX, Massachusetts, USA) was used for data acquisition and elaboration.

1.5 Toxicity tests

Three different methods were used in order to examine the toxicity of the three individual compounds' samples before and after treatment. Two *In vitro* bioassays based

on the acute toxicity effects on the crustacean *Thamnocephalus platyurus* and the bioluminescent bacteria *Alivibrio fischeri*, and one *In silico* method were followed.

1.5.1 *Thamnocephalus platyurus*

The commercially available Thamnotoxkit FTM microbiotest [192] was used for determining the lethal effects of toxicants on *Thamnocephalus platyurus* after freshly hatched larvae's exposure to samples before and after 30 minutes treatment, for 24h. The assays were performed according to the standard operational procedures declared in the form ISO 14380:2011(E) [193]. The lethal responses of the microorganisms were measured after 24h of incubation at 25°C.

1.5.2 *Alivibrio fischeri*

Toxicity screening of the three PFAS samples was examined with a standard inhibition test using the luminescent bacterium *Aliivibrio fischeri* (ISO 11348-1, 2009) [172]. Freeze-dried bacteria, reconstitution solution, diluent (2% NaCl) and an adjustment solution (non-toxic 22% sodium chloride) were obtained from Azur (Milan, Italy). Samples were prepared in a medium containing 2% sodium chloride. Changes in bioluminescence were recorded after 5, 15 and 30 minutes of incubation at 15 °C, using a Microtox Model 500 toxicity analyzer.

1.5.3 *In silico*

In silico toxicity was evaluated regarding LC50 values on fish (96h LC50 values), daphnids (48h LC50 values) and green algae (96h LC50 values) using the ECOSAR tool (developed by EPA) -based on Quantitative Structure-Activity Relationship (QSAR) mathematical models.

2. Results

2.1 Degradation rate

Individual solutions of PFOA, PFOS and PFHxA both in MilliQ and in groundwater matrices were treated using surface plasma discharge, performing all the experiments in triplicates for better evaluating the degradation rate. Figure 23 summarizes the degradation rates of the three compounds in individual solutions of both matrices. For all the substances, the degradation started immediately after treatment but followed different rates. More specifically, the best results were obtained for PFOS, showing a 100% removal in MilliQ and 85% in groundwater matrix after 30 minutes of treatment. PFHxA showed a 40% removal after 30 minutes of treatment in MilliQ and a 35% removal in real water, while PFOA showed a 50% removal at the end of the treatment time, regardless the matrix.

When the compounds were treated in a mixture, the degradation rate was slower for all of them, as shown in figure 23D, due to the competition among the substrates for the produced reactive species. For that reason, the treatment time was extended to 60 minutes. In general, the degradation profiles were similar to those obtained for individual treatments. More specifically, degradation of PFOS showed again the best results, with 85% of removal in MilliQ and 79% in groundwater after the 30 minutes of treatment, with a complete removal after 40 minutes of treatment in both matrices. PFOA in MilliQ water showed a 40% removal after 30 minutes of treatment, which arrived to 50% after 1 hour, and 32% removal after 30 minutes when prepared in groundwater, which eventually achieved a 44% removal after 1 hour. Finally, PFHxA showed the lowest removal when treated in the mix as well, with 26% after 30 minutes of treatment in MilliQ and 21% in groundwater, arriving to 45% and 32% removal after 1 hour respectively.

In all cases, an exponential decay of the concentration following a pseudo first order kinetics was observed, with those in groundwater showing a lower degradation rate, indicating effects from unknown constituents in the water. The results obtained from this study are in accordance with those reported in literature, with longer chain compounds (with more than 8 atoms of carbon) showing higher degradation rates, and those with shorter chains (with less than 8 atoms of carbon) being more recalcitrant.
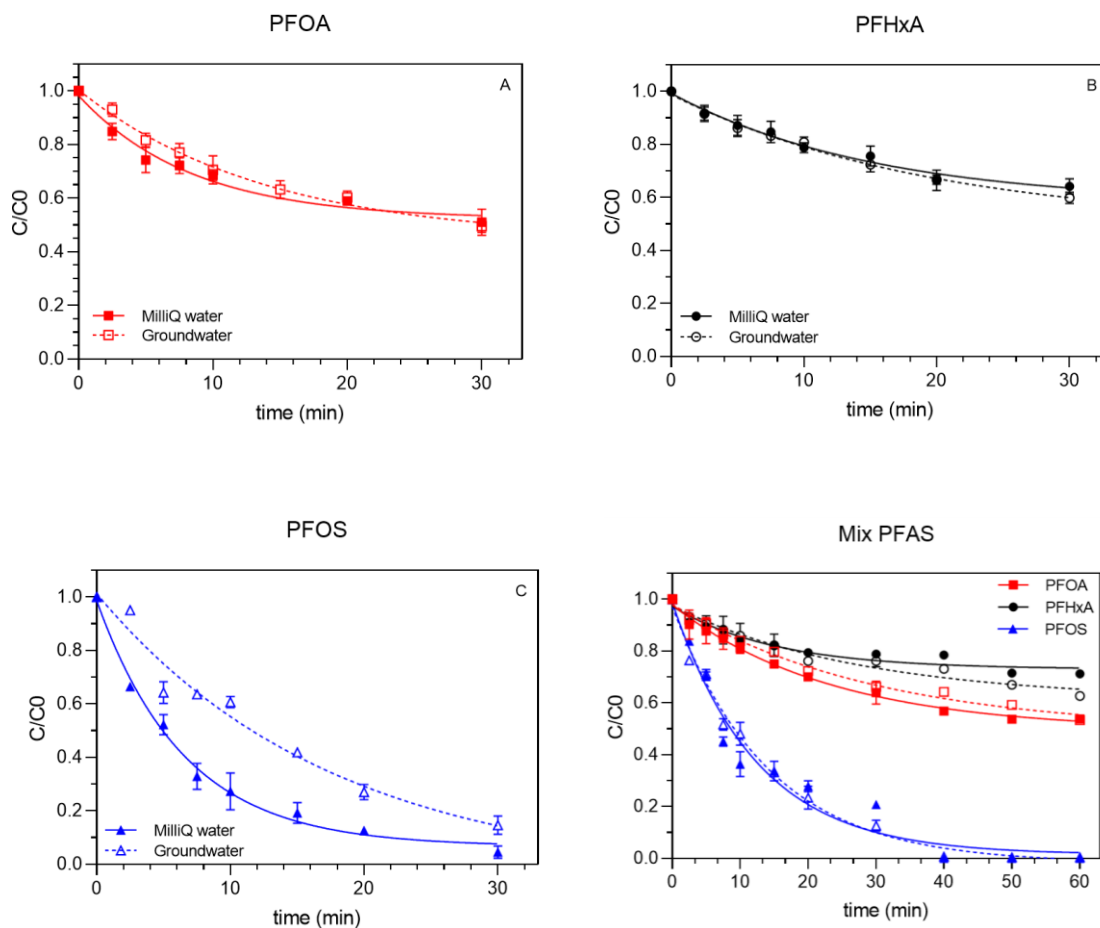
Figure 23: Degradation rate of PFOA (A), PFHxA (B) and PFOS (C) treated in individual solutions prepared in MilliQ water and groundwater matrices. Degradation of PFAS treated as mixture (D) in MilliQ (solid symbols, solid lines) and groundwater (open symbols, dashed lines).

2.2 Generation of byproducts

The identification of byproducts was challenging due to the lack of commercially available standards. However, after an HRMS analysis using the SWATH acquisition mode, identification of potential byproducts was achieved, based on mass accuracy of the measured molecular ions [M-H]$^-$ within 5 ppm, isotopic patterns, retention times and MS/MS spectra comparison between theoretical and found m/z features. A suspect screening method was followed, focusing on compounds with compatible elemental composition to PFASs. More specifically, only compounds with a maximum number of $C_8O_4F_{18}N_5H_{18}S_2$ were considered. For those that matches with online libraries were not found, *In silico* fragmentation was performed using ChemSpider.

Previous studies have shown that plasma water treatment technologies are able to degrade longer chain PFASs into short-chain Perfluorocarboxylic acids (PFCAs) and Perfluorosulfonic acids (PFSAs) [194]. The most common byproducts from PFOA and PFOS degradation are found to be the following: Perfluoroheptanoic acid (PFHpA), Perlfuorohexanoic acid (PFHxA), Perfluoropentanoic acid (PFHPeA), Perfluorobutanoic acid (PFBA), Perfluorobutane sulfonate (PFBS) and Perfluorohexane sulfonate (PFHxS), with the last two being reported only for PFOS.

In this study, the above mentioned shorter chain compounds were detected as well (Table 20), confirming the results found in literature. More specifically, after treatment of PFOA's solution treatment individually, all the four previously mentioned compounds were detected. PFHpA and PFHxA were firstly detected after 2,5 minutes of treatment, while for PFPeA and PFBA the first time was after 5 minutes of treatment. A quantification study of the byproducts was not possible – mainly due to lack of analytical standards - and hence specific information about their concentrations cannot be provided. However, the byproducts' peak areas were considered in order to understand their formation rate over time. A peak area increase was observed for all four byproducts until the end of the experiment (30 minutes) (Figure 24). These results confirm PFOA's already known degradation pathway, based on the deterioration of the carbon chain: PFOA (C8) > PFHpA (C7) > PFHxA (C6) > PFPeA (C5) > PFBA (C4).
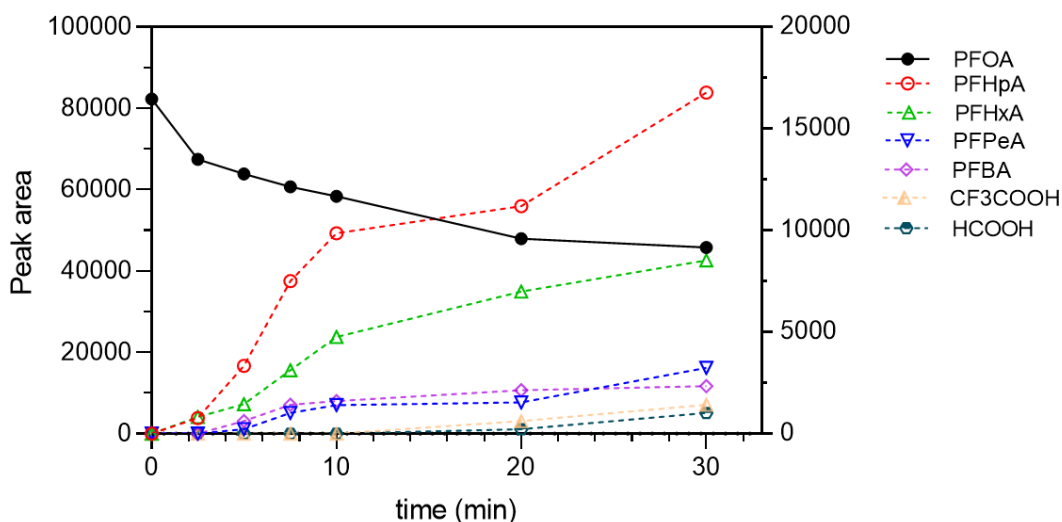

Figure 24: Generation of byproducts, after individual treatment of PFOA in MilliQ water matrix.

Concerning PFOS, the first byproduct was PFOA and was detected after 2,5 minutes, while after 5 minutes PFHpA, PFHpS and PFHxS were detected, and finally PFBA, PFPeS

and PFBS firstly occurred after 7,5 minutes. These results indicate a gradual degradation of the carbon chain as well, starting from eight atoms of carbon and arriving at four. However, PFHxA and PFPeA were not detected at all during PFOS treatment. The peak areas for all the identified byproducts were increasing with the treatment time, arriving at a highest point at 30 minutes of treatment (Figure 25). Finally, in treated solutions of both individual compounds, further mineralization of the byproducts was observed, since CF_3COOH and $HCOOH$ were detected after 20 minutes of treatment, with their peak areas increasing until the end of the treatment time (Figure 24,25).
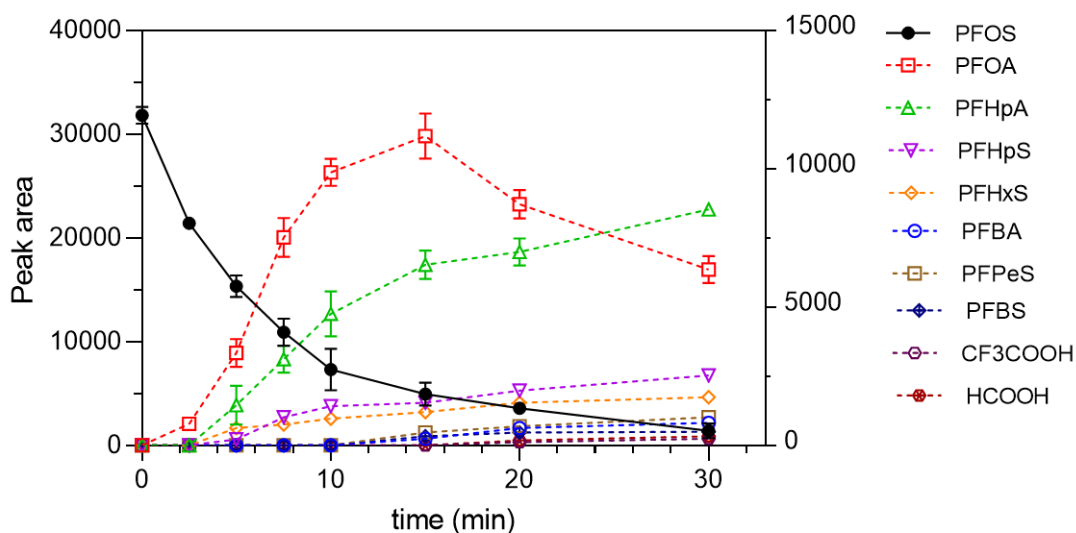

Figure 25: Generation of byproducts, after individual treatment of PFOS in MilliQ water matrix.

Concerning PFHxA - the shorter chain target molecule, which resulted to be also the most recalcitrant - PFBA and PFPeA were identified as its byproducts, molecules with carbon chains containing four and five atoms of carbon respectively. Both molecules were detected after 2,5 minutes of treatment and their peak areas were increasing with the treatment time as well. In this case CF_3COOH was detected after 20 minutes of treatment. Interestingly, the moiety •C_4F_9 was detected in the treated PFHxA solution after the 30 minutes interval, indicating the fact that further treatment could potentially result to a higher degradation rate.

Table 20. Detected byproducts after the individual treatment of PFOA, PFOS and PFHxA, in both MilliQ water and groundwater matrices.

Molecular formula	RT (min)	Observed m/z	Library Identification
PFOA byproducts			
$C_7HF_{13}O_2$	9,91	363,997	PFHpA
$C_6HF_{11}O_2$	6,89	313,992	PFHxA
$C_5HF_9O_2$	3,96	263,952	PFPeA
$C_4HF_7O_2$	1,53	214,002	PFBA
$C_2HF_3O_2$	1,99	114,939	CF_3COOH
CH_2O_2	1,16	46,028	HCOOH
C_2HO_2	1,02	57,031	-
$C_3H_5F_3O$	4,76	114,029	-
$C_3HF_7O_6$	1,03	265,967	-
$C_4H_5F_3O_2$	1,09	142,025	-
$C_5H_6F_6$	2,64	180,038	-
C_5HF_9O	1,05	247,989	-
$C_6H_{11}F_3$	9,13	140,150	-
$C_6H_2F_{10}$	7,01	279,996	-
$C_6H_2F_{13}O_2$	5,03	352,060	-
$C_6HF_9O_3$	9,15	291,060	-
$C_7H_6F_{10}O$	1,03	296,011	-
C_7HF_{15}	9,41	369,986	-
$C_8H_3F_9O_4$	9,06	333,989	-
$C_8H_5F_9O_3$	10,38	320,011	-
PFOS byproducts			
$C_8HF_{15}O_2$	10,44	413,985	PFOA
$C_7HF_{13}O_2$	9,93	365,001	PFHpA
$C_7HF_{15}O_3S$	13,26	450,790	PFHpS
$C_6HF_{13}O_3S$	12,72	401,127	PFHxS
$C_5HF_{11}O_3S$	11,74	351,163	PFPeS
$C_4HF_7O_2$	1,53	215,076	PFBA

Molecular formula	RT (min)	Observed m/z	Library Identification
$C_4HF_9O_3S$	6,62	301,056	PFBS
$C_3HF_5O_3S$	2,06	212,014	PFPrS
$C_2HF_3O_2$	1,85	114,522	CF_3COOH
CH_2O_2	1,13	45,998	HCOOH
C_2HO_2	1,02	56,998	-
$C_8H_7F_{11}O_3$	8,76	360,012	-
$C_8H_7F_{11}O_2S$	7,03	376,019	-
$C_8H_6F_8O_3S$	9,00	334,018	-
$C_6H_2F_6O_2S_2$	6,09	283,941	-
$C_5H_2F_8O_3S$	2,64	293,960	-
$C_4H_5F_3O_2$	6,05	142,083	-
$C_3H_7FO_2S$	3,01	126,015	-
$C_2H_4O_4S_2$	1,03	156,018	-
PFHxA byproducts			
$C_5HF_9O_2$	3,96	264,050	PFPeA
$C_4HF_7O_2$	1,53	215,076	PFBA
$C_6H_6F_5O_2$	6,87	205,103	-
C_5HF_{11}	8,98	270,004	-
$C_5H_2F_8O_2$	6,23	246,016	-
$C_4H_5F_3O_2$	5,32	142,084	-
$C_2HF_3O_2$	1,69	114,022	CF_3COOH
$C_3H_5F_3O$	3,85	114,071	-

Moieities like $\bullet C_8F_{17}$, $\bullet C_7F_{15}$, $\bullet C_6F_{13}$, $\bullet C_5F_{11}$, and $\bullet C_4F_9$ were observed in all the treated samples. Their existence can be explained by the fact that plasma or aqueous electrons – which are the main species responsible for degradation in the non-thermal plasma treatment technique, and those generating the hydroxyl radical species as well- attack the -COOH functional group of PFCAs, which may transform to the more stable alkane form (C_8HF_{17}, C_7HF_{15}, C_6HF_{13}, C_5HF_{11}, C_4HF_9), or after addition of the $\bullet OH^-$ radical may result to the formation of alcohols ($C_8HF_{17}O$, $C_7HF_{15}O$, $C_6HF_{13}O$, $C_5HF_{11}O$, C_4HF_9O) (Figure 26 a,b). Thermally unstable alcohols could be transformed into their more stable ketone

forms (-C=O), after elimination of an -HF, caused by e^- attack. Furthermore, hydrolysis of the ketones could yield the formation of carboxylic acids ($C_7HF_{13}O_2$, $C_6HF_{11}O_2$, $C_5HF_9O_2$, $C_4HF_7O_2$, $C_2HF_3O_2$) with a loss of another -HF molecule. In this way, chain deterioration reactions for longer chain PFCAs (PFOA, PFHxA) result into shorter chain compounds (Figure 26). The degradation pathway of PFSAs seems to be similar to the one of PFCAs. More specifically, the degradation mechanism of PFOS initiates with the attack of electrons to the C-S bond forming the $\bullet C_8F_{17}$ moiety and the SO_3^- group. The chain propagation reactions of $\bullet C_8F_{17}$ follow the mechanisms described above, resulting to shorter chain PFCAs. Moreover, the moieties such as $\bullet C_7F_{15}$, $\bullet C_6F_{13}$, $\bullet C_5F_{11}$, and $\bullet C_4F_9$, may react with the SO_3^- group and form shorter chain of PFSAs (PFHpS, PFHxS, PFPeS, PFBS). The byproducts from all the three (common) degradation mechanisms, were further mineralized to CF_3COOH, $HCOOH$, and CO_2. Figures 26a and 26b summarize these degradation pathways. The identified short-chain PFAS compounds were detected in both studied matrices, in both states – individual substances and their mix.

