

Department of

Earth and Environmental Sciences

PhD program in Chemical, Geological and Environmental Sciences Cycle XXXIII

Curriculum in Environmental Sciences

INNOVATIVE TECHNIQUES FOR THE REMOVAL OF ORGANIC MICROPOLLUTANTS FROM WASTEWATERS

Tasselli Stefano

Registration number **835496**

Tutor: **Prof. Antonio Finizio**

Supervisor: **Dr. Licia Maria Guzzella**

Coordinator: **Prof. Emilio Padoa-Schioppa**

ACADEMIC YEAR 2019-2020

The present thesis was carried out at the
National Research Council -Water Research Institute (CNR-IRSA)
Via del Mulino 19, 20861 Brugherio, MB

TABLE OF CONTENTS

| | |
|--|----|
| ABSTRACT | 1 |
| CHAPTER I..... | 2 |
| General introduction..... | 2 |
| 1.1 Historical trend of musk chemicals | 2 |
| 1.2 Production and usage..... | 3 |
| 1.3 Physicochemical properties and degradation product | 4 |
| 1.4 Polycyclic Musk Fragrances in Wastewater Treatment Plants..... | 5 |
| 1.5 Environmental contamination..... | 6 |
| 1.6 Bioaccumulation of PMFs in organisms | 9 |
| 1.7 Toxicity of polycyclic musk fragrances in organisms | 13 |
| 1.8 Analysis of polycyclic musk fragrances in environmental samples | 15 |
| 1.9 References | 17 |
| CHAPTER II | 21 |
| Thesis Aims and Structure..... | 21 |
| 2.1 Thesis objectives | 21 |
| 2.2 Thesis structure..... | 22 |
| CHAPTER III..... | 23 |
| Polycyclic Musk Fragrances (PMFs) in wastewater and activated sludge: analytical protocol and application to a real case study | 23 |
| Abstract | 23 |
| Keywords..... | 23 |
| Acknowledgements | 24 |
| 3.1 Introduction | 24 |
| 3.2 Materials and methods..... | 25 |
| 3.2.1 Chemicals | 25 |
| 3.2.2 Sampling..... | 26 |
| 3.2.3 Extraction procedure..... | 27 |
| 3.2.3.1 Water samples | 27 |
| 3.2.3.2 Sludge samples | 27 |
| 3.2.4 Quantification | 27 |

| | |
|---|----|
| 3.2.4.1 GC-MS/MS analysis for water samples | 27 |
| 3.2.4.2 GC-Ion trap analysis for sludge samples | 28 |
| 3.2.5 Detection and quantification limits..... | 29 |
| 3.2.6 Repeatability of the methods | 29 |
| 3.3. Results and discussion | 29 |
| 3.3.2 Method validation..... | 29 |
| 3.3.2.1 Linearity | 29 |
| 3.3.2.2 Detection and quantification limits..... | 29 |
| 3.3.2.3 Repeatability..... | 30 |
| 3.3.2.4 Specificity and contamination of the laboratory with polycyclic musks..... | 31 |
| 3.3.2.5 Recovery..... | 31 |
| 3.3.2.6 Comparison with other methods..... | 32 |
| 3.3.3 A case study: wastewater and sludge of a WWTP in Northern Italy | 32 |
| 3.4 Conclusions | 35 |
| 3.5 References | 36 |
| CHAPTER IV | 41 |
| Polycyclic musk fragrance (PMF) removal, adsorption and biodegradation in a conventional activated sludge wastewater treatment plant in Northern Italy | 41 |
| Abstract | 41 |
| Keywords..... | 41 |
| Funding..... | 41 |
| Acknowledgements | 42 |
| 4.1 Introduction | 42 |
| 4.2 Materials and methods..... | 44 |
| 4.2.1 Chemicals | 44 |
| 4.2.2 Sample collection, influent and WWTP characteristics | 44 |
| 4.2.3 Sample preparation and analysis | 45 |
| 4.2.4 Quality control..... | 45 |
| 4.3. Results and discussion..... | 46 |
| 4.3.1 Presence of PMFs in wastewater | 46 |
| 4.3.2 Adsorption and biotransformation of HHCB during secondary treatment..... | 47 |
| 4.3.3 PMFs concentrations in activated sludge | 50 |

| | |
|---|----|
| 4.3.4 Ratios of HHCB and AHTN..... | 51 |
| 4.3.5 Seasonality of PMF concentrations in wastewaters..... | 51 |
| 4.3.6 WWTP removal efficiency..... | 53 |
| 4.4 Conclusions | 55 |
| References | 55 |
| CHAPTER V..... | 58 |
| Preliminary results regarding the application of ozonation and adsorption on activated carbon in a wastewater treatment plant: new promising advanced technologies for Polycyclic Musk Fragrances (PMFs) removal..... | 58 |
| Abstract | 58 |
| Funding..... | 58 |
| Acknowledgements | 59 |
| 5.1 Introduction | 59 |
| 5.2 Materials and methods..... | 61 |
| 5.2.1 Wastewater treatment plants (WWTPs) | 61 |
| 5.2.2 Ozonation pilot plant | 62 |
| 5.2.3 Powdered activated carbon pilot plant..... | 63 |
| 5.2.4 Powdered activated carbon choice | 64 |
| 5.2.5 Sampling campaigns at pilot plants | 64 |
| 5.2.6 Chemicals and reagents | 64 |
| 5.2.7 Selection of PMFs and analytical method | 65 |
| 5.2.8 Other analysis | 66 |
| 5.3 Results and discussion..... | 66 |
| 5.3.1 Effect of ozone on PMF removal | 66 |
| 5.3.2 Ozonation pilot plant efficiency | 69 |
| 5.3.3 Powdered activated carbon choice and isotherm experiments | 70 |
| 5.3.4 Powdered activated carbon pilot plant setups..... | 71 |
| 5.3.4.1 Powdered activated carbon pilot plant setups with PAC 1 | 72 |
| 5.3.4.2 Powdered activated carbon pilot plant setups with PAC 2..... | 74 |
| 5.4 Conclusions | 76 |
| References | 77 |
| CHAPTER VI..... | 80 |
| General conclusions..... | 80 |

| | |
|---|----|
| 6.1 Development of the analytical protocol for the detection of Polycyclic Musk Fragrances (PMFs) in wastewater and activated sludge..... | 80 |
| 6.2 Monitoring of Polycyclic Musk Fragrance (PMFs) removal, adsorption and biodegradation in a conventional activated sludge wastewater treatment plant in Northern Italy | 81 |
| 6.3 Preliminary results regarding the application of two promising advanced wastewater treatments for Polycyclic Musk Fragrances (PMFs) removal in wastewater treatment plants..... | 81 |
| CHAPTER VII | 83 |
| Supporting information | 83 |
| Polycyclic musk fragrance (PMF) removal, adsorption and biodegradation in a conventional activated sludge wastewater treatment plant in Northern Italy | 83 |

ABSTRACT

Personal care products are a heterogeneous group of chemicals that include Polycyclic Musk Fragrances (PMFs) as Galaxolide (HHCB), Tonalide (AHTN), Celestolide (ADBI) and Phantolide (AHDI). PMFs are widely spread substances employed in perfumes, detergents and house-cleaning products. The massive use leads to PMF release into the environment mainly through Wastewater Treatment Plants (WWTPs) discharges. Even if Italy is the European country with the highest use of PMFs, only a few data are available about these products' occurrence in Italian water and about PMF fate in conventional WWTPs. In this, PhD was firstly focused on developing protocols for PMF determination in wastewater and in activated sludge. PMFs in water samples were determined by SPE extraction and analysis in GC-MS with a triple-quadrupole while for activated sludge analysis an ultrasonic bath and a GC-Ion trap were employed. Detection limits of selected compounds were considerably lower than sample concentrations. These methods allow the analysis of many samples in short times employing simple instrumentation and limiting the solvent volumes used. After that, PMFs inside an Italian conventional WWTPs was monitored. HHCB and its main by-product, Galaxolidone (HHCB-lactone), were found in concentrations of $\mu\text{g/L}$, one order of magnitude greater than AHTN, AHDI was always lower than LOD while ADBI was measured only at trace levels. No seasonal variability was recorded in PMF input onto WWTP. In the water phase, HHCB and AHTN evidenced a modest reduction during treatments (20% and 50%, respectively) mainly due to adsorption processes during the biological treatment as suggested by the high stable PMF concentrations in activated sludges. HHCB-lactone registered an increase up to 70% during treatments caused by HHCB biotransformation during biological treatment. This study revealed that current technologies are not enough efficient in removing PMFs from wastewaters which are discharged into the environment. Additional treatments are therefore necessary inside WWTPs. In this, the efficiency of ozonation and adsorption on activated carbon in removing PMFs from wastewaters was assessed. Treatments were tested through two pilot plants installed after the biological treatment in two WWTPs located in Northern Italy performing different operational conditions. Regarding ozonation, a medium-low dosage of O_3 was applied. HHCB was efficiently removed at low O_3 dosages and short hydraulic retention times while AHTN and HHCB-lactone were less reactive to oxidation. HHCB-lactone registered insufficient removals even with the highest O_3 dosage and hydraulic retention time. Ozonation was effective in removing PMFs from wastewater but high gas dosages may be required to avoid the formation of oxidation by-products. Adsorption on powdered activated carbon (PAC) was tested in a pilot plant consisting on a series of tanks in which PAC is added to the wastewater together with coagulant, microsand and flocculant and can be further recirculated. By operating on water flow, quantity of virgin PAC added to the system and concentration of PAC inside the pilot plant, different setups were studied achieving satisfactory removals for all studied compounds even at low concentrations of the two PACs. A dependence between PMF removal efficiency and PAC concentration, quantity of virgin PAC and hydraulic flow rate was highlighted. In our pilot plant, lower dosages of carbon can be further tested in order to obtain high removals together with lower management costs. By comparing two different PACs, the importance of associating the PAC type with the class of compound to remove was evidenced. Overall, both technologies were effective in removing PMFs from wastewaters even if adjustment of operational parameters is needed to obtain great performances with low operating costs.

CHAPTER I

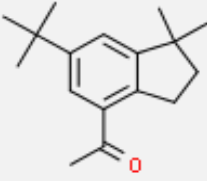
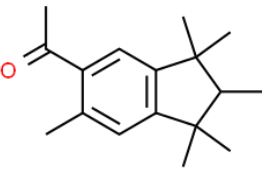
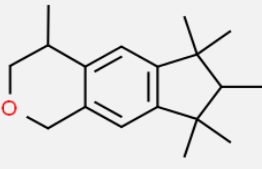
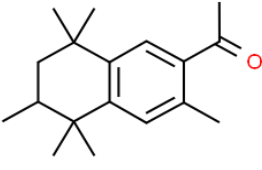
General introduction

1.1 Historical trend of musk chemicals

In the early 1900s, musk fragrances were originally collected and extracted from animals. For example, muscone was obtained from the male musk deer *Moschus moschiferus* while civetone was extracted from African and Asian civet cats *Viverra civetta* and *Viverra zibetha*. These animals produce, through specific glands, substances that were widely used in perfumes (Franke et al., 1999). Subsequently, other sources of musky scents were found, for example in more accessible animals (*Ondatra zibethica*) and plants (*Archangelica officinalis*). Researches on the active principles of musk and civet, driven by the restriction on the hunting of musk deer issued in 1979 by the Convention on International Trade in Endangered Species of Wild Fauna and Flora and additional laws, resulted in the replacement of the expensive natural products by synthetic chemicals. Musk xylene and musk ketone, synthetic nitro musks, were the first compounds started to be widely used in the perfume industry. Today's synthetic musks belong to three main chemically distinct groups: macrocyclic musks, nitro musks and polycyclic musk. Fragrances with macrocyclic structure failed economically because expensive to be produced and chemically not stable under alkaline conditions (Herrmann, 2005). Nitro musks showed to be toxic; musk ambrette showed neurotoxicity (Spencer et al., 1984) and musk xylol was carcinogenic in rats (Maekawa et al., 1990). Based on these issues, restrictions on their use were applied in Europe (ECHA – European Chemicals Agency, 2010; O.S.P.A.R. Commission, 2004) and their presence in personal care products as well as in the environment started to decrease. They were replaced by polycyclic musks (PMFs) since the 1970s and nowadays they are used globally in personal care and household cleaning products as shampoos, cosmetics, deodorants, soaps and detergents. Besides perfume properties, polycyclic musks act also as fixatives since they help maintain the integrity of commercial products and extend their expiration dates.

Among polycyclic musks, there are ADBI, or Celestolide, AHDI, or Phantolide, HHCB, or Galaxolide and AHTN, or Tonalide (Table I-1).

Table I-1 Identification of polycyclic musk fragrances (PMFs).

| <i>Identification</i> | |
|--|---|
| <p><i>ADBI – Celestolide</i></p>  | <p>CAS: 13171-00-1 $C_{17}H_{24}O$, 4-acetyl-6-tert. butyl-1,1-dimethylindan</p> |
| <p><i>AHDI – Phantolide</i></p>  | <p>CAS: 15323-35-0 $C_{17}H_{24}O$, 5-acetyl-1,1,2,3,3,6-hexamethylindan</p> |
| <p><i>HHCB – Galaxolide</i></p>  | <p>CAS: 1222-05-5 $C_{18}H_{26}O$, 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-[γ]-2-benzopyran</p> |
| <p><i>AHTN – Tonalide</i></p>  | <p>CAS: 21145-77-7/1506-02-1 $C_{18}H_{26}O$, 7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene or 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8-hexamethyl-2-naphthyl)ethan-1-one or 6-acetyl-1,1,2,4,4,7-hexamethyltetraline</p> |

1.2 Production and usage

HHCB and AHTN are the two most important polycyclic musks while the others are quantitatively less important: HHCB and AHTN represent 95% of all polycyclic musks used in the European Union market and 90% of the United-States market in 2004 with an estimated consumption of 1427 ton y^{-1} and 358 ton y^{-1} , respectively, in 2000 (Clara et al., 2011; O.S.P.A.R. Commission, 2004). The polycyclic musks HHCB and AHTN are included into the OSPAR list of Chemicals for Priority Action (HERA, 2004). In the EU, they are included in the fourth priority list within the context of the Existing Chemical Programme (Council Regulation EEC 793/93). Their production is monitored in Europe since 2003 by the Oslo-Paris Commission for Protection and Conservation of the North-East Atlantic and its resources. The European Union has currently only one manufacturer of HHCB, the International Flavors & Fragrances Inc (IFF), in an annual volume of 1000 to 5000 tonnes (2001). Even for AHTN, the European Union currently has only one manufacturer, the PFW Aroma Chemicals BV, which produces AHTN in an annual volume of 1000 to 5000 tonnes (2001). Other companies have terminated their production (HERA, 2004). A significant part of the production of both HHCB and AHTN is exported outside EU (20-30% as finished fragrance compounds or in consumer products). Used volumes of HHCB and AHTN in Europe, based on surveys carried out by the RIFM (Research Institute of Fragrance Materials) in 1993, 1995, 1998 and by IFRA (International Fragrance Association) in 2000, are shown in Table I-2. The use of HHCB is therefore 4 times greater than that of AHTN.

Table I-2 Use volumes in Europe (RIFM and IFRA surveys).

| <i>Year</i> | <i>HHCB (ton year⁻¹)</i> | <i>AHTN (ton year⁻¹)</i> |
|-------------|-------------------------------------|-------------------------------------|
| 1992 | 2400 | 885 |
| 1995 | 1482 | 585 |
| 1998 | 1473 | 385 |
| 2000 | 1427 | 358 |

However, regional differences based on detergent consumption are present in the EU member states regarding the use of PMFs; in Northern European countries the use of these substances has been greatly reduced while the higher consumptions are registered in Southern Europe, particularly in Italy, where there is the highest use of detergents with a volume of 7.23 g y⁻¹ per capita for HHCB and 1.81 g y⁻¹ for AHTN (HERA, 2004). The use of PMFs in cosmetics and detergents production decreased during the second half of the nineties. This trend was followed by Northern European countries as Germany, the Netherlands, Belgium, Switzerland, Austria and Scandinavia but, in the other parts of Europe, these chemicals are still widely used. Due to this, cosmetics and household cleaning products probably contain less quantity of PMFs in Northern Europe in respect to Southern Europe (HERA, 2004). However, monitoring data are available only for Northern European countries while in Southern Europe there is still a lack of information.

1.3 Physicochemical properties and degradation product

The structures of the polycyclic musks mentioned above consist mostly of non-polar functional groups. Partition coefficients indicate that these compounds are hydrophobic and possess a high affinity for organic carbon, such as sediments and lipids. They are also considered as semivolatiles compounds since they have vapor pressure between 10⁻⁹ and 10 Pa and may volatilize only at temperatures higher room temperature (Weschler and Nazaroff, 2008) (Table I-3).

Table I-3 Properties of Polycyclic musks fragrances (PMFs).

| | <i>ADBI</i> | <i>AHDI</i> | <i>HHCB</i> | <i>AHTN</i> |
|---|-------------|-------------|-------------|-------------|
| <i>Molecular Weight (g mol⁻¹)</i> | 244 | 244 | 258 | 258 |
| <i>Melting point (°C)</i> | 96 | >58 | -10 – 0 | >54 |
| <i>Boiling point (°C)</i> | 319 | 318 | 325 | 180 |
| <i>Water solubility (mg L⁻¹)</i> | 0.22 | 0.9 | 0.19 | 0.36 |
| <i>Vapor pressure at 25 °C (Pa)</i> | 0.019 | 0.196 | 0.0117 | 0.0074 |
| <i>Henry's Law Constant (Pa m³ mol⁻¹)</i> | 3.22 | 3.22 | 13.4 | 4.28 |
| <i>Log K_{ow}</i> | 5.4 | 4.90 | 5.3 | 5.4 |
| <i>Log K_{oc} in sludge</i> | - | - | 3.8 | 3.8 |
| <i>Log K_{oc} in sediment</i> | - | - | 3.9 | 4.0 |

HHCB-lactone, or Galaxolidone is a by-product of abiotic and biotic degradation of HHCB. The benzylic methylene group of HHCB is oxidized to a more polar form, resulting in a lactone group (Figure I-1). Because of the increase in polarity, HHCB-lactone is mainly detected in waters rather than in sediments or organic matter. To the best of our knowledge, no other degradation product of HHCB has been identified.

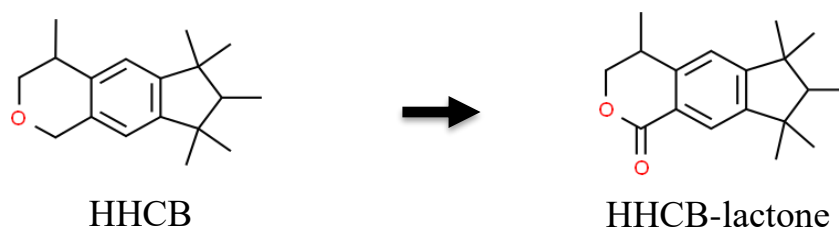


Figure I-1 Degradation of Galaxolide (HHCB) in Galaxolidone (HHCB-lactone).

1.4 Polycyclic Musk Fragrances in Wastewater Treatment Plants

The widespread use of synthetic musks leads to their release in large amounts through discharges of wastewater treatment plants (WWTPs) into the environment (Santiago-Morales et al., 2012; Gao et al., 2016). Generally, wastewaters produced by industrial and human activities contains concentration of $\mu\text{g L}^{-1}$ of synthetic musks, depending on the population dimension (Bester, 2005; Clara et al., 2011). In active sludge WWTPs, the biotransformation of HHCB into HHCB-lactone was observed (Bester, 2004) and, consequently, while concentrations of HHCB and AHTN generally decrease during wastewater treatment, HHCB-lactone increase: for example $1620\text{-}1740 \text{ ng L}^{-1}$ of HHCB-lactone were found in two WWTPs effluents receiving mainly domestic wastewaters from New York in comparison with $505\text{-}897 \text{ ng L}^{-1}$ detected in the respective influents (Reiner et al., 2007). Literature papers showed removal efficiency in WWTPs varying from 40 to 100% around the world: absorption on sludges is the main removal mechanism for PMFs. Many studies recorded unsatisfactory removal rates: below 50% HHCB for were registered in a WWTP located in Colombia (Arrubla et al., 2016) while Lishman et al. (2006) calculated a reduction of 43% for HHCB and 37% for AHTN during wastewater treatments in eight WWTPs located in Canada. A complete investigation of polycyclic musks fragrances fate in waters and sludges in an active sludge WWTP was carried out in Canada (Yang and Metcalfe, 2006) in order to understand the behavior of these compounds during treatment processes. Regarding waters, Yang & Metcalfe (2006) noticed a general reduction in synthetic musk concentrations during treatments: from 390 to 173 ng L^{-1} for HHCB, from 86 to 41 ng L^{-1} for AHTN, from 6 to 3 ng L^{-1} for ADBI and from 5 to 3 ng L^{-1} for AHDI. Otherwise, the same study highlighted an increase in PMFs concentrations from raw sludges to digested biosolids, from 3300 to $6800 \mu\text{g kg}^{-1} \text{ d.w.}$ for HHCB, from 720 to $1350 \mu\text{g kg}^{-1} \text{ d.w.}$ for AHTN, from 24 to $51 \mu\text{g kg}^{-1} \text{ d.w.}$ for ADBI and from 20 to $34 \mu\text{g kg}^{-1} \text{ d.w.}$ for AHDI. Those high concentrations confirmed the hypothesis that PMFs mainly accumulate in sludges due to their high hydrophobicity and increase their concentration during sludge treatment because this process reduce the solid mass up to 50-70% but do not degrade PMFs (Heberer, 2003). Therefore, digested sludge is more contaminated by PMFs than activated sludge and this could be a serious problem when sludges are employed for example in agricultural culture. Same patterns were observed in Europe in different states thus confirming that the incomplete removal of PMFs in WWTPs do

not strictly depends on concentrations in influents and, consequently, on the use of these compounds in the respective states, and the accumulation in sludges remain the main process which involves these compounds during wastewater treatment. In fact, same changes in concentration levels were observed in Switzerland, where Kupper et al. (2006) conducted a removal study analyzing HHCB, AHTN, ADBI and AHDI in water and sludge at different treatment steps of a WWTP. Concerning water line, results showed a general decrease in the concentration of all PMFs: HHCB and AHTN were the highest concentration compounds with 4420 and 1430 ng L⁻¹ in the influent, 3440 and 1110 ng L⁻¹ in the primary effluent and 770 and 320 ng L⁻¹ in the final effluent. ADBI and AHDI evidenced significantly lower values: 180 and 70 ng L⁻¹ in the influent, 120 and 50 ng L⁻¹ in the primary effluent and values below the limit of quantification in the final effluent. Regarding the sludge line, the highest values were found in the untreated raw sludge: 10350 µg kg⁻¹ d.w. for HHCB, 3420 µg kg⁻¹ d.w. for AHTN, 320 µg kg⁻¹ d.w. for ADBI and 130 µg kg⁻¹ d.w. for AHDI. The concentrations of all the polycyclic musks analyzed decreased during sludge treatment but, at the end of the process, they registered values similar to those of the untreated sludge (Kupper et al., 2006). In general, the concentration of PMFs in sludges is from three to four orders of magnitude higher than the concentration in the aqueous phase and the technologies used in conventional WWTPs are unable to completely degrade this type of micropollutants both in water and mostly in sludges. However, exhaustive monitoring data are available only for Germany, Netherlands and Switzerland while in Southern Europe, where the highest use of detergent was recorded, only few studies on PMFs in wastewater treatments were carried out. Because of the incomplete removal of these compounds already in countries with a lower consumption of PMFs, exhaustive monitoring studies appear extremely necessary in Southern Europe in order to assess the amount of PMFs that is received into the environment by WWTPs effluents. Researches regarding PMFs removal in WWTPs were carried out only in Spain (Godayol et al., 2015). HHCB and AHTN were monitored in two WWTPs located in Castell d'Aro and Girona (north-east Spain). Samples of primary effluent, biological treatment effluent and tertiary effluent were analyzed. In the three sampling stations of Castell d'Aro, mean values of HHCB were 2.92–3.94–3.56 µg L⁻¹ and 0.19–0.48–0.29 µg L⁻¹ of AHTN, respectively. These results showed that significant differences between the three sampling stations were measured for all compounds and that levels were always higher in the secondary rather than the primary effluent. Even at Girona WWTP, samples of primary and secondary effluents showed the same increase in PMFs concentration: from 2.50 to 3.36 µg L⁻¹ for HHCB and from 0.14 to 0.37 µg L⁻¹ for AHTN. The high concentrations of PMFs detected in influent samples reflects the extensive usage of these chemicals in this region (HERA, 2004). No data regarding removal of PMFs in WWTPs are available for Italy. Given the highest usage of these compounds detected in our country (HERA, 2004), it is extremely necessary to assess the removal grade of PMFs in WWTPs and the evaluation of possible environmental contamination.

1.5 Environmental contamination

As described above, most of PMFs are not removed by conventional wastewater treatments because they are not specifically designed for the removal of this kind of micropollutants. Therefore, WWTPs effluents represent the main source of pollution for the aquatic environment. In fact, PMFs are still present at ng L⁻¹ (sometimes even µg L⁻¹) in the WWTPs effluents and receiving aquatic ecosystems (Chase et al., 2012). Melymuk et al. estimated that loadings of PMFs (HHCB and AHTN) to Lake Ontario (Canada) were 83%

from WWTP effluent, 10% due to atmospheric deposition and only 7% to tributary discharge to the lake (Melymuk et al., 2014). Bester (2005) reported concentrations of polycyclic musks up to 600 ng L⁻¹ in Ruhr river at the discharge of a WWTP and of 60 ng L⁻¹ of HHCB and 10 ng L⁻¹ of AHTN in a collection point for drinking water in the same river. In Korea, a research on synthetic musk compounds in WWTPs and receiving surface water systems was carried out by Lee et al. (2010). The study focused on the occurrence and distribution patterns of musk xylene, musk ketone, HHCB and AHTN in 10 WWTPs located in Busan metropolitan city, their removal effects by various secondary treatment process and the presence of these compounds in the surface waters of urban and rural areas. HHCB, AHTN and musk ketone were detected in all influent end effluent samples, with HHCB as the predominant compound. The analysis of different types of wastewater, industrial and municipal, revealed once again that household sewage was the main source of synthetic musk compounds, as expected from their fields of application. Removal efficiencies in different types of WWTPs were calculated and compared to the classical activated sludge process in which average removals of HHCB and AHTN were 53±6% and 56±5%. Even if HHCB and AHTN removals of modified biological treatment processes were slightly higher than the conventional activated sludge process, a complete removal of these compounds was never detected. As a consequence, HHCB and AHTN were detected even in river waters, from 4 rivers and 10 streams near rural and urban areas (Lee et al., 2010). HHCB and AHTN were detected in all water samples at an average concentration of 4.91 µg L⁻¹ and 0.98 µg L⁻¹, respectively. HHCB concentrations were approximately 4–5 times higher than those of AHTN in all sampling sites according to their respective usage. Urban areas showed higher concentrations than rural areas thus confirming WWTPs effluents as the main source of PMFs in surface waters. In particular, sampling points near WWTPs discharge showed relatively high concentrations of PMFs. The continuous discharge of PMFs from WWTPs can even impact large aquatic ecosystem that, at a certain point, are no longer able to mitigate this type of pollution through the dilution process, the main way to disperse persistent organic pollutants as PMFs in the environment. This process was evidenced in Korea, in which freshwater and sediments from Nakdong River were analyzed to monitor nitro musks, HHCB and AHTN (Lee et al., 2014). PMFs were detected according to the respective consumption grade in Korea (NIER, 2009): HHCB was the most commonly detected compound (80 ± 15%) followed by musk ketone (10 ± 12%) and AHTN (10 ± 10%) while musk xylene was not detected. Authors evidenced higher fragrances concentrations in sampling sites closer to effluents of WWTPs, revealing them as contamination sources and suggesting that dilution plays an important role in the aquatic environment. The same trend was found in freshwater sediments in which PMFs tends to accumulate due to their lipophilic properties thus causing a higher detection frequency and concentration levels in respect to the water phase. In general, higher concentrations were found in sites closer to WWTPs effluents. This hypothesis was confirmed also through the analysis of coastal sediments: always in Korea, Lee et al. (2014) measured synthetic musk fragrances in coastal sediments of 25 sampling sites and detected these compounds with lower frequency and concentration than freshwater ecosystems confirming the high dilution effect in this matrix. The fact that presence and concentration levels of synthetic musks in the aquatic environment depend on the grade of wastewaters discharge and its dilution in the surface water was emphasized even by Lange et al. (2015). In this research, authors investigated the presence and fate of HHCB, its metabolite HHCB-lactone and AHTN in a small river located in south-west Germany which receives wastewaters from two municipal wastewater treatment plants. Once again, the concentration

profile of HHCB and AHTN clearly identified the municipal WWTPs as the main source of the synthetic musks; downstream WWTPs, concentrations increased significantly from $0.001 \mu\text{g L}^{-1}$ to $0.25 \mu\text{g L}^{-1}$ for HHCB, from $0.001 \mu\text{g L}^{-1}$ up to $0.06 \mu\text{g L}^{-1}$ for AHTN. Comparable to those compounds, the concentration of HHCB-lactone increased downstream WWTPs from $0.003 \mu\text{g L}^{-1}$ to $1 \mu\text{g L}^{-1}$ but decreased along the course of the river due to dilution and reduced HHCB degradation. In addition to these findings, a one year monitoring study allowed to point out the influence of temperature in degradation of HHCB in surface waters; HHCB-lactone/HHCB ratio was measured at different temperatures and revealed an increase in microbiological or chemical degradation of HHCB at higher temperatures (Lange et al., 2015). The same authors evidenced that since HHCB has a higher sorption tendency in comparison to HHCB-lactone, as expected from their physico-chemical properties. In general, surface water concentrations of PMFs downstream a WWTP reflect the dilution factor of the wastewater effluent into the receiving body and, in addition, with increasing distance from the WWTP, degradation, sorption and sedimentation processes become more important. Regarding Italian surface waters, only one monitoring study was carried out in 2010 in order to contribute to fill the gap of knowledge regarding the presence of PMFs in Italian rivers (Villa et al., 2012). The survey was conducted along the Molgora River, located in a very densely populated area of Lombardia Region. Water was sampled seasonally in 7 stations, starting from a rural site used as background level of pollution and crossing over three WWTPs present along the river. Water samples and suspended solids were analyzed for the presence of HHCB, AHTN and ADBI. Results indicated that the concentrations in Molgora River highly vary in the two matrices depending on the chemical compound considered, the sampling location and time. The median concentrations of PMFs in Molgora River were from two to four times higher than those measured in Northern Europe surface waters, as expected from PMFs usage data in Europe. Starting from sites considered as rural, an increase of PMFs concentration was observed for both water and suspended solids continuing along the axis of the river with peak concentrations of 1141 ng L^{-1} and $17993 \text{ ng g}^{-1} \text{ d.w.}$ for HHCB, 365 ng L^{-1} and $4321 \text{ ng g}^{-1} \text{ d.w.}$ for AHTN, 23.4 ng L^{-1} and $249 \text{ ng g}^{-1} \text{ d.w.}$ for ADBI registered downstream the three WWTPs. Higher PMFs levels were observed in spring; this phenomenon might be related to the reduced water flow of the river in this period but also to different activity regimes of the three WWTPs. Besides this work, other researches are absolutely needed in order to assess the current presence and concentration levels of PMFs in Italian surface water systems.