Moreover, chemical reactions of PFASs with plasma electrons, can result to the formation of many transient or stable compounds. A number of novel byproducts (Table 20) was detected after PFOA, PFOS and PFHxA degradation, with no spectral matches in the libraries. Their chemical formulas were confirmed based on accurate mass measurements and after comparison with results obtained from their *in silico* fragmentation with ChemSpider. However, such information was not adequate for building structural formulas for all the detected byproducts, due to for example isomeric patterns. These novel byproducts, showed the degradation of the strong C-F bond (Figure 26 a,b), followed by a substitution with a H atom ($C_3H_5F_3O$, $C_5H_6F_6$, $C_6H_{11}F_3$, $C_6H_2F_{10}$, $C_6H_2F_{13}$, $C_7H_6F_{10}$, $C_8H_5F_8O_3S$, $C_5H_2F_8O_3S$, $C_5H_2F_8O_3S$, $C_4H_4F_3O_2$, $C_6H_6F_5O_2$, $C_5H_2F_8O_2$, $C_4H_5F_3O_2$) providing us with satisfactory results about the bond's stability elimination, which would mean less bioaccumulative compounds in the water bodies. Analysis of total fluorine mass in the liquid phase was carried out, but the low initial concentrations of the solutions (1 µg/L) were significantly lower than the limits of detection of the method used, and thus confirmation of these results was not done.

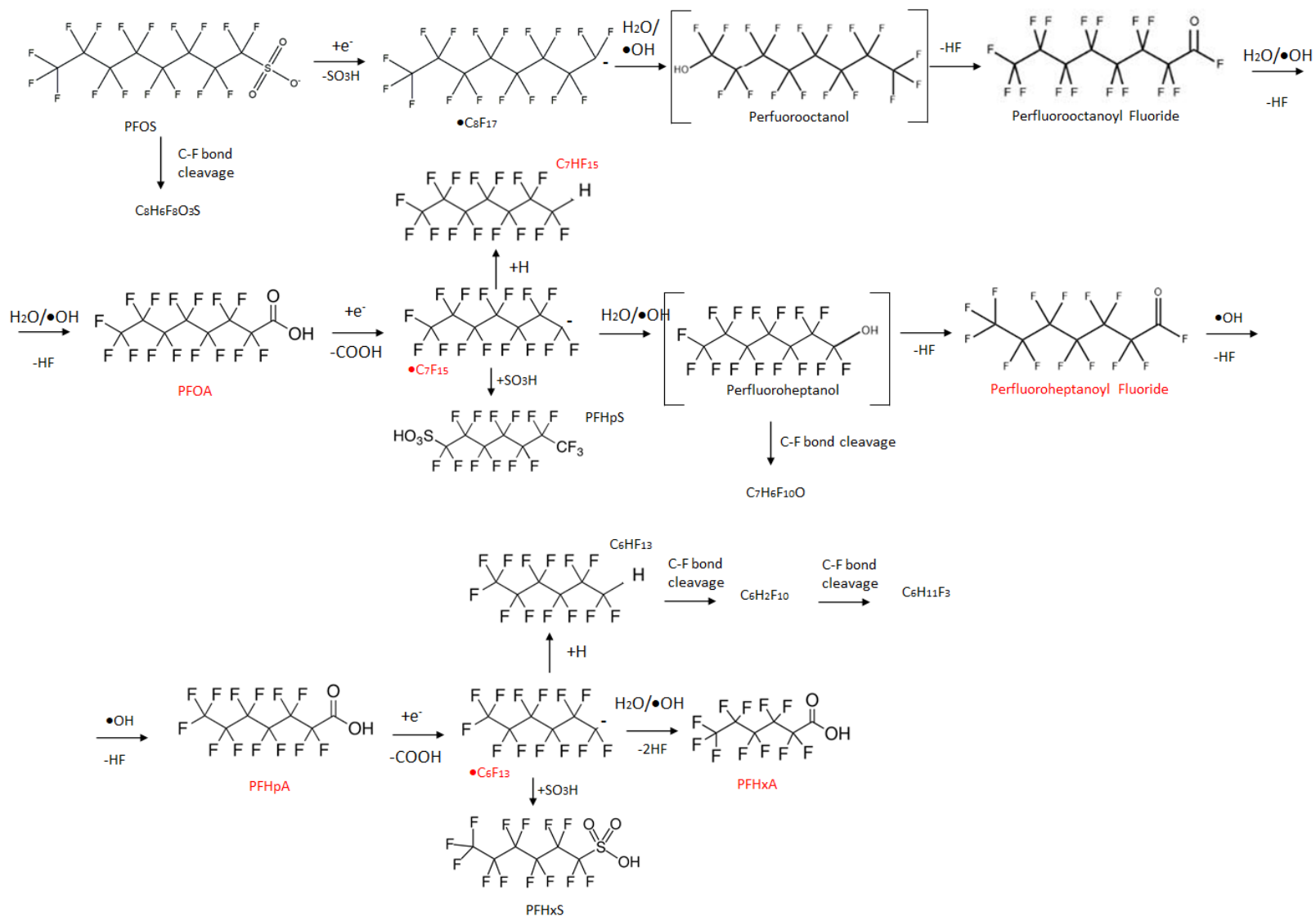**Figure 26(a):** Proposed degradation mechanism of PFOS, PFOA and PFHxA, as red are marked the common byproducts, between parenthesis are the unstable byproducts.
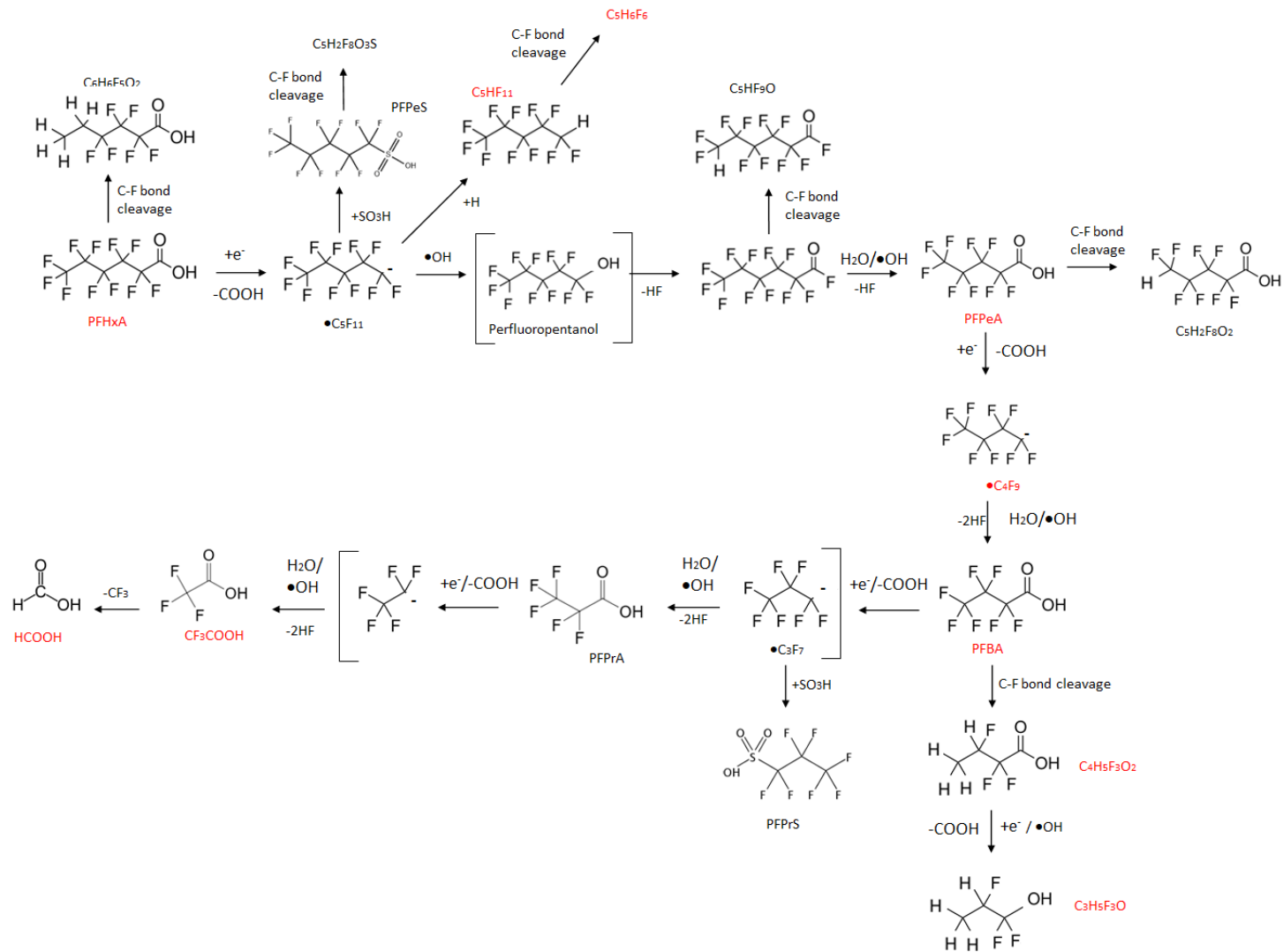
Figure 36(b): Proposed degradation mechanism of PFOS, PFOA and PFHxA, as red are marked the common byproducts, between parenthesis are the unstable byproducts.

2.3 Toxicity of byproducts

Toxicity of the samples before and after treatment was firstly evaluated using two different bioassays targeting to the crustacean *Thamnocephalus platyurus* and the bioluminescent bacteria *Alivibrio fischeri*, However, due to metals' release from the electrodes after the treatment [191], toxic effects on the two organisms after treatment was not possible to evaluate. For this reason, the potential toxicity of the 3 target analytes and their generated byproducts was predicted using the ECOSAR 2.0 program.

From the three target compounds, PFOA has been predicted as the most toxic to aquatic organisms with LC50 values of 10,1 mg/L for fish, 7,44 mg/L for Daphnid and 16,2 mg/L for Green Algae species (Table 21). The second most toxic compound was found to be PFOS, with LC50 values of 23,7 mg/L, 16,9 mg/L and 32,6 mg/L for fish, Daphnid and Green Algae species respectively (Table 21). The least toxic compound among the three target analytes was PFHxA, with LC50 values of 122 mg/L for fish, 79,3 mg/L for Daphnid and 104 mg/L for Green Algae species (Table 21). All these results are in accordance with those already published in the bibliography and are confirming the fact that shorter chain compounds have less harmful effects [46].

Moreover, a toxicity decrease was observed alongside the degradation of the carbon chain of the three target compounds, confirming once again the less harmful effects of short-chain PFAS (Table 21). In general, the toxic effects of all the detected PFSAs to aquatic organisms were predicted to be lower than their equivalent PFCAs. Interestingly, the shorter chain compound that was identified in PFOA and PFOS degradation after 20 minutes of treatment – HCOOH – was predicted to have more harmful effects than CF_3COOH. Finally, concerning the novel byproducts, toxicity information was difficult to be predicted due to data limitations in literature. Optimization of the reactor parameters for eliminating metals' release and analytical standards for identifying the novel byproducts are necessary for a more comprehensive evaluation of the toxic effects before and after treatment.

Table 21. Toxicity predictions for PFOA, PFOS, PFHxA and their detected byproducts using the ECOSAR software.

Molecular formula	Library Identification	LC50 (mg/L) Fish	LC50 (mg/L) Daphnid	EC50 (mg/L) Green Algae
$C_8HF_{15}O_2$	PFOA	10,10	7,44	16,20
$C_7HF_{13}O_2$	PFHpA	35,40	24,50	41,40
$C_6HF_{11}O_2$	PFHxA	122	79,30	104
$C_5HF_9O_2$	PFPeA	409	250	254
$C_4HF_7O_2$	PFBA	1,32E+3	761	597
$C_2HF_3O_2$	CF_3COOH	2,07E+4	1,02E+4	4,31E+3
CH_2O_2	HCOOH	6,13E+3	2,77E+3	807
C_2HO_2	-	11,80	46,70	2,32
$C_3H_5F_3O$	-	1,40E+3	705	319
$C_3HF_7O_6$	-	-	-	-
$C_4H_5F_3O_2$	-	50	112	53,60
$C_5H_6F_6$	-	5,94	3,89	5,25
C_5HF_9O	-	32,80	394	283
$C_6H_{11}F_3$	-	3,40	2,26	3,22
$C_6H_2F_{10}$	-	3,79	2,58	4,06
$C_6H_2F_{13}O_2$	-	-	-	-
$C_6HF_9O_3$	-	232	548	385
$C_7H_6F_{10}O$	-	-	-	-
C_7HF_{15}	-	0,76	0,57	1,28
$C_8H_3F_9O_4$	-	-	-	-
$C_8H_5F_9O_3$	-	41,30	85,70	36,30
$C_8HF_{17}O_3S$	PFOS	23,70	16,90	32,60
$C_7HF_{15}O_3S$	PFHpS	85	57,10	85,40
$C_6HF_{13}O_3S$	PFHxS	301	190	220
$C_5HF_{11}O_3S$	PFPeS	1,05E+3	625	560
$C_4HF_9O_3S$	PFBS	3,60E+3	2,01E+3	1,40E+3
$C_3HF_5O_3S$	PFPrS	-	-	-
$C_8H_7F_{11}O_3$	-	49,90	30,70	31,70

Molecular formula	Library Identification	LC50 (mg/L) Fish	LC50 (mg/L) Daphnid	EC50 (mg/L) Green Algae
$C_8H_7F_{11}O_2S$	-	-	-	-
$C_8H_6F_8O_3S$	-	-	-	-
$C_6H_2F_6O_2S_2$	-	-	-	-
$C_5H_2F_8O_3S$	-	0,51	0,37	0,72
$C_4H_5F_3O_2$	-	50	112	53,60
$C_3H_7FO_2S$	-	8,37	64,70	54,30
$C_2H_4O_4S_2$	-	502	1,34E+3	832
$C_6H_6F_5O_2$	-	-	-	-
C_5HF_{11}	-	8,91	5,84	7,88
$C_5H_2F_8O_2$	-	1,34E+3	775	623
$C_4H_5F_3O_2$	-	50	112	53,60
$C_3H_5F_3O$	-	1,20E+3	610	283

3. Conclusions

In this chapter, the degradation of PFOA, PFOS and PFHxA, as well as their mix, in different water matrices after treatment with a non-thermal plasma generator is reported. The compounds were chosen according to their occurrence levels in groundwater sources from the Metropolitan Area of Turin. The samples were prepared in trace level concentrations, in order to examine the efficiency of this technique in realistic conditions. The best results were obtained for PFOS in both matrices, while PFHxA showed the lowest removal. Identification of byproducts was done with LC-HRMS analysis. In summary, the identified molecules showed a reduction of the carbon chain, confirming the mechanisms already reported in literature obtained after the employment of other AOP techniques. *In silico* prediction of the toxicity before and after treatment showed that the generated byproducts have less harmful effects on aquatic organisms. Moreover, in this study, defluorination of the compounds was observed as well, highlighting the ability of this technique to break one of the strongest chemical bonds.