Environmental contamination by polycyclic musk fragrances is not only related to the aquatic compartment; biosolids deriving from WWTPs are increasingly applied as fertilizers in agricultural lands. On the one hand, biosolids applied to farmland can bring some benefits as improving soil properties and supplying nutrients essential for plant growth (Kimberley et al., 2004). On the other hand, the frequent use of wastewater biosolids that may contain a wide range of inorganic and organic toxic contaminants can pose a serious risk for environment and human health. As a consequence, these persistent organic chemicals were detected in large amounts even in agricultural soils (Biel-Maeso et al., 2019; Chen et al., 2014). The effects of the application of biosolids as fertilizers were studied for example in China (Chen et al., 2014) where soils with single and repeated biosolids application were compared with biosolids-free soils with the aim of detecting PMFs. AHTN and HHCB were detected in all biosolid-amended soils while no or trace amounts of these compounds were detected in soils in which biosolids were not applied. Concentrations of these compounds were significantly higher than those measured in soils with a single biosolid application. For instance, in

soils with one single biosolid application AHTN was found at concentration range of 2.4-9.7 $\mu\text{g kg}^{-1}$ while HHCB was found at 0.7-3.5 $\mu\text{g kg}^{-1}$ while in soils with a repeated biosolid application AHTN concentration range was 24.4-67.5 $\mu\text{g kg}^{-1}$ and HHCB concentration range was 6.3-29.0 $\mu\text{g kg}^{-1}$. This suggested that the presence of the target compounds in the biosolid-amended soils was due to the application of biosolids. Not only biosolids but even wastewater effluent can represent a pollution source for agriculture. Biel-Maeso et al. (2019) evidenced that the application of treated wastewater for irrigation of soils showed the detection of the same chemicals in both the two different matrices. Among all personal care products monitored, fragrances were the most predominant both in wastewater and in soil. After continuous irrigation with reclaimed wastewater, HHCB, AHTN and ADBI were detected in the soil column up to 150 cm of depth, with fragrances reaching maximum concentrations of up to 1290 ng g^{-1} of HHCB. From these studies, a serious environmental contamination from PMFs in WWTPs effluents and sludges is evidenced. However, the potential role of treated wastewater reuse as an alternative source of water supply is now well acknowledged, embedded within international, European and national strategies and included in the 17 Sustainable Development Goals (SDGs), the world's best plan to build a better world for people and our planet by 2030. Adopted by all United Nations Member States in 2015, the SDGs are a call for action by all countries - poor, rich and middle-income - to promote prosperity while protecting the environment. Sustainable Development Goal on Water (SDG 6) specifically targets a substantial improvement in water quality by reducing pollution, eliminating dumping and minimizing release of hazardous chemicals and materials, halving the proportion of untreated wastewater and substantially increasing recycling and safe reuse of water globally.

Water reuse is also a top priority activity the Strategic Implementation Plan of the European Innovation Partnership on Water, and maximization of water reuse is a specific objective in the Communication "Blueprint to safeguard Europe's water resources" (COM., 2012). In this framework, understand the actual impact of WWTPs discharges on receiving ecosystems in terms of PMF concentrations and detection of their presence in surface waters of a country in which the highest consumption rates of these chemicals were measured can be useful not only for the economic reasons mentioned above, but also for the protection of the environment and, ultimately, of human health.

1.6 Bioaccumulation of PMFs in organisms

As described above, PMFs are widely detected in environmental samples as surface waters, sediments and soils. Given their high persistence in their environment and their lipophilic characteristics, PMFs can be detected even in organisms and may be subjected to bioaccumulation and biomagnification processes which can pose a serious risk for the organisms themselves and, again, also for the human health. Since HHCB and AHTN are present in the environment at much higher concentration than other PMFs, the risk pose by these two compounds is greater. For this reason, most of the researches conducted on PMFs presence in organisms focused only on those two polycyclic musk compounds. As said before, today PMFs are detected ubiquitously in all environmental compartments as a consequence of both direct (disposal, wastage) and indirect (washing and swimming) applications (Daughton and Ternes, 1999). Among aquatic organisms, PMFs were detected for example in macroalgae reaching concentrations of 3.1 $\mu\text{g kg}^{-1}$ d.w. for HHCB (Cunha et al., 2015). In clams and mussels, HHCB, AHTN, ADBI and AHDI reached values of 6.0 mg kg^{-1} (Shek et al., 2008), 1.1 mg kg^{-1} (Gatermann et al., 1999), 11.5 mg kg^{-1} and 0.28 mg kg^{-1} (Mogensen et al.,

2004) corrected for lipid weight (l.w.), respectively. In different fish species HHCB and AHTN can be detected even at levels of hundred mg kg^{-1} l.w. (Reiner & Kannan, 2011; Ros et al., 2015). For instance, the presence of synthetic musk was also assessed in fish from Taihu Lake, the second largest freshwater lake in China (Zhang et al., 2013). Fishes were collected from three different stations in Taihu Lake and analyzed for the presence of HHCB, AHTN, ADBI and AHDI plus two nitromusks. HHCB and AHTN were the most dominant compounds, with a detection frequency of 63 % and 64 %, respectively while ADBI and AHDI were not detected. The concentrations of HHCB and AHTN in the fish samples ranged from <LOD to 52.9 ng g^{-1} l.w. and from <LOD to 7.5 ng g^{-1} l.w., respectively. These median concentrations were significantly lower than those in wild freshwater fish captured from the Haihe River (Hu et al., 2011), located always in China; here concentrations ranged from 107.9 ng g^{-1} to 823.3 ng g^{-1} l.w. for HHCB and 107.1 ng g^{-1} to 771.7 ng g^{-1} l.w. for AHTN. Differences in HHCB and AHTN concentrations detected reflect the different grade of usage of PMFs in the specific place and so it is very important to take into consideration this parameter especially when making comparison between different regions or States. However, a general trend was evidenced everywhere: highest concentrations of PMFs were detected in aquatic organisms living in water ecosystems which receive effluents from WWTPs, thus confirming wastewaters as the source of pollution. Other researches detected HHCB and AHTN in fish fillets from other locations around the world: in fish fillet from Vltava River, Czech Republic, concentration ranges were $663\text{--}1900 \text{ ng g}^{-1}$ l.w. for HHCB and $134\text{--}525 \text{ ng g}^{-1}$ l.w. for AHTN (Hájková et al., 2007), while in bream from Elbe River, in Germany, were $545\text{--}6400 \text{ ng g}^{-1}$ l.w. for HHCB and $48\text{--}2130 \text{ ng g}^{-1}$ l.w. for AHTN (Rüdel et al., 2006). PMFs were detected also in fishes from remote areas as Alpine Lakes in Switzerland located between 2000 and 2600 m a.s.l. where no WWTPs are present and, therefore, contamination come only by atmospheric transport deposition from polluted sites. Here, PMFs concentration ranges, expressed as ng g^{-1} l.w., were significantly lower as expected: 8.7–35 for ADBI, 0.79–6.3 for AHDI, 20–54 for AHTN and 42–230 for HHCB (Schmid et al., 2007). To summarize, these studies confirmed that PMFs discharged in surface waters from WWTPs effluents can be accumulated in all organisms living in the aquatic ecosystem as other persistent organic pollutants already subjected to restriction and specific regulations. Specifically, bioaccumulation is defined as the net result of the rate of chemical uptake (from respiratory uptake, diet) and the chemical elimination by several biological pathways (Arnot and Gobas, 2006). Some characteristics of HHCB and AHTN, such as slow elimination rates and tendency to be present in several environmental compartments, make them good candidates for bioaccumulation and bioconcentration. Bioconcentration (BCF) and bioaccumulation (BAF) factors of PMFs in aquatic organisms were calculated by several studies, both in laboratory and fields conditions (Table I-4).

Table I-4 Bioconcentration (lab conditions) and Bioaccumulation (field conditions) factors of polycyclic musks in various aquatic organisms based on lipid (BCF_L - BAF_L) and wet (BCF_w - BAF_w) weights taken from Tumová et al. (2019).

| <i>PMF</i> | <i>Species</i> | <i>Sample type</i> | <i>BCF_L</i> (lab) or <i>BAF_L</i> (field) [L kg ⁻¹] | <i>BCF_w</i> (lab) or <i>BAF_w</i> (field) [L kg ⁻¹] | <i>n</i> | <i>C_w</i> [µg L ⁻¹] ^c | <i>Reference</i> | | |
|-----------------------------|---|--------------------|---|---|-----------------------|--|--------------------------|---|---|
| <i>HHCB</i> <i>lab</i> | Zebrafish (<i>Danio rerio</i>) | whole body | NP | 945 ^b 799 ^b | 6 | 20 ^c 169 ^c | Schreurs et al. (2004) | | |
| | Zebra mussel (<i>Dreissena polymorpha</i>) | soft tissue | 891 ^b 447 ^b | NP | 10 | 0.10 ^d 0.34 ^d | Parolini et al. (2015) | | |
| <i>HHCB</i> <i>field</i> | Eel (<i>Anguilla anguilla</i>) | muscle | 6357 ^b 2860 ^b 4069 ^b 3504 ^e | 742 ^b 509 ^b 952 ^b 862 ^e | 54 53 58 165 | 0.07 0.23 1.59 0.63 ^{b,e} | Fromme et al. (2001) | | |
| | Common rudd (<i>Scardinius erythrophthalmus</i>) | whole body | 2600 | 20 | 15 | 2.65 ^e | Gatermann et al. (2002) | | |
| | Common tench (<i>Tinca tinca</i>) | | 56 300 | 510 | 4 | 2.65 ^e | | | |
| | Crucian carp (<i>Carassius carassius</i>) | | 24 300 | 580 | 7 | 2.65 ^e | | | |
| | Eel (<i>Anguilla anguilla</i>) | | 1700 | 290 | 11 | 2.65 ^e | | | |
| | Zebra mussel (<i>Dreissena polymorpha</i>) | | 44 400 | 620 | 50 | 2.65 ^e | | | |
| | Crucian carp (<i>Carassius auratus</i>) | muscle | 52 370 | NP | 5 | 0.01 ^e | Hu et al. (2011) | | |
| | Common carp (<i>Cyprinus carpio</i>) | | 66 030 | NP | 1 | 0.01 ^e | | | |
| | Silver carp (<i>Hypophthalmichthys molitrix</i>) | | 39 400 | NP | 1 | 0.01 ^e | | | |
| | White perch (<i>Morone americana</i>) | liver | 1664 ^e 546 ^e 5097 ^e | 21-333 ^b | 3 | 0.01 0.03 0.004 | Reiner and Kannan (2011) | | |
| | Channel catfish (<i>Ictalurus punctatus</i>) | | 2895 ^e | | | 18-371 ^c | | 3 | 0.01 ^e |
| | White catfish (<i>Ameiurus catus</i>) | | 261 1 466 | | | 18-371 ^c | | 1 | 0.03 ^e 0.004 ^e |
| | Smallmouth bass (<i>Micropterus dolomieu</i>) | | 1169 ^e | 31-106 | 2 | | | | 0.01 ^e |
| | Largemouth bass (<i>Micropterus salmoides</i>) | | 434 | 30-146 | 1 | 0.03 ^e | | | |
| | Zebra mussel (<i>Dreissena polymorpha</i>) | | 3324 ^e | NP | 4 | 0.004 ^e | | | |

Table I-4 (Continued).

| | | | | | | | |
|---|---|----------------|--|--------------------------------------|-----|--|-------------------------|
| AHTN lab | Zebrafish (<i>Danio rerio</i>) | whole body | NP | 878 ^b 630 ^b | 6 | 18 ^c 227 ^c | Schreurs et al. (2004) |
| | Zebra mussel (<i>Dreissena polymorpha</i>) | soft tissue | 3949 ^b 1850 ^b | NP | 10 | 0.02 ^d 0.06 ^d | Parolini et al. (2015) |
| AHTN field | Eel (<i>Anguilla anguilla</i>) | muscle | < LOQ | < LOQ | 54 | 0.02 | Fromme et al. (2001) |
| | | | 5415 ^b | 757 ^b | 53 | 0.07 | |
| | | | 5774 ^b | 1 364 ^b | 58 | 0.53 | |
| | | | 5017 ^e | 1069 ^e | 165 | 0.21 ^{b,e} | |
| | Common rudd (<i>Scardinius erythrophthalmus</i>) | whole body | 5100 | 40 | 15 | 1.07 ^e | Gatermann et al. (2002) |
| | Common tench (<i>Tinca tinca</i>) | | 31 600 | 280 | 4 | | |
| | Crucian carp (<i>Carassius carassius</i>) | | 29 300 | 670 | 7 | | |
| | Eel (<i>Anguilla anguilla</i>) | | 2400 | 400 | 11 | | |
| | Zebra mussel (<i>Dreissena polymorpha</i>) | | 40 100 | 570 | 50 | | |
| | Crucian carp (<i>Carassius auratus</i>) | muscle | 54 070 | NP | 5 | 0.01 ^e | Hu et al. (2011) |
| Common carp (<i>Cyprinus carpio</i>) | 72 220 | | 1 | | | | |
| Silver carp (<i>Hypophthalmichthys molitrix</i>) | 39 940 | | 1 | | | | |
| ADBI field | Eel (<i>Anguilla anguilla</i>) | muscle | NP | 9 ^b | 58 | 0.53 | Fromme et al. (2001) |
| AHDI field | Eel (<i>Anguilla Anguilla</i>) | muscle | NP | 213 ^b | 10 | 0.53 | Fromme et al. (2001) |

BCF and BAF = C_B/C_w , C_B = median or mean concentration of a target compound inside biota, C_w = median or mean concentration of precursor compound in exposure water, LOQ= limit of quantification, n = number of biota samples, NP= not provided, not analysed or not specified, ^a= low dose and high dose concentrations, ^b= value was calculated from data provided in the study, ^c= initial concentration at time 0 h, ^d= arithmetic mean of initial concentrations at time 0 h, ^e= arithmetic mean of field study

Differences in BCF and BAF values indicates species-dependent accumulation and metabolism in fish. The field wet-weight-based BAF values were from 18 to 952 L kg⁻¹ for HHCB and from 40 to 1364 L kg⁻¹, comparable with BCF values obtained in laboratory conditions. This indicate that PMFs are absorbed by organisms through freshwaters alone rather than from a combination of uptake from water and from food. PMFs have been detected even in aquatic top predators (Nakata, 2005) and this may indicate a possible biomagnification of PMFs along the trophic chain. However, only few studies were conducted on the possible biomagnification of PMFs and results are contradictory and do not provide a strong positive or negative effect related to biomagnification. Because the extensive application of biosolids as fertilizers in agricultural fields, PMFs can be potentially accumulated not only in living organisms but also in organisms living in soil. Transfer of PMFs from soils amended with biosolids to soil dwelling organisms was already demonstrated (Kinney et al., 2008). In this study, samples of earthworms, soils and applied biosolids or swine manure were analyzed for the presence of organic micropollutants including PMFs. HHCB and AHTN

were detected in biosolids at 427 and 177 mg kg⁻¹, respectively, while they were not detected in swine manure. In agreement with this evidence, PMFs were detected in soils amended with biosolids up to 2770 µg kg⁻¹ (HHCB) and 773 µg kg⁻¹ (AHTN) while both were not detected in manure amended soil. Earthworms followed the same pattern, with concentration up to 3340 µg kg⁻¹ for HHCB and up to 279 µg kg⁻¹ for AHTN in biosolid amended soils and no detection in earthworms coming from manure amended soils. These results demonstrated that earthworms in common agricultural soils can bioaccumulate organic contaminants originating from biosolids. Because the application of municipal biosolids on agricultural soils is a common worldwide practice, the environmental risk of bioaccumulation of these organic contaminants into the food web is relevant. Artola-Garicano et al. (2003) evaluated bioconcentration factor of AHTN and HHCB in *Lumbriculus variegatus*. The organisms were exposed in a flow-through system with PMFs concentrations of 4 µg L⁻¹. The uptake in worms reached a plateau level after 3 days and respective BCF was 2692. For AHTN a plateau level was reached only after 8 days and so a bioconcentration factor of 6918 was calculated. Bioaccumulation and biomagnification processes can pose a serious risk for the organisms themselves and for the human health because they can lead to toxic effects in organisms and can be transferred to humans through the food web chain.

1.7 Toxicity of polycyclic musk fragrances in organisms

Many researches have been conducted to evaluate the toxicity effects of PMF on model organisms that may be representative for all organisms living in ecosystems in which PMF have been detected. These are species that can be utilized for studying specific biological processes, such as toxicity of xenobiotics, in order to obtain information on biological functioning in other organisms that live in the same environment. Different types of toxicity tests can be performed. Acute toxicity involves harmful effects on an organism through a single or short-term exposure to a xenobiotic and is generally the first test performed on a new chemical. It allows the estimation of the potential hazards of chemicals by evaluating their LC₅₀, the xenobiotic concentration which can cause a mortality of 50% in the exposed organisms. Assessment of the acute toxic potential of substances is required to determine their adverse effects that might occur due to accidental or deliberate short-term exposure. Acute toxicity is distinguished from subchronic and chronic toxicity, which describe the adverse health effects from repeated exposures, often at lower levels, to a substance over a longer time period (months or years). Subchronic toxicity is the ability of a toxic substance to cause negative effects over more than one year but less than the lifetime of the exposed organism. Chronic toxicity is the ability of a substance or mixture of substances to cause harmful effects over an extended period, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism. Adverse effects associated with chronic toxicity may be directly lethal effect but are more commonly sublethal, including changes in growth, reproduction, or behaviour. Aquatic chronic toxicity tests are commonly used to determine water quality guidelines and regulations for protection of aquatic organisms. Acute toxicity of PMFs to aquatic organisms ranges from hundreds of µg L⁻¹ to amounts of <20 mg L⁻¹ (Tumová et al., 2019). For example, Wollenberger et al., (2003) evaluated the acute toxicity of PMFs in the marine copepod *Acartia tonsa*. The LC₅₀ values were 0.47 mg L⁻¹ for HHCB, 0.71 mg L⁻¹ for AHTN and 2.50 mg L⁻¹ for ADBI. Acute toxicity was calculated even for the crustacean *Artemia salina* in a 48-h exposure test, resulting in LC₅₀ value of 1.88 mg L⁻¹ for HHCB 1.53 mg L⁻¹ for AHTN. In *Thamnocephalus platyurus*, another

crustacean, a 24 h exposure test showed a LC_{50} value of 1.23 mg L^{-1} for HHCB and 1.53 mg L^{-1} for AHTN (Doležalová Weissmannová et al., 2013). Concerning fish, acute toxicity of HHCB and AHTN was evaluated in the Bluegill sunfish *Lepomis macrochirus*. In this case, exposure lasted for 21 days and LC_{50} were $452 \text{ } \mu\text{g L}^{-1}$ for HHCB and $314 \text{ } \mu\text{g L}^{-1}$ for AHTN (Balk and Ford, 1999). Few studies focused on subchronic effects were published and no data are currently available regarding chronic toxicity. The lowest concentrations of PMFs causing subchronic effects were in the range from 50 to $184 \text{ } \mu\text{g L}^{-1}$. In a long-term study, it has been shown that HHCB and AHTN affect negatively the reproduction of the crustacean *Daphnia magna* at concentrations of 205 and $244 \text{ } \mu\text{g L}^{-1}$ (Balk & Ford, 1999). Exposure for 21 days of the freshwater zebra mussel *Dreissena polymorpha* to concentrations of HHCB and AHTN of 500 ng L^{-1} and 80 ng L^{-1} , respectively, caused oxidative stress by increasing lipid peroxide, protein carbonyl levels and changes in DNA structure (Parolini et al., 2015). Other modifications in oxidative stress parameters were observed in adult goldfish of *Carassius auratus* after 21 days of exposure to $15 \text{ } \mu\text{g L}^{-1}$ of HHCB (Chen et al., 2012) and in juvenile zebrafish *Danio rerio* after 28 days of exposure to 50 ng L^{-1} of AHTN (Blahova et al., 2018). In the copepod *Arcatia tonsa*, the early copepodite stage development was inhibited at $59 \text{ } \mu\text{g L}^{-1}$ of HHCB and $26 \text{ } \mu\text{g L}^{-1}$ of AHTN during 5 days of exposure (Wollenberger et al., 2003). Toxicity of PMFs was observed at the multixenobiotic transporters, cell membrane proteins responsible for elimination of xenobiotics (Luckenbach et al., 2004). These protein transporters were inhibited in marine mussels *Mytilus californianus* with a dose-dependently mechanism depending on PMFs. Inhibition of these transporters allows the accumulation of xenobiotics in the cell which may increase their toxic action. Besides inducing an oxidative stress response in exposed organisms, PMFs reported to have endocrine disrupting activities in male and female fish. The induction of vitellogenin was reported in male Medaka fish *Oryzias latipes* exposed to concentrations of $500 \text{ } \mu\text{g L}^{-1}$ of HHCB and AHTN (Yamauchi et al., 2008). Enzyme activity changes were observed by Fernandes et al. (2012) in the male European sea bass *Dicentrarchus labrax* exposed to HHCB: enzymes Cyp17 and Cyp11 β , which are involved in the production of testosterone, were both inhibited. Changes in these enzymes during the early stages of sexual maturation may alter androgen related processes as spermatogenesis, reproductive behaviour and development of secondary sexual characteristics. The inhibition of these two enzymes caused by HHCB was observed also in male carp (Schnell et al., 2009). However, when analysing laboratory toxicological studies, it must be considered that nominal concentrations of PMFs may not correspond to real concentrations of these substances as assessed by Tumová et al. (2019). Decrease in PMFs concentration may occur especially in aerated conditions reaching 80% of the initial values. Thus, organisms will be exposed to variable concentrations rather than stable as expected in nature and, consequently, the interpretation of the observed effects and their extrapolations may be compromised. For this reason, a continuous check of the compound stability though the course of the experiment is extremely important (Tumová et al., 2019). Since PMFs were detected at high concentrations also in agricultural soils amended with biosolids derived from WWTPs as described above, an evaluation of their potential harmful effects on soil living organisms is essential in order to protect the environment and also the biodiversity of this ecosystem, extremely important for agriculture activity. Toxicity of PMFs was evaluated in the free-living soil nematode *Caenorhabditis elegans* exposed to sewage sludges (Čadková et al., 2020). In this case, the effect of HHCB was analysed on the feeding activity; an inhibition of the feeding activity caused by HHCB at concentration of $12.2 \pm 2.2 \text{ mg L}^{-1}$ was evidenced and supported with reactive oxygen

species (ROS) measurement that highlighted oxidative stress in *C. elegans* at a HHCb concentration above 1 mg L⁻¹. The ecotoxicity assessment of HHCb and AHTN was investigated even in the earthworm *Eisenia fetida* (Chen et al., 2011) using acute and chronic toxicity tests. In the acute study, earthworms were exposed to HHCb and AHTN concentrations of 0-100-140-196-274-384-538-753-1054 and 1476 mg kg⁻¹ (air-dried soil). No mortalities occurred at the lowest concentrations of 100 and 140 mg kg⁻¹. However, there was 100% of mortality at concentrations higher than 1054 mg kg⁻¹. HHCb was considerably more toxic to *E. fetida* than AHTN. In the chronic test, earthworms were exposed to concentrations of HHCb and AHTN of 3-10-30-50 and 100 mg kg⁻¹ (air-dried soil). No mortality occurred in this experiment, but the growth rate was inhibited by 68% for earthworms exposed to 100 mg kg⁻¹ of AHTN and 40% for worms exposed to 100 mg kg⁻¹ of HHCb in respect to the control. Additionally, a significant decrease in reproduction rate and cocoon production in earthworms exposed to 50 and 100 mg kg⁻¹ of both AHTN and HHCb were remarked. Besides traditional endpoints of toxicological studies, even gene expression response of antioxidant enzymes was considered in order to assess adverse effects of very low chemical concentrations. In fact, chemicals can induce oxidative stress in organisms with the consequent generation of reactive oxygen species (ROS) which may affect the expression of numerous genes encoding ROS scavengers such as superoxide dismutase (SOD) and catalase (CAT). Chen et al. (2011) highlighted that expression levels of SOD gene in *E. fetida* were significantly increased after a 28-day exposure in the treatments exposed to 30-50 and 100 mg kg⁻¹ AHTN while, concerning HHCb, after a 28-day exposure a significant increase in expression levels was observed in 10-30-50 and 100 mg kg⁻¹. CAT and SOD gene expression showed a similar pattern in response to AHTN and HHCb. In conclusion, since PMFs showed acute and subchronic toxic effects on both aquatic and terrestrial organisms, their ubiquitous presence at high levels, relative persistence, tendency for bioconcentration and potential hormonal activities raised significant concerns about their impact on the environment and, at the end, even on the human health. Once again, monitoring actual environmental concentrations of PMFs and current WWTPs efficiency in removing these compounds from wastewaters and sludges in a country which registered, between Europe, the highest use of these products, is extremely crucial to achieve Sustainable Development Goals of the UN by 2030 regarding the possibility to reuse wastewaters and sludges for other applications, but also in a context of environmental and human health protection.

1.8 Analysis of polycyclic musk fragrances in environmental samples

Different methods are currently performed for the analysis of polycyclic musk fragrances according to laboratory instrumental availability and to analyzed matrix samples. The main problem regarding the analysis of musk compounds are blanks; PMFs concentration should be very low in these samples that must not be contaminated by products present inside the lab. The analysis of fragrances, therefore, requires some precautions for laboratory personnel. Soaps, creams, shower-gel, shampoos, detergents and all products which might contain fragrances have to be avoided from labs (Homem et al., 2016). Contamination may also derive from room cleaning agents, such as floor or window polisher cleaner; the same precautions must be adopted even during sampling campaigns, together with avoiding, for example, the use of sunscreen protection creams and lotions. These guidelines may be crucial when analyzing matrices which are not contaminated by fragrances as surface or drinking waters. Other problems come up when analyzing wastewater samples, since they contain large amounts of surfactants and suspended particles that may

interfere with the analysis of organic micro-pollutants (Bester, 2009). This kind of interferences can be avoided by a sample pre-treatment consisting in filtration, centrifugation or by using high quantity of extraction materials or extraction devices with larger diameter, for example extraction disk instead of extraction cartridges. Musks have been extracted from water samples using different systems as solid phase extraction (SPE) by C₁₈ Empore disk (Simonich et al., 2002), Biobeads SM-2 (Buerge et al., 2003), divinylbenzene based polymers (Osemwengie and Gerstenberger, 2004) or by using liquid-liquid extraction (LLE) with various solvents (Bester, 2004; Reiner et al., 2007). All these methods can give reliable results. Sludges can be treated both as a liquid or dry sample. One critical aspect for the analysis of sludges is the complete extraction of PMFs from the matrix since reference spiked material is not available. Another problem is due to the removal of all possible matrix interferences that might reduce the sensitivity of analytical instrumentations. LLE can also be used with the addition of salts as NaCl to improve phase separation (Reiner et al., 2007). To obtain a solid matrix for extraction, sludges can be also previously dried by a lyophilization step and then extracted. Lyophilization overnight, followed by Soxhlet extraction proved to give higher recovery rates than a slurry extraction using high volumes of solvents or than drying samples with sodium sulphate before extraction (Bester, 2004). Regarding sediment, this matrix is usually analyzed dried. These samples are usually Soxhlet extracted (Peck et al., 2006) but also a microwave assisted solvent extraction was successfully applied (Rice and Mitra, 2007). After extraction, co-extracted impurities must be removed. The clean-up procedure for trace analysis of PMFs in environmental samples are very similar to those for PCB residual analysis, because of the comparable lipophilicity and polarity of these substances. Thus, clean-up often includes adsorption chromatography on various adsorbents, such as silica gel, florisil or aluminum oxide while high losses were observed when using concentrated sulphuric acid for the clean-up of raw extract (Rimkus & Wolf, 1997). Also adsorption column chromatography on florisil or silica gel resulted in variable high losses of some polycyclic musk fragrances due to apparently increased adsorption (Fromme et al., 2001). Thus, in order to obtain satisfactory results with clean-up techniques, the elution conditions must be modified according to analytes and different type of extraction. In addition, it should be considered that metabolites as HHCB-lactone are more polar than parental compounds and therefore they need slightly polar solvents to be eluted from cleaning columns. Regarding instrumental analysis, the combination of capillary gas-chromatography and mass spectrometry (GC-MS) proved to be versatile for the detection of PMFs. The Electron Impact (EI) mass spectra show several characteristic mass fragments which can be used for identification and quantification of the compounds (Rimkus & Wolf, 1997). In addition, even indicative mass ions are available for the analysis of this compound using Selected Ion Monitoring (SIM) or Selected Reaction Monitoring (SRM) mode. These analytical procedures allow to reach a high level of sensitivity and specificity for PMFs analysis. For the trace analysis of PMFs various MS systems can be used, such as quadrupole mass spectrometers (Schmidt-Bäumler et al., 1999), ion trap system (Winkler et al., 1998) and high resolution (HR) mass spectrometer (Müller et al., 1996), the latter one is rarely used, because the sensitivity of the low resolution mass spectrometer is sufficient for the analysis of these compounds. In literature, there are no indications for compounds that co-elute with musk fragrances giving similar spectra. Based on these studies and given the growing concern about the presence of fragrances in the environment, the development of a rapid, inexpensive and easily usable analytical method for most laboratories represents a fundamental point for achieving a homogeneous analytical monitoring of PMFs between countries.

1.9 References

- Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ. Rev.* 14, 257–297. <https://doi.org/10.1139/A06-005>
- Arrubla, J.P., Cubillos, J.A., Ramírez, C.A., Arredondo, J.A., Arias, C.A., Paredes, D., 2016. Pharmaceutical and personal care products in domestic wastewater and their removal in anaerobic treatment systems: Septic tank – Up flow anaerobic filter. *Ing. e Investig.* 36, 70–78. <https://doi.org/10.15446/ing.investig.v36n1.53076>
- Artola-Garicano, E., Sinnige, T.L., Van Holsteijn, I., Vaes, W.H.J., Hermens, J.L.M., 2003. Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (*Chironomus riparius* and *Lumbriculus variegatus*). *Environ. Toxicol. Chem.* 22, 1086–1092. [https://doi.org/10.1897/1551-5028\(2003\)022<1086:BAATOP>2.0.CO;2](https://doi.org/10.1897/1551-5028(2003)022<1086:BAATOP>2.0.CO;2)
- Balk, F., Ford, R.A., 1999. Environmental risk assessment for the polycyclic musks, AHTN and HHCB. II. Effect assessment and risk characterisation. *Toxicol. Lett.* 111, 81–94. [https://doi.org/10.1016/S0378-4274\(99\)00170-8](https://doi.org/10.1016/S0378-4274(99)00170-8)
- Bester, K., 2009. Analysis of musk fragrances in environmental samples. *J. Chromatogr. A* 1216, 470–480. <https://doi.org/10.1016/j.chroma.2008.08.093>
- Bester, K., 2005. Polycyclic musks in the Ruhr catchment area - Transport, discharges of waste water, and transformations of HHCB, AHTN and HHCB-lactone. *J. Environ. Monit.* 7, 43–51. <https://doi.org/10.1039/b409213a>
- Bester, K., 2004. Retention characteristics and balance assessment for two polycyclic musk fragrances (HHCB and AHTN) in a typical German sewage treatment plant. *Chemosphere* 57, 863–870. <https://doi.org/10.1016/j.chemosphere.2004.08.032>
- Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. *Water Res.* 150, 129–139. <https://doi.org/10.1016/j.watres.2018.11.045>
- Blahova, J., Divisova, L., Plhalova, L., Enevova, V., Hostovsky, M., Doubkova, V., Marsalek, P., Fictum, P., Svobodova, Z., 2018. Multibiomarker Responses of Juvenile Stages of Zebrafish (*Danio rerio*) to Subchronic Exposure to Polycyclic Musk Tonalide. *Arch. Environ. Contam. Toxicol.* 74, 568–576. <https://doi.org/10.1007/s00244-017-0484-8>
- Buerge, I.J., Buser, H.R., Müller, M.D., Poiger, T., 2003. Behavior of the Polycyclic Musks HHCB and AHTN in Lakes, Two Potential Anthropogenic Markers for Domestic Wastewater in Surface Waters. *Environ. Sci. Technol.* 37, 5636–5644. <https://doi.org/10.1021/es0300721>
- Čadková, Z., Száková, J., Mukhtorova, D., Hlava, J., Pulkrabová, J., Balík, J., Tlustoš, P., Vadlejch, J., 2020. The response of soil nematode *Caenorhabditis elegans* on the sewage sludge-derived micropollutants. *J. Hazard. Mater.* 384. <https://doi.org/10.1016/j.jhazmat.2019.121468>
- Chase, D.A., Karnjanapiboonwong, A., Fang, Y., Cobb, G.P., Morse, A.N., Anderson, T.A., 2012. Occurrence of synthetic musk fragrances in effluent and non-effluent impacted environments. *Sci. Total Environ.* 416, 253–260. <https://doi.org/10.1016/j.scitotenv.2011.11.067>
- Chen, F., Gao, J., Zhou, Q., 2012. Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in *Carassius auratus* using oxidative stress biomarkers. *Environ. Pollut.* 162, 91–97. <https://doi.org/10.1016/j.envpol.2011.10.016>
- Chen, F., Ying, G.G., Ma, Y.B., Chen, Z.F., Lai, H.J., Peng, F.J., 2014. Field dissipation and risk assessment of typical personal care products TCC, TCS, AHTN and HHCB in biosolid-amended soils. *Sci. Total Environ.* 470–471, 1078–1086. <https://doi.org/10.1016/j.scitotenv.2013.10.080>
- Clara, M., Gans, O., Windhofer, G., Krenn, U., Hartl, W., Braun, K., Scharf, S., Scheffknecht, C., 2011. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. *Chemosphere*

- 82, 1116–1123. <https://doi.org/10.1016/j.chemosphere.2010.11.041>
- Cunha, S.C., Fernandes, J.O., Vallecillos, L., Cano-Sancho, G., Domingo, J.L., Pocurull, E., Borrull, F., Maulvault, A.L., Ferrari, F., Fernandez-Tejedor, M., Van den Heuvel, F., Kotterman, M., 2015. Co-occurrence of musk fragrances and UV-filters in seafood and macroalgae collected in European hotspots. *Environ. Res.* 143, 65–71. <https://doi.org/10.1016/j.envres.2015.05.003>
- Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and Personal Care Products in the Environment : Agents of Subtle Change ? 107, 907–938.
- ECHA – European Chemicals Agency, 2010. Candidate List of Substances of Very High Concern for Authorisation.
- Franke, S., Meyer, C., Heinzl, N., Gatermann, R., Hühnerfuss, H., Rimkus, G., König, W.A., Francke, W., 1999. Enantiomeric composition of the polycyclic musks HHCB and AHTN in different aquatic species. *Chirality* 11, 795–801. [https://doi.org/10.1002/\(SICI\)1520-636X\(1999\)11:10<795::AID-CHIR10>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1520-636X(1999)11:10<795::AID-CHIR10>3.0.CO;2-S)
- From, C., Commission, T.H.E., The, T.O., Council, T.H.E., Economic, T.H.E.E., Committee, T.H.E., The, O.F., 2012. Communication COM(2012)673.
- Fromme, H., Otto, T., Pilz, K., 2001. Polycyclic Musk Fragrances in the Aquatic Environment. *ACS Symp. Ser.* 773, 203–222. <https://doi.org/10.1021/bk-2001-0773.ch015>
- Gao, Y., Ji, Y., Li, G., Mai, B., An, T., 2016. Bioaccumulation and ecotoxicity increase during indirect photochemical transformation of polycyclic musk tonalide: A modeling study. *Water Res.* 105, 47–55. <https://doi.org/10.1016/j.watres.2016.08.055>
- Gatermann, R., Hehot, J., Hiihnetiss, H., Rimkus, G., Zitko, V., 1999. Polycyclic and nitro musks in the environment: a comparison between Canadian and European aquatic biota. 38, 3431–3441.
- Godayol, A., Besalú, E., Anticó, E., Sanchez, J.M., 2015. Monitoring of sixteen fragrance allergens and two polycyclic musks in wastewater treatment plants by solid phase microextraction coupled to gas chromatography. *Chemosphere* 119, 363–370. <https://doi.org/10.1016/j.chemosphere.2014.06.072>
- H. Doležalová Weissmannová, I.Š., M. Vávrová, and A.L., 2013. Determination of Toxicity of Musk Compounds Using Alternative Tests of Ecotoxicity. *Chem. List.* 107.
- Hájková, K., Pulkrabová, J., Hajšlová, J., Randák, T., Žlábek, V., 2007. Chub (*Leuciscus cephalus*) as a bioindicator of contamination of the Vltava River by synthetic musk fragrances. *Arch. Environ. Contam. Toxicol.* 53, 390–396. <https://doi.org/10.1007/s00244-006-0190-4>
- Heberer, T., 2003. Occurrence, fate, and assessment of polycyclic musk residues in the aquatic environment of urban areas - A review. *Acta Hydrochim. Hydrobiol.* 30, 227–243. <https://doi.org/10.1002/aheh.200390005>
- HERA, 2004. HERA Environmental Risk Assessment Polycyclic musks AHTN and HHCB 1–80.
- Herrmann, A., 2005. Chemistry and Technology of Flavors and Fragrances. Edited by David J. Rowe. *Angew. Chemie Int. Ed.* 44, 3650–3651. <https://doi.org/10.1002/anie.200585283>
- Homem, V., Alves, Alice, Alves, Arminda, Santos, L., 2016. Ultrasound-assisted dispersive liquid-liquid microextraction for the determination of synthetic musk fragrances in aqueous matrices by gas chromatography-mass spectrometry. *Talanta* 148, 84–93. <https://doi.org/10.1016/j.talanta.2015.10.049>
- Hu, Z., Shi, Y., Cai, Y., 2011. Concentrations, distribution, and bioaccumulation of synthetic musks in the Haihe River of China. *Chemosphere* 84, 1630–1635. <https://doi.org/10.1016/j.chemosphere.2011.05.013>
- Kimberley, M.O., Wang, H., Wilks, P.J., Fisher, C.R., Magesan, G.N., 2004. Economic analysis of growth response from a pine plantation forest applied with biosolids. *For. Ecol. Manage.* 189, 345–351. <https://doi.org/10.1016/j.foreco.2003.09.003>
- Kupper, T., Plagellat, C., Brändli, R.C., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. *Water Res.* 40, 2603–2612. <https://doi.org/10.1016/j.watres.2006.04.012>
- Lange, C., Kuch, B., Metzger, J.W., 2015. Occurrence and fate of synthetic musk fragrances in a small German river. *J.*

- Hazard. Mater. 282, 34–40. <https://doi.org/10.1016/j.jhazmat.2014.06.027>
- Lee, I.S., Kim, U.J., Oh, J.E., Choi, M., Hwang, D.W., 2014. Comprehensive monitoring of synthetic musk compounds from freshwater to coastal environments in Korea: With consideration of ecological concerns and bioaccumulation. *Sci. Total Environ.* 470–471, 1502–1508. <https://doi.org/10.1016/j.scitotenv.2013.07.070>
- Lee, I.S., Lee, S.H., Oh, J.E., 2010. Occurrence and fate of synthetic musk compounds in water environment. *Water Res.* 44, 214–222. <https://doi.org/10.1016/j.watres.2009.08.049>
- Lishman, L., Smyth, S.A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos, M., Beland, M., Seto, P., 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. *Sci. Total Environ.* 367, 544–558. <https://doi.org/10.1016/j.scitotenv.2006.03.021>
- Luckenbach, T., Corsi, I., Epel, D., 2004. Fatal attraction: Synthetic musk fragrances compromise multixenobiotic defense systems in mussels. *Mar. Environ. Res.* 58, 215–219. <https://doi.org/10.1016/j.marenvres.2004.03.017>
- Maekawa, A., Matsushima, Y., Onodera, H., Shibutani, M., Ogasawara, H., Kodama, Y., Kurokawa, Y., Hayashi, Y., 1990. Long-term toxicity/carcinogenicity of musk xylol in B6C3F1 mice. *Food Chem. Toxicol.* 28, 581–586. [https://doi.org/10.1016/0278-6915\(90\)90159-K](https://doi.org/10.1016/0278-6915(90)90159-K)
- Melymuk, L., Robson, M., Csiszar, S.A., Helm, P.A., Kaltenecker, G., Backus, S., Bradley, L., Gilbert, B., Blanchard, P., Jantunen, L., Diamond, M.L., 2014. From the city to the lake: Loadings of PCBs, PBDEs, PAHs and PCMs from Toronto to Lake Ontario. *Environ. Sci. Technol.* 48, 3732–3741. <https://doi.org/10.1021/es403209z>
- Mogensen, B.B., Pritzl, G., Rastogi, S., Glense, O., Hedlund, B., Hirvi, J.P., Lundgren, A., Sigurdsson, A., 2004. Musk compounds in the Nordic environment. *TemaNord Rep.* 1–70.
- Müller, S., Schmid, P., Schlatter, C., 1996. Occurrence of nitro and non-nitro benzenoid musk compounds in human adipose tissue. *Chemosphere* 33, 17–28. [https://doi.org/10.1016/0045-6535\(96\)00160-9](https://doi.org/10.1016/0045-6535(96)00160-9)
- Nakata, H., 2005. Occurrence of synthetic musk fragrances in marine mammals and sharks from Japanese coastal waters. *Environ. Sci. Technol.* 39, 3430–3434. <https://doi.org/10.1021/es050199i>
- NIER, 2009. Development of analytical method and monitoring of personal care products in the Nakdong River system.
- O.S.P.A.R. Commission, 2004. Hazardous substances series, musk xylene and other musks. London, UK. <https://doi.org/ISBN 1-904426-36-0>
- Osemwengie, L.I., Gerstenberger, S.L., 2004. Levels of synthetic musk compounds in municipal wastewater for potential estimation of biota exposure in receiving waters. *J. Environ. Monit.* 6, 533–539. <https://doi.org/10.1039/b400514g>
- Parolini, M., Magni, S., Traversi, I., Villa, S., Finizio, A., Binelli, A., 2015. Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in *Dreissena polymorpha*. *J. Hazard. Mater.* 285, 1–10. <https://doi.org/10.1016/j.jhazmat.2014.11.037>
- Peck, A.M., Linebaugh, E.K., Hornbuckle, K.C., 2006. Synthetic musk fragrances in Lake Erie and Lake Ontario sediment cores. *Environ. Sci. Technol.* 40, 5629–5635. <https://doi.org/10.1021/es060134y>
- Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass flow of polycyclic musks in two wastewater treatment plants. *Arch. Environ. Contam. Toxicol.* 52, 451–457. <https://doi.org/10.1007/s00244-006-0203-3>
- Reiner, J.L., Kannan, K., 2011. Polycyclic musks in water, sediment, and fishes from the upper Hudson River, New York, USA. *Water, Air, Soil Pollut.* 214, 335–342. <https://doi.org/10.1007/s11270-010-0427-8>
- Rice, S.L., Mitra, S., 2007. Microwave-assisted solvent extraction of solid matrices and subsequent detection of pharmaceuticals and personal care products (PPCPs) using gas chromatography-mass spectrometry. *Anal. Chim. Acta* 589, 125–132. <https://doi.org/10.1016/j.aca.2007.02.051>
- Rimkus, G. G., & Wolf, R., 1997. Polycyclic musk fragrances in biota from aquatic ecosystems. *Chemosphere*.
- Ros, O., Izaguirre, J.K., Olivares, M., Bizarro, C., Ortiz-Zarragoitia, M., Cajaraville, M.P., Etxebarria, N., Prieto, A., Vallejo, A., 2015. Determination of endocrine disrupting compounds and their metabolites in fish bile. *Sci. Total*

- Environ. 536, 261–267. <https://doi.org/10.1016/j.scitotenv.2015.07.074>
- Rüdel, H., Böhmer, W., Schröter-Kermani, C., 2006. Retrospective monitoring of synthetic musk compounds in aquatic biota from German rivers and coastal areas. *J. Environ. Monit.* 8, 812–823. <https://doi.org/10.1039/b602389b>
- Santiago-Morales, J., Gómez, M.J., Herrera, S., Fernández-Alba, A.R., García-Calvo, E., Rosal, R., 2012. Oxidative and photochemical processes for the removal of galaxolide and tonalide from wastewater. *Water Res.* 46, 4435–4447. <https://doi.org/10.1016/j.watres.2012.05.051>
- Schmid, P., Kohler, M., Gujer, E., Zennegg, M., Lanfranchi, M., 2007. Persistent organic pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes in Switzerland. *Chemosphere* 67, 16–21. <https://doi.org/10.1016/j.chemosphere.2006.05.080>
- Schmidt-Bäumler, K., Heberer, T., Stan, H.J., 1999. Occurrence and distribution of organic contaminants in the aquatic system in Berlin. Part II: Substituted phenols in Berlin surface water. *Acta Hydrochim. Hydrobiol.* 27, 143–149. [https://doi.org/10.1002/\(SICI\)1521-401X\(199905\)27:3<143::AID-AHEH143>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1521-401X(199905)27:3<143::AID-AHEH143>3.0.CO;2-9)
- Schnell, S., Martin-Skilton, R., Fernandes, D., Porte, C., 2009. The interference of nitro- and polycyclic musks with endogenous and xenobiotic metabolizing enzymes in carp: An in vitro study. *Environ. Sci. Technol.* 43, 9458–9464. <https://doi.org/10.1021/es902128x>
- Shek, W.M., Murphy, M.B., Lam, J.C.W., Lam, P.K.S., 2008. Polycyclic musks in green-lipped mussels (*Perna viridis*) from Hong Kong. *Mar. Pollut. Bull.* 57, 373–380. <https://doi.org/10.1016/j.marpolbul.2008.02.037>
- Simonich, S.L., Federle, T.W., Eckhoff, W.S., Rottiers, A., Webb, S., Sabaliunas, D., De Wolf, W., 2002. Removal of fragrance materials during U.S. and European wastewater treatment. *Environ. Sci. Technol.* 36, 2839–2847. <https://doi.org/10.1021/es025503e>
- Spencer, P.S., Bischoff-Fenton, M.C., Moreno, O.M., Opdyke, D.L., Ford, R.A., 1984. Neurotoxic properties of musk ambrette. *Toxicol. Appl. Pharmacol.* 75, 571–575. [https://doi.org/10.1016/0041-008X\(84\)90194-7](https://doi.org/10.1016/0041-008X(84)90194-7)
- Tumová, J., Šauer, P., Golovko, O., Koba Učun, O., Grabic, R., Máčková, J., Kocour Kroupová, H., 2019. Effect of polycyclic musk compounds on aquatic organisms: A critical literature review supplemented by own data. *Sci. Total Environ.* 651, 2235–2246. <https://doi.org/10.1016/j.scitotenv.2018.10.028>
- Villa, S., Assi, L., Ippolito, A., Bonfanti, P., Finizio, A., 2012. First evidences of the occurrence of polycyclic synthetic musk fragrances in surface water systems in Italy: Spatial and temporal trends in the Molgora River (Lombardia Region, Northern Italy). *Sci. Total Environ.* 416, 137–141. <https://doi.org/10.1016/j.scitotenv.2011.11.027>
- Weschler, C.J., Nazaroff, W.W., 2008. Semivolatile organic compounds in indoor environments. *Atmos. Environ.* 42, 9018–9040. <https://doi.org/10.1016/j.atmosenv.2008.09.052>
- Winkler, M., Kopf, G., Hauptvogel, C., Neu, T., 1998. Fate of artificial musk fragrances associated with suspended particulate matter (SPM) from the River Elbe (Germany) in comparison to other organic contaminants. *Chemosphere* 37, 1139–1156. [https://doi.org/10.1016/S0045-6535\(98\)00110-6](https://doi.org/10.1016/S0045-6535(98)00110-6)
- Wollenberger, L., Breitholtz, M., Kusk, K.O., ..., 2003. Inhibition of larval development of the marine copepod. *Acartia tonsa* 305, 53–64.
- Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K., 2008. Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (*Oryzias latipes*). *Aquat. Toxicol.* 90, 261–268. <https://doi.org/10.1016/j.aquatox.2008.09.007>
- Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. *Sci. Total Environ.* 363, 149–165. <https://doi.org/10.1016/j.scitotenv.2005.06.022>
- Zhang, X., Xu, Q., Man, S., Zeng, X., Yu, Y., Pang, Y., Sheng, G., Fu, J., 2013. Tissue concentrations, bioaccumulation, and biomagnification of synthetic musks in freshwater fish from Taihu Lake, China. *Environ. Sci. Pollut. Res.* 20, 311–322. <https://doi.org/10.1007/s11356-012-1095-6>

CHAPTER II

Thesis Aims and Structure

2.1 Thesis objectives

In the last few years, a growing interest concerning pollution derived from personal care products (PCPs) has emerged. Between PCPs are Polycyclic Musk Fragrances (PMFs), synthetic compounds included in the wording of a wide range of products such as soaps, creams, detergents and house-cleaning products in order to add perfume (Heberer, 2003). Many researches evidenced that these compounds are released in large quantities into the environment mainly through the discharges of WWTPs which are not specifically designed for the removal of these micropollutants (Santiago-Morales et al., 2012). Moreover, due to their persistence in the environment, they can be accumulated in sediments and in lipid tissue of organisms thus representing a potential danger to both aquatic ecosystems and, ultimately, to human health (Daughton and Ternes, 1999; Reiner and Kannan, 2011). For these reasons, it is extremely crucial to study PMFs during wastewater treatments in order to clearly understand their behavior inside WWTPs and the main mechanisms that determine their fate. Several methods for the analysis of PMFs in waters and sludges have been published but, to our knowledge, most of them require a large consumption of time, a use of significant quantities of solvents and, above all, highly sensitive and expensive instruments. For these reasons the present thesis aims to achieve three fundamental goals. Firstly to build methods for the analysis of PMFs in wastewaters and sludge that are rapid, sensitive, which require low volumes of solvents and the use of common laboratory instrumentations along with the avoidance of cross-contamination between samples and the contamination of expensive instruments from highly contaminated matrices such as sludges. The second objective of this thesis is to analyze the behavior of PMFs inside a WWTP that uses conventional wastewater treatments in order to highlight the possible effectiveness of these technologies regarding the removal of this category of micropollutants. This type of monitoring study appears to be of significant importance in Italy, the European country with the highest rate of consumption of detergents recorded (HERA, 2004) and in which a study of this type, according to our knowledge, has not yet been carried out. Finally, this thesis aims to evaluate the effectiveness of the two main technologies for the advanced removal of micropollutants in use today, ozonation and adsorption on activated carbon, as regards the removal of fragrances. By testing different operating conditions in the two pilot plants installed inside two conventional WWTPs in Italy, the foundations were laid for the achievement of optimal operating conditions that can lead to an effective removal of these compounds without constituting a huge energy and economic commitment for the managers of the treatment plants.

2.2 Thesis structure

The results of the present doctoral study are summarized in three papers dealing with the detection of polycyclic musk fragrances in wastewaters and sludges, their occurrence and fate in a conventional wastewater treatment plant and the evaluation of their removal after the addition of two new tertiary treatments in order to remove this class of micropollutants from wastewater.

In particular:

- In **Chapter III**, novel analytical methods were developed in order to detect polycyclic musk fragrances in wastewaters and sludges. The aim of this study was to develop new reliable analytical methods that were easily applicable by most laboratories using simple instrumentation, fast in terms of analytical timing and that did not require the use of large quantities of solvents, harmful for the environment. The present work was published on *Environmental Science and Pollution Research*.
- In **Chapter IV** the fate of polycyclic musk fragrances was assessed in a conventional activated sludge wastewater treatment plant located in Northern Italy. With this study, the first monitoring in a WWTP was carried out in Italy. Given the large use of products containing these compounds in our country, an evaluation of their removal degree using conventional wastewater treatment technologies was necessary in order to assess their possible impact on the receiving water systems. The present work was submitted to *Environmental Science and Pollution Research*.
- **Chapter V** finally has the objective of evaluating the two advanced wastewater treatment technologies most widely used today in order to understand if they can also be effective as regards the removal of the group of organic micropollutants investigated in this research. Two pilot plants that respectively use ozonation and adsorption on activated carbon have been installed after the secondary activated sludge treatment of two conventional WWTPs located in northern Italy. Different operating conditions were then tested for both pilot plants in order to achieve the best environmental and economical efficiencies. Corresponding scientific paper is now in preparation.

2.3 References

- Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and Personal Care Products in the Environment : Agents of Subtle Change ? 107, 907–938.
- Heberer, T., 2003. Occurrence, fate, and assessment of polycyclic musk residues in the aquatic environment of urban areas - A review. *Acta Hydrochim. Hydrobiol.* 30, 227–243. <https://doi.org/10.1002/aheh.200390005>
- HERA, 2004. HERA Environmental Risk Assessment Polycyclic musks AHTN and HHCB 1–80.
- Reiner, J.L., Kannan, K., 2011. Polycyclic musks in water, sediment, and fishes from the upper Hudson River, New York, USA. *Water, Air, Soil Pollut.* 214, 335–342. <https://doi.org/10.1007/s11270-010-0427-8>
- Santiago-Morales, J., Gómez, M.J., Herrera, S., Fernández-Alba, A.R., García-Calvo, E., Rosal, R., 2012. Oxidative and photochemical processes for the removal of galaxolide and tonalide from wastewater. *Water Res.* 46, 4435–4447. <https://doi.org/10.1016/j.watres.2012.05.051>

CHAPTER III

Polycyclic Musk Fragrances (PMFs) in wastewater and activated sludge: analytical protocol and application to a real case study

Stefano Tasselli^{1-2,*} and Licia Guzzella¹

¹ National Research Council - Water Research Institute (CNR-IRSA), S.S. Brugherio via del Mulino 19, Brugherio (MB), 20861, Italy

² Department of Environmental Sciences, University of Milano Bicocca, Piazza della Scienza 1, 20126 Milano, Italy

* Corresponding author: tasselli@irsa.cnr.it Telephone: +39 039 21694223

Environmental Science and Pollution Research 2020, 1-10; doi: 10.1007/s11356-019-06767-7

Abstract

Two different analytical methods for the determination of polycyclic musk fragrances (PMFs) in wastewater and in activated sludge were developed. PMFs in filtered water samples were determined by gas chromatography coupled with a triple-quadrupole mass spectrometer in selected reaction monitoring (SRM) mode. Activated sludge samples were extracted using an ultrasonic bath and analyzed using a GC-Ion trap. The developed methods respected a linear model ($R^2 > 0.995$). Detection limits of selected compounds (Celestolide, Galaxolide, Galaxolidone, Phantolide and Tonalide) varied from 1.7 to 80 ng L⁻¹ for water and from 0.1 ng g⁻¹ to 210 ng g⁻¹ for activated sludge considering laboratory contamination for each PMF. Recovery studies were performed on spiked water samples and, for sludges, on procedural blanks, showing recoveries above 70% for all the considered compounds while recovery of the internal standard was always above limit of acceptance (30%). Proposed methods were used to determine PMFs concentrations in wastewaters and activated sludges of a wastewater treatment plant (WWTP) located in Northern Italy. Concentrations in the range of µg L⁻¹ of Galaxolide and of its metabolite Galaxolidone were detected even in the WWTP effluent. Biotransformation of Galaxolide into Galaxolidone occurred during biological treatment with the consequent release of this compound through WWTP effluents. In activated sludges concentrations of all PMFs except Galaxolidone were one order of magnitude higher than waste waters, as expected according to their physio-chemical properties. Present wastewater treatment technologies were confirmed to not be efficient in removing PMFs from influent wastewaters since with only ≈30% of Celestolide and Tonalide were removed.

Keywords

Polycyclic musk fragrances (PMFs), emerging pollutants, wastewater treatment plant, activated sludge, solid phase extraction, ultrasonic bath extraction, gas chromatography-mass spectrometry

Acknowledgements

The project was partially funded by PerFORMWATER 2030 project ID 240750 (POR FESR 2014-2020 Asse I -Accordi per la Ricerca e l'Innovazione). We like to thank SIAD Group (Partner of the project) for its support in this investigation and Laura Marziali (CNR-IRSA) for her editing revision.