The results obtained for the tested compounds, significantly assisted in understanding the degradation of PFASs after the non-thermal plasma treatment, and suggest that this technology could efficiently be used for the removal of PFASs found in water sources used for drinking water production. Moreover, as the degradation results were satisfactory for one of the most recalcitrant classes of CECs, this technique could be efficient also on the removal of other contaminants. Further studies in order to understand if a large scale application of this technique, for example after the granulated activated carbon (GAC) filters in a DWTP line where the concentrations of these molecules are extremely low, are needed. A human health risk assessment for the existence of these compounds in drinking water sources was not carried out, since lack of information about the effects these compounds on human health can have. Further investigation in this field is necessary.

Section V

Conclusions

This thesis is part of the AQUAlity-ETN project, which aims to find solutions for the removal of Contaminants of Emerging Concern, present in water bodies in trace level concentrations. The main research activities of the individual project were carried out in the Research Center of Società Metropolitana Acque Torino, while secondment periods at the Chemistry and Biology Department of Aalborg University, Denmark and the Chemistry Department of Turin University and further collaborations within the consortium contributed to its conclusion.

The main goal of this thesis was to develop green and without waste generation, advanced analytical tools for evaluating the presence of CECs in different water sources and improve conventional monitoring approaches which resulted insufficient in evaluating water bodies' quality, towards the adoption of a circular economy approach. In order to take control measures for a safe and sustainable water supply, it is important to evaluate the holistic quality of water sources and identify the hazardous components, their occurrence areas, and the points at higher contamination risk. We achieved this goal by combining target, suspect and non-target chemical analyses with risk-based approaches and toxicological assessments.

Firstly, a new green, fast and cost-efficient method – following the principles of Green Analytical Chemistry - with high sensitivity in detecting a mix of sixteen different PFAS compounds in drinking water samples at trace level concentrations was developed and validated. The key characteristic of this method, was the absence of an extraction step and a direct injection into the analytical system. This method was used in an assessment of PFASs occurrence levels in the Metropolitan Area of Turin in Italy. A correlation of the "positive" sampling points and the potential pollution sources in the territory based on multivariate and spatial statistical tools, was done in order to understand their influence on the pollution levels and take decisions for reduction of contamination at source. The results showed that the number of point sources within a watershed significantly affects PFASs occurrence levels, providing us with significant predictors for guiding future choice of sampling points at higher risk.

Based on that "smart" monitoring tool and in order to avoid the costs, efforts and environmental impact of large-scale, blind monitoring assessments, a prioritization of the sites at major risks of pollution with pharmaceuticals and hormones was done for the second monitoring assessment in the study area included in this thesis. A new method following the principles of Green Analytical Chemistry as well, targeting at sixteen compounds was developed achieving low Quantification Limits for every analyte,

and validated. The method was applied in the analyses of raw water samples taken from the areas at higher risks, including surface, groundwater and treated water. The results confirmed the presence of the target compounds in the area, in concentrations of the ng/L scale, with those geographically closer to the considered pollution sources showing higher detection rates. Analysis of the samples after the treatment line showed sufficient removal of the target pollutants, minimizing human health risks and a risk assessment study was then carried out in order to evaluate the potential effects on human health, taking into account the cocktail effects of the compounds' occurrence in mixtures.

However, target analyses alone, have been proved insufficient in estimating the total quality of water bodies. For this reason, in this thesis we included a non-target screening of surface samples collected from three different locations in Greece and Italy. High resolution Mass Spectrometry using the SWATH-MS acquisition mode showed the occurrence of a vast variety of pollutants, mainly pharmaceuticals, pesticides and herbicides, PFAS and personal care products. Multivariate statistical analysis tools were used in order to identify pollution patterns and similarities among the samples, as well as to identify the compounds that are responsible for the discrimination among the samples. The use of non-target screening highlighted the need of including it as a first step in monitoring assessments, in order to evaluate the quality of water sources, prioritize the contaminants to be included in quantification studies and take decisions for treatment needs.

On the second part of this thesis, based on the results obtained from the three monitoring assessments, the removal efficiency of different degradation methods was studied. Towards a sustainable and toxic-free environment the implementation of fast, cost-efficient and environmental friendly techniques that don't generate waste or more toxic byproducts, are necessary. In this way, Advanced Oxidation Processes (AOPs) were studied as degradation techniques, within the context of Green Chemistry, taking into account byproducts generation and toxicity effects.

More specifically, first the effects of UV-A, UV-B, and UV-C irradiation on the degradation of glyphosate were studied. Different parameters that could affect the process were taken into account and their effects on toxicity to aquatic organisms from different trophic levels were studied. The effect-based monitoring approach for the evaluation of the method's efficiency was combined with chemical analyses in order to identify transformation byproducts. Toxicity assays were used as an important supplement to chemical analyses in order to assess water quality, as bioassays can integrate changes in

water chemistry and bioactivity before and after water treatment. The results suggested that UV-C and to some extend UV-B photolysis of glyphosate in water could decrease concentrations of this pesticide and reduce overall ecotoxicity by generating less toxic transformation products. Moreover, even if UV doses used in treatment plants are mainly used for disinfection processes, being not adequate to degrade organic contaminants, UV photolysis doesn't generate waste and could be a perfect candidate for implementation in WWTPs and DWTPs.

Finally, the degradation of PFOA, PFOS and PFHxA, as well as their mix, in different water matrices after treatment with a non-thermal plasma generator was studied. This technique basically applies on one or several very high voltage (HV) pulses of very short duration to a reactor containing the contaminated water samples, generating in this way pressure waves, UV light and formation of chemically active species such as $\bullet OH$, $\bullet H$, $\bullet O$, $\bullet O_2^-$, $\bullet HO_2$, $\bullet H_2O_2$, $\bullet O_3$ that can break the organic molecules. The results showed degradation of the compounds after 30 minutes of treatment individually, and 60 minutes as a mix. Identification of the byproducts was done with HRMS analysis and defluorination of the compounds was observed as well, highlighting the ability of this method to break one of the strongest chemical bonds. Toxicity before and after treatment was predicted with the ECOSAR software and showed less harmful effects on aquatic organisms alongside with reduction of the carbon chain.

In conclusion, moving towards a circular economy concept, a more sustainable water management is necessary, including access to tools for identification and detection of potentially hazardous compounds and efficient treatment techniques that don't generate additional waste, with the upper aim of water reuse. The results presented in this thesis highlight the importance of improving water quality monitoring assessments. Target analyses alone are not able to sufficiently evaluate the pollution of water bodies. Combination with non-target and risk-based approaches is fundamental in order to evaluate water bodies status more comprehensively. In the future, in order to take control measures for a safe and sustainable water supply, is important to identify the hazardous components, their occurrence areas and the points at higher contamination risk, including toxicological methods in order to minimize risk to human health and ecosystems.

References

[1]   M. Smol, C. Adam, and M. Preisner, "Circular economy model framework in the European water and wastewater sector," *J Mater Cycles Waste Manag*, vol. 22, no. 3, pp. 682–697, May 2020, doi: 10.1007/s10163-019-00960-z.

[2]   D. Abu-Ghunmi, L. Abu-Ghunmi, B. Kayal, and A. Bino, "Circular economy and the opportunity cost of not 'closing the loop' of water industry: the case of Jordan," *Journal of Cleaner Production*, vol. 131, pp. 228–236, Sep. 2016, doi: 10.1016/j.jclepro.2016.05.043.

[3]   W. R. Stahel, "History of the Circular Economy. The Historic Development of Circularity and the Circular Economy," in *The Circular Economy in the European Union: An Interim Review*, S. Eisenriegler, Ed. Cham: Springer International Publishing, 2020, pp. 7–19.

[4]   Ellen Macarthur Foundation, "Water-and-Circular-Economy-White-paper" Available online : https://www.ellenmacarthurfoundation.org/assets/downloads/ce100/Water-and-Circular-Economy-White-paper-WIP-2018-04-13.pdf (accessed on 28 January 2021).

[5]   N. S. Kakwani and P. P. Kalbar, "Review of Circular Economy in urban water sector: Challenges and opportunities in India," *Journal of Environmental Management*, vol. 271, p. 111010, Oct. 2020, doi: 10.1016/j.jenvman.2020.111010.

[6]   European Commission, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions "Towards a circular economy: A zero waste programme for Europe". 02 July 2014.

[7]   European Commission, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions "Closing the loop - An EU action plan for the Circular Economy". 02 December 2015.

[8]   J. C. G. Sousa, A. R. Ribeiro, M. O. Barbosa, M. F. R. Pereira, and A. M. T. Silva, "A review on environmental monitoring of water organic pollutants identified by EU guidelines," *J Hazard Mater*, vol. 344, pp. 146–162, Feb. 2018, doi: 10.1016/j.jhazmat.2017.09.058.

[9] W. H. Organization, *World health statistics 2019: monitoring health for the SDGs, sustainable development goals*. World Health Organization, 2019.

[10] UNICEF, WHO "1 in 3 people globally do not have access to safe drinking water." Available online: https://www.who.int/news/item/18-06-2019-1-in-3-people-globally-do-not-have-access-to-safe-drinking-water-unicef-who (accessed on 15 January 2021).

[11] OECD, "Managing Water for All, AN OECD PERSPECTIVE ON PRICING AND FINANCING KEY MESSAGES FOR POLICY MAKERS".

[12] "Use of freshwater resources in Europe — European Environment Agency." https://www.eea.europa.eu/data-and-maps/indicators/use-of-freshwater-resources-3/assessment-4 (accessed on 04 February 2021).

[13] UNESCO, WWAP (United Nations World Water Assessment Programme). 2016. The United NationsWorld Water Development Report 2016: Water and Jobs.

[14] C. J. Vörösmarty *et al.*, "Global threats to human water security and river biodiversity," *Nature*, vol. 467, no. 7315, Art. no. 7315, Sep. 2010, doi: 10.1038/nature09440.

[15] M. T. H. van Vliet, M. Flörke, and Y. Wada, "Quality matters for water scarcity," *Nature Geoscience*, vol. 10, no. 11, Art. no. 11, Nov. 2017, doi: 10.1038/ngeo3047.

[16] B. Essex, S. H. A. Koop, and C. J. Van Leeuwen, "Proposal for a National Blueprint Framework to Monitor Progress on Water-Related Sustainable Development Goals in Europe," *Environmental Management*, vol. 65, no. 1, pp. 1–18, Jan. 2020, doi: 10.1007/s00267-019-01231-1.

[17] "SDG 6 Synthesis Report 2018 on Water and Sanitation Archives," *UN-Water*. Available online: https://www.unwater.org/publication_categories/sdg-6-synthesis-report-2018-on-water-and-sanitation/ (accessed on 04 February 2021).

[18] UN General Assembly, "A/RES/70/1 Transforming our world: the 2030 Agenda for Sustainable Development," p. 35, 2030.

[19] United Nations, "Integrated monitoring guide for Sustainable Development Goal 6 on water and sanitation: targets and global indicators. The water cycle in the sustainable development goals.

[20] S. Tsani, P. Koundouri, and E. Akinsete, "Resource management and sustainable development: A review of the European water policies in accordance with the

United Nations' Sustainable Development Goals," *Environmental Science & Policy*, vol. 114, pp. 570–579, Dec. 2020, doi: 10.1016/j.envsci.2020.09.008.

[21] European Commission, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions "A European Green Deal". 11 December 2019.

[22] Interreg Europe, "A Policy Brief from the Policy Learning Platform on Environment and resource efficiency ".

[23] L. W. Mays, *Water Resources Sustainability*. McGraw Hill Professional, 2007.

[24] P. Stanchev, V. Vasilaki, J. Dosta, and E. Katsou, "Measuring the circular economy of water sector in the three-fold linkage of water, energy and materials," vol. 30, no. 04, p. 2020, 2017.

[25] P. Schroeder, K. Anggraeni, and U. Weber, "The Relevance of Circular Economy Practices to the Sustainable Development Goals," *Journal of Industrial Ecology*, vol. 23, no. 1, pp. 77–95, 2019, doi: https://doi.org/10.1111/jiec.12732.

[26] I. Eilks and F. Rauch, "Sustainable development and green chemistry in chemistry education," *Chemistry Education Research and Practice*, vol. 13, no. 2, pp. 57–58, 2012, doi: 10.1039/C2RP90003C.

[27] P. T. Anastas and J. C. Warner, *Green Chemistry: Theory and Practice*. Oxford University Press, 1998.

[28] T.-L. Chen, H. Kim, S.-Y. Pan, P.-C. Tseng, Y.-P. Lin, and P.-C. Chiang, "Implementation of green chemistry principles in circular economy system towards sustainable development goals: Challenges and perspectives," *Science of The Total Environment*, vol. 716, p. 136998, May 2020, doi: 10.1016/j.scitotenv.2020.136998.

[29] US EPA, "Basics of Green Chemistry," *US EPA*, Feb. 12, 2013. Available Online: https://www.epa.gov/greenchemistry/basics-green-chemistry (accessed on 09 February 2021).

[30] F. J. Lozano *et al.*, "New perspectives for green and sustainable chemistry and engineering: Approaches from sustainable resource and energy use, management, and transformation," *Journal of Cleaner Production*, vol. 172, pp. 227–232, Jan. 2018, doi: 10.1016/j.jclepro.2017.10.145.

[31] Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off. J. Eur. Communities L327, 1–72.

[32] Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Off. J. Eur. Union L348, 84–97.

[33] Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy. Off. J. Eur. Union L226, 1–17.

[34] Commission Implementing Decision (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council (notified under document C(2015) 1756) Text with EEA relevance, vol. 078. 2015.

[35] P. C. von der Ohe and V. Dulio, *NORMAN Prioritisation framework for emerging substances*. 2013.

[36] Directive 2020/2184/EU of the European Parliament and of the Council of 16 December 2020 on the quality of water intended for human consumption, vol. OJ L. 2020.

[37] W. Yang, H. Zhou, and N. Cicek, "Treatment of Organic Micropollutants in Water and Wastewater by UV-Based Processes: A Literature Review," *Critical Reviews in Environmental Science and Technology*, vol. 44, no. 13, pp. 1443–1476, Jul. 2014, doi: 10.1080/10643389.2013.790745.

[38] US EPA, "White Paper Aquatic Life Criteria For Contaminants of Emerging Concern PART I, General Challenges and Recommendations". 03 June 2008.

[39] D. B. Miklos, C. Remy, M. Jekel, K. G. Linden, J. E. Drewes, and U. Hübner, "Evaluation of advanced oxidation processes for water and wastewater treatment – A critical review," *Water Research*, vol. 139, pp. 118–131, Aug. 2018, doi: 10.1016/j.watres.2018.03.042.

[40] A. Stefanakis and J. Becker, "A Review of Emerging Contaminants in Water: Classification, Sources, and Potential Risks," 2015, pp. 57–82.

[41] J. W. Birkett and J. N. Lester, *Endocrine Disrupters in Wastewater and Sludge Treatment Processes*. IWA Publishing, 2002.

[42] W. Song, M. Huang, W. Rumbeiha, and H. Li, "Determination of amprolium, carbadox, monensin, and tylosin in surface water by liquid chromatography/tandem mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 21, no. 12, pp. 1944–1950, 2007, doi: https://doi.org/10.1002/rcm.3042.

[43] S. D. Richardson and S. Y. Kimura, "Water Analysis: Emerging Contaminants and Current Issues," *Anal. Chem.*, vol. 92, no. 1, pp. 473–505, Jan. 2020, doi: 10.1021/acs.analchem.9b05269.

[44] M. Haukås, U. Berger, H. Hop, B. Gulliksen, and G. W. Gabrielsen, "Bioaccumulation of per- and polyfluorinated alkyl substances (PFAS) in selected species from the Barents Sea food web," *Environ Pollut*, vol. 148, no. 1, pp. 360–371, Jul. 2007, doi: 10.1016/j.envpol.2006.09.021.

[45] Hedlund, J. Per- and Polyfluoroalkyl Substances (PFASs) in Swedish Waters. 10 June 2016. Available online: https://stud.epsilon.slu.se/9187/ (accessed on 13 December 2018).

[46] Dasu, K.; Kempisty, D.M.; Millis, M.A. Per- and polufluoroalkyl substance analysis to support site characterization, exposure, and risk management. In Perfluoroalkyl Substances in the Environment | Theory, Practice, and Innovation, 1st ed.; Series: Environmental and occupational health series; Kempisty, D.M., Xing, Y., Racz, L., Eds.; CRC Press Taylor & Francis: Boca Raton, FL, USA, 2018; pp. 35–66. ISBN 9781498764186.

[47] R. C. Buck *et al.*, "Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins," *Integr Environ Assess Manag*, vol. 7, no. 4, pp. 513–541, Oct. 2011, doi: 10.1002/ieam.258.

[48] L. Ahrens, "Polyfluoroalkyl compounds in the aquatic environment: a review of their occurrence and fate," *J Environ Monit*, vol. 13, no. 1, pp. 20–31, Jan. 2011, doi: 10.1039/c0em00373e.

[49] F. Xiao, "Emerging poly- and perfluoroalkyl substances in the aquatic environment: A review of current literature," *Water Res*, vol. 124, pp. 482–495, Nov. 2017, doi: 10.1016/j.watres.2017.07.024.