3.1 Introduction

Personal care products (PCPs) are a heterogeneous and commonly used group of chemicals (Avila and Garcia 2015). Among PCPs, there are polycyclic musk fragrances (PMFs) as Galaxolide (HHCB), Tonalide (AHTN), Celestolide (ADBI) and Phantolide (AHDI); their common use regards production of perfumes, soaps, house-cleaning products and detergents (Heberer 2002). HHCB and AHTN are, among PMFs, the most widely used products, representing more than 95% of the European market (Clara et al. 2011) with an estimated consumption of 1427 ton y⁻¹ and 358 ton y⁻¹ in 2000 (OSPAR Commission 2004). Both substances have experienced a reduction in their use in EU during the nineties from 2400 ton y⁻¹ to 1427 ton y⁻¹ for HHCB and from 885 ton y⁻¹ to 358 ton y⁻¹ for AHTN (ECB 2008). However, there are regional differences in the use of PMFs throughout the EU member states with higher consumptions in Southern Europe, particularly in Italy, where there is the highest use of detergents with a volume of 7.23 g y⁻¹ per capita for HHCB and 1.81 g y⁻¹ for AHTN (ECB 2008; HERA 2008). Among fragrances, Limonene is a terpene hydrocarbon which exists in two isomers, *d*-limonene and *l*-limonene. Purity of commercial *d*-Limonene is 90-98% approximately. It has a low solubility in water and a high value of Henry's constant which implicate a high rate of vaporization. Limonene is employed in degreasing metals before industrial painting, for cleaning in electronic and painting industries, in paint products as solvent and in many household cleaning detergents (Filipsson et al. 1998). The widespread use of synthetic musks leads to their release in large amounts through discharges of Wastewater Treatment Plants (WWTPs) into the environment (Santiago-Morales et al. 2012; Gao et al. 2016). These compounds are persistent and therefore can be accumulated in sediments and in lipid tissues of aquatic organisms (Daughton and Ternes 1999; Reiner and Kannan 2011). Present environmental concentrations of PMFs in aquatic ecosystem are lower than EC₅₀ values in acute toxicity tests (0.153-0.83 mg L⁻¹ HHCB and 0.108-1.034 mg L⁻¹ AHTN) (US-EPA. 2014; Gooding et al. 2006); however, aquatic organisms may be affected by long-term exposure of PMFs, due to continual discharges of these compounds into the environment (Parolini et al. 2015). Main PMF pollution sources are anthropic as industrial and domestic discharges. These compounds can reach aquatic ecosystem via household wastes and WWTP (US-EPA 2014); most of the compounds are not removed by conventional wastewater treatments and can be transported even far away from pollution sites (Wu et al. 2009; Walters et al. 2010). As result, they are present at ng L⁻¹ concentrations in wastewater treatment plants (WWTPs) effluent and receiving aquatic ecosystems (Chase et al. 2012). Besides native compounds, biotransformation of HHCB into Galaxolidone (HHCB-lactone) in activated sludge wastewater treatment plants was demonstrated by Bester in 2004. This phenomenon was highlighted by the fact that while HHCB and AHTN concentrations decreased during water treatments while those of HHCB-lactone increased in the final effluents of WWTP. Reiner et al. (2007) measured concentrations of HHCB-lactone from 505-897 ng L⁻¹ to 1620-1740 ng L⁻¹ before and after a biological WWTP with activated sludge. There is still limited information concerning the removal of Limonene in WWTPs. Limonene was measured in a WWTP near

Paris (Bruchet and Janex-Habibi, 2007) at concentrations of $17.2 \mu\text{g L}^{-1}$ in raw water, $37.2 \mu\text{g L}^{-1}$ in raw water mixed with water deriving from sludge dehydration, $22.7 \mu\text{g L}^{-1}$ after primary settling and $< 0.1 \mu\text{g L}^{-1}$ in final effluent after activated sludge treatment and secondary clarifiers. According to this research, secondary treatment seemed to be efficient in removing Limonene in wastewaters. Godayol et al. (2015) evaluated the removal of Limonene in Castell d'Aro and Girona WWTPs. They measured Limonene only in the primary effluent of the two WWTPs confirming that there was a quantitative removal during secondary treatments.

Several methods for analyzing PMFs in waters and sludges have been published. For water samples, extraction with solid phase (SPE) was mainly used, sometimes followed by a clean-up step depending on the quantity of impurities in extracts. Different solid phase adsorption phases and elution solvents were applied to extract PMFs from wastewaters (He et al. 2013; Chase et al. 2012; Hu et al. 2011). Solid-liquid partitioning with organic solvents, followed by extract clean-up can be considered the standard extraction technique for PMFs in sludge samples. Mechanical shaking and Soxhlet extraction were also widely employed (Kupper et al. 2006; Poulsen and Bester 2010) but the usage of large amount of solvents and the long time needed for analysis limit these traditional methods. The continuous production and use of polycyclic musk fragrances will probably lead, in a not so far future, to an increase of environmental concentrations in aquatic ecosystems; here the reason why it is important to develop validated protocols for PMF analyses.

The aim of the present investigation is the evaluation of a rapid and sensitive methods for PMF analyses in wastewater and sludge samples by solid phase extraction (SPE) and ultrasonic bath extraction, respectively, followed by quantification in GC-MS/MS or GC-Ion trap. Present methods were applied to a real case study, measuring PMFs concentrations in wastewaters and sludges in a WWTP located nearby the metropolitan city of Milan (Italy), indicated with MS1 code.

3.2 Materials and methods

3.2.1 Chemicals

Polycyclic musk standards Galaxolide (HHCB), Galaxolidone (HHCB-lactone), Tonalide (AHTN), Celestolide (ADBI), Phantolide (AHDI) and Limonene in iso-octane were purchased from Chemical Research 2000. (Rome, IT). Internal standard Tonalide (AHTN) D3 in iso-octane was purchased from LGC Standards (Manchester, USA). From standards, working solutions in iso-octane were prepared to obtain final concentrations of 0.5 mg mL^{-1} . AHTN-D3 was diluted in acetone to a final concentration of $20 \text{ ng } \mu\text{L}^{-1}$. Solutions were stored at $-30 \text{ }^\circ\text{C}$ in 25 mL flasks in the dark to prevent photolysis. Methanol, n-hexane, dichloromethane, acetone, iso-octane and Milli-Q water were obtained from VWR International (Radnor, PA, USA). Superclean ENVI-Carb 120/400 was purchased from Supelco (Bellefonte, PA, USA) and Acrodisc syringe filters of 25 mm diameter with $1 \mu\text{m}$ glass fiber pore size were obtained from VWR International (Radnor, PA, USA).

3.2.2 Sampling

Wastewater and sludge samples were collected at MS1 WWTP, an average size WWTP (usually from 10,000 to 50,000 served inhabitants) collecting wastewaters from two of the main cities directly surrounding Milan urban area. It is an activated sludge WWTP with simultaneous oxidation/nitrification and anaerobic digestion of sludge. Regarding water line (Fig. III-1), after primary physical treatments of grilling, sand and oil removal, water goes into the primary sedimentation tank and in the denitrification/nitrification tanks. Secondary sedimentation, filtration and final UV disinfection are employed.

24-hour water samples were collected with an automatic sampler (1 grab sample per hour) in March 2019 on three consecutive days. Four different point of the treatment process were sampled: WWTP inlet (IN IDA), biological treatment inlet (IN BIO), biological treatment outlet (OUT BIO) and WWTP outlet (OUT IDA) after the final disinfection step. Water samples were firstly filtered on glass microfiber filters of 47 mm diameter and particle retention of 1 μm (VWR International, Radnor, PA, USA). Eluates were subsequently filtered on the same type of filters with 0.7 μm particle retention. Samples were kept at 4°C for a maximum of 24 h before analysis.

Instantaneous sample of activated sludge was collected from the middle of the nitrification tank. Sludge was transferred to the laboratory and kept overnight at 4°C to let it settle. The excess of water was removed, and sludge was freeze-dried and kept at room temperature in amber glass bottle until analysis.

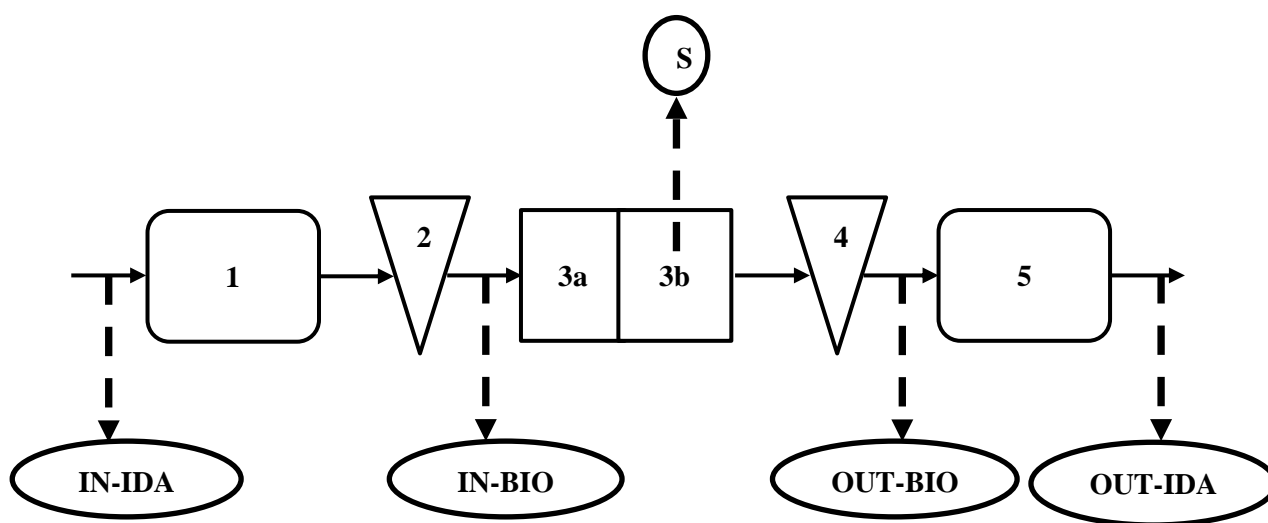


Fig. III-1 MS1 WWTP water line. 1=mechanical treatments 2=primary sedimentation; 3a=anoxic denitrification; 3b=oxic nitrification; 4= secondary sedimentation; 5= filtration and UV disinfection. IN IDA= water samples of WWTP inlet; IN BIO= water samples of biological inlet; OUT BIO= water samples of biological outlet; OUT IDA= water samples of WWTP outlet; S= activated sludge samples of oxic nitrification.

3.2.3 Extraction procedure

3.2.3.1 Water samples

Water samples were spiked with 25 μL of AHTN-D3 20 $\text{ng } \mu\text{L}^{-1}$ and extracted with a manual SPE system, employing Bakerbond Speedisks Octadecyl C_{18} 50 mm (Avantor, Center Valley, PA, USA). Cartridges were conditioned with 10 mL of methanol and 10 mL of Milli-Q water. Samples (1 L) were concentrated, under vacuum pressure, on C_{18} cartridges at a flow rate of 15 mL min^{-1} . After the extraction, cartridges were air dried under vacuum pressure for 1 h and eluted with 10 mL of n-hexane:dichloromethane 1:1 v/v and 10 mL of n-hexane. At the end, 1 mL of iso-octane was added to the extracts which were concentrated to 1 mL using N_2 , transferred to GC micro-vials and analyzed by GC-MS.

3.2.3.2 Sludge samples

Sludge samples (0.2 g d.w.) were poured in 250 mL glass flask, spiked with 25 μL of AHTN-D3 20 $\text{ng } \mu\text{L}^{-1}$ and extracted with 20 mL of n-hexane:acetone 1:1 v/v for 30 min at 30°C in an ultrasonic water bath at 40 Hz intensity (Falc Instruments, Treviglio, IT). Extracts were transferred in tubes and centrifuged at 3000 rpm for 5 min at room temperature and the supernatant solvent was collected with Pasteur pipettes. Extracts were then concentrated to 5 mL using N_2 flux at 30°C. Concentrated extracts were then transferred in centrifuge tubes and 100 mg of Superclean ENVI-Carb 120/400 were added. After vortexing for 30 sec, samples were centrifuged again as previously reported and the supernatant solvent was collected with Pasteur pipettes. After adding 1 mL of iso-octane, samples were concentrated to 800 μL , filtered with Acrodisc syringe filters and transferred to GC micro-vials for GC-MS analysis.

3.2.4 Quantification

3.2.4.1 GC-MS/MS analysis for water samples

GC-MS/MS analysis was performed using a Trace 1310 (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatograph equipped with an automatic injector Model AS 1310, and a triple-quadrupole mass spectrometer Model TSQ 8000 Evo. A fused silica capillary column (SLBTM-5ms, 60 m x 0.25 mm x 0.25 μm film thickness) supplied by Sigma-Aldrich (Saint Louis, MO, USA), was employed. Operating conditions were as follows: injector port temperature starting at 50°C and raising at 1°C min^{-1} until 200°C when samples were transferred into the column; helium as gas carrier at a programmed flow-rate of 1.3 mL min^{-1} for 10 min and 1.5 mL min^{-1} for 20 min and argon as collision gas. Column temperature was maintained at 50°C for 1 min, programmed at 12°C min^{-1} until 170°C and 6°C min^{-1} until 230°C, held for 3 min, then raised at 20°C min^{-1} at 290°C and held for 3 min. The total analysis time was 30 min. A 2 μL sample volume was injected splitless at 50°C. Mass spectrometer operated in electron impact ionization mode with an ionizing energy of 70 eV. The ion source temperature and the MS transfer line temperature were both 290°C. Analysis was performed with selected reaction monitoring (SRM). Table III-1 lists retention times, precursor and product ions and collision energies used for quantification of each polycyclic musk fragrances. Target ions selected have the highest intensity in the mass spectra of the polycyclic musk fragrances.

Table III-1 PMF names, formulas, retention times, ions and collision energies used for quantification with GC-MS/MS.

| Name | Formula | t_R (min) | Precursor ions (m/z) | Product ions (m/z) | Collision energy (eV) |
|--------------------------------|-------------------|-------------|-----------------------------|---------------------------|--------------------------|
| Celestolide (ADBI) | $C_{17}H_{24}O$ | 20.18 | 244.1 229.1 | 229.2 173.1 | 10 5 |
| Galaxolide (HHCB) | $C_{18}H_{26}O$ | 22.48 | 243.1 213.1 | 213.1 171.1 | 10 10 |
| Galaxolidone (HHCB-lactone) | $C_{18}H_{24}O_2$ | 27.64 | 258.1 257.1 | 240.2 239.2 | 10 10 |
| Limonene | $C_{10}H_{16}$ | 10.83 | 93.1 68.1 | 77 53 | 10 10 |
| Phantolide (AHDI) | $C_{17}H_{24}O$ | 20.87 | 244.2 229.1 | 229.2 187.1 | 10 5 |
| Tonalide (AHTN) | $C_{18}H_{26}O$ | 22.57 | 243.1 243.1 | 187.1 159.1 | 5 10 |

3.2.4.2 GC-Ion trap analysis for sludge samples

GC analysis for sludge samples were performed using a Thermo Electron TraceGC 2000 coupled with a PolarisQ Ion Trap (ThermoElectron - Austin, Texas) mass spectrometer and equipped with a PTV injector and an AS 3000 auto sampler. Separation of PMFs was achieved using a SLB®-5ms capillary column, 30 m x 0.25 mm i.d. x 0.25 μ m film thickness (Supelco, Bellefonte, PA, USA) in the following conditions: carrier gas helium at 1.3 mL min⁻¹; injector temperature starting at 50°C then ramped to 200°C at 1°C s⁻¹; split mode with a split flow of 10 mL min⁻¹ and a split ratio of 8; initial oven temperature set at 50°C (held 1 min), then ramped first to 170°C at 12 °C min⁻¹ and then to 230°C at 6°C min⁻¹ (held 20 min). A final cleaning ramp at 310°C (25°C min⁻¹) was applied for 5 min to clean the column from sludge impurities. Samples were analyzed using tandem mass spectrometry under the following instrumental conditions: EI mode with standard electron energy of 70 eV; the transfer line was maintained at 300°C, the damping gas at 1 mL min⁻¹, and the ion source at 250°C. Retention times, ions and voltages used for quantification of each polycyclic musk fragrances are reported in Table III-2.

Table III-2 For each PMFs, retention times, ions and collision energies used for PMF quantification with GC-Ion trap.

| Name | t_R (min) | Ions (m/z) | Voltage (eV) |
|--------------------------------|-------------|--|--------------|
| Celestolide (ADBI) | 14.72 | 188.1; 229.1 | 1.4 |
| Galaxolide (HHCB) | 16.63 | 213.1 | 1.6 |
| Galaxolidone (HHCB-lactone) | 21.35 | 201.1; 213.2; 215.1; 239.1 | 1.5 |
| Phantolide (AHDI) | 15.29 | 131.1; 145.1; 159.1; 173.1; 185.1; 187.1; 201.2; 214 | 1.6 |
| Tonalide (AHTN) | 16.74 | 157.1; 171.1; 185.1; 187.1; 201; 225.1 | 1.5 |

3.2.5 Detection and quantification limits

Limits of detection (LOD) and limits of quantification (LOQ) of this method were calculated considering mean (μ) and standard deviation (σ) of four blank samples according to EU Directive on water monitoring (EU 2009):

$$\text{LOD} = \mu + 3 * \sigma$$

$$\text{LOQ} = \mu + 10 * \sigma$$

3.2.6 Repeatability of the methods

The instrumentation repeatability of the two GC-MS methods was evaluated by performing the analysis of the same sample 10 times with an automatic injector and calculating the relative standard deviation (R.S.D.) for each compound. Repeatability of all the analytical steps were determined by four replicate analysis of a WWTP inlet water sample and of an activated sludge sample. Relative Coefficient of Variation for each compound was calculated. The coefficient of variation is defined as the ratio of the standard deviation σ to the mean μ (Everitt 1998):

$$\text{CV} = \sigma / \mu$$

The coefficient of variation is a useful statistic for comparing the variation degree of one data series with another, even if the means are drastically different from each other. In general, $\text{CV} \leq 0.1$ corresponds to weak variability; $0.1 < \text{CV} < 1$ corresponds to moderate variability; and $\text{CV} \geq 1$ corresponds to strong variability.

3.3. Results and discussion

3.3.2 Method validation

3.3.2.1 Linearity

The linearity of all target compounds was determined with standard solutions in iso-octane in the range from 0.1 to 5 mg L⁻¹, corresponding to the concentration range of the considered samples. A four-points calibration curve forced by the origin was set up for every polycyclic musk. The response of the mass spectrometer was linear in the concentration range considered with a determination coefficient higher than 0.99 for all PMFs. Calibration data are reported in Table III-3.

3.3.2.2 Detection and quantification limits

Table III-3 summarizes detection and quantification limits obtained for all the PMFs in water and sludge samples. The analysis of Limonene was not carried out because of high and variable concentrations in blank samples.

Table III-3 Limits of detection and quantification (LOD, LOQ respectively) of PMFs, and calibration data of the methods.

| PMFs | Calibration data | | | | Water (ng L ⁻¹) | | Sludge (ng g ⁻¹) | |
|---------------------|------------------|----------------|-------------|----------------|-----------------------------|-----|------------------------------|-----|
| | GC-MS/MS | | GC-Ion trap | | LOD | LOQ | LOD | LOQ |
| | Equation | R ² | Equation | R ² | | | | |
| <i>ADBI</i> | y= 1.614x | 0.997 | y=1.183x | 0.997 | 2.4 | 6.3 | 0.1 | 1 |
| <i>HHCb</i> | y= 2.181x | 0.997 | y=2.474x | 0.999 | 61 | 124 | 210 | 526 |
| <i>HHCb-lactone</i> | y= 0.923x | 0.996 | y=4.706x | 0.999 | 39 | 90 | 56 | 120 |
| <i>Limonene</i> | y=1.246x | 0.995 | - | - | - | - | - | - |
| <i>AHDI</i> | y= 1.756x | 0.998 | y=2.142x | 0.999 | 1.7 | 5 | 0.1 | 1 |
| <i>AHTN</i> | y= 1.386x | 0.999 | y=0.935x | 0.997 | 80 | 137 | 67 | 137 |

Regarding GC-MS/MS, calibration data showed a high instrumental sensitivity for HHCb which had the highest angular coefficient while GC-Ion trap showed a high sensitivity even for HHCb-lactone and AHDI. With this last technique AHTN decreased in sensitivity because of the presence of common ions with HHCb. All calibration curves had a R²> 0.99. For both methods, LOQ of HHCb, HHCb-lactone and AHTN were higher than 50 ng L⁻¹ due to the extensive usage of those compounds which cause their presence even in blank samples. LOD, LOQ and calibration data (GC-Ion trap) of Limonene were not calculated.

3.3.2.3 Repeatability

Repeatability of the two GC-MS methods are listed in Table III-4. Results showed a high instrumental sensitivity of the two methods for each compound. R.S.D. of AHDI was higher than other compounds because it was measured in water only in trace while in sludge was not detected.

Table III-4 Repeatability of the two GC-MS methods. Values of R.S.D. are expressed in %.

| PMFs | GC-MS/MS (water) | GC-Ion trap (sludge) |
|---------------------|---------------------|-------------------------|
| <i>ADBI</i> | 1.75 | 13.65 |
| <i>HHCb</i> | 1.80 | 7.84 |
| <i>HHCb-lactone</i> | 4.00 | 3.81 |
| <i>AHDI</i> | 31.24 | Not done |
| <i>AHTN</i> | 2.08 | 10.28 |

In Table III-5 results concerning the repeatability of the all analytical methods are listed. Results showed a limited variability for all compounds in both methods. Only the analysis of AHTN in sludge evidenced a slightly moderate variability. Repeatability of AHDI was not calculated because its concentrations were below the limit of detection in all samples.

Table III-5 Repeatability of the method for water and sludge samples. Values are expressed in ng L⁻¹ for water samples and in ng g⁻¹ for sludge samples.

| PMFs | Water | | | | | Sludge | | | | |
|--------------|-------|------|------|------|-----------------|--------|-------|-------|-------|-----------------|
| | A | B | C | D | Cv ^a | A | B | C | D | Cv ^a |
| ADBI | 9.6 | 9.1 | 8.5 | 10.4 | 0.09 | 90 | 97 | 105 | 100 | 0.06 |
| HHCB | 2421 | 2505 | 2279 | 2543 | 0.05 | 18590 | 19287 | 20457 | 17007 | 0.08 |
| HHCB-lactone | 589 | 533 | 495 | 595 | 0.09 | 3047 | 3212 | 3472 | 3104 | 0.06 |
| AHDI | <LOD | <LOD | <LOD | <LOD | - | <LOD | <LOD | <LOD | <LOD | - |
| AHTN | 236 | 236 | 221 | 241 | 0.04 | 1246 | 1446 | 1338 | 1028 | 0.14 |

^a= Coefficient of variation.

3.3.2.4 Specificity and contamination of the laboratory with polycyclic musks

Due to the widespread use of musks in different personal care products (soaps, hand cream, detergents, etc.) it is essential to reduce laboratory contamination by limiting the use of common detergents, preferring alcohol-based detergent and using dedicated acetone-washed glassware. Milli-Q water was used for water blank samples instead of tap water employing the same glassware and solvents of the other samples. Blank samples of Milli-Q water (1L) were analyzed every 4 samples. Concerning sludge, because of unavailability of a reference material, procedural blanks were used.

3.3.2.5 Recovery

For water analyses, 1 L of Milli-Q water was spiked with 100 ng L⁻¹ of ADBI, AHDI and AHTN and with 500 ng L⁻¹ of HHCB and HHCB-lactone with the aim of simulating a realistic environmental sample in terms of relative compound concentrations. Four replicates of spiked Milli-Q water (1 L) was used for evaluating the different chemical recoveries. 500 ng of internal standard AHTN-D3 were added to each sample before extraction. Recovery rates were considered acceptable if the recovery of internal standard was >30% and chemical concentrations were in the range of 50-150% of the original fortified samples (Table III-5). For sludge analyses, four replicate extractions were conducted on the samples, using internal standard AHTN-D3 for the evaluation of the chemical recovery rates. Recovery rates of AHTN-D3 were always all above 77%.

Table III-5 Recovery rates in water samples. Values are expressed in percentage.

| PMFs | A | B | C | D | Mean |
|--------------|-------|-------|-------|-------|-------|
| ADBI | 73.81 | 95.53 | 63.20 | 88.85 | 80.35 |
| HHCB | 86.12 | >100 | 54.02 | 96.40 | 84.13 |
| HHCB-lactone | >100 | >100 | 80.65 | >100 | 95.16 |
| AHDI | 78.48 | 97.91 | 63.97 | 94.12 | 83.6 |
| AHTN | 56.78 | 63.98 | 93.79 | 69.65 | 71.05 |

3.3.2.6 Comparison with other methods

Comparison of different analytical methods for PMFs is not possible focusing on LOD and LOQ because they depend on laboratory specific contamination. The proposed method evidences some important advantages such as reduction of time of analysis, quantity of used reagents and finally the employing of simple and commonly used instrumentation. Considering water samples, our method was slightly modified from Hu et al. (2011) decreasing the amount of solvent used. For sludge samples, the application of an ultrasonic bath instead of other extraction techniques reduces quantity of solvents (20 mL), time of extraction (30 min) and avoids the possibility of cross-contamination among samples. For example, Shek et al. (2008) analyzed PMFs in sludge using Soxhlet with an extraction time of 10 hours and a solvent consumption of 400 mL for each sample while Clara et al. (2011) carried out the extraction step on a rotation-shaker twice for 150 min. When analyzing complex and unknown matrices such as sludges, the use of simple, cheap and specifically dedicated extraction material must be preferred. This is the reason that lead us to avoid more sensitive tools as Soxhlet during samples preparation. Ultrasonic bath technique for sludge extraction is present in most chemical labs allowing a simple and easy preparation of samples for GC-MS analyses.

3.3.3 A case study: wastewater and sludge of a WWTP in Northern Italy

These methods were applied for the analysis of PMFs in wastewater and sludge samples at MS1 WWTP. PMFs were measured in all wastewater samples with AHDI sometimes <LOQ; in this case “trace” is reported (Table III-6).

Table III-6 PMFs concentrations in MS1 WWTP wastewater. Values are expressed in ng L⁻¹.

| <i>PMFs</i> | <i>IN IDA</i> | <i>IN BIO</i> | <i>OUT BIO</i> | <i>OUT IDA</i> |
|---------------------|---------------|---------------|----------------|----------------|
| Day 1 | | | | |
| <i>ADBI</i> | 39 | 35 | 33 | 30 |
| <i>HHCB</i> | 6404 | 9233 | 5895 | 6940 |
| <i>HHCB-lactone</i> | 1129 | 1883 | 5388 | 5386 |
| <i>AHDI</i> | <LOD | trace | <LOD | trace |
| <i>AHTN</i> | 518 | 684 | 316 | 298 |
| Day 2 | | | | |
| <i>ADBI</i> | 58 | 29 | 23 | 29 |
| <i>HHCB</i> | 5539 | 7431 | 4805 | 6698 |
| <i>HHCB-lactone</i> | 1025 | 1447 | 4397 | 5087 |
| <i>AHDI</i> | <LOD | <LOD | <LOD | trace |
| <i>AHTN</i> | 373 | 512 | 252 | 285 |
| Day 3 | | | | |
| <i>ADBI</i> | 25 | 48 | 41 | 37 |
| <i>HHCB</i> | 5255 | 10923 | 7104 | 7324 |
| <i>HHCB-lactone</i> | 616 | 1835 | 5695 | 4786 |
| <i>AHDI</i> | <LOD | trace | trace | trace |
| <i>AHTN</i> | 339 | 783 | 366 | 328 |

AHTN, HHCB and its metabolite HHCB-lactone showed the highest concentrations, as expected from their extensive usage. Mean concentrations of 7000 ng L⁻¹ and 5000 ng L⁻¹ of HHCB and HHCB-lactone were measured in the WWTP effluent (Fig.III-2). AHTN was one order of magnitude lower in comparison with HHCB with a median concentration of 300 ng L⁻¹ while ADBI had a median concentration of 32 ng L⁻¹ and AHDI was detected only at low concentrations (2.5 ng L⁻¹). Considering HHCB and AHTN in WWTPs influents, lower HHCB concentrations were reported in other studies (Carballa et al. 2004; Clara et al. 2005; Hu et al. 2011) while AHTN showed the same range of concentration of our data. Hu et al. (2011) measured concentrations of 31-3039 ng L⁻¹ of HHCB and 28-1486 ng L⁻¹ of AHTN in China, in Austria Clara et al. (2005) reported values of 830-4443 ng L⁻¹ for HHCB and 210-1106 ng L⁻¹ and Carballa et al. (2004) detected polycyclic musk in Spain in the range of 2100-3400 ng L⁻¹ for HHCB and 900-1700 ng L⁻¹ for AHTN. These differences might be explained by the diverse regional usage of those compound by resident population (ECB 2008; HERA 2008).

During wastewater treatments, biotransformation of HHCB into its metabolite HHCB-lactone occurred. This had been demonstrated also by Bester (2004) who measured an increase in wastewater concentrations of HHCB-lactone in an activated sludge treatment. This oxidation, in fact, occurred in the biological tank of our case study (Fig. III-2) with an increase of 300% of HHCB-lactone concentrations from the inlet to the outlet of the tank and a corresponding reduction of HHCB concentration.

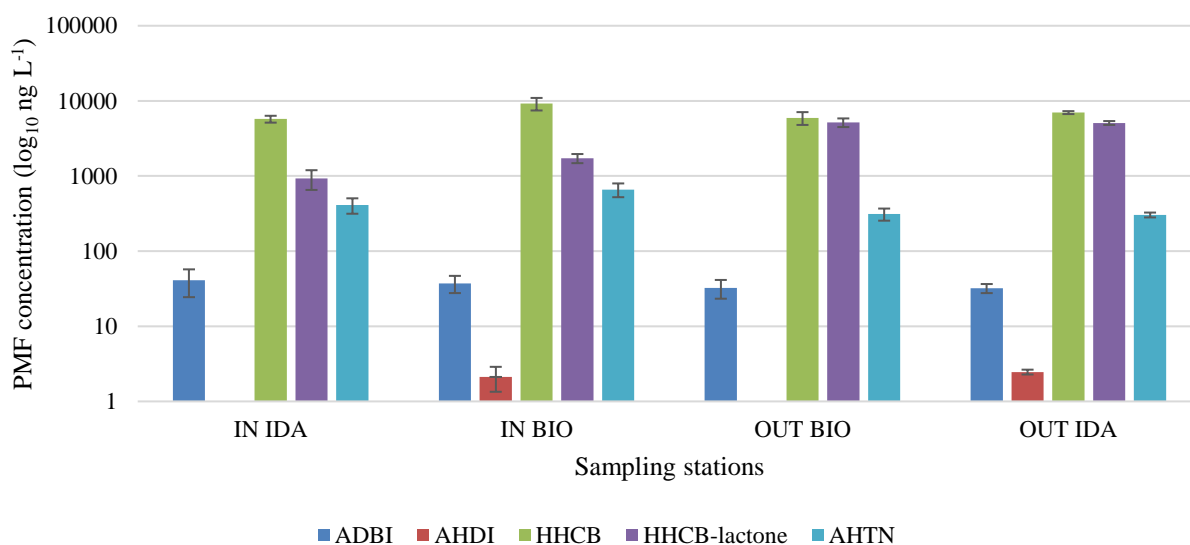


Fig. III-2 Mean concentrations of PMFs (ng L⁻¹) in WWTP inlet (IN IDA), biological tank inlet (IN BIO), biological tank outlet (OUT BIO) after secondary sedimentation and in WWTP outlet (OUT IDA). Y-axis is in logarithmic scale.

Considering a water retention time of 24 h in the WWTP, % of removal efficiencies were obtained both in two experiments for main compounds (Fig. III-3) comparing WWTP inlet and WWTP outlet. The considered wastewater treatment technologies seemed to be inefficient in removing polycyclic musk fragrances from wastewater. From our results, only ≈30% of ADBI and AHTN (musk fragrances with lower concentrations) were partially removed, while other PMFs increased their concentrations in the WWTP effluent in comparison with the influent. This increase might be due to a partial release of these compounds from sludges to the aqueous matrix after biological treatment caused probably by the inability of activated sludges

to adsorb such high PMFs influent concentrations. Other studies demonstrated that polycyclic musk fragrances are not completely removed in conventional wastewater treatments. Zhou et al. (2009) reported efficiency of 58% in HHCB removal in three WWTPs while Joss et al. (2005) reported 50% removal efficiency of HHCB. Even Lishman et al. (2006) calculated a median reduction of 43% for HHCB and 37% for AHTN during wastewater treatment in 8 WWTPs in Canada. Overall, removal efficiency seems to be affected not only from influent concentrations but also from WWTP dimension and adopted wastewater treatments. Due to this removal incapacity, PMFs can be detected even at concentrations of $\mu\text{g L}^{-1}$ in WWTP effluents transferring PMF pollution from wastewaters to receiving water bodies such as in the case of rivers and lakes. Bester (2005) reported concentrations up to 600 ng L^{-1} of polycyclic musk in Ruhr River near the outlet of a WWTP and concentrations of 60 ng L^{-1} for HHCB and 10 ng L^{-1} for AHTN in another station along the River. Lee et al. (2010) reported concentration of $100\text{-}272 \text{ ng L}^{-1}$ for HHCB and $30\text{-}52 \text{ ng L}^{-1}$ for AHTN in Korean superficial water resources.

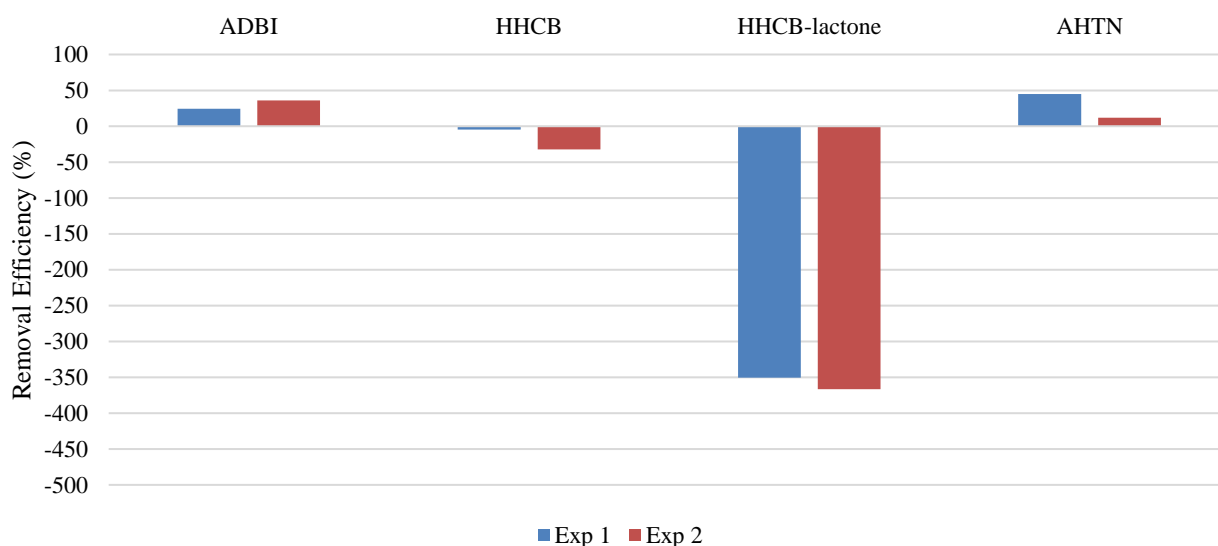


Fig. III-3 % of removal efficiency of PMFs in MS1 WWTP calculated in two experiments. Positive values indicate removal efficiency, while negative values indicate increase in concentrations during wastewater treatments.

The validated method was used for the determination of polycyclic musk fragrances in an activated sludge sample taken during the sampling campaign. Three sludge replicates were analyzed, and mean concentration was calculated (Table III-7).

Table III-7 PMFs concentrations in three replicates of activated sludges (A, B, C). Values are expressed in ng g^{-1} .

| PMFs | A | B | C | Mean \pm S.D. |
|--------------|-------|-------|-------|------------------|
| ADBI | 65 | 45 | 31 | 47 ± 17 |
| HHCB | 21294 | 14367 | 13030 | 16230 ± 4436 |
| HHCB-lactone | 2830 | 1993 | 1786 | 2203 ± 553 |
| AHDI | <LOD | <LOD | <LOD | <LOD |
| AHTN | 1509 | 1037 | 915 | 1153 ± 314 |

Figure III-4 shows mean concentrations and composition of activated sludge. Once again, HHCB was the main compound (82.7%) with concentration of $16230 \pm 4436 \text{ ng g}^{-1}$ while HHCB-lactone and AHTN were of one order of magnitude lower, representing respectively only 11.2% and 5.9% of the sludge sample. ADBI was measured only in trace while AHDI was under the limit of detection.

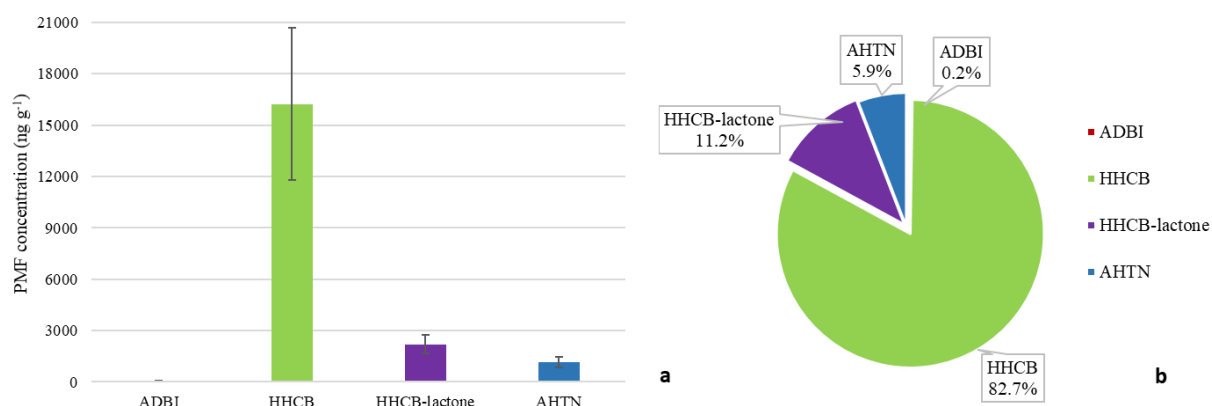


Fig. III-4 Mean concentrations in ng g^{-1} of PMFs in activated sludge (a) and relative sample composition (b).

In Switzerland, AHTN and HHCB were measured in activated sludges at concentrations ranging from 2300 to 8500 ng g^{-1} and in digested sludge between 6600 and 15,000 $\mu\text{g g}^{-1}$ (Ternes et al. 2005). Sludge digestion reduces the solid mass up to 50-70% but do not degrade PMFs. Therefore, digested sludge is more contaminated by PMFs than activated sludges (Ternes et al. 2005).

Yang and Metcalfe (2006) evaluated PMFs concentrations in Peterborough WWTP sewage sludges, such as raw sludge from primary sedimentation, recirculated activated sludge deriving from final sedimentation and back going sludges before the aeration tank and digested biosolids. In this case PMFs concentrations were, respectively, 3302-3309-6788 ng g^{-1} for HHCB, 720-776-1349 ng g^{-1} for AHTN, 23-29-51 ng g^{-1} for ADBI and 20-27-33 ng g^{-1} for AHDI. In the sludge treatment line, there was a clear trend of synthetic musks to be accumulated in recirculated activated sludge and finally to be concentrated into digested biosolids. Even in our case, activated sludge treatment was not able to remove and degrade PMFs and, probably, a step of accumulation may occur during sludge anaerobic digestion. If looking at sample composition of recirculated activated sludges, even in Peterborough WWTP HHCB represents the 80% of the whole sample while AHTN only the 19%, as showed by our results. Regarding PMF concentrations, these high values support the hypothesis of Heberer (2002) and Kupper et al. (2006) that polycyclic musks mainly accumulate in sludges because of their elevated hydrophobicity.

3.4 Conclusions

Two rapid and sensitive methods were developed for PMF analyses in wastewaters and activated sludges; for wastewaters, SPE extraction followed by quantification in GC-MS/MS was used while for activated sludges ultrasonic bath extraction and a cleaning step coupled with GC-Ion trap quantification was applied. Using these analytical protocols, high recoveries were obtained notwithstanding the presence of matrix impurities; low detection and quantification limits were achieved in respect to the concentrations in real samples. Cross-contamination of samples and high solvent consumption were limited employing ultrasound system instead

of Soxhlet extraction. These methods were applied for PMF analyses in wastewaters and activated sludges of a WWTP in Northern Italy showing concentrations of HHCB and HHCB-lactone in the effluent in the range of $\mu\text{g L}^{-1}$; HHCB-lactone compound was formed through oxidation of HHCB. As expected, biotransformation of HHCB into its metabolite HHCB-lactone occurred in the biological tank, with an increase in HHCB-lactone concentrations of 300% after this step. In the WWTP effluents AHTN showed concentrations of about 300 ng L^{-1} , while ADBI and AHDI were detected at trace levels. In activated sludges, concentration of HHCB reached about $16 \mu\text{g g}^{-1}$ while HHCB-lactone and AHTN were one order of magnitude lower. Since only $\approx 30\%$ of AHTN and ADBI were removed, it can be concluded that current technologies are not enough efficient for the removal of PMFs from wastewaters. To our knowledge, this is the first evaluation of PMFs in Italian wastewaters and activated sludges which can be considered a starting point for a monitoring program of these compounds in Italy. Higher concentrations in respect to other EU member States reflected the widespread consumption of these compounds in Southern Europe, particularly in Italy, where it was estimated the highest use of detergents.

3.5 References

- Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ. Rev.* 14, 257–297. <https://doi.org/10.1139/A06-005>
- Arrubla, J.P., Cubillos, J.A., Ramírez, C.A., Arredondo, J.A., Arias, C.A., Paredes, D., 2016. Pharmaceutical and personal care products in domestic wastewater and their removal in anaerobic treatment systems: Septic tank – Up flow anaerobic filter. *Ing. e Investig.* 36, 70–78. <https://doi.org/10.15446/ing.investig.v36n1.53076>
- Artola-Garicano, E., Sinnige, T.L., Van Holsteijn, I., Vaes, W.H.J., Hermens, J.L.M., 2003. Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (*Chironomus riparius* and *Lumbriculus variegatus*). *Environ. Toxicol. Chem.* 22, 1086–1092. [https://doi.org/10.1897/1551-5028\(2003\)022<1086:BAATOP>2.0.CO;2](https://doi.org/10.1897/1551-5028(2003)022<1086:BAATOP>2.0.CO;2)
- Balk, F., Ford, R.A., 1999. Environmental risk assessment for the polycyclic musks, AHTN and HHCB. II. Effect assessment and risk characterisation. *Toxicol. Lett.* 111, 81–94. [https://doi.org/10.1016/S0378-4274\(99\)00170-8](https://doi.org/10.1016/S0378-4274(99)00170-8)
- Bester, K., 2009. Analysis of musk fragrances in environmental samples. *J. Chromatogr. A* 1216, 470–480. <https://doi.org/10.1016/j.chroma.2008.08.093>
- Bester, K., 2005. Polycyclic musks in the Ruhr catchment area - Transport, discharges of waste water, and transformations of HHCB, AHTN and HHCB-lactone. *J. Environ. Monit.* 7, 43–51. <https://doi.org/10.1039/b409213a>
- Bester, K., 2004. Retention characteristics and balance assessment for two polycyclic musk fragrances (HHCB and AHTN) in a typical German sewage treatment plant. *Chemosphere* 57, 863–870. <https://doi.org/10.1016/j.chemosphere.2004.08.032>
- Biel-Maeso, M., Corada-Fernández, C., Lara-Martín, P.A., 2019. Removal of personal care products (PCPs) in wastewater and sludge treatment and their occurrence in receiving soils. *Water Res.* 150, 129–139. <https://doi.org/10.1016/j.watres.2018.11.045>
- Blahova, J., Divisova, L., Plhalova, L., Enevova, V., Hostovsky, M., Doubkova, V., Marsalek, P., Fictum, P., Svobodova, Z., 2018. Multibiomarker Responses of Juvenile Stages of Zebrafish (*Danio rerio*) to Subchronic Exposure to Polycyclic Musk Tonalide. *Arch. Environ. Contam. Toxicol.* 74, 568–576. <https://doi.org/10.1007/s00244-017-0484-8>
- Buerge, I.J., Buser, H.R., Müller, M.D., Poiger, T., 2003. Behavior of the Polycyclic Musks HHCB and AHTN in

- Lakes, Two Potential Anthropogenic Markers for Domestic Wastewater in Surface Waters. *Environ. Sci. Technol.* 37, 5636–5644. <https://doi.org/10.1021/es0300721>
- Čadková, Z., Száková, J., Mukhtorova, D., Hlava, J., Pulkrabová, J., Balík, J., Tlustoš, P., Vadlejch, J., 2020. The response of soil nematode *Caenorhabditis elegans* on the sewage sludge-derived micropollutants. *J. Hazard. Mater.* 384. <https://doi.org/10.1016/j.jhazmat.2019.121468>
- Chase, D.A., Karnjanapiboonwong, A., Fang, Y., Cobb, G.P., Morse, A.N., Anderson, T.A., 2012. Occurrence of synthetic musk fragrances in effluent and non-effluent impacted environments. *Sci. Total Environ.* 416, 253–260. <https://doi.org/10.1016/j.scitotenv.2011.11.067>
- Chen, F., Gao, J., Zhou, Q., 2012. Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in *Carassius auratus* using oxidative stress biomarkers. *Environ. Pollut.* 162, 91–97. <https://doi.org/10.1016/j.envpol.2011.10.016>
- Chen, F., Ying, G.G., Ma, Y.B., Chen, Z.F., Lai, H.J., Peng, F.J., 2014. Field dissipation and risk assessment of typical personal care products TCC, TCS, AHTN and HHCB in biosolid-amended soils. *Sci. Total Environ.* 470–471, 1078–1086. <https://doi.org/10.1016/j.scitotenv.2013.10.080>
- Clara, M., Gans, O., Windhofer, G., Krenn, U., Hartl, W., Braun, K., Scharf, S., Scheffknecht, C., 2011. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. *Chemosphere* 82, 1116–1123. <https://doi.org/10.1016/j.chemosphere.2010.11.041>
- Cunha, S.C., Fernandes, J.O., Vallecillos, L., Cano-Sancho, G., Domingo, J.L., Pocurull, E., Borrull, F., Maulvault, A.L., Ferrari, F., Fernandez-Tejedor, M., Van den Heuvel, F., Kotterman, M., 2015. Co-occurrence of musk fragrances and UV-filters in seafood and macroalgae collected in European hotspots. *Environ. Res.* 143, 65–71. <https://doi.org/10.1016/j.envres.2015.05.003>
- Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and Personal Care Products in the Environment : Agents of Subtle Change ? 107, 907–938.
- ECHA – European Chemicals Agency, 2010. Candidate List of Substances of Very High Concern for Authorisation. [WWW Document].
- Franke, S., Meyer, C., Heinzl, N., Gatermann, R., Hühnerfuss, H., Rimkus, G., König, W.A., Francke, W., 1999. Enantiomeric composition of the polycyclic musks HHCB and AHTN in different aquatic species. *Chirality* 11, 795–801. [https://doi.org/10.1002/\(SICI\)1520-636X\(1999\)11:10<795::AID-CHIR10>3.0.CO;2-S](https://doi.org/10.1002/(SICI)1520-636X(1999)11:10<795::AID-CHIR10>3.0.CO;2-S)
- From, C., Commission, T.H.E., The, T.O., Council, T.H.E., Economic, T.H.E.E., Committee, T.H.E., The, O.F., 2012. Communication COM(2012)673.
- Fromme, H., Otto, T., Pilz, K., 2001. Polycyclic Musk Fragrances in the Aquatic Environment. *ACS Symp. Ser.* 773, 203–222. <https://doi.org/10.1021/bk-2001-0773.ch015>
- Gao, Y., Ji, Y., Li, G., Mai, B., An, T., 2016. Bioaccumulation and ecotoxicity increase during indirect photochemical transformation of polycyclic musk tonalide: A modeling study. *Water Res.* 105, 47–55. <https://doi.org/10.1016/j.watres.2016.08.055>
- Gatermann, R., Hehot, J., Hiihnetiss, H., Rimkus, G., Zitko, V., 1999. Polycyclic and nitro musks in the environment: a comparison between Canadian and European aquatic biota. 38, 3431–3441.
- Godayol, A., Besalú, E., Anticó, E., Sanchez, J.M., 2015. Monitoring of sixteen fragrance allergens and two polycyclic musks in wastewater treatment plants by solid phase microextraction coupled to gas chromatography. *Chemosphere* 119, 363–370. <https://doi.org/10.1016/j.chemosphere.2014.06.072>
- H. Doležalová Weissmannová, I.Š., M. Vávrová, and A.L., 2013. Determination of Toxicity of Musk Compounds Using Alternative Tests of Ecotoxicity. *Chem. List.* 107.
- Hájková, K., Pulkrabová, J., Hajšlová, J., Randák, T., Žlábek, V., 2007. Chub (*Leuciscus cephalus*) as a bioindicator of contamination of the Vltava River by synthetic musk fragrances. *Arch. Environ. Contam. Toxicol.* 53, 390–396.

<https://doi.org/10.1007/s00244-006-0190-4>

- Heberer, T., 2003. Occurrence, fate, and assessment of polycyclic musk residues in the aquatic environment of urban areas - A review. *Acta Hydrochim. Hydrobiol.* 30, 227–243. <https://doi.org/10.1002/aheh.200390005>
- HERA, 2004. HERA Environmental Risk Assessment Polycyclic musks AHTN and HHCB 1–80.
- Herrmann, A., 2005. Chemistry and Technology of Flavors and Fragrances. Edited by David J. Rowe. *Angew. Chemie Int. Ed.* 44, 3650–3651. <https://doi.org/10.1002/anie.200585283>
- Homem, V., Alves, Alice, Alves, Arminda, Santos, L., 2016. Ultrasound-assisted dispersive liquid-liquid microextraction for the determination of synthetic musk fragrances in aqueous matrices by gas chromatography-mass spectrometry. *Talanta* 148, 84–93. <https://doi.org/10.1016/j.talanta.2015.10.049>
- Hu, Z., Shi, Y., Cai, Y., 2011. Concentrations, distribution, and bioaccumulation of synthetic musks in the Haihe River of China. *Chemosphere* 84, 1630–1635. <https://doi.org/10.1016/j.chemosphere.2011.05.013>
- Kimberley, M.O., Wang, H., Wilks, P.J., Fisher, C.R., Magesan, G.N., 2004. Economic analysis of growth response from a pine plantation forest applied with biosolids. *For. Ecol. Manage.* 189, 345–351. <https://doi.org/10.1016/j.foreco.2003.09.003>
- Kupper, T., Plagellat, C., Brändli, R.C., de Alencastro, L.F., Grandjean, D., Tarradellas, J., 2006. Fate and removal of polycyclic musks, UV filters and biocides during wastewater treatment. *Water Res.* 40, 2603–2612. <https://doi.org/10.1016/j.watres.2006.04.012>
- Lange, C., Kuch, B., Metzger, J.W., 2015. Occurrence and fate of synthetic musk fragrances in a small German river. *J. Hazard. Mater.* 282, 34–40. <https://doi.org/10.1016/j.jhazmat.2014.06.027>
- Lee, I.S., Kim, U.J., Oh, J.E., Choi, M., Hwang, D.W., 2014. Comprehensive monitoring of synthetic musk compounds from freshwater to coastal environments in Korea: With consideration of ecological concerns and bioaccumulation. *Sci. Total Environ.* 470–471, 1502–1508. <https://doi.org/10.1016/j.scitotenv.2013.07.070>
- Lee, I.S., Lee, S.H., Oh, J.E., 2010. Occurrence and fate of synthetic musk compounds in water environment. *Water Res.* 44, 214–222. <https://doi.org/10.1016/j.watres.2009.08.049>
- Lishman, L., Smyth, S.A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos, M., Beland, M., Seto, P., 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. *Sci. Total Environ.* 367, 544–558. <https://doi.org/10.1016/j.scitotenv.2006.03.021>
- Luckenbach, T., Corsi, I., Epel, D., 2004. Fatal attraction: Synthetic musk fragrances compromise multixenobiotic defense systems in mussels. *Mar. Environ. Res.* 58, 215–219. <https://doi.org/10.1016/j.marenvres.2004.03.017>
- Maekawa, A., Matsushima, Y., Onodera, H., Shibutani, M., Ogasawara, H., Kodama, Y., Kurokawa, Y., Hayashi, Y., 1990. Long-term toxicity/carcinogenicity of musk xylol in B6C3F1 mice. *Food Chem. Toxicol.* 28, 581–586. [https://doi.org/10.1016/0278-6915\(90\)90159-K](https://doi.org/10.1016/0278-6915(90)90159-K)
- Melymuk, L., Robson, M., Csiszar, S.A., Helm, P.A., Kaltenecker, G., Backus, S., Bradley, L., Gilbert, B., Blanchard, P., Jantunen, L., Diamond, M.L., 2014. From the city to the lake: Loadings of PCBs, PBDEs, PAHs and PCMs from Toronto to Lake Ontario. *Environ. Sci. Technol.* 48, 3732–3741. <https://doi.org/10.1021/es403209z>
- Mogensen, B.B., Pritzl, G., Rastogi, S., Glense, O., Hedlund, B., Hirvi, J.P., Lundgren, A., Sigurdsson, A., 2004. Musk compounds in the Nordic environment. *TemaNord Rep.* 1–70.
- Müller, S., Schmid, P., Schlatter, C., 1996. Occurrence of nitro and non-nitro benzenoid musk compounds in human adipose tissue. *Chemosphere* 33, 17–28. [https://doi.org/10.1016/0045-6535\(96\)00160-9](https://doi.org/10.1016/0045-6535(96)00160-9)
- Nakata, H., 2005. Occurrence of synthetic musk fragrances in marine mammals and sharks from Japanese coastal waters. *Environ. Sci. Technol.* 39, 3430–3434. <https://doi.org/10.1021/es050199l>
- NIER, 2009. Development of analytical method and monitoring of personal care products in the Nakdong River system.
- O.S.P.A.R. Commission, 2004. Hazardous substances series, musk xylene and other musks. London, UK.

<https://doi.org/ISBN 1-904426-36-0>

- Osemwengie, L.I., Gerstenberger, S.L., 2004. Levels of synthetic musk compounds in municipal wastewater for potential estimation of biota exposure in receiving waters. *J. Environ. Monit.* 6, 533–539. <https://doi.org/10.1039/b400514g>
- Parolini, M., Magni, S., Traversi, I., Villa, S., Finizio, A., Binelli, A., 2015. Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in *Dreissena polymorpha*. *J. Hazard. Mater.* 285, 1–10. <https://doi.org/10.1016/j.jhazmat.2014.11.037>
- Peck, A.M., Linebaugh, E.K., Hornbuckle, K.C., 2006. Synthetic musk fragrances in Lake Erie and Lake Ontario sediment cores. *Environ. Sci. Technol.* 40, 5629–5635. <https://doi.org/10.1021/es060134y>
- Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass flow of polycyclic musks in two wastewater treatment plants. *Arch. Environ. Contam. Toxicol.* 52, 451–457. <https://doi.org/10.1007/s00244-006-0203-3>
- Reiner, J.L., Kannan, K., 2011. Polycyclic musks in water, sediment, and fishes from the upper Hudson River, New York, USA. *Water, Air, Soil Pollut.* 214, 335–342. <https://doi.org/10.1007/s11270-010-0427-8>
- Rice, S.L., Mitra, S., 2007. Microwave-assisted solvent extraction of solid matrices and subsequent detection of pharmaceuticals and personal care products (PPCPs) using gas chromatography-mass spectrometry. *Anal. Chim. Acta* 589, 125–132. <https://doi.org/10.1016/j.aca.2007.02.051>
- Rimkus, G. G., & Wolf, R., 1997. Polycyclic musk fragrances in biota from aquatic ecosystems. *Chemosphere*.
- Ros, O., Izaguirre, J.K., Olivares, M., Bizarro, C., Ortiz-Zarragoitia, M., Cajaraville, M.P., Etxebarria, N., Prieto, A., Vallejo, A., 2015. Determination of endocrine disrupting compounds and their metabolites in fish bile. *Sci. Total Environ.* 536, 261–267. <https://doi.org/10.1016/j.scitotenv.2015.07.074>
- Rüdel, H., Böhmer, W., Schröter-Kermani, C., 2006. Retrospective monitoring of synthetic musk compounds in aquatic biota from German rivers and coastal areas. *J. Environ. Monit.* 8, 812–823. <https://doi.org/10.1039/b602389b>
- Santiago-Morales, J., Gómez, M.J., Herrera, S., Fernández-Alba, A.R., García-Calvo, E., Rosal, R., 2012. Oxidative and photochemical processes for the removal of galaxolide and tonalide from wastewater. *Water Res.* 46, 4435–4447. <https://doi.org/10.1016/j.watres.2012.05.051>
- Schmid, P., Kohler, M., Gujer, E., Zennegg, M., Lanfranchi, M., 2007. Persistent organic pollutants, brominated flame retardants and synthetic musks in fish from remote alpine lakes in Switzerland. *Chemosphere* 67, 16–21. <https://doi.org/10.1016/j.chemosphere.2006.05.080>
- Schmidt-Bäumler, K., Heberer, T., Stan, H.J., 1999. Occurrence and distribution of organic contaminants in the aquatic system in Berlin. Part II: Substituted phenols in Berlin surface water. *Acta Hydrochim. Hydrobiol.* 27, 143–149. [https://doi.org/10.1002/\(SICI\)1521-401X\(199905\)27:3<143::AID-AHEH143>3.0.CO;2-9](https://doi.org/10.1002/(SICI)1521-401X(199905)27:3<143::AID-AHEH143>3.0.CO;2-9)
- Schnell, S., Martin-Skilton, R., Fernandes, D., Porte, C., 2009. The interference of nitro- and polycyclic musks with endogenous and xenobiotic metabolizing enzymes in carp: An in vitro study. *Environ. Sci. Technol.* 43, 9458–9464. <https://doi.org/10.1021/es902128x>
- Shek, W.M., Murphy, M.B., Lam, J.C.W., Lam, P.K.S., 2008. Polycyclic musks in green-lipped mussels (*Perna viridis*) from Hong Kong. *Mar. Pollut. Bull.* 57, 373–380. <https://doi.org/10.1016/j.marpolbul.2008.02.037>
- Simonich, S.L., Federle, T.W., Eckhoff, W.S., Rottiers, A., Webb, S., Sabaliunas, D., De Wolf, W., 2002. Removal of fragrance materials during U.S. and European wastewater treatment. *Environ. Sci. Technol.* 36, 2839–2847. <https://doi.org/10.1021/es025503e>
- Spencer, P.S., Bischoff-Fenton, M.C., Moreno, O.M., Opdyke, D.L., Ford, R.A., 1984. Neurotoxic properties of musk ambrette. *Toxicol. Appl. Pharmacol.* 75, 571–575. [https://doi.org/10.1016/0041-008X\(84\)90194-7](https://doi.org/10.1016/0041-008X(84)90194-7)
- Tumová, J., Šauer, P., Golovko, O., Koba Ucu, O., Grabic, R., Máchová, J., Kocour Kroupová, H., 2019. Effect of polycyclic musk compounds on aquatic organisms: A critical literature review supplemented by own data. *Sci. Total Environ.* 651, 2235–2246. <https://doi.org/10.1016/j.scitotenv.2018.10.028>