[50] M. Babut *et al.*, "Per- and poly-fluoroalkyl compounds in freshwater fish from the Rhône River: Influence of fish size, diet, prey contamination and biotransformation," *Science of The Total Environment*, vol. 605–606, pp. 38–47, Dec. 2017, doi: 10.1016/j.scitotenv.2017.06.111.

[51] F. Gosetti *et al.*, "Determination of perfluorochemicals in biological, environmental and food samples by an automated on-line solid phase extraction ultra high performance liquid chromatography tandem mass spectrometry method," *J Chromatogr A*, vol. 1217, no. 50, pp. 7864–7872, Dec. 2010, doi: 10.1016/j.chroma.2010.10.049.

[52] S. F. Nakayama *et al.*, "Worldwide trends in tracing poly- and perfluoroalkyl substances (PFAS) in the environment," *TrAC Trends in Analytical Chemistry*, vol. 121, p. 115410, Dec. 2019, doi: 10.1016/j.trac.2019.02.011.

[53] Z. Abunada, M. Y. D. Alazaiza, and M. J. K. Bashir, "An Overview of Per- and Polyfluoroalkyl Substances (PFAS) in the Environment: Source, Fate, Risk and Regulations," *Water*, vol. 12, no. 12, Art. no. 12, Dec. 2020, doi: 10.3390/w12123590.

[54] M. L. Brusseau, R. H. Anderson, and B. Guo, "PFAS concentrations in soils: Background levels versus contaminated sites," *Science of The Total Environment*, vol. 740, p. 140017, Oct. 2020, doi: 10.1016/j.scitotenv.2020.140017.

[55] M. Kotthoff *et al.*, "Per- and polyfluoroalkyl substances in the German environment – Levels and patterns in different matrices," *Science of The Total Environment*, vol. 740, p. 140116, Oct. 2020, doi: 10.1016/j.scitotenv.2020.140116.

[56] A. G. Paul, K. C. Jones, and A. J. Sweetman, "A first global production, emission, and environmental inventory for perfluorooctane sulfonate," *Environ Sci Technol*, vol. 43, no. 2, pp. 386–392, Jan. 2009, doi: 10.1021/es802216n.

[57] W. Zhang *et al.*, "Distribution and fate of perfluoroalkyl substances in municipal wastewater treatment plants in economically developed areas of China," *Environ Pollut*, vol. 176, pp. 10–17, May 2013, doi: 10.1016/j.envpol.2012.12.019.

[58] European Commission, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions "Chemicals Strategy for Sustainability Towards a Toxic-Free Environment". 14 October 2020.

[59] M. Patel, R. Kumar, K. Kishor, T. Mlsna, C. U. Pittman, and D. Mohan, "Pharmaceuticals of Emerging Concern in Aquatic Systems: Chemistry, Occurrence, Effects, and Removal Methods," *Chem. Rev.*, vol. 119, no. 6, pp. 3510–3673, Mar. 2019, doi: 10.1021/acs.chemrev.8b00299.

[60] S. Castiglioni, R. Fanelli, D. Calamari, R. Bagnati, and E. Zuccato, "Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy," *Regul Toxicol Pharmacol*, vol. 39, no. 1, pp. 25–32, Feb. 2004, doi: 10.1016/j.yrtph.2003.10.002.

[61] P. Calza, C. Medana, E. Padovano, V. Giancotti, and C. Minero, "Fate of selected pharmaceuticals in river waters," *Environ Sci Pollut Res*, vol. 20, no. 4, pp. 2262–2270, Apr. 2013, doi: 10.1007/s11356-012-1097-4.

[62] C. J. Houtman, J. Kroesbergen, K. Lekkerkerker-Teunissen, and J. P. van der Hoek, "Human health risk assessment of the mixture of pharmaceuticals in Dutch drinking water and its sources based on frequent monitoring data," *Sci Total Environ*, vol. 496, pp. 54–62, Oct. 2014, doi: 10.1016/j.scitotenv.2014.07.022.

[63] E. Zuccato *et al.*, "Pharmaceuticals in the environment in Italy: causes, occurrence, effects and control," *Environ Sci Pollut Res Int*, vol. 13, no. 1, pp. 15–21, Jan. 2006, doi: 10.1065/espr2006.01.004.

[64] P. Verlicchi, M. Al Aukidy, A. Jelic, M. Petrović, and D. Barceló, "Comparison of measured and predicted concentrations of selected pharmaceuticals in wastewater and surface water: A case study of a catchment area in the Po Valley (Italy)," *Science of The Total Environment*, vol. 470–471, pp. 844–854, Feb. 2014, doi: 10.1016/j.scitotenv.2013.10.026.

[65] L. Patrolecco, S. Capri, and N. Ademollo, "Occurrence of selected pharmaceuticals in the principal sewage treatment plants in Rome (Italy) and in the receiving surface waters," *Environ Sci Pollut Res Int*, vol. 22, no. 8, pp. 5864–5876, Apr. 2015, doi: 10.1007/s11356-014-3765-z.

[66] M. Pedrouzo, F. Borrull, E. Pocurull, and R. M. Marcé, "Presence of Pharmaceuticals and Hormones in Waters from Sewage Treatment Plants," *Water Air Soil Pollut*, vol. 217, no. 1, pp. 267–281, May 2011, doi: 10.1007/s11270-010-0585-8.

[67] P. F. Kamba, B. Kaggwa, E. I. Munanura, T. Okurut, and F. E. Kitutu, "Why regulatory indifference towards pharmaceutical pollution of the environment could be a missed opportunity in public health protection. a holistic view," *Pan Afr Med J*, vol. 27, Jun. 2017, doi: 10.11604/pamj.2017.27.77.10973.

[68] K. S. Rajmohan, R. Chandrasekaran, and S. Varjani, "A Review on Occurrence of Pesticides in Environment and Current Technologies for Their Remediation and Management," *Indian J Microbiol*, vol. 60, no. 2, pp. 125–138, Jun. 2020, doi: 10.1007/s12088-019-00841-x.

[69] R. Z. Rissoli, F. C. Abdalla, M. J. Costa, F. T. Rantin, D. J. McKenzie, and A. L. Kalinin, "Effects of glyphosate and the glyphosate based herbicides Roundup Original ® and Roundup Transorb ® on respiratory morphophysiology of bullfrog tadpoles," *Chemosphere*, vol. 156, pp. 37–44, Aug. 2016, doi: 10.1016/j.chemosphere.2016.04.083.

[70] A. H. C. Van Bruggen *et al.*, "Environmental and health effects of the herbicide glyphosate," *Science of The Total Environment*, vol. 616–617, pp. 255–268, Mar. 2018, doi: 10.1016/j.scitotenv.2017.10.309.

[71] J. Albaigés, "Environmental organic chemistry, 3rd edition, by R. P. Schwarzenbach, Ph. M. Gschwend and D. M. Imboden, New Jersey, USA, Wiley-Interscience, 2017, 1026 pp., € 144 (paperback), € 129.99 (E-book), ISBN 978-1-118-76723-8," *International Journal of Environmental Analytical Chemistry*, vol. 97, no. 4, pp. 398–399, Mar. 2017, doi: 10.1080/03067319.2017.1318869.

[72] A. Székács, M. Mörtl, and B. Darvas, "Monitoring Pesticide Residues in Surface and Ground Water in Hungary: Surveys in 1990–2015," *Journal of Chemistry*, vol. 2015, p. e717948, Sep. 2015, doi: 10.1155/2015/717948.

[73] T. Dolan, P. Howsam, D. J. Parsons, and M. J. Whelan, "Is the EU Drinking Water Directive Standard for Pesticides in Drinking Water Consistent with the Precautionary Principle?," *Environ. Sci. Technol.*, vol. 47, no. 10, pp. 4999–5006, May 2013, doi: 10.1021/es304955g.

[74] Directive 91/414/EEC of the European Parliament and of the Council of 15 July 1991 as regards the placing of plant protection products on the market.

[75] M. E. Richmond, "Glyphosate: A review of its global use, environmental impact, and potential health effects on humans and other species," *J Environ Stud Sci*, vol. 8, no. 4, pp. 416–434, Dec. 2018, doi: 10.1007/s13412-018-0517-2.

[76] C. M. Junges *et al.*, "Effectiveness evaluation of glyphosate oxidation employing the H2O2/UVC process: Toxicity assays with Vibrio fischeri and Rhinella arenarum tadpoles," *Journal of Environmental Science and Health, Part B*, vol. 48, no. 3, pp. 163–170, Feb. 2013, doi: 10.1080/03601234.2013.730011.

[77] R. Z. Rissoli, F. C. Abdalla, M. J. Costa, F. T. Rantin, D. J. McKenzie, and A. L. Kalinin, "Effects of glyphosate and the glyphosate based herbicides Roundup Original® and Roundup Transorb® on respiratory morphophysiology of bullfrog tadpoles," *Chemosphere*, vol. 156, pp. 37–44, Aug. 2016, doi: 10.1016/j.chemosphere.2016.04.083.

[78] J. Jönsson, R. Camm, and T. Hall, "Removal and degradation of glyphosate in water treatment: a review," *Journal of Water Supply: Research and Technology-Aqua*, vol. 62, no. 7, pp. 395–408, Sep. 2013, doi: 10.2166/aqua.2013.080.

[79] A. H. C. Van Bruggen *et al.*, "Environmental and health effects of the herbicide glyphosate," *Science of The Total Environment*, vol. 616–617, pp. 255–268, Mar. 2018, doi: 10.1016/j.scitotenv.2017.10.309.

[80] M. J. Davoren and R. H. Schiestl, "Glyphosate-based herbicides and cancer risk: a post-IARC decision review of potential mechanisms, policy and avenues of research," *Carcinogenesis*, vol. 39, no. 10, pp. 1207–1215, Oct. 2018, doi: 10.1093/carcin/bgy105.

[81] European Environment Agency, "European waters — Assessment of status and pressures 2018." Available online https://www.eea.europa.eu/publications/state-of-water (accessed on 15 February 2021).

[82] C. E. Nika, V. Vasilaki, A. Expósito, and E. Katsou, "Water Cycle and Circular Economy: Developing a Circularity Assessment Framework for Complex Water Systems," *Water Research*, vol. 187, p. 116423, Dec. 2020, doi: 10.1016/j.watres.2020.116423.

[83] S. K. Bopp *et al.*, "Current EU research activities on combined exposure to multiple chemicals," *Environment International*, vol. 120, pp. 544–562, Nov. 2018, doi: 10.1016/j.envint.2018.07.037.

[84] Md. M. Rahman, A. R. Md. T. Islam, and M. Shammi, "14 - Emerging trends of water quality monitoring and applications of multivariate tools," in *Water Engineering Modeling and Mathematic Tools*, P. Samui, H. Bonakdari, and R. Deo, Eds. Elsevier, 2021, pp. 271–283.

[85] J. Hollender *et al.*, "High resolution mass spectrometry-based non-target screening can support regulatory environmental monitoring and chemicals management," *Environmental Sciences Europe*, vol. 31, no. 1, p. 42, Jul. 2019, doi: 10.1186/s12302-019-0225-x.

[86] V. Dulio *et al.*, "Emerging pollutants in the EU: 10 years of NORMAN in support of environmental policies and regulations," *Environmental Sciences Europe*, vol. 30, no. 1, p. 5, Feb. 2018, doi: 10.1186/s12302-018-0135-3.

[87] A. M. Brunner *et al.*, "Integration of target analyses, non-target screening and effect-based monitoring to assess OMP related water quality changes in drinking water treatment," *Sci Total Environ*, vol. 705, p. 135779, Feb. 2020, doi: 10.1016/j.scitotenv.2019.135779.

[88] W. Brack *et al.*, "Effect-based methods are key. The European Collaborative Project SOLUTIONS recommends integrating effect-based methods for diagnosis and monitoring of water quality," *Environmental Sciences Europe*, vol. 31, no. 1, p. 10, Feb. 2019, doi: 10.1186/s12302-019-0192-2.

[89] B. I. Escher *et al.*, "Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays," *Environ Sci Technol*, vol. 48, no. 3, pp. 1940–1956, 2014, doi: 10.1021/es403899t.

[90] E. Boelee, G. Geerling, B. van der Zaan, A. Blauw, and A. D. Vethaak, "Water and health: From environmental pressures to integrated responses," *Acta Tropica*, vol. 193, pp. 217–226, May 2019, doi: 10.1016/j.actatropica.2019.03.011.

[91] S. Garrigues and M. de la Guardia, Challenges in Green Analytical Chemistry. The Royal Society of Chemistry, 2020, p. 378. doi: 10.1039/9781788016148.

[92] P. J. V. den Brink *et al.*, "Toward sustainable environmental quality: Priority research questions for Europe," *Environmental Toxicology and Chemistry*, vol. 37, no. 9, pp. 2281–2295, 2018, doi: https://doi.org/10.1002/etc.4205.

[93] M. Čelić, A. Jaén-Gil, S. Briceño-Guevara, S. Rodríguez-Mozaz, M. Gros, and M. Petrović, "Extended suspect screening to identify contaminants of emerging concern in riverine and coastal ecosystems and assessment of environmental risks," *Journal of Hazardous Materials*, vol. 404, p. 124102, Feb. 2021, doi: 10.1016/j.jhazmat.2020.124102.

[94] A. Agüera, M. J. Martínez Bueno, and A. R. Fernández-Alba, "New trends in the analytical determination of emerging contaminants and their transformation products in environmental waters," *Environ Sci Pollut Res Int*, vol. 20, no. 6, pp. 3496–3515, Jun. 2013, doi: 10.1007/s11356-013-1586-0.

[95] K. M. G. Mostofa, C.-Q. Liu, D. Vione, K. Gao, and H. Ogawa, "Sources, factors, mechanisms and possible solutions to pollutants in marine ecosystems," *Environ Pollut*, vol. 182, pp. 461–478, Nov. 2013, doi: 10.1016/j.envpol.2013.08.005.

[96] M. Huerta-Fontela, M. T. Galceran, and F. Ventura, "Occurrence and removal of pharmaceuticals and hormones through drinking water treatment," *Water Res*, vol. 45, no. 3, pp. 1432–1442, Jan. 2011, doi: 10.1016/j.watres.2010.10.036.

[97] A. V. Santos *et al.*, "Occurrence and risk assessment of pharmaceutically active compounds in water supply systems in Brazil," *Science of The Total Environment*, vol. 746, p. 141011, Dec. 2020, doi: 10.1016/j.scitotenv.2020.141011.

[98] H. Mestankova, K. Schirmer, B. I. Escher, U. von Gunten, and S. Canonica, "Removal of the antiviral agent oseltamivir and its biological activity by oxidative processes," *Environmental Pollution*, vol. 161, pp. 30–35, Feb. 2012, doi: 10.1016/j.envpol.2011.09.018.

[99] M. Taheran, S. K. Brar, M. Verma, R. Y. Surampalli, T. C. Zhang, and J. R. Valero, "Membrane processes for removal of pharmaceutically active compounds (PhACs) from water and wastewaters," *Science of The Total Environment*, vol. 547, pp. 60–77, Mar. 2016, doi: 10.1016/j.scitotenv.2015.12.139.

[100] Y. Wang *et al.*, "A Novel Tracheobronchial Stent Loaded with 125I Seeds in Patients with Malignant Airway Obstruction Compared to a Conventional Stent: A Prospective Randomized Controlled Study," *EBioMedicine*, vol. 33, pp. 269–275, Jul. 2018, doi: 10.1016/j.ebiom.2018.06.006.

[101] N. Voulvoulis, "Water reuse from a circular economy perspective and potential risks from an unregulated approach," *Current Opinion in Environmental Science & Health*, vol. 2, pp. 32–45, Apr. 2018, doi: 10.1016/j.coesh.2018.01.005.

[102] E. M. Cuerda-Correa, M. F. Alexandre-Franco, and C. Fernández-González, "Advanced Oxidation Processes for the Removal of Antibiotics from Water. An Overview," *Water*, vol. 12, no. 1, Art. no. 1, Jan. 2020, doi: 10.3390/w12010102.

[103] M. A. Oturan and J.-J. Aaron, "Advanced Oxidation Processes in Water/Wastewater Treatment: Principles and Applications. A Review," *Critical Reviews in Environmental Science and Technology*, vol. 44, no. 23, pp. 2577–2641, Dec. 2014, doi: 10.1080/10643389.2013.829765.

[104] D. Ghime and P. Ghosh, *Advanced Oxidation Processes: A Powerful Treatment Option for the Removal of Recalcitrant Organic Compounds*. IntechOpen, 2020.

[105] A. Dirany, I. Sirés, N. Oturan, A. Özcan, and M. A. Oturan, "Electrochemical Treatment of the Antibiotic Sulfachloropyridazine: Kinetics, Reaction Pathways, and Toxicity Evolution," *Environ. Sci. Technol.*, vol. 46, no. 7, pp. 4074–4082, Apr. 2012, doi: 10.1021/es204621q.

[106] T. Katagi, "Direct photolysis mechanism of pesticides in water," *Journal of Pesticide Science*, vol. 43, no. 2, pp. 57–72, 2018, doi: 10.1584/jpestics.D17-081.

[107] B. A. Wols and C. H. M. Hofman-Caris, "Review of photochemical reaction constants of organic micropollutants required for UV advanced oxidation processes in water," *Water Research*, vol. 46, no. 9, pp. 2815–2827, Jun. 2012, doi: 10.1016/j.watres.2012.03.036.

[108] Y. Chen, F. Wu, Y. Lin, N. Deng, N. Bazhin, and E. Glebov, "Photodegradation of glyphosate in the ferrioxalate system," *Journal of Hazardous Materials*, vol. 148, no. 1, pp. 360–365, Sep. 2007, doi: 10.1016/j.jhazmat.2007.02.044.

[109] G. R. M. Echavia, F. Matzusawa, and N. Negishi, "Photocatalytic degradation of organophosphate and phosphonoglycine pesticides using TiO2 immobilized on silica gel," *Chemosphere*, vol. 76, no. 5, pp. 595–600, Jul. 2009, doi: 10.1016/j.chemosphere.2009.04.055.

[110] S. Malato, J. Blanco, M. I. Maldonado, P. Fernández-Ibáñez, and A. Campos, "Optimising solar photocatalytic mineralisation of pesticides by adding inorganic oxidising species; application to the recycling of pesticide containers," *Applied Catalysis B: Environmental*, vol. 28, no. 3, pp. 163–174, Dec. 2000, doi: 10.1016/S0926-3373(00)00175-2.

[111] A. Manassero, C. Passalia, A. C. Negro, A. E. Cassano, and C. S. Zalazar, "Glyphosate degradation in water employing the H2O2/UVC process," *Water Research*, vol. 44, no. 13, pp. 3875–3882, Jul. 2010, doi: 10.1016/j.watres.2010.05.004.

[112] B. R. Locke, M. Sato, P. Sunka, M. R. Hoffmann, and J.-S. Chang, "Electrohydraulic Discharge and Nonthermal Plasma for Water Treatment," *Ind. Eng. Chem. Res.*, vol. 45, no. 3, pp. 882–905, Feb. 2006, doi: 10.1021/ie050981u.

[113] M. Tezuka and M. Iwasaki, "Plasma induced degradation of chlorophenols in an aqueous solution," *Thin Solid Films*, vol. 316, no. 1, pp. 123–127, Mar. 1998, doi: 10.1016/S0040-6090(98)00401-5.

[114] P. Murugesan, E. M. V., J. A. Moses, and C. Anandharamakrishnan, "Water decontamination using non-thermal plasma: Concepts, applications, and

prospects," *Journal of Environmental Chemical Engineering*, vol. 8, no. 5, p. 104377, Oct. 2020, doi: 10.1016/j.jece.2020.104377.

[115] W. Sang *et al.*, "Degradation of liquid phase N,N-dimethylformamide by dielectric barrier discharge plasma: Mechanism and degradation pathways," *Chemosphere*, vol. 236, p. 124401, Dec. 2019, doi: 10.1016/j.chemosphere.2019.124401.

[116] M. A. Malik, "Water Purification by Plasmas: Which Reactors are Most Energy Efficient?," *Plasma Chem Plasma Process*, vol. 30, no. 1, pp. 21–31, Feb. 2010, doi: 10.1007/s11090-009-9202-2.

[117] S. T. Wolf and W. K. Reagen, "Method and validation for the analysis of perfluorinated compounds in water by pre-sampling isotope dilution-direct injection-LC/MS/MS," *Anal. Methods*, vol. 5, no. 10, pp. 2444–2454, May 2013, doi: 10.1039/C3AY26347A.

[118] V. I. Furdui, P. W. Crozier, E. J. Reiner, and S. A. Mabury, "Trace level determination of perfluorinated compounds in water by direct injection," *Chemosphere*, vol. 73, no. 1, Supplement, pp. S24–S30, Aug. 2008, doi: 10.1016/j.chemosphere.2007.07.085.

[119] L. Ciofi *et al.*, "Applicability of the direct injection liquid chromatographic tandem mass spectrometric analytical approach to the sub-ngL-1 determination of perfluoro-alkyl acids in waste, surface, ground and drinking water samples," *Talanta*, vol. 176, pp. 412–421, Jan. 2018, doi: 10.1016/j.talanta.2017.08.052.

[120] H. Gu, G. Liu, J. Wang, A.-F. Aubry, and M. E. Arnold, "Selecting the correct weighting factors for linear and quadratic calibration curves with least-squares regression algorithm in bioanalytical LC-MS/MS assays and impacts of using incorrect weighting factors on curve stability, data quality, and assay performance," *Anal Chem*, vol. 86, no. 18, pp. 8959–8966, Sep. 2014, doi: 10.1021/ac5018265.

[121] S. Taniyasu *et al.*, "Analysis of fluorotelomer alcohols, fluorotelomer acids, and short- and long-chain perfluorinated acids in water and biota," *J Chromatogr A*, vol. 1093, no. 1–2, pp. 89–97, Nov. 2005, doi: 10.1016/j.chroma.2005.07.053.

[122] N. Yamashita *et al.*, "Analysis of perfluorinated acids at parts-per-quadrillion levels in seawater using liquid chromatography-tandem mass spectrometry," *Environ Sci Technol*, vol. 38, no. 21, pp. 5522–5528, Nov. 2004, doi: 10.1021/es0492541.

[123] K. Wille *et al.*, "A validated analytical method for the determination of perfluorinated compounds in surface-, sea- and sewagewater using liquid chromatography coupled to time-of-flight mass spectrometry," *J Chromatogr A*, vol. 1217, no. 43, pp. 6616–6622, Oct. 2010, doi: 10.1016/j.chroma.2010.03.054.

[124] ISO 25101:2009. Water quality — Determination of perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) — Method for unfiltered samples using solid phase extraction and liquid chromatography/mass spectrometry, International Standards Organisation, Geneva, 2019.

[125] O. of R. & Development, "Method 537.1: Determination of Selected Per- and Polyfluorinated Alkyl Substances in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." https://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=343042&Lab= NERL (accessed May 09, 2021).

[126] ISO/IEC 17025. Testing and calibration laboratories, International Standards Organisation, Geneva, 2017.

[127] S. A. Snyder, *Removal of EDCs and pharmaceuticals in drinking and reuse treatment processes*. IWA Publishing, 2007.

[128] K. A. Heys, R. F. Shore, M. G. Pereira, K. C. Jones, and F. L. Martin, "Risk assessment of environmental mixture effects," *RSC Adv.*, vol. 6, no. 53, pp. 47844–47857, May 2016, doi: 10.1039/C6RA05406D.

[129] M. Al Aukidy, P. Verlicchi, A. Jelic, M. Petrovic, and D. Barcelò, "Monitoring release of pharmaceutical compounds: occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy," *Sci Total Environ*, vol. 438, pp. 15–25, Nov. 2012, doi: 10.1016/j.scitotenv.2012.08.061.

[130] Y. Yang, Y. S. Ok, K.-H. Kim, E. E. Kwon, and Y. F. Tsang, "Occurrences and removal of pharmaceuticals and personal care products (PPCPs) in drinking water and water/sewage treatment plants: A review," *Sci Total Environ*, vol. 596–597, pp. 303–320, Oct. 2017, doi: 10.1016/j.scitotenv.2017.04.102.

[131] S. Foteinis, A. G. L. Borthwick, Z. Frontistis, D. Mantzavinos, and E. Chatzisymeon, "Environmental sustainability of light-driven processes for wastewater treatment applications," *Journal of Cleaner Production*, vol. 182, pp. 8–15, May 2018, doi: 10.1016/j.jclepro.2018.02.038.

[132] S. J, B. W, and Y. X, "Organic contaminants removal by the technique of pulsed high-voltage discharge in water.," *J Hazard Mater*, vol. 171, no. 1–3, pp. 924–931, Jul. 2009, doi: 10.1016/j.jhazmat.2009.06.134.

[133] ISO 5667-1:2020. Water quality — Sampling, International Standards Organisation, Geneva, 2020.

[134] GeoPortale ARPA Piemonte. Available online: http://webgis.arpa.piemonte.it/geoportale/ (accessed on 10 October 2018).

[135] Story Map Series. Available online: http://webgis.arpa.piemonte.it/monitoraggio_qualita_acque_mapseries/monitor aggio_qualita_acque_webapp/ (accessed on 13 October 2018).

[136] DIVA-GIS | Free, Simple & Effective. Available online: http://www.diva-gis.org/ (accessed on 11 November 2018).

[137] X. C. Hu *et al.*, "Detection of Poly- and Perfluoroalkyl Substances (PFASs) in U.S. Drinking Water Linked to Industrial Sites, Military Fire Training Areas, and Wastewater Treatment Plants," *Environ. Sci. Technol. Lett.*, vol. 3, no. 10, pp. 344–350, Oct. 2016, doi: 10.1021/acs.estlett.6b00260.

[138] H. Gu, G. Liu, J. Wang, A.-F. Aubry, and M. E. Arnold, "Selecting the Correct Weighting Factors for Linear and Quadratic Calibration Curves with Least-Squares Regression Algorithm in Bioanalytical LC-MS/MS Assays and Impacts of Using Incorrect Weighting Factors on Curve Stability, Data Quality, and Assay Performance," *Anal. Chem.*, vol. 86, no. 18, pp. 8959–8966, Sep. 2014, doi: 10.1021/ac5018265.

[139] Guideline, ICH Harmonised Tripartite. Validation of Analytical Procedures: Text and Methodology Q2 (R1); International Conference on Harmonization: Geneva, Switzerland, 2005; pp. 11–12.

[140] D19 Committee, "Test Method for Determination of Perfluorinated Compounds in Water, Sludge, Influent, Effluent and Wastewater by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)," ASTM International. doi: 10.1520/D7979-17.

[141] D. Gamerman and A. R. B. Moreira, "Multivariate spatial regression models," *Journal of Multivariate Analysis*, vol. 91, no. 2, pp. 262–281, Nov. 2004, doi: 10.1016/j.jmva.2004.02.016.

[142] ARPA Piemonte. Available online: http://www.arpa.piemonte.it/approfondimenti/temi-ambientali/aia/documentazione-e-dati-ambientali (accessed on 10 March 2021).

[143] M. Schriks, M. B. Heringa, M. M. E. van der Kooi, P. de Voogt, and A. P. van Wezel, "Toxicological relevance of emerging contaminants for drinking water quality," *Water Res*, vol. 44, no. 2, pp. 461–476, Jan. 2010, doi: 10.1016/j.watres.2009.08.023.

[144] US EPA. Estimation Programs Interface Suite™ for Microsoft® Windows; v 4.11; United States Environmental Protection Agency: Washington, DC, USA, 2012.

[145] L. J. Carter, B. Chefetz, Z. Abdeen, and A. B. A. Boxall, "Emerging investigator series: towards a framework for establishing the impacts of pharmaceuticals in wastewater irrigation systems on agro-ecosystems and human health," *Environ. Sci.: Processes Impacts*, vol. 21, no. 4, pp. 605–622, Apr. 2019, doi: 10.1039/C9EM00020H.

[146] S. C. Walpole, D. Prieto-Merino, P. Edwards, J. Cleland, G. Stevens, and I. Roberts, "The weight of nations: an estimation of adult human biomass," *BMC Public Health*, vol. 12, no. 1, p. 439, Jun. 2012, doi: 10.1186/1471-2458-12-439.

[147] C. M. de Jongh, P. J. F. Kooij, P. de Voogt, and T. L. ter Laak, "Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water," *Sci Total Environ*, vol. 427–428, pp. 70–77, Jun. 2012, doi: 10.1016/j.scitotenv.2012.04.010.

[148] M. C. N. Pais and E. de S. Nascimento, "Guideline values and human risk assessment for the presence of anti-inflammatory drugs remaining in drinking water after lab scale treatment," *Brazilian Journal of Pharmaceutical Sciences*, vol. 54, no. 1, 2018, doi: 10.1590/s2175-97902018000117320.

[149] L.-T. Qin *et al.*, "Ecological and human health risk of sulfonamides in surface water and groundwater of Huixian karst wetland in Guilin, China," *Sci Total Environ*, vol. 708, p. 134552, Mar. 2020, doi: 10.1016/j.scitotenv.2019.134552.

[150] S. Castiglioni *et al.*, "Data on occurrence and fate of emerging contaminants in a urbanised area," *Data in Brief*, vol. 17, pp. 533–543, Apr. 2018, doi: 10.1016/j.dib.2018.01.029.

[151] R. Loos *et al.*, "Pan-European survey on the occurrence of selected polar organic persistent pollutants in ground water," *Water Res*, vol. 44, no. 14, pp. 4115–4126, Jul. 2010, doi: 10.1016/j.watres.2010.05.032.

[152] L. M. Bexfield, P. L. Toccalino, K. Belitz, W. T. Foreman, and E. T. Furlong, "Hormones and Pharmaceuticals in Groundwater Used As a Source of Drinking Water Across the United States," *Environ. Sci. Technol.*, vol. 53, no. 6, pp. 2950–2960, Mar. 2019, doi: 10.1021/acs.est.8b05592.

[153] A. J. Ebele, T. Oluseyi, D. S. Drage, S. Harrad, and M. Abou-Elwafa Abdallah, "Occurrence, seasonal variation and human exposure to pharmaceuticals and personal care products in surface water, groundwater and drinking water in Lagos State, Nigeria," *Emerging Contaminants*, vol. 6, pp. 124–132, Jan. 2020, doi: 10.1016/j.emcon.2020.02.004.

[154] J. Lv, L. Zhang, Y. Chen, B. Ye, J. Han, and N. Jin, "Occurrence and distribution of pharmaceuticals in raw, finished, and drinking water from seven large river basins in China," *J Water Health*, vol. 17, no. 3, pp. 477–489, Jun. 2019, doi: 10.2166/wh.2019.250.

[155] X. Jiang *et al.*, "PPCPs in a drinking water treatment plant in the Yangtze River Delta of China: Occurrence, removal and risk assessment," *Front. Environ. Sci. Eng.*, vol. 13, no. 2, p. 27, Mar. 2019, doi: 10.1007/s11783-019-1109-4.

[156] E. Vulliet, C. Cren-Olivé, and M.-F. Grenier-Loustalot, "Occurrence of pharmaceuticals and hormones in drinking water treated from surface waters," *Environ Chem Lett*, vol. 9, no. 1, pp. 103–114, Mar. 2011, doi: 10.1007/s10311-009-0253-7.

[157] S. D, K. R, S. T, A. M, N. T, and K. S, "Occurrence of selected pharmaceuticals at drinking water purification plants in Japan and implications for human health.," *Water Res*, vol. 76, pp. 187–200, Mar. 2015, doi: 10.1016/j.watres.2015.02.059.

[158] I. Fuoco *et al.*, "Geochemical modeling of chromium release in natural waters and treatment by RO/NF membrane processes," *Chemosphere*, vol. 254, p. 126696, Sep. 2020, doi: 10.1016/j.chemosphere.2020.126696.

[159] K. A. Baken, R. M. A. Sjerps, M. Schriks, and A. P. van Wezel, "Toxicological risk assessment and prioritization of drinking water relevant contaminants of emerging concern," *Environ Int*, vol. 118, pp. 293–303, Sep. 2018, doi: 10.1016/j.envint.2018.05.006.

[160] Y. Ben, C. Fu, M. Hu, L. Liu, M. H. Wong, and C. Zheng, "Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review," *Environmental Research*, vol. 169, pp. 483–493, Feb. 2019, doi: 10.1016/j.envres.2018.11.040.

[161] T. Backhaus and M. Faust, "Predictive environmental risk assessment of chemical mixtures: a conceptual framework," *Environ Sci Technol*, vol. 46, no. 5, pp. 2564–2573, Mar. 2012, doi: 10.1021/es2034125.

[162] K. A. Heys, R. F. Shore, M. G. Pereira, K. C. Jones, and F. L. Martin, "Risk assessment of environmental mixture effects," *RSC Adv.*, vol. 6, no. 53, pp. 47844–47857, May 2016, doi: 10.1039/C6RA05406D.

[163] G. M. Bruce, R. C. Pleus, and S. A. Snyder, "Toxicological Relevance of Pharmaceuticals in Drinking Water," *Environ. Sci. Technol.*, vol. 44, no. 14, pp. 5619–5626, Jul. 2010, doi: 10.1021/es1004895.

[164] Caffeine—ECHA. Available online: https://echa.europa.eu/registration-dossier/-/registered-dossier/10085/7/6/2 (accessed on 28 January 2021).

[165] World Health Organization, *Pharmaceuticals in drinking-water.* Geneva, Switzerland: World Health Organization, 2012.

[166] Drugs@FDA: FDA-Approved Drugs. Available online: https://www.accessdata.fda.gov/scripts/cder/daf/ (accessed on 28 January 2021).

[167] T. Pluskal, S. Castillo, A. Villar-Briones, and M. Orešič, "MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data," *BMC Bioinformatics*, vol. 11, no. 1, p. 395, Jul. 2010, doi: 10.1186/1471-2105-11-395.

[168] D. Kessner, M. Chambers, R. Burke, D. Agus, and P. Mallick, "ProteoWizard: open source software for rapid proteomics tools development," *Bioinformatics*, vol. 24, no. 21, pp. 2534–2536, Nov. 2008, doi: 10.1093/bioinformatics/btn323.

[169] E. L. Schymanski *et al.*, "Non-target screening with high-resolution mass spectrometry: critical review using a collaborative trial on water analysis," *Anal Bioanal Chem*, vol. 407, no. 21, pp. 6237–6255, Aug. 2015, doi: 10.1007/s00216-015-8681-7.