- Villa, S., Assi, L., Ippolito, A., Bonfanti, P., Finizio, A., 2012. First evidences of the occurrence of polycyclic synthetic musk fragrances in surface water systems in Italy: Spatial and temporal trends in the Molgora River (Lombardia Region, Northern Italy). *Sci. Total Environ.* 416, 137–141. <https://doi.org/10.1016/j.scitotenv.2011.11.027>
- Weschler, C.J., Nazaroff, W.W., 2008. Semivolatile organic compounds in indoor environments. *Atmos. Environ.* 42, 9018–9040. <https://doi.org/10.1016/j.atmosenv.2008.09.052>
- Winkler, M., Kopf, G., Hauptvogel, C., Neu, T., 1998. Fate of artificial musk fragrances associated with suspended particulate matter (SPM) from the River Elbe (Germany) in comparison to other organic contaminants. *Chemosphere* 37, 1139–1156. [https://doi.org/10.1016/S0045-6535\(98\)00110-6](https://doi.org/10.1016/S0045-6535(98)00110-6)
- Wollenberger, L., Breitholtz, M., Kusk, K.O., ..., 2003. Inhibition of larval development of the marine copepod. *Acartia tonsa* 305, 53–64.
- Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K., 2008. Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (*Oryzias latipes*). *Aquat. Toxicol.* 90, 261–268. <https://doi.org/10.1016/j.aquatox.2008.09.007>
- Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. *Sci. Total Environ.* 363, 149–165. <https://doi.org/10.1016/j.scitotenv.2005.06.022>
- Zhang, X., Xu, Q., Man, S., Zeng, X., Yu, Y., Pang, Y., Sheng, G., Fu, J., 2013. Tissue concentrations, bioaccumulation, and biomagnification of synthetic musks in freshwater fish from Taihu Lake, China. *Environ. Sci. Pollut. Res.* 20, 311–322. <https://doi.org/10.1007/s11356-012-1095-6>

CHAPTER IV

Polycyclic musk fragrance (PMF) removal, adsorption and biodegradation in a conventional activated sludge wastewater treatment plant in Northern Italy

Stefano Tasselli^{1-2,*}, Erica Valenti¹ and Licia Guzzella¹

¹ National Research Council - Water Research Institute (CNR-IRSA), S.S. Brugherio via del Mulino 19, Brugherio (MB), 20861, Italy

² Department of Environmental Sciences, University of Milano Bicocca, Piazza della Scienza 1, 20126 Milano, Italy

* Corresponding author: tasselli@irsa.cnr.it Telephone: +39 039 21694223

Abstract

The first Italian annual monitoring study was carried out in Northern Italy to analyze the fate and removal of polycyclic musk fragrances (PMFs) in a wastewater treatment plant (WWTP) with conventional activated sludge (CAS) system. Water was sampled in four different stations along wastewater treatments in order to better understand the behaviour of PMFs along different steps of the plant. Galaxolide (HHCB) and Galaxolidone (HHCB-lactone) were found in concentrations of $\mu\text{g L}^{-1}$, one order of magnitude greater than Tonalide (AHTN), while Phantolide (AHDI) was never detected and Celestolide (ADBI) was measured only at trace levels. Considering water concentrations, HHCB and AHTN evidenced a slight reduction, 20% and 50% respectively, during wastewater treatments thus resulting in a modest removal efficiency, mainly due to adsorption processes during the biological treatment. This was also confirmed by the high PMF concentrations measured in activated sludges which remained stable throughout the year. On the contrary, HHCB-lactone registered an increase up to 70% during wastewater treatments caused by the biotransformation of the parental compound HHCB during the biological treatment, as showed by the different HHCB-lactone/HHCB ratio measured before and after this step. No significant differences were recorded between seasons in terms of PMF input onto WWTP, in accordance with the common use of these chemicals in civil houses. Overall, this study suggests that current technologies employed in conventional WWTP are not enough efficient in removing these organic micropollutants from wastewaters and, therefore, WWTP effluents represent possible point sources of pollution for aquatic ecosystems. Additional treatments are necessary to enhance the removal of PMFs in order to increase the quality of the WWTP effluents.

Keywords

Personal care products (PCPs); emerging pollutants; wastewater treatment plant; activated sludge process; HHCB biotransformation; removal efficiency

Funding

This work was financially supported by PerFORMWATER 2030 project ID 240750 (POR FESR 2014-2020 Asse I -Accordi per la Ricerca e l'Innovazione).

Acknowledgements

We like to thank Società Italiana Acetilene E Derivati (SIAD), partner of the project, for its technical support during sampling activities and article drafting.

4.1 Introduction

Recently, a growing interest concerning pollution derived from personal care products (PCPs) has emerged. PCPs are chemical compounds frequently used all over the world (Ávila and García 2015) and, due to their extensive use, they are released in large amounts into sewage systems. Thus, wastewater effluents become their main source of pollution into the environment (Posada-Ureta et al. 2012). Within PCPs, there are four chemical classes of synthetic musks: nitro, polycyclic, macrocyclic and alicyclic musks (Posada-Ureta et al. 2012). Synthetic musks are incorporated in a wide range of products such as perfumes, soaps, house-cleaning products and detergents (Heberer 2003). Nitro musks and polycyclic musks (PMFs) are the most representative. Nitro musks, as xylene and ketone, are co-genotoxicants (Mersch-Sundermann et al. 2001) and, for this reason, restrictions of their use were applied in Europe (O.S.P.A.R. Commission, 2004; ECHA – European Chemicals Agency, 2010). Consequently, their presence decreased in personal care products as well as in the environment and they were replaced by polycyclic musks (Lung et al., 2011; Zhang et al., 2008) which are still globally used. Between polycyclic musks there are Galaxolide (HHCB), Tonalide (AHTN), Celestolide (ADBI) and Phantolide (AHDI). HHCB and AHTN represent about the 95% of the fragrances European market (Clara et al. 2011) and, in the EU, they are included in the fourth priority list within the context of the Existing Chemical Programme (Council Regulation EEC 793/93). Their production is monitored in Europe since 2003 by the Oslo-Paris Commission for Protection and Conservation of the North-East Atlantic and its resources. Among EU member states, Italy registered the highest consumptions of PMFs with volumes of 7.23 g y⁻¹ per capita for HHCB (European Commission 2003) and 1.81 g y⁻¹ for AHTN (EU-RAR 2008). Other polycyclic musks as ADBI and AHDI are quantitatively less important. Alongside parental compounds, even transformation products might represent hazard risk to the environment; between PMFs, the best known is HHCB-lactone or Galaxolidone, the by-product derived from abiotic degradation or biotransformation of HHCB. In this reaction, the benzylic methylene group of HHCB is oxidized to a more polar and recalcitrant compound with a lactone group. As a consequence of this reaction, Galaxolidone shows a greater polarity and higher affinity for water rather than for the organic matter and, therefore, its concentrations in wastewater treatment plant (WWTP) effluents are often higher than those in the respective influents; this was also showed by Reiner et al. (2007) who measured concentrations of HHCB-lactone of 505-897 ng L⁻¹ in the influent and of 1620-1740 ng L⁻¹ in the effluent of a biological WWTP with Conventional Activated Sludge system (CAS). Our group evaluated that this biotransformation takes place during the secondary treatment in activated sludge WWTPs (Tasselli and Guzzella 2020).

PMFs evidenced a potentially risk for humans and aquatic environment because they have been detected in different matrices, including air (Weinberg et al. 2011), freshwater (Villa et al. 2012), sediments (Lou et al. 2016), aquatic organisms (Sapozhnikova et al. 2010) and even in human blood (Hu et al. 2010). In fact, synthetic musk compounds are only partially degradable, and for this reason they are not removed by conventional WWTPs. As a consequence, they have been detected in rivers in which WWTP effluents are

present (Lange et al. 2015) but also in agricultural fields fertilized with WWTP biosolids (Yang and Metcalfe 2006). Previous studies showed that acute toxicity of PMFs to aquatic organisms ranges from hundreds of $\mu\text{g L}^{-1}$ to amounts of $<20 \text{ mg L}^{-1}$ (Tumová et al., 2019). No data are currently available regarding chronic toxicity while only few studies focusing on subchronic effects were published; Parolini et al., (2015) evidenced an oxidative stress through the increasing in lipid peroxide, protein carbonyl levels and changes in DNA structure after an exposure for 21 days of the freshwater zebra mussel *Dreissena polymorpha* to concentrations of HHCB and AHTN of 500 ng L^{-1} and 80 ng L^{-1} , respectively. Other modifications in oxidative stress parameters were observed in adult goldfish of *Carassius auratus* after 21 days of exposure to $15 \mu\text{g L}^{-1}$ of HHCB (Chen et al. 2012) and in juvenile zebrafish *Danio rerio* after 28 days of exposure to 50 ng L^{-1} of AHTN (Blahova et al. 2018). These first evidences combined with PMF ubiquitous presence at high concentrations, relative persistence, tendency for bioconcentration and potential hormonal activities raised significant concerns about their impact on the environment and, at the end, even on the human health. To our knowledge, environmental regulatory limits have not been set for these compounds and so it is extremely important to study their behaviour in WWTPs and their actual concentrations in the environment in order to evaluate their potential hazardous properties for the environment and the human health. Reports on polycyclic musks concentrations at various stages of wastewater treatments are also limited and seasonal variation was not been investigated yet. Monitoring studies were carried out only in Northern Europe while concerning Southern Europe data are very scarce. Furthermore, predictions cannot be made given the difference in use of these compounds among different countries also in terms of volumes. For what we know, only one monitoring study regarding the presence of HHCB, AHTN and ADBI was carried out in Italy (Villa et al. 2012), evaluating their concentrations along the axis of a river located in Lombardy where discharges of effluents from WWTPs are present. To our knowledge, studies regarding the behaviour of PMFs inside a conventional WWTPs in Italy have not been performed yet and this can represent a significant lack of knowledge as the concentrations of PMFs in our wastewaters, much higher than in those recorded in other countries, could lead to different behaviours inside WWTPs than those studied elsewhere. Moreover, this study analyse not only the effectiveness of WWTPs in PMF removal but also the main treatments employed in WWTPs in order to understand which are the mechanisms that determine the fate of these compounds and which ultimately lead to the WWTP removal ability, already investigated by other studies and which can therefore act as a support to our findings.

The aim of the present investigation is therefore to monitor the fate of polycyclic musk fragrances in wastewater and activated sludges of an Italian wastewater treatment plant with CAS system located in Lombardy, Northern Italy. Different water treatment steps were evaluated and the overall WWTP removal ability was assessed. By considering one year of monitoring, seasonal variations of PMF concentrations in the WWTPs were evaluated and some considerations regarding usage and measured concentrations of the main PMFs were argued.

4.2 Materials and methods

4.2.1 Chemicals

Galaxolide (HHCB), Tonalide (AHTN), Galaxolidone (HHCB-lactone), Phantolide (AHDI) and Celestolide (ADBI) standards were obtained from Chemical Research 2000 (Rome, IT). Tonalide (AHTN) D3 in iso-octane was purchased from LGC Standards (Manchester, USA). All solvents were purchased from VWR International (Radnor, PA, USA). Superclean ENVI-Carb 120/400 was obtained from Supelco (Bellefonte, PA, USA) and Acrodisc syringe filters (1 μm glass fibre pore size) were purchased from VWR International (Radnor, PA, USA).

4.2.2 Sample collection, influent and WWTP characteristics

Wastewater and activated sludge samples were taken from a WWTP located in a densely urbanized area, in the metropolitan area of Milan, between June 2019 and February 2020. The plant has a nominal capacity of 30000 population equivalent and treats a mixture of municipal and industrial wastewater. The WWTP water line consists in primary treatments (coarse and primary settling), a CAS system (anoxic tank followed by an aerobic tank) and tertiary treatment (filtration and UV disinfection). The average inlet flow during the studied period was 13000 $\text{m}^3 \text{d}^{-1}$, while the wastewater characteristics are reported in Table IV-1.

Table IV-1 WWTP influent characteristics during the studied period. Means and standard deviations are reported.

| <i>WWTP influent characteristics</i> | | |
|--------------------------------------|------------------------------|----------------------|
| <i>Parameter</i> | <i>Units</i> | <i>Concentration</i> |
| <i>COD</i> | $\text{mgO}_2 \text{L}^{-1}$ | 111 ± 38 |
| <i>BOD5</i> | $\text{mgO}_2 \text{L}^{-1}$ | 47 ± 30 |
| <i>NH4</i> | mgN L^{-1} | 9 ± 2.6 |
| <i>TKN</i> | mgN L^{-1} | 14.7 ± 7 |

Composite samples were daily collected during three consecutive days with an automatic sampler (1 grab sample per hour) every three months for one year considering the hydraulic retention time of the WWTP. Four different stations of the treatment process were sampled (Fig. 1): WWTP inlet (1), biological treatment inlet (2), biological treatment outlet (3) and WWTP outlet (4) after the final UV-disinfection step. One instantaneous activated sludge samples were collected from the nitrification tank during each three-day campaign (S).

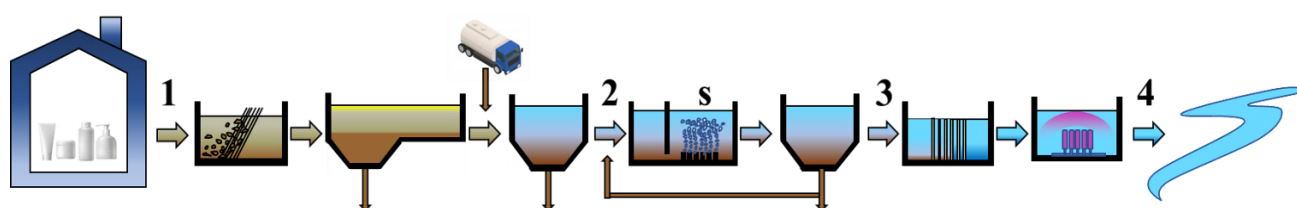


Fig. IV-1 Sampling stations along the wastewater treatment process: WWTP inlet (1), biological treatment inlet (2), biological treatment outlet (3) and WWTP outlet (4) after the final UV-disinfection step. The activated sludge sampling station (s) is also reported.

Water samples were pre-treated in lab using a two-stages filtration steps with glass microfiber filters (VWR International, Radnor, PA, USA) of 47 mm diameter and nominal particle retention of 1 µm and 0.7 µm, subsequently. Samples were kept at 4°C in the dark and analysis were carried out within 24 h.

Concerning sludge, after removing the excess of water, the matrix was freeze-dried and kept in amber glass bottle at room temperature until analysis. Sampling campaigns were planned to avoid both heavy rain events and August period that could modify wastewater trends.

4.2.3 Sample preparation and analysis

Analysis of water and sludge samples were performed according to (Tasselli and Guzzella 2020). Briefly, water samples were extracted with solid phase extraction, concentrated to 1 mL using N₂ and transferred to GC micro-vials for GC-MS/MS analysis. Sludge samples were extracted in an ultrasonic water bath, concentrated to 5 mL using N₂ flux and transferred in centrifuge tubes with Superclean ENVI-Carb 120/400 (Supelco, Bellefonte, PA, USA). Supernatants were concentrated to 800 µL, filtered with Acrodisc syringe filters and transferred to GC micro-vials for GC-MS analysis. Both GC-MS analysis were performed using methods described in (Tasselli and Guzzella 2020). Additional data on sample preparation are reported on the Supporting Information (Chapter VII-S1).

4.2.4 Quality control

Details on validation parameters of the analytical methods employed in this study (linearity ranges, coefficients of determination R², repeatability and recoveries) are reported in Tasselli & Guzzella, (2020). Laboratory contamination was reduced by limiting the use of common detergents, using dedicated acetone-washed glassware and preferring alcohol-based detergent. Laboratory personnel was cautious in not wearing personal care products during sample processing. Blank samples of Milli-Q water were analyzed every 4 samples and all samples were blank subtracted. Procedural blanks were used for the analysis of sludge because of unavailability of reference material. Limits of detection (LOD) were calculated considering mean (µ) and standard deviation (σ) of four blank samples analyzed in the same days of other samples (Shrivastava and Gupta 2011):

$$LOD = \mu + 3 * \sigma$$

Table IV-2 summarizes detection limits for every PMF in water and sludge samples.

Table IV-2 Limits of detection (LOD) of PMFs in water and sludge.

| <i>PMFs</i> | <i>LOD Water (ng L⁻¹)</i> | <i>LOD Sludge (ng g⁻¹)</i> |
|---------------------|--------------------------------------|---------------------------------------|
| <i>HHCB</i> | 393 | 48 |
| <i>HHCB-lactone</i> | 164 | 23 |
| <i>AHTN</i> | 78 | 78 |
| <i>ADBI</i> | 21 | 0.1 |
| <i>AHDI</i> | 17 | 0.1 |

4.3. Results and discussion

4.3.1 Presence of PMFs in wastewater

The measured concentrations of PMFs in the four different stations of the WWTP are summarized in the Supporting Information (Chapter VII-Table S1) and Fig. IV-2. Data of HHCB, AHTN and HHCB-lactone were plotted in boxplot diagrams to track changes in concentrations at different stations along the WWTP treatment process (Fig. IV-2).

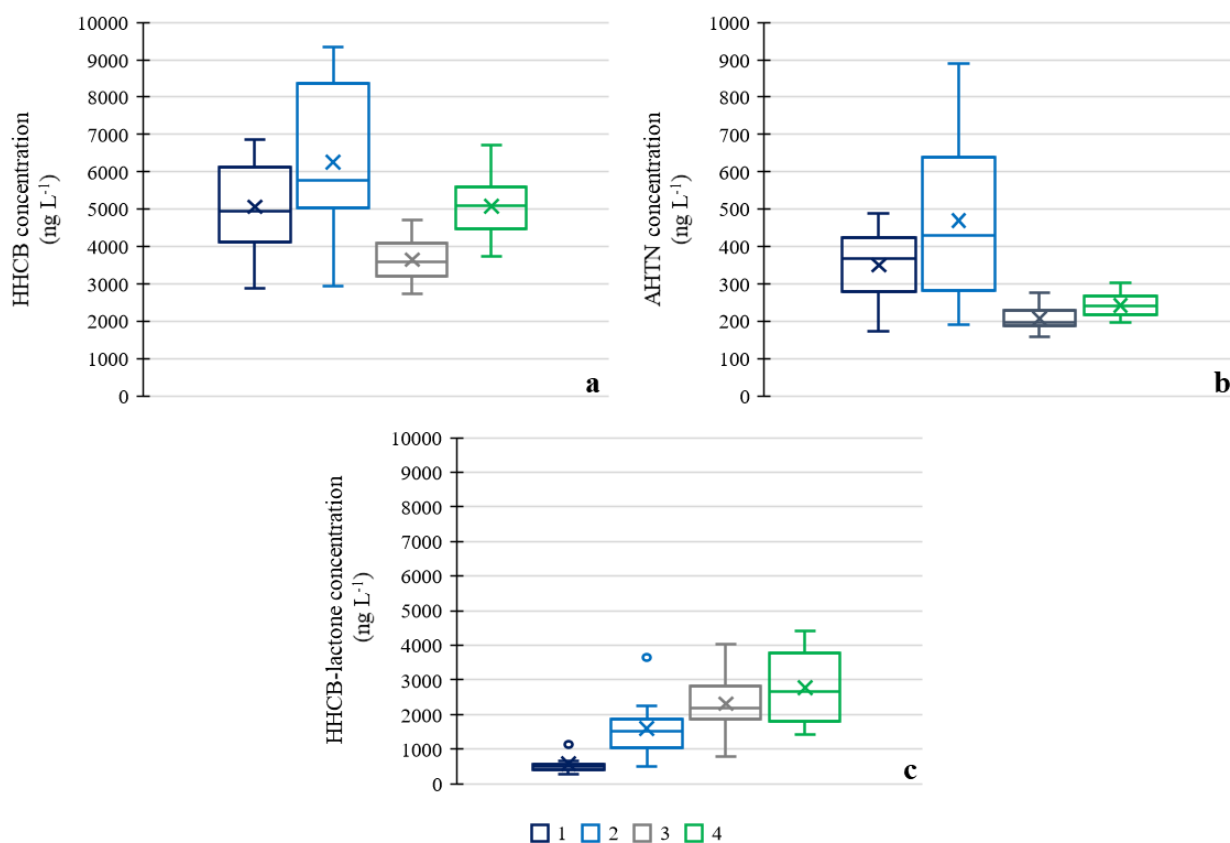


Fig. IV-2 Boxplot diagrams representing min, max, mean and median of HHCB (a), AHTN (b) and HHCB-lactone (c) concentrations detected in WWTP inlet (1), biological treatment inlet (2), biological treatment outlet (3) and WWTP outlet (4). Values are expressed in ng L^{-1} .

HHCB, AHTN and HHCB-lactone were detected in all samples of wastewaters of the WWTP while ADBI only in stations 1 and 2. AHDI was never detected in all the analyzed samples. HHCB has been the compound with the highest concentrations and, together with HHCB-lactone, has been in the $\mu\text{g L}^{-1}$ range while AHTN showed concentrations always below $1 \mu\text{g L}^{-1}$. Considering the same PMFs analyzed in influents of other WWTPs, some differences can be highlighted. For example, Clara et al. (2011) measured HHCB and AHTN concentration in 14 different WWTP influents in Austria, at levels between 0.65 and $1.8 \mu\text{g L}^{-1}$ of AHTN and between 1.4 and $13 \mu\text{g L}^{-1}$ of HHCB. In China, Hu et al. (2011) reported concentrations in WWTP influents at very high levels: 31-3039 ng L^{-1} of HHCB and 28-1486 ng L^{-1} of AHTN. Despite these findings, other studies detected PMFs in WWTP influents at lower concentrations than our studies; Yang & Metcalfe, (2006) evaluated the annual average concentrations of PMFs in the influent of the Peterborough WWTP. Authors measured average concentrations of 5.8 ng L^{-1} for ADBI, 4.8 ng L^{-1} for

AHDI, 390.2 ng L⁻¹ for HHCB and 85.9 ng L⁻¹ for AHTN. Another research carried out in Georgia (Horii et al. 2007) detected annual concentrations of only 420 ng L⁻¹ of HHCB, 388 ng L⁻¹ of AHTN and 371 ng L⁻¹ of HHCB-lactone. As showed by these studies, there is a great variability in PMF concentrations detected in the influents of WWTPs that follows the different usage of these chemicals between countries. In our study, all detected PMFs except HHCB-lactone showed the highest concentrations in station 2, thus evidencing a modest increase between station 1 and station 2 due to a further addition of mud and wastewater deriving from sewage trucks directly discharged before the primary sedimentation tank. The contribution from sewage trucks was not constant and extremely variable; in fact, the highest variability in measured concentrations, expressed as the coefficient of variation (CV%), was registered in station 2. Regarding secondary treatment, a decrease in concentrations in the water phase was noticed for HHCB and AHTN between station 2 to station 3 (Fig. IV-2a and IV-2b). The reason is, as showed below, the adsorption of these hydrophobic molecules, together with suspended solids and organic matter, to activated sludges which decreased their concentrations in the aqueous phase. A final slight increase in HHCB and AHTN concentrations was observed between station 3 and station 4, caused by UV-induced alterations of the chemical equilibrium between dissolved organic carbon content and PMF concentrations in waters during the UV-disinfection treatment as previously verified by Yang & Metcalfe, (2006). HHCB-lactone concentrations should be evaluated by considering a different approach (Fig. IV-2c): this compound had the highest annual mean values in station 4 and showed a constant increase in concentrations in the water phase along wastewater treatments from 581 ng L⁻¹ in station 1 up to 2773 ng L⁻¹ in station 4. HHCB-lactone is a by-product of abiotic and biotic degradation of HHCB and it is more polar than the parental compound. For this reason, once formed, this compound is mainly detected in the water phase rather than in the solid one. The biotransformation of HHCB into HHCB-lactone was firstly reported to take place in activated sludge WWTP by Bester, (2004) who measured an average concentration of 230 ng L⁻¹ in the WWTP influent and of 370 ng L⁻¹ in the effluent. After, Reiner et al. (2007) highlighted the increase in HHCB-lactone concentration in wastewaters by comparing inputs and outputs of two activated sludge WWTPs: 1620-1740 ng L⁻¹ of HHCB-lactone were found in two WWTP effluents receiving mainly domestic wastewaters from New York in comparison with 505-897 ng L⁻¹ detected in the respective influents. Besides, Bester et al. (2004) evidenced that HHCB-lactone might be sometimes present also in the technical HHCB product (about 10% of HHCB), and for this reason it can be found even at the entrance of WWTPs. The percentage was evaluated even in this study by calculating HHCB-lactone/HHCB ratio in all samples from WWTP inlet and a mean value of 11 ± 4% was calculated, thus confirming the observations of Bester et al., (2004). The slight decrease in HHCB-lactone/HHCB ratio between station 3 and station 4 might be due to a partial desorption of HHCB from dissolved organic carbon caused by UV disinfection as discussed above.

4.3.2 Adsorption and biotransformation of HHCB during secondary treatment

During the secondary treatment in the aerated tank, different mechanisms are involved in the removal of micropollutants: volatilization, adsorption and biodegradation (Besha et al. 2017). Concerning PMFs, volatilization may be considered very limited if compared with other possible removal processes as adsorption (Luo et al. 2014). This mechanism is generally referred as the process in which chemicals are adsorbed to solid phases and is correlated with solid water distribution coefficient (K_d), defined as the ratio

of the concentration of the chemical in the solid and in the aqueous phase at equilibrium (Ternes et al. 2004). Always Ternes et al. (2004) defined that compounds with a K_d value minor of 500 L kg^{-1} do not be significantly removed via adsorption and calculated K_d values for HHCB one order of magnitude higher than this threshold. These findings, combined with the high hydrophobicity of PMFs, suggest that adsorption may play a significant role in the removal of this compound during secondary treatment. Biodegradation is the degradation of compounds operated by microorganisms and fungus and it depends both on the redox conditions of the system and on adsorption of compounds to sludge matrix and microbial populations present in the system (Cirja et al. 2008). Our group previously evidenced (Tasselli & Guzzella, 2020) that the biotransformation of HHCB into HHCB-lactone takes place during the secondary treatment in the WWTP by comparing concentrations of the parental compound and its metabolite in water samples before and after this treatment step. In order to confirm these data, a focus on the concentration levels of HHCB and HHCB-lactone in the four stations was undertaken in this study by calculating HHCB-lactone/HHCB ratio in each station (Fig. IV-3).

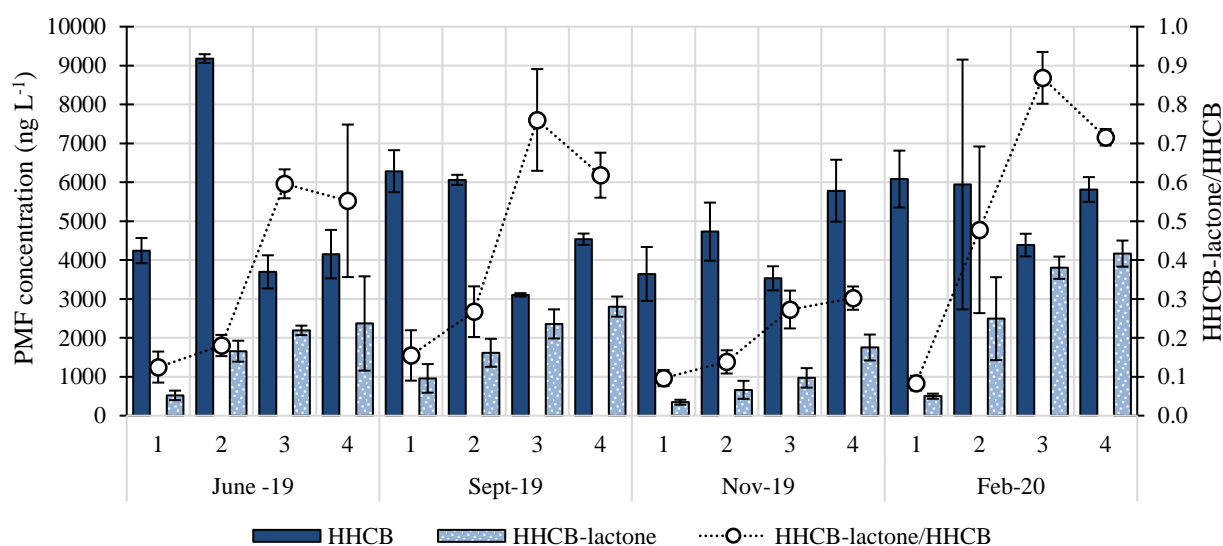


Fig. IV-3 Mean concentrations of HHCB and HHCB-lactone in the water samples at sampling stations 1-4 of the WWTP in different seasons. Values are expressed as ng L^{-1} on the left y-axes. For each station, the mean HHCB-lactone/HHCB ratio was also calculated and reported on the right y-axes.

Results showed a clear increase in HHCB-lactone/HHCB ratio between station 2 and station 3 which represents the inlet and the outlet of the biological tank caused by the decrease of HHCB with the simultaneous increase of HHCB-lactone indicating the biotransformation process of HHCB into HHCB-lactone. In addition to this, previous studies highlighted that PMFs were mainly removed by both biodegradation or biotransformation and adsorption processes (Ternes et al. 2004; Fernandez-Fontaina et al. 2013). The contribution of these processes was evaluated in this study by comparing concentrations of HHCB and its metabolite HHCB-lactone at the inlet (station 2) and the outlet (station 3) of the biological tank (Table IV-3).

Table IV-3 Mean differences in HHCB and HHCB-lactone concentrations between station 2 and station 3. Negative values indicate removal and positive values indicate formation. pH, temperature and Total Suspended Solids (TSS) values in the biological tank are also reported.

| <i>Sampling campaigns</i> | <i>Biological tank conditions</i> | | | <i>HHCB</i> | <i>HHCB-lactone</i> |
|---------------------------|-----------------------------------|---------------|-------------------------------|-------------|---------------------|
| | <i>pH</i> | <i>T (°C)</i> | <i>TSS (g L⁻¹)</i> | | |
| <i>June 2019</i> | 6.8 | 27.4 | 6.8 | -60% ± 5% | 32% ± 23% |
| <i>September 2019</i> | 7.1 | 25.3 | 5.7 | -49% ± 1% | 46% ± 40% |
| <i>November 2019</i> | 7.0 | 18.3 | 5.5 | -25% ± 13% | 46% ± 64% |
| <i>February 2020</i> | 6.8 | 17.2 | 4.7 | -26% ± 40% | 53% ± 66% |

Results showed that the decrease in HHCB concentration was much more pronounced than the formation of its metabolite and so adsorption might be the main process driving the fate of HHCB while only a minor part depends on its biodegradation into HHCB-lactone. Mean differences in concentrations of HHCB and HHCB-lactone between station 2 and station 3 in each sampling campaigns were plotted together with the temperature of the biological tank in order to highlight a possible correlation between PMF concentrations and tank temperature: results showed that removal of HHCB increased with temperature ($R^2 = 0.992$). A probable explanation may be found in the rising role of the activated sludge adsorption process. Although adsorption itself is an exothermic process, the biological activity of microorganism in the sludge can increase from 15 to 35 °C, which is considered the best temperature range for the biological treatment of wastewaters. Therefore, the enhanced sorption capacities at higher temperatures might be related to the higher biological activity of the sludge. The same trend was observed even in batch experiments for the evaluation of per- and polyfluoroalkyl substances (PFOS and PFOA) removal by activated sludge in which a higher efficiency was observed at 25 °C rather than at 15 °C (Zhou et al. 2010). The influence of temperature was evaluated even regarding HHCB-lactone and mean % of HHCB-lactone formation in each sampling campaign were plotted with the temperature registered in the biological tank but, in this case, the correlation resulted to be not significant ($R^2 = 0.675$). Therefore, a possible influence of temperature on the formation of HHCB-lactone cannot be confirmed. On the other hand, a strong positive correlation was found by plotting Total Suspended Solids (TSS) registered in the biological tank with mean % of HHCB removal ($R^2=0.75$) and of HHCB-lactone formation ($R^2=0.97$) thus confirming adsorption as the main mechanism driving the fate of the two compounds in the biological tank. However, these preliminary correlations must be confirmed by further data.

4.3.3 PMFs concentrations in activated sludge

An instantaneous sample of activated sludge was taken in each campaign and PMF concentrations were measured. (Fig IV-4a).

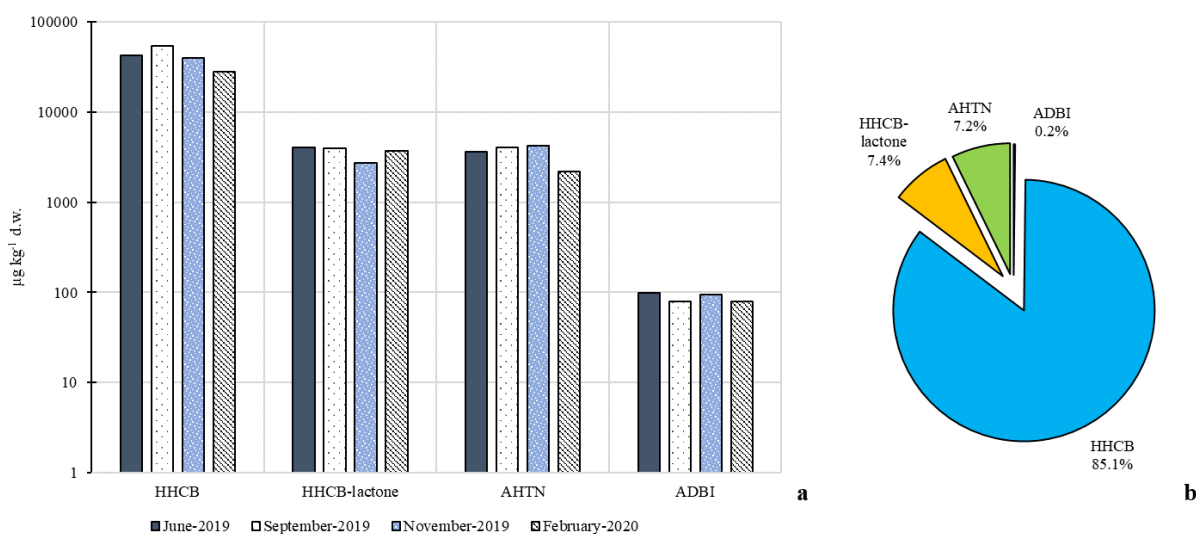


Fig. IV-4 PMF concentrations ($\mu\text{g kg}^{-1}$ d.w.) detected in activated sludge samples in each campaign (a). y-axis is in logarithmic scale. Mean PMF % composition of the activated sludge (b).

Concentrations of PMFs in sludges remained constants during the year. HHCB was the main PMF detected in all samples, with concentrations always above 10 mg kg^{-1} while HHCB-lactone and AHTN were one order of magnitude lower and ADBI was measured only at trace levels. AHTN was never detected even in this matrix thus revealing that this compound was not present in the studied WWTP. Given the similar values of each PMF detected in the activated sludge of each campaign, a mean sample composition can be calculated (Fig. IV-4b). HHCB represent the 86% of the total amount in sludge sample while HHCB-lactone and AHTN are only the 7% of the total PMFs. As in the water phase, this difference in concentration might be explained by the presence of these compounds in house-cleaning and personal care products. For example, a study conducted in Switzerland on fragrances in washing agents, which account for about one third of the total amount of products containing these compounds (Wiegel et al., 2000), showed concentrations of HHCB to be three times higher than those of AHTN (Biniossek 2000). Yang & Metcalfe, (2006) measured concentration of PMFs in return activated sludge of Peterborough WWTP one order of magnitude lower than our study while similar concentrations were measured in China (HHCB: $5.4\text{--}21 \text{ mg kg}^{-1}$ d.w.; AHTN: $0.72\text{--}6.2 \text{ mg kg}^{-1}$ d.w.) (Zeng et al. 2005), in Kentucky and Georgia (HHCB: $0.02\text{--}36 \text{ mg kg}^{-1}$ d.w.; AHTN: $0.02\text{--}7.2 \text{ mg kg}^{-1}$ d.w.) (Horii et al. 2007) and even in Switzerland (HHCB: $7.4\text{--}36 \text{ mg kg}^{-1}$ d.w.; AHTN: $2.5\text{--}11 \text{ mg kg}^{-1}$ d.w.) (Kupper et al. 2004). From these evidences, it can be concluded that PMFs accumulate in large quantities already in activated sludges which, if not properly treated and disposed, could be an additional source of PMF release to the terrestrial environment through its subsequent application to farmlands for soil amendment (DiFrancesco et al. 2004).

4.3.4 Ratios of HHCB and AHTN

Concentration ratios of HHCB and AHTN in different aquatic compartments have been used in the past as an effective tool to characterize differences in their partitioning (Dsikowitzky et al. 2002; Yang and Metcalfe 2006). In this study a significant positive correlation ($R^2= 0.72$) was observed between concentrations of HHCB and AHTN in wastewaters samples indicating similar behaviour during the all treatment process. This was also confirmed by analyzing HHCB/AHTN ratio in wastewater entering the biological tank and the same ratio in activated sludge samples. Results showed no significant difference between HHCB/AHTN ratio in wastewater (13.66 ± 2.18) and in activated sludge (11.55 ± 2.00). As in Yang & Metcalfe (2006), this indicates that HHCB and AHTN have similar partition coefficient between aqueous and solid matrices, as expected from their similar KOW values, 5.9 for HHCB and 5.7 for AHTN (HERA 2004).

4.3.5 Seasonality of PMF concentrations in wastewaters

Seasonal variations of PMFs in the input of the WWTP were evaluated considering measured concentrations in station 1 of the WWTP (Fig IV-5).

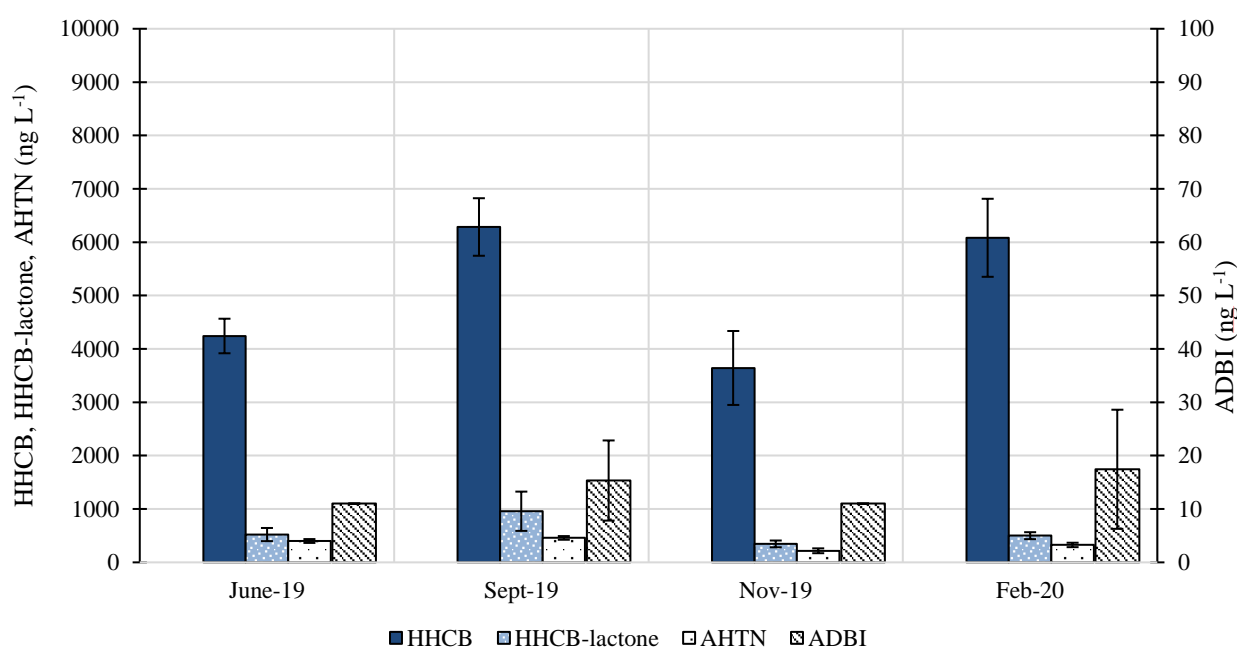


Fig. IV-5 Seasonal variation in total concentrations of PMFs detected in the inlet of the WWTP. HHCB, HHCB-lactone and AHTN are referred to the left y-axes while ADBI on the right y-axes.

HHCB was the compound always detected at higher concentration, always above 3000 ng L⁻¹. Being more than the 80% of all the fragrances contained in each sample, the total concentration of the fragrances detected in wastewater samples is closely linked to the presence of this compound. Because PMFs are present in products such as detergents, house cleaning and personal care products (Heberer 2003), their origin is identified in domestic discharges that are usually characterized by a higher stability respect to industrial wastewaters. For this reason, significant variations during the year are not expected unless of exceptional events as sewer overflows during heavy rain events or reduction of population in summer. To confirm this, water flows and weather conditions were registered in each campaign (Table IV-4) and

correlations were calculated between PMF concentrations and respective water fluxes (Fig. IV-6). Results showed that the increase of flow rate decreased PMF concentrations thus meaning that rain events recorded in proximity of sampling campaigns might had a dilution effect on wastewaters.

Table IV-4 Mean water flows and weather conditions registered during sampling campaigns.

| Sampling campaign | Mean water flow ($m^3 d^{-1}$) | Weather conditions | |
|-------------------|-------------------------------------|-----------------------------------|------------------|
| | | Week before sampling | Sampling week |
| June 2019 | 14282 | Heavy rain on Saturday | Sunny |
| September 2019 | 10206 | Scattered rains and thunderstorms | Sunny |
| November 2019 | 19226 | Frequent heavy rains | Cloudy and rainy |
| February 2020 | 14498 | Sunny | Sunny |

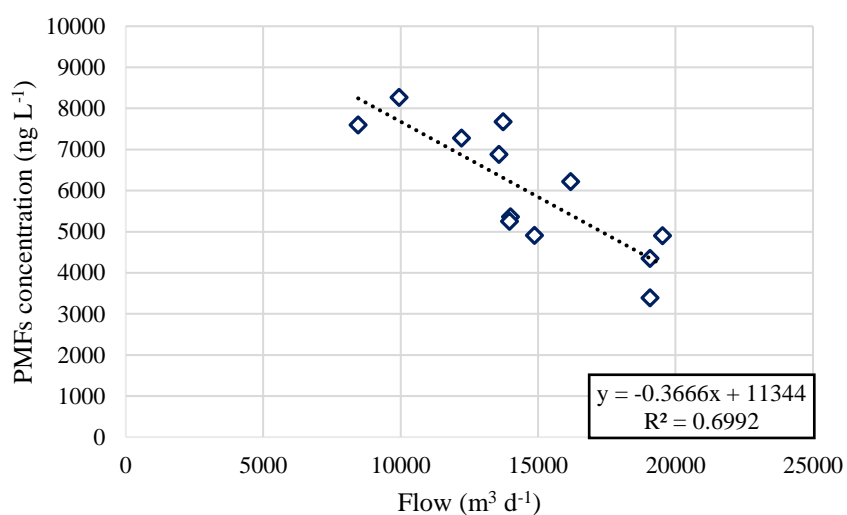


Fig. IV-6 Relation between PMF concentration and water flow in WWTP influent.

4.3.6 WWTP removal efficiency

In order to assess the removal efficiency of the WWTP regarding the three main PMFs measured, variations between water samples of the inlet and the outlet of the WWTP were calculated for each season (Fig. IV-7).

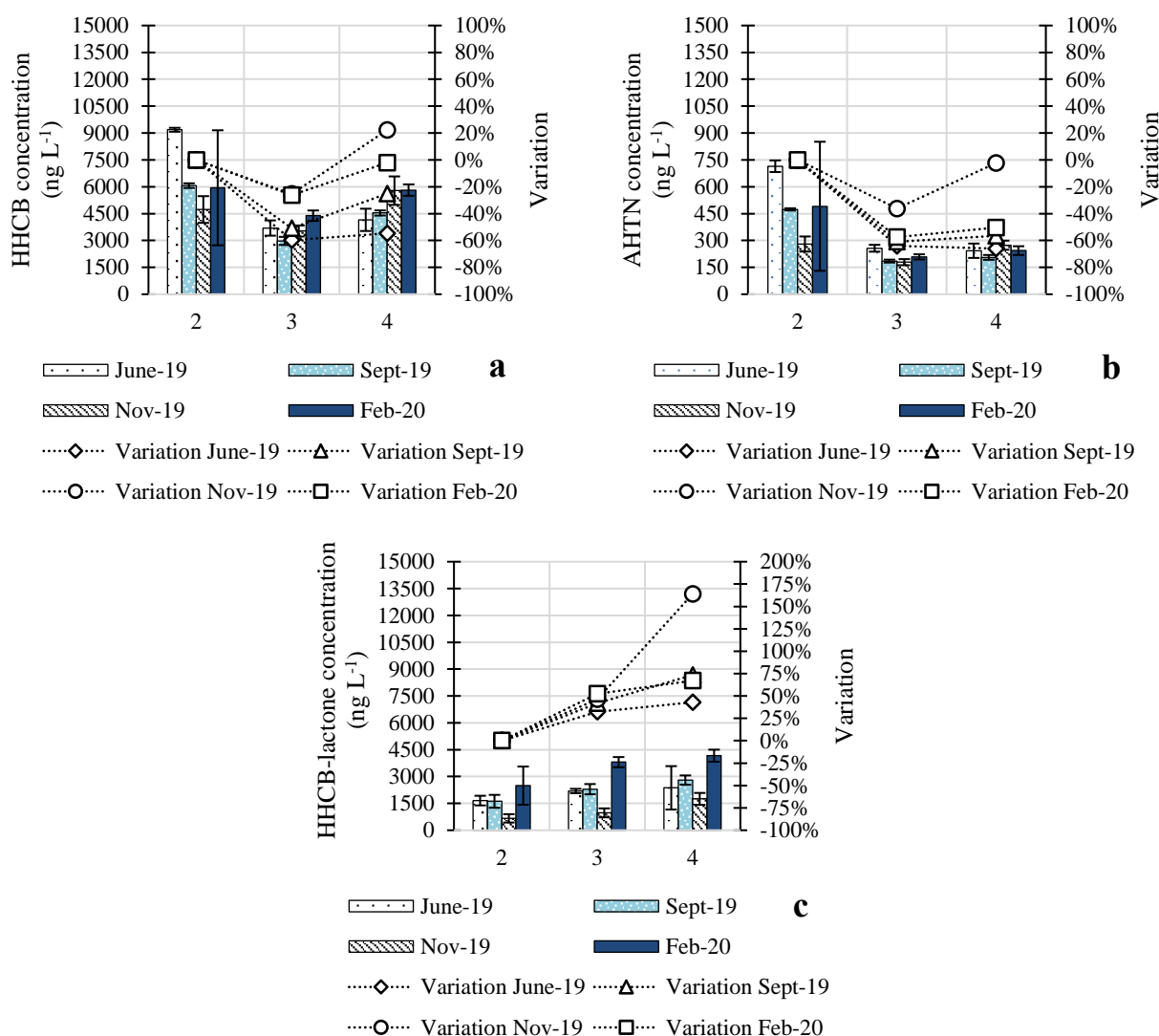


Fig. IV-7 Seasonal variations of HHCB (a), AHTN (b) and HHCB-lactone (c) during treatments. Bars represent mean seasonal concentrations (left y-axes) registered in stations 2-4 (x-axes) while lines represent % of variation during treatments respect to WWTP inlet (right y-axes): positive values indicate formation while negative values indicate removal.

Station 2 was set as the inlet of the WWTP in order to consider also sewage trucks contribution. HHCB (Fig. IV-7a) and AHTN (Fig. IV-7b) showed the same behaviour in the WWTP as expected from their similar physio-chemical properties; the best removal rates were registered in June 2019 while in November 2019 there was the worst removal of both compounds. In this campaign an increase of 20% in HHCB concentration was registered by comparing the inlet to the outlet of the WWTP probably due to a dilution effect of the wastewater entering the WWTP together with a release of compounds previously adsorbed on the activated sludge; this matrix may have accumulated PMFs from the wastewater that reached the WWTP before the rain event and released them later thus causing higher concentrations at the WWTP outlet respect to the inlet. The release from the activated sludge is supported event by data of HHCB-lactone (Fig. IV-7c).

Positive values were always registered for this compound, indicating that HHCB-lactone concentration increased from the inlet to the outlet of the WWTP, in all the sampling campaigns. Moreover, the highest formation of this compound was registered in November 2019 and this suggest a possible release of the metabolite from the activated sludge. However, to better estimate the efficiency of the WWTP in removing PMFs from wastewaters, the overall removal rates were estimated considering the whole year (Fig. IV-8).

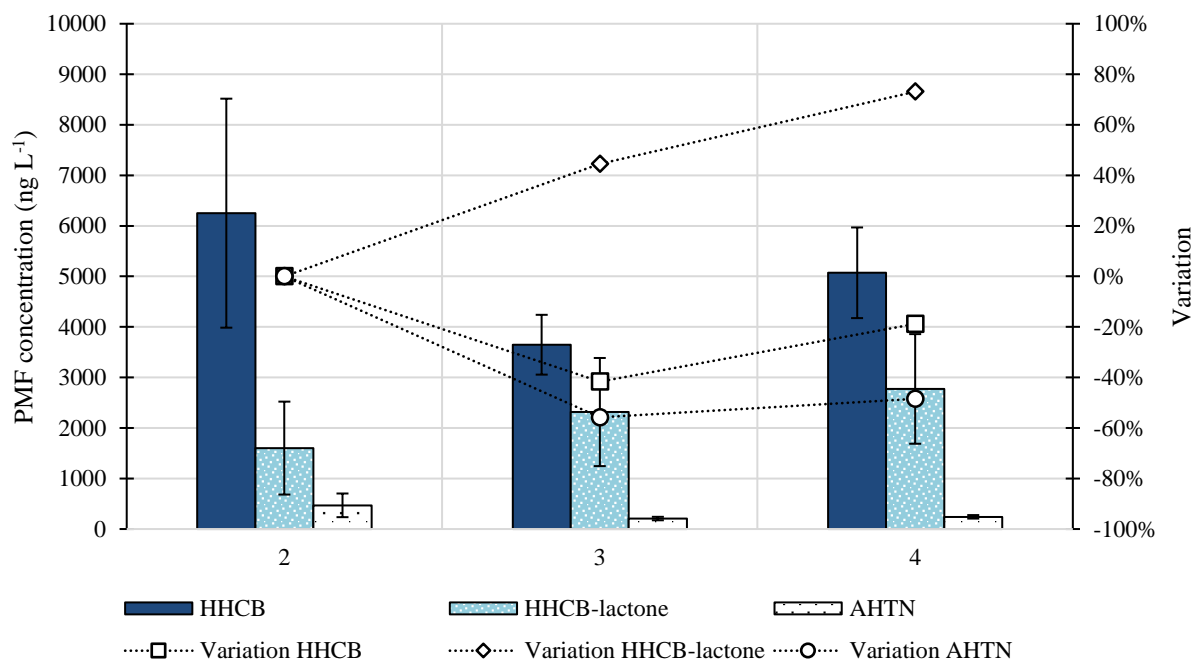


Fig. IV-8 Annual variation of HHCB, AHTN and HHCB-lactone during treatments. Bars represent mean seasonal concentrations (left y-axis) registered in stations 2-4 (x-axis) while lines represent % of variation during treatments respect to WWTP inlet (right y-axis): positive values indicate formation while negative values indicate removal.

Results showed a mean removal of 50% for AHTN and of 20% for HHCB. AHTN was better removed from wastewaters rather than HHCB probably because of the lower concentrations registered in all wastewater's samples, one order of magnitude lower than HHCB. HHCB-lactone showed an increase up to 70% of the mean value registered in station 2. These data confirmed our previously published results (Tasselli and Guzzella 2020) which evidenced in the same WWTP a removal of only AHTN (30%) but not of HHCB. However, it should be underlined that previous published data referred only to a single campaign conducted over three consecutive days, so only the same removal pattern of the various compounds can be compared. Other authors registered comparable removals of PMFs in WWTPs: for example Arrubla et al. (2016) registered in a CAS WWTP located in Colombia removal rates below 50% for HHCB and below 15% for AHTN, meanwhile Luo et al., (2014) reported removals of HHCB and AHTN between 15% and 30%. In contrast, higher removal rates of HHCB and AHTN ranging from 72% to 98% were detected in WWTPs located in Kentucky and Georgia (Horii et al. 2007) and values above 50% until 95% regarding HHCB and AHTN removal were registered even in Austria (Clara et al. 2011). However, Clara et al., (2011) highlighted that concerning PMF removal efficiency, literature values vary within a very wide range, from 10% to 99%. In fact, many factors can influence the removal of micropollutants in a WWTP as plant configuration and operating conditions of the WWTP itself, the chemical characteristics of wastewater entering the plant and

the concentration of micropollutants at the inlet of the WWTP that depends on the site specific PMF use. For this reason, it is very difficult to compare WWTP of different areas and even of different countries and, consequently, in-depth studies on the individual WWTP referring to their specific conditions are to be preferred.

4.4 Conclusions

The first one-year monitoring study was carried out in Northern Italy to analyze the fate of polycyclic musk fragrances in water and activated sludges during treatments applied in an Italian conventional WWTP with CAS system. Four different stations along wastewaters treatments were considered in order to better understand the behaviour of PMFs inside a conventional WWTP. Results showed higher PMF concentrations respect to other countries, as expected from the widest use of detergent registered in Italy. While AHDI was never detected and ADBI was measured only at trace levels, HHCB and HHCB-lactone were found in concentrations of $\mu\text{g L}^{-1}$, one order of magnitude greater than AHTN. HHCB and AHTN evidenced a slight reduction in water concentrations during wastewater treatments. This was mainly due to adsorption processes on activated sludge during the biological treatment, confirmed also by high PMF concentrations in the activated sludge, but also to alterations of the chemical equilibrium between dissolved organic carbon content and PMF concentrations in waters caused by the UV final disinfection. An opposite trend was measured for HHCB-lactone which registered an increase up to 70% in respect to concentrations caused by the biotransformation of HHCB during the biological treatment. Only slightly differences were recorded between seasons in terms of PMF input the WWTP, in accordance with the constant use of these chemicals. Overall, this study showed that current technologies are not enough efficient to remove from wastewaters these organic micropollutants, which are released from the WWTP effluents thus representing a potential risk primarily for aquatic ecosystems. Additional treatments are therefore necessary to enhance the removal of PMFs in order to increase the quality of the WWTP effluents and their possible reuse in industrial and agricultural fields.

References

- Arrubla JP, Cubillos JA, Ramírez CA, et al (2016) Pharmaceutical and personal care products in domestic wastewater and their removal in anaerobic treatment systems: Septic tank – Up flow anaerobic filter. *Ing e Investig* 36:70–78. <https://doi.org/10.15446/ing.investig.v36n1.53076>
- Ávila C, García J (2015) Pharmaceuticals and Personal Care Products (PPCPs) in the Environment and Their Removal from Wastewater through Constructed Wetlands. *Compr Anal Chem* 67:195–244. <https://doi.org/10.1016/B978-0-444-63299-9.00006-5>
- Besha AT, Gebreyohannes AY, Tufa RA, et al (2017) Removal of emerging micropollutants by activated sludge process and membrane bioreactors and the effects of micropollutants on membrane fouling: A review. *J Environ Chem Eng* 5:2395–2414. <https://doi.org/10.1016/j.jece.2017.04.027>
- Bester K (2004) Retention characteristics and balance assessment for two polycyclic musk fragrances (HHCB and AHTN) in a typical German sewage treatment plant. *Chemosphere* 57:863–870. <https://doi.org/10.1016/j.chemosphere.2004.08.032>
- Binossek M (2000) Identifizierung und Quantifizierung von Riechstoffen und anderen flüchtigen Verbindungen in Waschmitteln mittels simultaner Destillations—Extraktion und Gaschromatographie/Massenspektrometrie.