[170] P. Gago-Ferrero, E. L. Schymanski, A. A. Bletsou, R. Aalizadeh, J. Hollender, and N. S. Thomaidis, "Extended Suspect and Non-Target Strategies to Characterize Emerging Polar Organic Contaminants in Raw Wastewater with LC-HRMS/MS,"

Environ. Sci. Technol., vol. 49, no. 20, pp. 12333–12341, Oct. 2015, doi: 10.1021/acs.est.5b03454.

[171] L. L. Hohrenk, M. Vosough, and T. C. Schmidt, "Implementation of Chemometric Tools To Improve Data Mining and Prioritization in LC-HRMS for Nontarget Screening of Organic Micropollutants in Complex Water Matrixes," *Anal. Chem.*, vol. 91, no. 14, pp. 9213–9220, Jul. 2019, doi: 10.1021/acs.analchem.9b01984.

[172] ISO 11348-1. Determination of the Inhibitory Effect of Water Samples on the Light Emission of Vibrio fischeri (Luminescent bacteria test), International Standards Organisation, Geneva, 2007.

[173] ISO 8692. Water Quality; Fresh Water Algal Growth Inhibition Test with Unicellular Green Algae, International Standards Organisation, Geneva, 2012.

[174] ISO 6341. Water Quality—Determination of the Inhibition of the Mobility of Daphnia magna Straus (Cladocera, Crustacea)—Acute Toxicity Test, International Standards Organisation, Geneva, 2012.

[175] L. R. Hansen and P. Roslev, "Behavioral responses of juvenile Daphnia magna after exposure to glyphosate and glyphosate-copper complexes," *Aquatic Toxicology*, vol. 179, pp. 36–43, Oct. 2016, doi: 10.1016/j.aquatox.2016.08.010.

[176] C. A. Smith *et al.*, "METLIN: A Metabolite Mass Spectral Database," *Therapeutic Drug Monitoring*, vol. 27, no. 6, pp. 747–751, Dec. 2005, doi: 10.1097/01.ftd.0000179845.53213.39.

[177] C. Guijas *et al.*, "METLIN: A Technology Platform for Identifying Knowns and Unknowns," *Anal. Chem.*, vol. 90, no. 5, pp. 3156–3164, Mar. 2018, doi: 10.1021/acs.analchem.7b04424.

[178] ChemSpider. Available online: http://www.chemspider.com/Chemical-Structure.1906.html (accessed on 05 May 2021).

[179] Beate Escher and Frederic Leusch, "Bioanalytical Tools in Water Quality Assessment | IWA Publishing." eISBN: 9781780400778.

[180] M. Ørsted and P. Roslev, "A fluorescence-based hydrolytic enzyme activity assay for quantifying toxic effects of Roundup® to *Daphnia magna*: Fluorescence-based enzyme activity assay for *D. magna*," *Environ Toxicol Chem*, vol. 34, no. 8, pp. 1841–1850, Aug. 2015, doi: 10.1002/etc.2997.

[181] M. Cuhra, T. Traavik, and T. Bøhn, "Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in Daphnia magna," *Ecotoxicology*, vol. 22, no. 2, pp. 251–262, Mar. 2013, doi: 10.1007/s10646-012-1021-1.

[182] R. Annett, H. R. Habibi, and A. Hontela, "Impact of glyphosate and glyphosate-based herbicides on the freshwater environment: Impact of glyphosate-based herbicides," *J. Appl. Toxicol.*, vol. 34, no. 5, pp. 458–479, May 2014, doi: 10.1002/jat.2997.

[183] M. T. K. Tsui and L. M. Chu, "Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors," *Chemosphere*, vol. 52, no. 7, pp. 1189–1197, Aug. 2003, doi: 10.1016/S0045-6535(03)00306-0.

[184] M. E. Sáenz, W. D. D. Marzio, J. L. Alberdi, and M. del C. Tortorelli, "Effects of Technical Grade and a Commercial Formulation of Glyphosate on Algal Population Growth," *Bulletin of Environmental Contamination and Toxicology*, vol. 59, no. 4, pp. 638–644, Oct. 1997, doi: 10.1007/s001289900527.

[185] J. Ma, S. Wang, P. Wang, L. Ma, X. Chen, and R. Xu, "Toxicity assessment of 40 herbicides to the green alga Raphidocelis subcapitata," *Ecotoxicology and Environmental Safety*, vol. 63, no. 3, pp. 456–462, Mar. 2006, doi: 10.1016/j.ecoenv.2004.12.001.

[186] N. Cedergreen and J. C. Streibig, "The toxicity of herbicides to non-target aquatic plants and algae: assessment of predictive factors and hazard," *Pest. Manag. Sci.*, vol. 61, no. 12, pp. 1152–1160, Dec. 2005, doi: 10.1002/ps.1117.

[187] Philip McCleaf, et al., ''Removal efficiency of multiple poly- and perfluoroalkyl substances (PFASs) in drinking water using granular activated carbon (GAC) and anion exchange (AE) column tests'', Water Research, vol. 120, pp. 77-87, Sep. 2017, doi: 10.1016/j.watres.2017.04.057.

[188] Valeria Ochoa-Herrera, Reyes Sierra-Alvarez, ''Removal of perfluorinated surfactants by sorption onto granular activated carbon, zeolite and sludge'', Chemosphere, vol. 72, no. 10, pp. 1588-1593, Aug. 2008, doi: 10.1016/j.chemosphere.2008.04.029.

[189] A. J. Lewis *et al.*, "Rapid degradation of PFAS in aqueous solutions by reverse vortex flow gliding arc plasma," *Environ. Sci.: Water Res. Technol.*, vol. 6, no. 4, pp. 1044–1057, 2020, doi: 10.1039/C9EW01050E.

[190] C. E. Schaefer *et al.*, "Electrochemical treatment of perfluorooctanoic acid and perfluorooctane sulfonate: Insights into mechanisms and application to groundwater treatment," *Chemical Engineering Journal*, vol. 317, pp. 424–432, Jun. 2017, doi: 10.1016/j.cej.2017.02.107.

[191] D. Palma *et al.*, "PFAS Degradation in Ultrapure and Groundwater Using Non-Thermal Plasma," *Molecules*, vol. 26, no. 4, Art. no. 4, Jan. 2021, doi: 10.3390/molecules26040924.

[192] "Thamnocephalus toxicity test with Thamnotoxkit F," *MicroBioTests*. https://www.microbiotests.com/toxkit/freshwater-thamnocephalus-toxicity-test-with-thamnotoxkit-f/ (accessed on 05 May 2021).

[193] ISO 14380:2011. Water quality — Determination of the acute toxicity to Thamnocephalus platyurus (Crustacea, Anostraca), International Standards Organisation, Geneva, 2011.

[194] R. Kamal Singh et al., '' Breakdown Products from Perfluorinated Alkyl Substances (PFAS) Degradation in a Plasma-Based Water Treatment Process'', Environmental Science & Technology vol. 53, no. 5, pp. 2731-2738,2019, doi: 10.1021/acs.est.8b07031.

Appendix
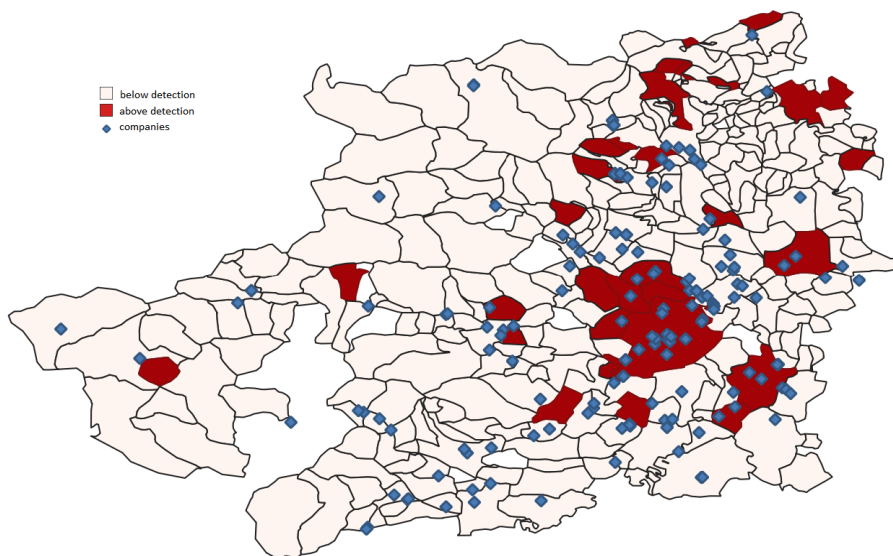
(**a**)
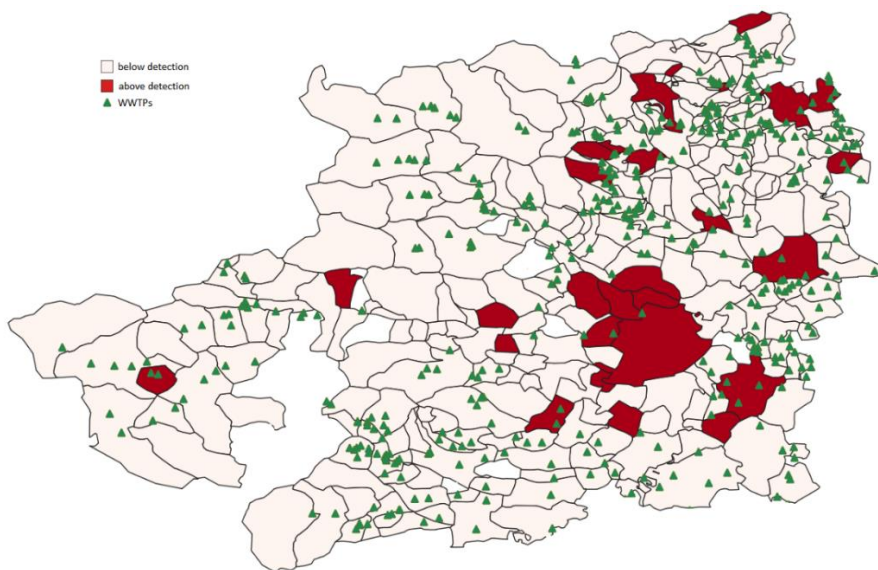
(**b**)

Figure A1: Map of the pollution levels of PFAS as a sum (limit 10 ng L^{-1}) and the selected point sources present in the studied area: (**a**) industrial sites and (**b**) waste water treatment plants, Chapter 3.

Table A1. List of the municipalities in the Metropolitan Area of Turin, Italy included in the studies reported in Chapters 3 and 4

Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID
AGLIE'	1	CHIVASSO	74	MONCALIERI	147	SAN CARLO CANAVESE	220
AIRASCA	2	CICONIO	75	MONCENISIO	148	SAN COLOMBANO BELMONTE	221
ALA DI STURA	3	CINTANO	76	MONTALDO TORINESE	149	SAN DIDERO	222
ALBIANO D'IVREA	4	CINZANO	77	MONTALENGHE	150	SAN FRANCESCO AL CAMPO	223
ALICE SUPERIORE	5	CIRIE'	78	MONTALTO DORA	151	SAN GERMANO CHISONE	224
ALMESE	6	CLAVIERE	79	MONTANARO	152	SAN GILLIO	225
ALPETTE	7	COASSOLO TORINESE	80	NICHELINO	153	SAN GIORGIO CANAVESE	226
ANDEZENO	8	COAZZE	81	NOASCA	154	SAN GIORIO DI SUSA	227
ANDRATE	9	COLLEGNO	82	NOLE	155	SAN GIUSTO CANAVESE	228
ANGROGNA	10	COLLERETTO CASTELNUOVO	83	NOMAGLIO	156	SAN MARTINO CANAVESE	229
ARIGNANO	11	COLLERETTO GIACOSA	84	NONE	157	SAN MAURIZIO CANAVESE	230
AVIGLIANA	12	CONDOVE	85	NOVALESA	158	SAN MAURO TORINESE	231

Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID
BAIRO	13	CORIO	86	OGLIANICO	159	SAN PIETRO VAL LEMINA	232
BALANGERO	14	COSSANO CANAVESE	87	ORBASSANO	160	SAN PONSO	233
BALDISSERO CANAVESE	15	CUCEGLIO	88	ORIO CANAVESE	161	SAN RAFFAELE CIMENA	234
BALDISSERO TORINESE	16	CUMIANA	89	OSASCO	162	SAN SEBASTIANO DA PO	235
BALME	17	CUORGNE'	90	OSASIO	163	SAN SECONDO DI PINEROLO	236
BANCHETTE	18	DRUENTO	91	OULX	164	SANGANO	237
BARBANIA	19	EXILLES	92	OZEGNA	165	SANT'AMBROGIO DI TORINO	238
BARDONECCHIA	20	FAVRIA	93	PANCALIERI	166	SANT'ANTONINO DI SUSA	239
BARONE CANAVESE	21	FELETTO	94	PARELLA	167	SANTENA	240
BEINASCO	22	FIANO	95	PAVAROLO	168	SAUZE DI CESANA	241
BIBIANA	23	FIORANO CANAVESE	96	PAVONE CANAVESE	169	SAUZE D'OULX	242
BOBBIO PELLICE	24	FOGLIZZO	97	PECCO	170	SCALENGHE	243
BOLLENGO	25	FORNO CANAVESE	98	PECETTO TORINESE	171	SCARMAGNO	244
BORGARO TORINESE	26	FRASSINETTO	99	PEROSA ARGENTINA	172	SCIOLZE	245
BORGIALLO	27	FRONT	100	PEROSA CANAVESE	173	SESTRIERE	246

Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID
BORGOFRANCO D'IVREA	28	FROSSASCO	101	PERTUSIO	174	SETTIMO ROTTARO	247
BORGOMASINO	29	GARZIGLIANA	102	PESSINETTO	175	SETTIMO TORINESE	248
BORGONE SUSA	30	GASSINO TORINESE	103	PIANEZZA	176	SETTIMO VITTONE	249
BOSCONERO	31	GERMAGNANO	104	PINASCA	177	SPARONE	250
BRANDIZZO	32	GIAGLIONE	105	PINEROLO	178	STRAMBINO	251
BRICHERASIO	33	GIAVENO	106	PINO TORINESE	179	SUSA	252
BROSSO	34	GIVOLETTO	107	PIOBESI TORINESE	180	TAVAGNASCO	253
BRUINO	35	GRAVERE	108	PIOSSASCO	181	TORINO	254
BURIASCO	36	GROSCAVALLO	109	PISCINA	182	TORRAZZA PIEMONTE	255
BUSANO	37	GROSSO	110	POIRINO	183	TORRE CANAVESE	256
BUSSOLENO	38	GRUGLIASCO	111	POMARETTO	184	TORRE PELLICE	257
BUTTIGLIERA ALTA	39	INGRIA	112	PONT CANAVESE	185	TRANA	258
CAFASSE	40	INVERSO PINASCA	113	PORTE	186	TRAUSELLA	259
CALUSO	41	ISOLABELLA	114	PRAGELATO	187	TRAVERSELLA	260
CAMBIANO	42	ISSIGLIO	115	PRALORMO	188	TROFARELLO	261
CAMPIGLIONE FENILE	43	IVREA	116	PRAMOLLO	189	USSEAUX	262
CANDIA CANAVESE	44	LA CASSA	117	PRAROSTINO	190	USSEGLIO	263
CANDIOLO	45	LA LOGGIA	118	PRASCORSANO	191	VAIE	264
CANISCHIO	46	LANZO TORINESE	119	PRATIGLIONE	192	VAL DELLA TORRE	265
CANTALUPA	47	LEINI'	120	QUAGLIUZZO	193	VALGIOIE	266
CANTOIRA	48	LEMIE	121	QUASSOLO	194	VALPERGA	267
CAPRIE	49	LESSOLO	122	QUINCINETTO	195	VAUDA CANAVESE	268

Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID
CARAVINO	50	LEVONE	123	REANO	196	VENARIA REALE	269
CAREMA	51	LOCANA	124	RIBORDONE	197	VENAUS	270
CARIGNANO	52	LOMBARDORE	125	RIVA PRESSO CHIERI	198	VEROLENGO	271
CARMAGNOLA	53	LOMBRIASCO	126	RIVALBA	199	VESTIGNE'	272
CASALBORGONE	54	LORANZE'	127	RIVALTA DI TORINO	200	VIALFRE'	273
CASCINETTE D'IVREA	55	LUGNACCO	128	RIVARA	201	VICO CANAVESE	274
CASELETTE	56	LUSERNA SAN GIOVANNI	129	RIVAROLO CANAVESE	202	VIDRACCO	275
CASELLE TORINESE	57	LUSERNETTA	130	RIVAROSSA	203	VIGONE	276
CASTAGNETO PO	58	LUSIGLIE'	131	RIVOLI	204	VILLAFRANCA PIEMONTE	277
CASTAGNOLE PIEMONTE	59	MACELLO	132	ROBASSOMERO	205	VILLANOVA CANAVESE	278
CASTELLAMONTE	60	MAGLIONE	133	ROCCA CANAVESE	206	VILLAR DORA	279
CASTELNUOVO NIGRA	61	MAPPANO	134	ROLETTO	207	VILLAR PELLICE	280
CASTIGLIONE TORINESE	62	MARENTINO	135	ROMANO CANAVESE	208	VILLAR PEROSA	281
CAVOUR	63	MASSELLO	136	RONCO CANAVESE	209	VILLARBASSE	282
CERCENASCO	64	MATHI	137	RONDISSONE	210	VILLAREGGIA	283
CERES	65	MATTIE	138	RORA'	211	VILLASTELLONE	284
CERESOLE REALE	66	MAZZE'	139	ROSTA	212	VINOVO	285

Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID	Municipality Name ID	Number ID
CESANA TORINESE	67	MEANA DI SUSA	140	RUBIANA	213	VIRLE PIEMONTE	286
CHIALAMBERTO	68	MERCENASCO	141	RUEGLIO	214	VISCHE	287
CHIANOCCO	69	MEUGLIANO	142	SALASSA	215	VISTRORIO	288
CHIERI	70	MEZZENILE	143	SALBERTRAND	216	VIU'	289
CHIESANUOVA	71	MOMBELLO DI TORINO	144	SALERANO CANAVESE	217	VOLPIANO	290
CHIOMONTE	72	MOMPANTERO	145	SAMONE	218	VOLVERA	291
CHIUSA DI SAN MICHELE	73	MONASTERO DI LANZO	146	SAN BENIGNO CANAVESE	219		

Table A2. LC Gradient conditions of the method reported in Chapter 3.

Time (min)	Flow Rate (mL/min)	A %	B %
0,00	0,550	98	2
0,00	0,550	98	2
0,50	0,550	98	2
1,00	0,550	70	30
6,00	0,550	0	100
7,50	0,550	0	100
7,60	0,550	98	2
10,00	0,550	98	2

Table A3. Electrospray Ionization Mode (ESI) source parameters of the method reported in Chapter 3.

Parameter	Value
Polarity	Negative
Curtains Gas	30 psi
Collision Gas	30 psi
Ionspray Voltage	−4500 V
Temperature	350 °C
GS1	50 psi
GS2	55 psi

Table A4. Multiple Reaction Monitoring (MRM) transitions and the retention time (RT) for analytes and internal standards included in the method reported in Chapter 3.

Compound	Q1 *m/z*	Q3 *m/z*	RT (min)
PFBA	213	169	2,10
PFPeA	263	219	3,10
PFHxA	131	269	3,80
PFHpA	363	319	4,30
PFOA	413	369	4,60
PFNA	463	419	5,00
PFDA	513	469	6,90
PFUdA	563	519	5,50
PFDoA	613	569	5,70
PFTrDA	663	619	5,90
PFTeDA	713	669	6,00
PFHxDA	813	769	6,30
PFODA	913	869	6,50
L-PFBS	299	99	3,30
L-PFHxS	399	99	4,30
L-PFOS	499	99	5,00
L-PFDS	599	99	5,40
MPFHxS	403	10	4,30
MPFOS	503	99	5,00
MPFBA	217	172	2,10
MPFHxA	315	270	3,80
MPFOA	417	372	4,60
MPFNA	468	423	4,90
MPFDA	515	470	5,20
MPFUdA	565	520	5,50
MPFDoA	615	570	5,70

Table A5. MRM transitions and retention time (RT) for the target compounds included in the method reported in Chapter 4.

Compound	Ionization mode	Q1 *m/z*	Q3 *m/z*	RT (min)
Atenolol	ESI (+)	266,80 266,80	145,00 190,00	5,29
Azithromycin	ESI (+)	749,50 749,50 749,50	591,30 158,10 573,30	6,51
Caffeine	ESI (+)	195,10 195,10 195,10	138,10 110,00 123,00	6,37
Carbamazepine	ESI (+)	237,10 237,10	194,10 191,80	7,78
Erythromycin	ESI (+)	734,50 734,50	158,30 83,00	7,33
Sulfamethoxazole	ESI (+)	254,20 254,20 254,20	155,90 107,90 146,90	6,52
Trimethoprim	ESI (+)	291,10 291,10	230,20 275,00	5,88
Clarithromycin	ESI (+)	748,50 748,50 748,50	83,00 116,10 590,20	7,56
Ketoprofen	ESI (+)	255,10 255,10	105,00 77,10	8,13
Diclofenac	ESI (+)	296,00 296,00	215,00 215,00	8,71
Ofloxacin	ESI (+)	362,40 362,40 362,40	318,00 261,00 344,00	6,09
Ciprofloxacin	ESI (+)	332,20 332,20 332,20	314,00 231,00 288,30	6,27
Cyclophosphamide	ESI (+)	261,30 261,30	139,80 106,00	7,37
17-beta estradiol	ESI (-)	271,10	145,10	6,51
Estrone	ESI (-)	269,10	145,20	6,48
Ibuprofen	ESI (-)	205,20 205,20	161,20 177,00	6,88

Table A6. Details of the samples used in the study of Chapter 5.

No.	Sample code	Lake/River (Country)	Sampling date	Site Location GPS	Environ. Condition	Description
1	S1	Orta (Italy)	9/5/2020	45,7774133, 8,4077208	Cloud cover	Lake beach
2	S2	Orta (Italy)	9/5/2020	45,8733481, 8,4075652	Rainy	Lake pier
3	S3	Orta (Italy)	9/5/2020	45,8753988, 8,4092584	Rainy	Near Toce river
4	S4	Orta (Italy)	9/5/2020	45,8744660, 8,4101274	Cloud cover	Near Hospital
5	S5	Orta (Italy)	9/5/2020	45,7948270, 8,4156655	Cloud cover	Lake beach
6	S6	Comabbio (Italy)	9/5/2020	45,7612867, 8,6801196	Cloud cover	Lake beach
7	S7	Comabbio (Italy)	9/5/2020	45,7772281, 8,6868874	Cloud cover	Lake pier
8	S8	Comabbio (Italy)	9/5/2020	45,7789047, 8,6967841	Cloud cover	Lake beach
9	S9	Comabbio (Italy)	9/5/2020	45,7721398, 8,7001449	Cloud cover	Lake beach
10	S10	Comabbio (Italy)	9/5/2020	45,7673782, 8,7020929	Cloud cover	Lake beach
11	S11	Po (Italy)	7/31/2020	45,02359, 7,40508	Sunny (~28 °C)	Near Hospital
12	S12	Po (Italy)	9/17/2020	45,02359, 7,40508	Sunny (~26 °C)	Near Hospital
13	S13	Pamvotis (Greece)	9/5/2020	39,633149, 20,898402	Cloud cover	Lake side, bridge
14	S14	Pamvotis (Greece)	9/5/2020	39,673641, 20,858234	Cloud cover	Lake side, touristic area
15	S15	Pamvotis (Greece)	9/5/2020	39,689113, 20,840971	Cloud cover	Near a bridge
16	S16	Pamvotis (Greece)	9/5/2020	39,683082, 20,878095	Cloud cover	Lake side, recreation area
17	S17	Pamvotis (Greece)	9/5/2020	39,677277, 20,907347	Cloud cover	Lake side

Table A7. Details about the standard compounds that were used as suspect analytes in Chapter 5.

Compound	CAS Number	Compound	CAS Number
Dichlorvos		Epoxiconazole	
Methyl parathion		Ethofumesate	
Parathion (ethyl)		Fenamidone	
Penconazole		Fenbuconazole	
Pendimethalin		Fenhexamid	CUS 18511
Pethoxamid		Hexazinone	
Phosalone		Indoxacarb	
Pirimicarb		Kresoxim-methyl	
Prochloraz		Lenacil	
Procymidone		Mepanipyrim	
Prometryn		Alachlor	
Pronamide		Ametryn	
Propachlor		Atrazine	
Propazine		Atrazine-desethyl	
Propiconazole		Cyanazine	
Pyraclostrobin	CUS 19925	Metolachlor	CUS 12525
Pyrimethanil		Molinate	
Simazine		Oxadiazon	
Spirotetramat		Prometryn	
Spiroxamine		Propazine	
Tebufenozide		Simazine	
Terbuthylazine		Terbuthylazine	
Terbuthylazine-desethyl		Chlorpyriphos	
Tetraconazole		Chlorpyriphos-methyl	CUS17405
Thiacloprid		Diazinon	
Thiamethoxam		Pendimethalin	
Thiobencarb		Clarithromycin	81103-11-9
Tolylfluanid		Erythromycin	114-07-8
Trifloxystrobin		Caffeine	58-05-2
Trifluralin		Carbamazepine	298-46-4
Triticonazole		Ciprofloxacin	85721-33-1
Vinclozolin		Ofloxacin	82419-36-1
Zoxamide		Cyclophosphamide	50-18-0
Atenolol	29122-68-7	Diclofenac	15307-79-6
Azithromycin	83905-01-5	Ketoprofen	22071-15-4

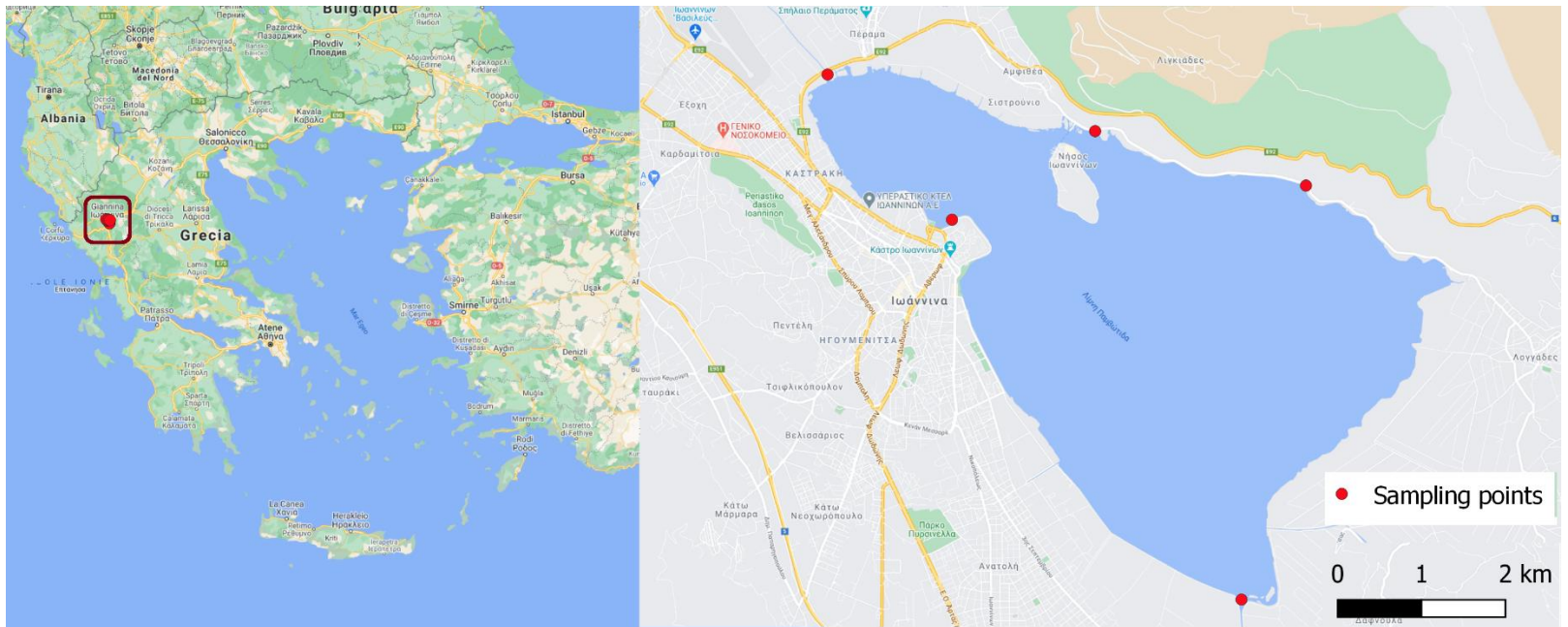| Compound	CAS Number	Compound	CAS Number
Ibuprofen	15687-27-1	Estrone	53-16-7
Sulfamethoxazole	723-46-6	PFOA	307-24-4
Trimethoprim	738-70-5	PFOS	1763-23-1
17-beta Estradiol	50-28-2	PFHxA	335-67-1

Figure A2a: A map of the sampling points from the lake Pamvotis, in Ioannina, Greece, included in Chapter 5, and its position on the national territory.
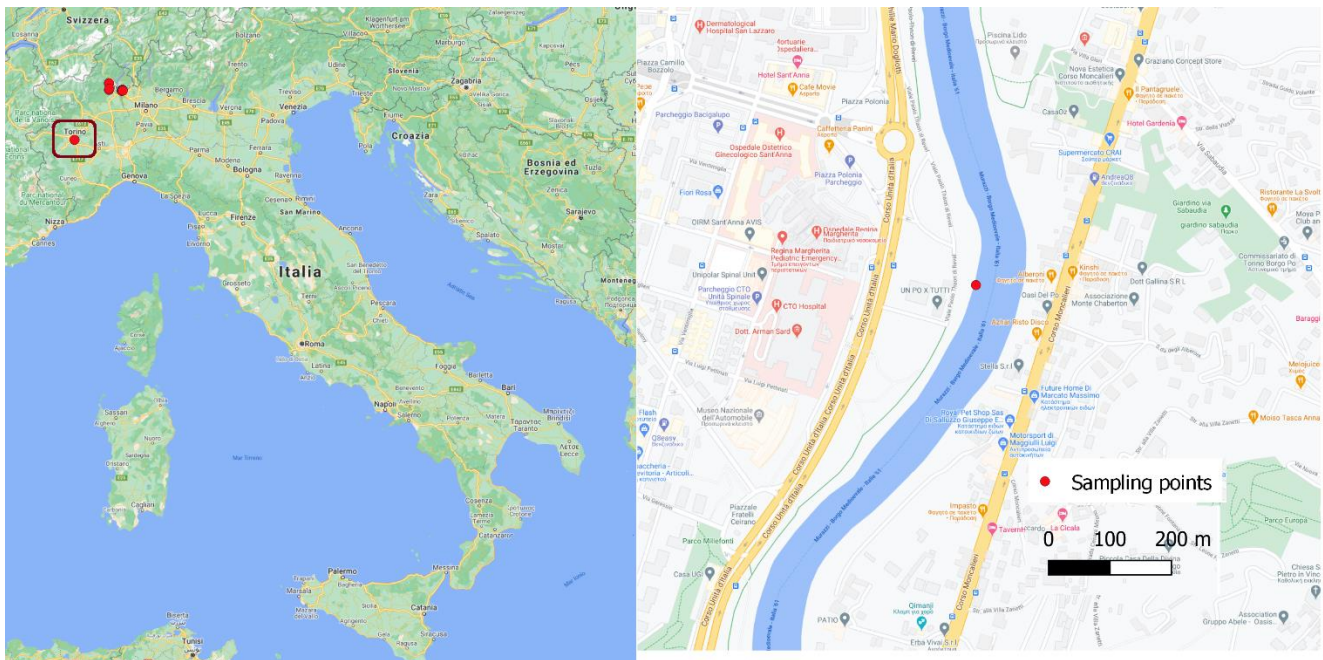
Figure A2b: A map of the sampling point from the Po river, in Turin, Italy included in Chapter 5, and its position on the national territory.
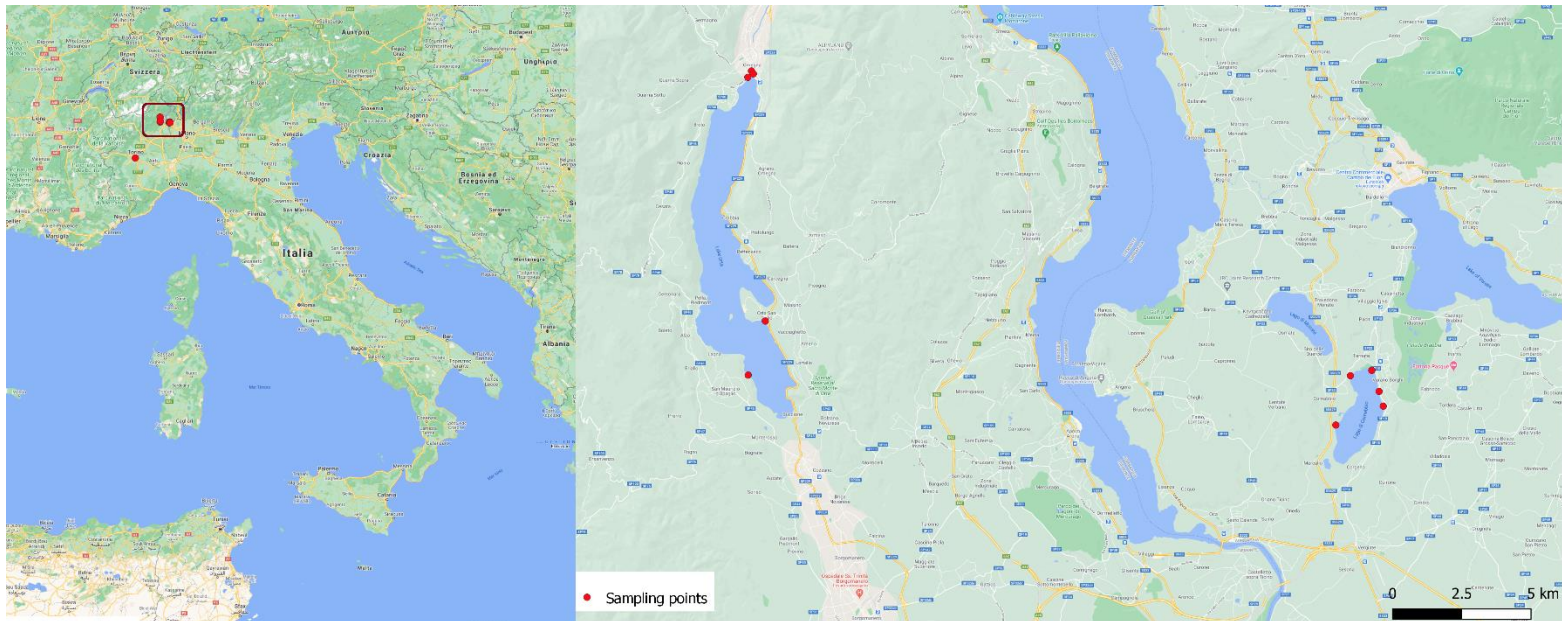
Figure A2c: A map of the sampling points from the lakes Orta and Comabbio in Italy included in Chapter 5, and their positions on the national territory.

Table A8. MzMine Parameters, used for the elaboration of the data in Chapter 5.

| Step	Parameter	Setting
Mass detection	Retention Time	0-20 min
	MS level	1
	Polarity	+ for ESI (+) - for ESI (-)
	Spectrum type	Profile
	Mass detector	Exact mass
	Noise level	2,00E+02 (ESI-) 5,00E+02 (ESI+)
Chromatogram builder	Retention time	0-20 min
	MS level	1
	Polarity	+ for ESI (+) - for ESI (-)
	Spectrum type	Profile
	Min time span (min)	0,01
	Min height	2,10E+02 (ESI-) 5,10E+02 (ESI+)
	m/z tolerance	0,001 *m/z* or 5 ppm
Smoothing	Filter width	7
Chromatogram deconvolution	Algorithm	Noise amplitude
	Min peak height	3,00E+02 (ESI-) 6,00E+02 (ESI+)
	Peak duration range	0,05-1,00 min
	Amplitude of noise	2,00E+02 (ESI-) 5,00E+02 (ESI+)
Isotopic peak filter	*m/z* tolerance	0,001 *m/z* or 5 ppm
	Retention time tolerance	0,01 min
	Maximum charge	1
	Representative isotope	Lowest *m/z*
Join aligner	*m/z* tolerance	0,001 *m/z* or 5 ppm
	Weight for *m/z*	70
	Retention time tolerance	0,3 (absolute) min
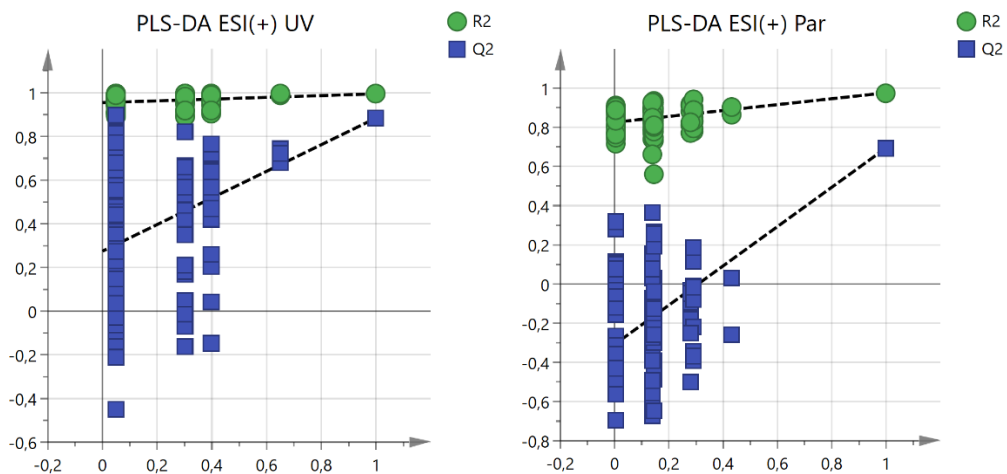
Figure A3: Validation tests for the PLS-DA model in UV scaling for the features detected in ESI (+) based on 100 permutations/replicated (left), and the PLS-DA model in Pareto scaling for the features detected in ESI (+) based on 100 permutations/replicated (right).

Training Activities, Seminars and Conferences Attended

- **14-19/03/2018, 07/05/2018**: Health and safety rules and laws in Italy, risks and hazards in the laboratory, SMAT, Turin, Italy. *24 hours*
- **27/03/2018, 18/04/2018**: UHPLC-QTRAP MS course, SMAT, Turin, Italy. *12 hours*
- **10-12/10/2018**: XENOWAC II "Challenges and Solutions related to Xenobiotics and Antimicrobial Resistance in the Framework of Urban Wastewater Reuse: Towards a Blue Circle Society' conference", Limassol, Cyprus.
- **24/01/2019, 28/01/2019**: Transizione alla norma UNI EN ISO/IEC 17025:2018 e requisiti Accredia (RT-08 per i CAB e RT-25 per i LAT): principali novità, approccio al rischio, requisiti della norma nella nuova edizione, SMAT, Turin, Italy. *8 hours*
- **10/03/2019**: Corso di Formazione Generale alla Salute e Sicurezza per i Lavoratori, Online, UniTO. *4 hours*
- **26-30/05/2019**: SETAC Europe 29th Annual Meeting, Helsinki, Finland.
- **04-06/09/2019**: International Conference on Chemical Energy and Semiconductor Photochemistry (CESCOP 2019), Trabzon, Turkey.
- **16-20/06/2019**: 17th International Conference on Chemistry and the Environment (ICCE 2019), Thessaloniki, Greece.
- **30/09/2019**: Piano di Sicurezza dell'acqua per la città di Torino, Presentazione e Pianificazione delle attività, SMAT, Turin, Italy. *4 hours*
- **17-18/02/2020**: Colloquium "Machine Learning meets Chemistry", University of Turin, Turin, Italy. *8 hours*
- **31/03/2020**: Corso Privacy per le persone autorizzate al trattamento dei dati personali, Online. *4 hours*
- **16/04/2020, 20/04/2020**: Italian Mass Spectrometry Society (IMaSS) webinar on Data Analysis, Online. *8 hours*
- **01-12/06/2020**: American Society of Mass Spectrometry (ASMS) 2020 Reboot Conference, Online.
- **12/06/2020**: Sample Preparation, Quo Vadis: Current Status of Sample Preparation Approaches (Molecules MDPI) webinar, Online. *4 hours*
- **15-16/06/2020**: ASMS Untargeted Metabolomics short course, Online. *16 hours*
- **20-24/07/2020**: UPLC-QTOF MS course, SMAT, Online. *16 hours*
- **18/01/2021**: EU versus Italian water management, Online. *3ECTS*
- **01/02/2021**: Basics of project writing: hands-on workshop session, Online. *4 hours*
- **05/02/2021**: Antibiotic Resistant Bacteria: Occurrence and removal from Urban Wastewater, Online. *2 hours*

PhD Courses Attended

University	Course Title	Hours
University of Turin	Introduction in Technological Sector of Circular Economy / Water Treatment Plan	4
University of Turin	Circular Economy and Social Innovation	3
University of Turin	New Technologies and Climate Change– ''Rethinking the Design of Climate Stabilization Policy''	6
University of Turin	Economic Fundamental Principles	3
University of Turin	Economics of Innovation and Green Technologies	12
University of Turin	Organization, Innovation and Value Measures	3
University of Turin	Data Science and Circular Economy	4
University of Turin	Circular Economy and Public Policies	4
University of Turin	Public Policies for the Environment	6
University of Turin	Innovation, sustainability and new business models	6
University of Turin	Resources and Raw Materials	9
University of Turin	LCA - Life Cycle Assessment – Principles, Plastic Materials in the Circular Economy Paradigm, Metallic Materials in the Circular Economy Paradigm	4
University of Turin	OpenLCA - The open source software for LCA, LCA analysis of plastic and metallic materials	4
University of Turin	Interactive laboratory to stimulate an attitude to outreach activities on basic physico-chemical phenomena	12

PhD Schools Attended

Date	Title	Place
25/04/2018-27/04/2018	Summer School on Photochemistry and Depollution	Clermont-Ferrand, France
27/08/2018-29/08/2018	International Summer School on "Micropollutant Analysis and Abatement"	Aalborg Denmark
04/03/2019-05/03/2019	International Winter School on Mass Spectrometry and Workshop on substances prioritization	Palaiseau France
03/06/2019-07/06/2019	3rd European Summer School on Environmental Applications of Advanced Oxidation Processes	Alcoy Spain
23/09/2020-24/09/2020	Summer school on "Introduction to Basic Statistical Tools and Data Analysis in Research"	Online

Presentations

1. Oral Presentation: **D. Papagiannaki**, P. Calza, R. Binetti, "General Introduction-ESR6", 1st AQUAlity Meeting, Clermont-Ferrand, France, 23-24 April 2018.

2. Oral Presentation: **D. Papagiannaki**, P. Calza, R. Binetti, "PFAS determination in real water samples by UHPLC/MS/MS", Scientific Storm Meeting, Turin, Italy, 30 May 2018.

3. Oral Presentation: **D. Papagiannaki**, F. Barsotti, M. Fungi, R. Binetti, "PFAS determination in real water samples", 2nd AQUAlity Meeting, Aalborg, Denmark, 30-31 August 2019.

4. Poster Presentation: **D. Papagiannaki**, F. Barsotti, A. Salaris, M. Fungi, R. Binetti, "Fate of Metolachlor and Terbuthylazine in surface water and related drinking water treatment plant", XENOWAC II, Limassol, Cyprus, 10-12 October 2018.

5. Oral Presentation: **D. Papagiannaki**, R. Binetti, ''Trace level analysis of CECs in drinking water using Mass Spectrometry'', International Winter School on Mass Spectrometry, Palaiseau, France, 4-5 March 2019.

6. Oral Presentation: **D. Papagiannaki**, S. Morgillo, P. Calza, R. Binetti, "PFAS an overview", 3rd AQUAlity Meeting, Palaiseau, France, 7-8 March 2019.

7. Poster Presentation: **D. Papagiannaki**, S. Morgillo, G. Costantino, M. Fungi, R. Binetti, "Perfluoroalkyl Substances Assessment in Turin Metropolitan area and correlation with potential sources of pollution according to the Water Safety Plan risk management approach", SETAC Europe 29th Annual Meeting, Helsinki, Finland, 26-30 May 2019.

8. Poster Presentation: **D. Papagiannaki**, S. Morgillo, G. Costantino, M. Fungi, R. Binetti, "Trace level analysis of perfluoroalkyl substances in drinking water and their assessment in Metropolitan Area of Turin", 17th International Conference on Chemistry and the Environment, Thessaloniki, Greece, 16-20 June 2019.

9. Oral Presentation: **D. Papagiannaki**, P. Roslev, "Effect of UV-A, UV-B and UV-C irradiation on Biotoxicity of Glyphosate in Drinking Water Samples", 4th AQUAlity Meeting, Trabzon, Turkey, 2-3 September 2019.

10. Oral Presentation: **D. Papagiannaki**, P. Calza, R. Binetti, "Evaluation of CECs in drinking water including toxicological assessment of their degradation by-products", Horizon 2020 MSCA-ITN cluster event "Clean Water", Girona, Spain, 22 October 2019.

11. Oral Presentation: **D. Papagiannaki**, S. Morgillo, R. Binetti, "Screening-level risk assessment of selected pharmaceuticals in the Metropolitan Area of Turin", 5th AQUAlity Meeting, Online, 6 April 2020.

12. Poster Presentation: **D. Papagiannaki**, R.Binetti, P. Calza, P. Roslev, "UV Irradiation Decreases Ecotoxicity of Glyphosate", SETAC SciCon, SETAC Europe 30th Annual Meeting, online, 3-7 May 2020.

13. Oral Presentation**: D. Papagiannaki**, Z. Varga, A. Cedrino, R. Binetti "Monitoring, understanding and predicting contamination of groundwater sources destined for drinking water supply", 6th AQUAlity Meeting, Online, 22 September 2020.

14. Oral Presentation: **D. Papagiannaki**, D, Fabbri, P. Calza, R. Binetti, "Non-target screening analysis of water samples using LC-HRMS and GC-MS", 7th AQUAlity Meeting, Online, 1-2 March 2021.

15. Oral Presentation: **D. Papagiannaki**, D. Palma, A. Cedrino, G. Molinari, M. Lai, M. Minella, R. Binetti "Removal Of Contaminants Of Emerging Concern From Water Using High Voltage Pulsed Electric Field Discharge", SPEA 2020 postponed, planned.

Outreach Activities

1. E. Robotti, M.H. Belay, N.P.F. Gonçalves, **D. Papagiannaki**, F.E.B Coelho, "AQUAlity Project: Removal of contaminants of emerging concern", European Researcher's night 2018, Alessandria, Italy, 28 September 2018.

2. D. Papagiannaki, A. Pavanello "AQUAlity Project: Removal of contaminants of emerging concern", Festival dell'Innovazione e della Scienza 2018, Settimo Torinese, Italy, 20 October 2018.

3. M.H. Belay, N.P.F. Gonçalves, **D. Papagiannaki**, I. Berruti, K. Janowska, F.E.B Coelho "AQUAlity Lab", laboratory experiments for secondary school students, Turin, Italy, 19 February 2019.

4. D. Papagiannaki, R. Binetti "PFAS Assessment in Metropolitan Area of Turin, Italy", TV Interview, TG Leonardo, Rai News, 17 April 2019.

5. N.P.F. Goncalves, **D. Papagiannaki**, D. Fabbri, P. Calza, "Il trattamento dell'acqua: abbattimento degli inquinanti", European Researcher's night 2019, Turin, Italy, 27 September 2019.

6. N.P.F. Gonçalves, Z. Varga, **D. Papagiannaki**, "AQUAlity Lab", laboratory experiments for secondary school students, Turin, Italy, 12 February 2020.

7. I. Berruti, M.H. Belay, **D. Papagiannaki**, "Il suolo come filtro naturale per gli inquinanti", Video presentation (translated into Italian), European Researcher's night 2020, online, 23 November 2020.

8. D. Papagiannaki, C. Jimenez Holgado, D. Fabbri, P. Calza, "La sfida di AQUAlity: rimuovere i contaminanti di ultima generazione dalle acque", Video presentation, European Researcher's night 2020, online, 24 November 2020.

9. D. Papagiannaki, R. Binetti, "L'acqua", Online lesson to secondary school students, European Researcher's night 2020, online, 27 November 2020.

10. N.P.F. Gonçalves, **D. Papagiannaki**, I. Berruti, P. Calza, "Pandemia e ricerca", TV interview, TGR Piemonte, TG Leonardo, 6 May 2021.

Periods as Visiting Researcher

1. 01/05/2019-31/08/2019: Department of Chemistry and Bioscience, Aalborg University, Aalborg, Denmark. Tutor: Prof. Peter Rolsev.

2. 01/11/2020-28/02/2021: Department of Chemistry, University of Turin, Turin, Italy. Tutor: Prof. Debora Fabbri.

Acknowledgments

A PhD title is awarded to a single person but many people and institutions are contributing to the success of such work. In this section I would like to thank all the people that supported the completion of this thesis.

Firstly, I would like to thank my supervisors, Dr. Rita Binetti and Prof. Paola Calza, for giving me this great opportunity, for their endless support and help during these three years that led to our excellent collaboration. To Dr. Rita Binetti I am really grateful for our everyday interaction, all the knowledge that she offered me in the several fields that we worked on, for always being active, helping with doubts, problems and questions, and most importantly for helping me grow as an independent scientist. I wish also to thank her for always fighting for me, and for introducing me in the reality of the industrial working environment without never forgetting the beautiful experience of a PhD abroad. You are a role model for me. From the bottom of my heart thank you.

A deep thanks goes to all my colleagues at the Research Center of SMAT, for providing a healthy working environment. I wish to give a big thanks especially to Marilena, Maurizio, Andrea, Agnese, Stefania, Emilia, Mauro, Sergio, Matteo, Cristina, Francesco for assisting with all my experiments and problems of my everyday life in Italy, teaching me Italian and making this experience special.

I wish to express my gratitude to my two tutors from the months I spent in other universities: Prof. Peter Roslev for hosting me in Aalborg, for guiding me through a completely different field of my studies and for introducing me in the local community, and Prof. Debora Fabbri, for our excellent collaboration during my secondment at UniTO, for all the knowledge and advice that she gave me and for her positive aura in the laboratory. Moreover, I would like to thank all the colleagues at Aalborg University and at University of Turin, for all the help they provided me with equipment and experiments and for introducing me in the local communities.

A big, happy thanks goes to my fellow ESRs and friends Nuno, Zsuzsanna, Cristina, Alice, Masho, Ivan, Ilaria, Davide, Esra, Oihane, Fabricio, Katarzyna, Dennis, Bethel for the collaboration and networking and for all the nice times that we had during these three years. I am really honored to have met and worked with all of you.

Moreover, I would like to say thanks to all my friends back home for supporting my decision to leave everything behind and follow the dream of a career abroad, and to the

new ones I met during this journey. Nuno, Mirtha, Betsy, and Jose I am so grateful for meeting you, for all the moments that we spent together, for the travels that we did and for all the food that you made me try, for all the help that you offered me during these years. It wouldn't be the same without you.

I am truly thankful to my boyfriend Kostas, who has always believed in me, supported me and stayed by my side throughout this journey, helped me find motivation and energy to overcome every problem, every obstacle. Thank you for tour love and your patience for all those times that I could not spent with you because of this thesis.

Finally and most importantly, I wish to thank my family: my sister Isidora, for being the light of positivity in my life, and my parents for everything that they have done and gave up for making me who I am today. There are no words to express my gratitude to you. This work is yours. Thank you.