- Blahova J, Divisova L, Plhalova L, et al (2018) Multibiomarker Responses of Juvenile Stages of Zebrafish (*Danio rerio*) to Subchronic Exposure to Polycyclic Musk Tonalide. *Arch Environ Contam Toxicol* 74:568–576. <https://doi.org/10.1007/s00244-017-0484-8>
- Chen F, Gao J, Zhou Q (2012) Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in *Carassius auratus* using oxidative stress biomarkers. *Environ Pollut* 162:91–97. <https://doi.org/10.1016/j.envpol.2011.10.016>
- Cirja M, Ivashechkin P, Schäffer A, Corvini PFX (2008) Factors affecting the removal of organic micropollutants from wastewater in conventional treatment plants (CTP) and membrane bioreactors (MBR). *Rev Environ Sci Bio/Technology* 7:61–78. <https://doi.org/10.1007/s11157-007-9121-8>
- Clara M, Gans O, Windhofer G, et al (2011) Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. *Chemosphere* 82:1116–1123. <https://doi.org/10.1016/j.chemosphere.2010.11.041>
- DiFrancesco AM, Chiu PC, Standley LJ, et al (2004) Dissipation of Fragrance Materials in Sludge-Amended Soils. *Environ Sci Technol* 38:194–201. <https://doi.org/10.1021/es034618v>
- Dsikowitzky L, Schwarzbauer J, Littke R (2002) Distribution of polycyclic musks in water and particulate matter of the Lippe River (Germany). *Org Geochem* 33:1747–1758. [https://doi.org/10.1016/S0146-6380\(02\)00115-8](https://doi.org/10.1016/S0146-6380(02)00115-8)
- ECHA – European Chemicals Agency (2010) Candidate List of Substances of Very High Concern for Authorisation. EU-RAR (2008) 1-(5,6,7,8-Tetrahydro-3,5,5,6,8,8-Hexamethyl-2-Naphthyl)Ethan-1-One (Ahtn). Rep AHTN 259. <https://doi.org/10.2788/40195>
- European Commission (2003) European Union Risk Assessment Report, Naphthalene. EUR 20763 EN, 1st Prior List
- Fernandez-Fontaina E, Pinho I, Carballa M, et al (2013) Biodegradation kinetic constants and sorption coefficients of micropollutants in membrane bioreactors. *Biodegradation* 24:165–177. <https://doi.org/10.1007/s10532-012-9568-3>
- Heberer T (2003) Occurrence, fate, and assessment of polycyclic musk residues in the aquatic environment of urban areas - A review. *Acta Hydrochim Hydrobiol* 30:227–243. <https://doi.org/10.1002/aheh.200390005>
- HERA (2004) HERA Environmental Risk Assessment Polycyclic musks AHTN and HHCB. 1–80
- Horii Y, Reiner JL, Loganathan BG, et al (2007) Occurrence and fate of polycyclic musks in wastewater treatment plants in Kentucky and Georgia, USA. *Chemosphere* 68:2011–2020. <https://doi.org/10.1016/j.chemosphere.2007.04.054>
- Hu Z, Shi Y, Cai Y (2011) Concentrations, distribution, and bioaccumulation of synthetic musks in the Haihe River of China. *Chemosphere* 84:1630–1635. <https://doi.org/10.1016/j.chemosphere.2011.05.013>
- Hu Z, Shi Y, Niu H, et al (2010) Occurrence of synthetic musk fragrances in human blood from 11 cities in China. *Environ Toxicol Chem* 29:1877–1882. <https://doi.org/10.1002/etc.258>
- Kupper T, Berset JD, Etter-Holzer R, et al (2004) Concentrations and specific loads of polycyclic musks in sewage sludge originating from a monitoring network in Switzerland. *Chemosphere* 54:1111–1120. <https://doi.org/10.1016/j.chemosphere.2003.09.023>
- Lange C, Kuch B, Metzger JW (2015) Occurrence and fate of synthetic musk fragrances in a small German river. *J Hazard Mater* 282:34–40. <https://doi.org/10.1016/j.jhazmat.2014.06.027>
- Lou Y hua, Wang J, Wang L, et al (2016) Determination of Synthetic Musks in Sediments of Yellow River Delta Wetland, China. *Bull Environ Contam Toxicol* 97:78–83. <https://doi.org/10.1007/s00128-016-1814-7>
- Luo Y, Guo W, Ngo HH, et al (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci Total Environ* 473–474:619–641. <https://doi.org/10.1016/j.scitotenv.2013.12.065>
- Mersch-Sundermann V, Schneider H, Freywald C, et al (2001) Musk ketone enhances benzo(a)pyrene induced

- mutagenicity in human derived Hep G2 cells. *Mutat Res - Genet Toxicol Environ Mutagen* 495:89–96. [https://doi.org/10.1016/S1383-5718\(01\)00202-9](https://doi.org/10.1016/S1383-5718(01)00202-9)
- O.S.P.A.R. Commission (2004) Hazardous substances series, musk xylene and other musks. London, UK
- Parolini M, Magni S, Traversi I, et al (2015) Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in *Dreissena polymorpha*. *J Hazard Mater* 285:1–10. <https://doi.org/10.1016/j.jhazmat.2014.11.037>
- Posada-Ureta O, Olivares M, Navarro P, et al (2012) Membrane assisted solvent extraction coupled to large volume injection-gas chromatography-mass spectrometry for trace analysis of synthetic musks in environmental water samples. *J Chromatogr A* 1227:38–47. <https://doi.org/10.1016/j.chroma.2011.12.104>
- Reiner JL, Berset JD, Kannan K (2007) Mass flow of polycyclic musks in two wastewater treatment plants. *Arch Environ Contam Toxicol* 52:451–457. <https://doi.org/10.1007/s00244-006-0203-3>
- Sapozhnikova Y, Liebert D, Wirth E, Fulton M (2010) Polycyclic musk fragrances in sediments and shrimp tissues. *Polycycl Aromat Compd* 30:298–308. <https://doi.org/10.1080/10406638.2010.525160>
- Shrivastava A, Gupta V (2011) Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles Young Sci* 2:21. <https://doi.org/10.4103/2229-5186.79345>
- Tasselli S, Guzzella L (2020) Polycyclic musk fragrances (PMFs) in wastewater and activated sludge: analytical protocol and application to a real case study. *Environ Sci Pollut Res*. <https://doi.org/10.1007/s11356-019-06767-7>
- Ternes TA, Herrmann N, Bonerz M, et al (2004) A rapid method to measure the solid–water distribution coefficient (K_d) for pharmaceuticals and musk fragrances in sewage sludge. *Water Res* 38:4075–4084. <https://doi.org/10.1016/j.watres.2004.07.015>
- Villa S, Assi L, Ippolito A, et al (2012) First evidences of the occurrence of polycyclic synthetic musk fragrances in surface water systems in Italy: Spatial and temporal trends in the Molgora River (Lombardia Region, Northern Italy). *Sci Total Environ* 416:137–141. <https://doi.org/10.1016/j.scitotenv.2011.11.027>
- Weinberg I, Dreyer A, Ebinghaus R (2011) Landfills as sources of polyfluorinated compounds, polybrominated diphenyl ethers and musk fragrances to ambient air. *Atmos Environ* 45:935–941. <https://doi.org/10.1016/j.atmosenv.2010.11.011>
- Wiegel, S., Harms, H., Stachel B (2000) Synthetische Moschus-Duftstoffe in der Elbe. Arbeitsgemeinschaft für die Reinhaltung der Elbe
- Yang JJ, Metcalfe CD (2006) Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. *Sci Total Environ* 363:149–165. <https://doi.org/10.1016/j.scitotenv.2005.06.022>
- Zeng X, Sheng G, Xiong Y, Fu J (2005) Determination of polycyclic musks in sewage sludge from Guangdong, China using GC–EI-MS. *Chemosphere* 60:817–823. <https://doi.org/10.1016/j.chemosphere.2005.04.026>
- Zhou Q, Deng S, Zhang Q, et al (2010) Sorption of perfluorooctane sulfonate and perfluorooctanoate on activated sludge. *Chemosphere* 81:453–458. <https://doi.org/10.1016/j.chemosphere.2010.08.009>

CHAPTER V

Preliminary results regarding the application of ozonation and adsorption on activated carbon in a wastewater treatment plant: new promising advanced technologies for Polycyclic Musk Fragrances (PMFs) removal

Stefano Tasselli^{1-2,*}, Erica Valenti¹ and Licia Guzzella¹

¹ National Research Council - Water Research Institute (CNR-IRSA), S.S. Brugherio via del Mulino 19, Brugherio (MB), 20861, Italy

² Department of Environmental Sciences, University of Milano Bicocca, Piazza della Scienza 1, 20126 Milano, Italy

Abstract

Two different advanced wastewater treatments, ozonation and adsorption on powdered activated carbon, were tested on pilot plant scale in two wastewater treatment plants (WWTPs) located in Northern Italy in order to remove polycyclic musks fragrances (PMFs) from the effluent of the biological treatment. Manipulating the principal operating parameters of the two pilot plants, different conditions were tested in order to achieve the best compromise between removal efficiencies and management costs. In the ozonation pilot plant ozone was dosed from 1.20 to 3.73 mg L⁻¹, corresponding to a typical medium-low dosage. The plant structure, consisting of two columns in series that blow the gas directly into the wastewater, allows to simultaneously test different experimental conditions as well as the possibility of lengthening the contact times without operating on additional ozone dosages. Using this technique, Galaxolide (HHCB) was efficiently removed (80%) even with low O₃ dosages applied in the first column while Tonalide (AHTN) and Galaxolidone (HHCB-lactone) were less reactive to oxidation. The latter compound registered insufficient removals of 60% even with the highest O₃ dosage and hydraulic retention time. Considering typical applications in WWTPs, higher O₃ dosages can be tested in order to increase removal values. Adsorption on powdered activated carbon (PAC) was tested on a pilot plant consisting on a series of tanks in which PAC is added to the wastewater and can be recirculated. Operating on water flow, quantity of virgin PAC added and concentration of PAC inside the plant, satisfactory removals were achieved for all the studied compounds even at the lowest concentrations of PAC. By comparing the efficiency of two different PACs, the importance of associating the choice of the PAC type with the class of compound to be removed was highlighted, together with the correct setting of the pilot plant main operational parameters. In this case, lower dosages of carbon can be further tested in order to obtain high removals together with lower management costs.

Funding

This work was financially supported by PerFORMWATER 2030 project ID 240750 (POR FESR 2014-2020 Asse I -Accordi per la Ricerca e l'Innovazione).

Acknowledgements

We like to thank Società Italiana Acetilene E Derivati (SIAD) and Veolia Water Technologies, partners of the project, for their technical support during sampling activities and article drafting.

5.1 Introduction

Synthetic musks are chemicals widely used as low-cost additives in many commercial products for house cleanings and personal care (soaps, detergents, air-fresheners, perfumes, body lotions and cosmetics). They are artificial compounds, commercially appreciated for their pleasant perfume and their fixative properties (Marchal and Beltran, 2016). Based on the chemical structure, they can be grouped in 4 classes: nitro, polycyclic, macrocyclic and alicyclic musks. Polycyclic musks currently dominate the fragrances global market, although their use in cosmetics is under discussion (Homem et al., 2015) since they have been recognized as persistent, bioaccumulative and potentially toxic to organisms (Fromme et al., 2001; Parolini et al., 2015; Schreurs et al., 2004). Among polycyclic musks fragrances (PMFs), Galaxolide (HHCB) and Tonalide (AHTN) are employed in larger volumes, while other PMFs, such as Celestolide (ADBI) and Phantolide (AHDI), are used to a lesser extent (O.S.P.A.R. Commission, 2004). Synthetic fragrances are employed in small % w/w in the end products (HERA, 2004; Reiner and Kannan, 2006) but a daily and worldwide use leads to a constant release of these chemicals into the environment. For this reason, in addition to their chemical-physical properties, synthetic musks have been recognized as micropollutants. In fact, considering structural features, all PMFs are highly methylated tetraline or indane derivatives: methyl groups are scarcely polar, and this increases PMF affinity for organic matter. Measured log K_{ow} for PMFs range from 5.4 to 5.9, values with the same magnitude order of organochlorine pesticides and PCBs (Fromme et al., 2001).

Considering the usage of products in which these chemicals are incorporated (showering, bathing, household cleaning), their diffusion into environment mainly occurs via the discharge in the sewer systems. Domestic wastewater treatment plants (WWTPs) have been identified as the main sources for synthetic musks, since conventional treatment systems, based on biological processes, only partially remove these chemicals from sewage (Heberer, 2003). PMFs are poorly biodegradable and adsorption on sludge seems to be the preferred route for their removal (Bester, 2004; Reiner et al., 2007; Clara et al., 2011;) in accordance with the hydrophobic nature of these chemicals.

Removal rates in WWTPs are very different, depending on the studied plant: in literature they can range from 10 to 99% (Clara et al., 2011). Variability might be related to differences in the plant design and in the per capita load of musks (Simonich et al., 2002), as well as to differences in organic carbon content of the sewage or in hydraulic retention times of the WWTPs (Artola-Garicano et al., 2003). Therefore, PMFs can be detected up to the $\mu\text{g L}^{-1}$ order even in treated effluents (Homem et al., 2015). Our group also measured mean concentrations of 7000, 300 and 32 ng L^{-1} for HHCB, AHTN and ADBI respectively, in the treated effluent of a WWTP located in Northern Italy (Tasselli and Guzzella, 2020). In addition to parental compounds, Galaxolidone (HHCB-lactone), the main oxidation product of HHCB, was also detected in the effluents of the WWTPs (Bester, 2004; Reiner et al., 2007) but also in the influents since it is present even in the technical Galaxolide product (Bester, 2004). Unlike others PMFs, HHCB-lactone concentration in water

has been shown to increase from the inlet to the outlet of WWTPs mainly due to the biodegradation of HHCB during the biological treatment (Reiner et al., 2007; Tasselli and Guzzella, 2020).

From WWTPs, synthetic musks are then discharged into receiving water bodies and frequently detected in surface water systems, both in the dissolved phase and adsorbed on suspended solids and sediments (Bester, 2005; Fromme et al., 2001; Villa et al., 2012; Winkler et al., 1998). Besides, the application of WWTP biosolids in agricultural fields represents a further contamination pathway for the terrestrial ecosystem (Yang and Metcalfe, 2006). As semi-volatile compounds, PMFs can also be detected in the atmosphere, partitioned nearly exclusively into the gas-phase (Upadhyay et al., 2011; Weinberg et al., 2011). WWTPs contribute to atmospheric pollution mainly with stripping that takes place in the oxidation tanks by bubbling air into the wastewater (Upadhyay et al., 2011). HHCB and AHTN have thus been found both in urban and in pristine areas as the Arctic Sea, with gas-phase concentrations in the order of 1000-5000 pg (m³)⁻¹ and of 4-18 pg (m³)⁻¹ respectively (Peck and Hornbuckle, 2006; Xie et al., 2007). Considering their potential bioaccumulation and related ecotoxicological risks (Tumová et al., 2019), the occurrence of musk fragrances in all environmental compartments represents a reason for concern.

In order to increase micropollutant removal from wastewaters, researches on different advanced wastewater treatments are being carried on. Two major removal processes are being tested worldwide in bench, pilot and full scale: ozonation and adsorption onto activated carbon. The efficiency of ozonation regarding micropollutant removal is based on two different reactive processes: direct oxidation with the high selective ozone with many organic compounds having π bonds or aromatic rings as PMFs by means of electrophilic addition or aromatic electrophilic substitution reactions and secondary reactions with less selective hydroxyl radicals simultaneously produced during this treatment (Dodd et al., 2009). Minimal differences in micropollutants chemical structures can lead to a different reactivity towards ozone or hydroxyl radical and different reaction rates. However, oxidation with ozone can generate intermediate products even more toxic than the parental compounds (Molinos-Senante et al., 2013). The implementation of technologies based on adsorption processes could be an alternative to ozonation in removing even the most stable compounds, since activated carbon allows removal of a wide spectrum of persistent micropollutants via adsorption to its high specific surface area (Margot et al., 2013). Activated carbon has been applied in numerous advanced wastewater treatment steps (Boehler et al., 2012; Margot et al., 2013) but it is preferably located after the secondary wastewater treatment which removes the majority of Dissolved Organic Carbon (DOC) which is in competition with micropollutants for the adsorption sites on the activated carbon (Hu et al., 2016). Granular activated carbon (GAC) was employed in drinking water purification (Babi et al., 2007) and during the treatment of secondary effluents of different quality in WWTPs (Paredes et al., 2018) given its simple application and the possibility of its regeneration and reuse. Alternatively, powdered activated carbon (PAC) can be applied as a tertiary treatment step or dosed directly into the biological treatment of the WWTP (Boehler et al., 2012). Due to its smaller particle size, PAC is superior in regard to adsorption kinetics and might be more efficient if compared to GAC (Nowotny et al., 2007). PAC is suitable to adsorb a wide range of micropollutants, but it works properly with hydrophobic compounds and its efficiency has been related to the specific K_{ow} of the studied micropollutant. Since their hydrophobic nature and values of $\log K_{ow} > 4$, PMFs could have a high adsorption potential on activated carbon (Rogers, 1996). PAC is composed of

carbon particles activated by a specific and expensive process, giving it a high surface area available per gram of material, thus allowing the removal of important concentrations of undesirable compounds even with a small amount of PAC. Usually, PAC is simply injected in the raw water to remove contaminants with short contact times and, for this reason, the adsorption capacity of the carbon is not fully used before its disposal. However, activated carbon recirculation even in the oxidation tank may be useful. Boehler et al. (2012), for example, have tested on pilot and full scale the removal efficiency of various micropollutants from secondary effluent by applying PAC with recirculation showing an increase in micropollutants removal by 10-50% respect to the application of PAC without recirculation. Contact time between PAC and wastewater represent another important control parameter of this treatment and must be adjusted in order to ensure the adsorption of contaminants and avoid their desorption at the same time.

Compared to other emerging contaminants, such as pharmaceuticals or pesticides, few data are currently present in literature regarding the behaviour of PMFs during ozonation processes and their removal efficiency from wastewaters with activated carbon. The aim of our study was therefore to evaluate and compare PMFs removal rates at pilot-scale by two different types of tertiary treatment technologies, ozone and powdered activated carbon, which were installed in two different WWTPs located in Northern Italy in the metropolitan area of Milan. For both technologies, different operating conditions were tested in order to achieve the best environmental and economical efficiencies.

5.2 Materials and methods

5.2.1 Wastewater treatment plants (WWTPs)

Two municipal WWTP (MS1 and MS2) in Lombardy collecting urban wastewaters near the metropolitan area of Milan (Northern Italy) were selected for the installation of the two different additional pilot-plant tertiary treatments. The two WWTPs differ for dimension and average quantity of daily water treated. MS1 has a people equivalent (PE) of 17,000 individuals and a water influent of 11000 m³ d⁻¹ while MS2 has a capacity of circa 120,000 PE and treats on average 27000 m³ d⁻¹. Both plants have conventional activated sludge as secondary treatment system with simultaneous oxidation/nitrification. Regarding water line, after primary physical treatments of grilling, sand and oil removal, water goes into the primary sedimentation tank and then in the denitrification/nitrification tanks. Secondary sedimentation, filtration and final disinfection (UV at MS1 and peracetic acid at MS2) are employed.

5.2.2 Ozonation pilot plant

The ozonation pilot plant was installed at the MS1 WWTP (Fig. V-1).

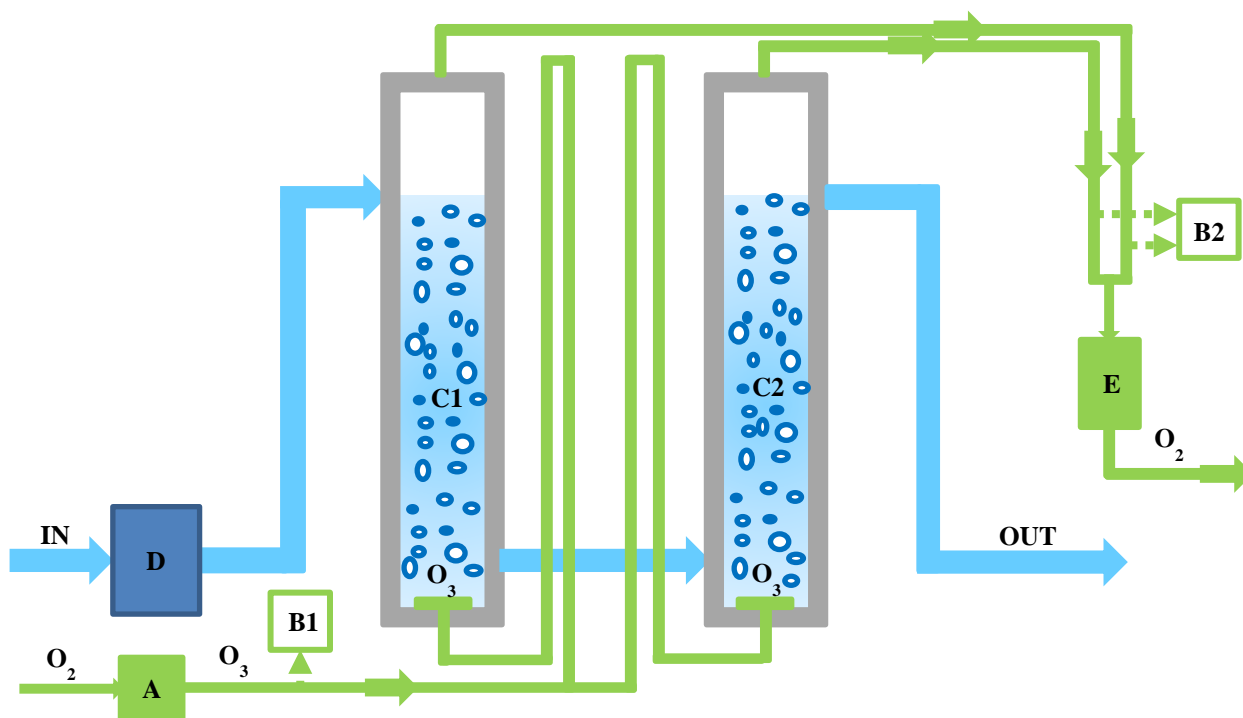


Fig. V-1 Scheme of the ozonation pilot plant. A= ozone generator; B1 and B2= ozone analysers; C1 and C2= reaction columns; D= filter for suspended solids; E= thermocatalytic destroyer.

The effluent of the WWTP filtration tank feed the ozonation pilot plant at a maximum flowrate of $2 \text{ m}^3 \text{ h}^{-1}$. The pilot plant consisted of two double stainless still columns (C1 and C2) with a useful volume of 0.65 m^3 , equipped with porous ceramic diffusers for ozone supply. Regarding the hydrodynamic configuration, the first column has a counter-current gas-liquid flow while the second column worked with a co-current flow. Ozone-containing gas was produced by an ozone generator (A) (Wedeco GSO40) feed with pure oxygen. The ozone concentration in the feed and off gas was measured using BMT 964 C, BMT 964 OG ozone analysers (B1 and B2) and the destruction of the residual ozone was carried out using a thermocatalytic O_3 destroyer (E) (WEDECO CODw3). The tested operating conditions are reported in Table V-1.

Table V-1 Operating conditions of ozonation pilot plant tested in the experimental work.

| | $Q \text{ IN}^a$ $\text{m}^3 \text{ h}^{-1}$ | $\text{O}_3 \text{ IN}^b$ $\text{mg O}_3 \text{ L}^{-1}$ | HRT^c (1 ST column) min | HRT (total) min | O_3 consumed ^d (1 ST column) mg L^{-1} | O_3 consumed (total) mg L^{-1} | O_3 dosed ^e in water (1 ST column) (mg L^{-1}) min | O_3 dosed in water (total) (mg L^{-1}) min |
|---------|---|---|---|--------------------------------|---|---|---|---|
| Min | 1.30 | 2.85 | 9.75 | 19.50 | 1.20 | 2.22 | 12.67 | 46.74 |
| Max | 2.00 | 5.42 | 15.00 | 30.00 | 2.19 | 3.73 | 27.73 | 106.71 |
| Average | 1.63 | 4.09 | 12.24 | 24.47 | 1.69 | 3.06 | 20.76 | 75.42 |

^a = inlet wastewater flow, ^b = total ozone mass inlet, ^c = hydraulic retention time, ^d = net ozone consumed calculated as O_3 fed in the pilot subtracted the O_3 lost in the off-gas and the O_3 remained dissolved in water, ^e = total ozone dose consumed in water.

5.2.3 Powdered activated carbon pilot plant

At MS2 WWTP an Actiflo® Carb pilot plant has been installed for the removal of micropollutants, treating a fraction (through a submersible pump) of the wastewater downstream the activated sludge clarification tank. The Actiflo® technology (patented by Veolia) is a compact chemical-physical treatment of clarification, which takes place through the dosage of chemicals and microsands that enhances the process. The Actiflo® Carb evolution provide the adding to the process of Powdered Activated Carbon (PAC): a “fresh” virgin PAC is dosed upstream and, in the system, there is a high PAC concentration due to its recirculation that maximizes removal performances and reduces operational costs. In details the technology has the following plant configuration (Fig. V-2): an initial contact tank (1) where the water to be treated is put in contact with “fresh” (A) and recirculated (B) PAC, a coagulation tank (2), where a coagulant (C), in this case FeCl_3 , is dosed for the particles destabilization, an injection tank (3) where microsands (D) and a flocculant (E) (TILLFLOCK 6480, Tillmanns) are dosed in order to aggregate all the suspended solids and colloids, a maturation tank (4) in which flocs formation takes places and a lamella sedimentation tank (5) in which the sedimented material accumulates on the bottom while in the upper part there is the outlet for the clarified effluent. The microsands acts as a ballast, weighing down the particles and speeding up their sedimentation. The mixture of sludge in the bottom of clarifier is finally pumped into an hydrocyclone (6) that separates the microsands, recirculated in the injection tank form sludge and PAC; this stream is partially recirculated into the contact tank and partially purged from the plant. The pilot plant works with an inlet flow rate between 4 and 8.6 $\text{m}^3 \text{h}^{-1}$ while the concentration of microsands is stable around 10 g L^{-1} .

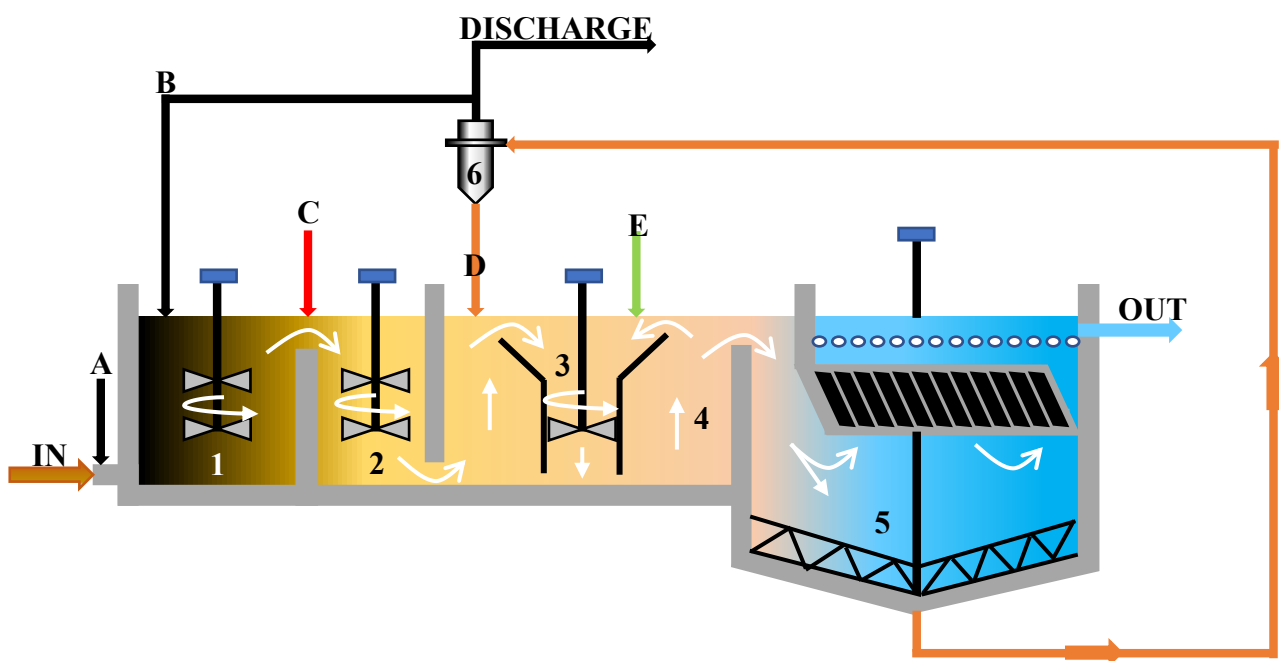


Fig. V-2 Scheme of Actiflo(R) Carb pilot plant. 1= contact tank; 2= coagulation tank; 3= injection tank; 4=maturation tank; 5= sedimentation tank; 6= hydrocyclone. A= fresh PAC; B= recirculated PAC; C= coagulant; D=microsands; E=flocculant.

Laboratory Jar tests were carried out in order to find optimal dosages of the polyelectrolyte (1 mg L^{-1}) and the ferric chloride (7 mg L^{-1}) which are dosed continuously by special dosing pumps which keep their

concentrations constant in the plant. In the present work, different setups were tested by varying the operating parameters of the pilot plant such as the flow rate, the quantity of fresh PAC and the concentration of PAC inside the pilot plant.

5.2.4 Powdered activated carbon choice

Before the application in the pilot plant, three types of PAC were tested in batch experiments in order to evaluate the variation in the micropollutant concentration in the water entering the MS2 pilot plant by varying the type of Activated Carbon and the relative contact time. Principal characteristics of tested PACs are summarized in Table V-2. Wastewater samples from the effluent of MS2 activated sludge clarification tank were kept in contact with a fixed PAC concentration of 1.5 g L⁻¹ using 5L beakers in a thermostatic tank for different times, from 30 min until 6 d. At fixed times, samples were filtered at 0.7 µm using glass fiber filters (VWR International, Radnor, PA, USA), and analyzed for PMF concentration.

Table V-2 Characterization of the selected PACs.

| <i>PAC</i> | <i>Iodine number mg/g</i> | <i>Total surface area m²/g</i> | <i>Apparent density kg/m³</i> | <i>Particle size D₅₀ µm</i> |
|---|-------------------------------|---|--|--|
| <i>Norit SAE SUPER (PAC1)</i> | 950 | 1050 | 375 | 20 |
| <i>AquaSorb AFP 25, PACS (PAC2)</i> | 1160 | 1150 | 390 | 16 |
| <i>EcoSorb BP2, PAC-S (PAC3)</i> | 897 | 987 | 560 | n.a. ^a |

^a= not available

5.2.5 Sampling campaigns at pilot plants

Concerning ozonation pilot plant, during the 4 sampling campaigns, pilot plant continuously operated for 3 days. Daily composite samples were collected at pilot inlet (outlet of WWTP filtration compartment) after the filtering unit, first column outlet and second column outlet. Composite sampling involved the collection of a sample every 20 minutes for 4 hours for each sampling point, starting from the achievement of the system operating balance. Collected samples were spiked with sodium thiosulfate to quench residual dissolved ozone, before PMF analysis.

Regarding PAC pilot plant, each analytical campaign involved the collection of daily composite samples at the pilot plant inlet (outlet of WWTP activated sludge clarification tank) and pilot plant outlet, for two consecutive days, representing two replicates of same operative conditions to be analyzed for PMF potential removal. The daily sample taken from the pilot plant was a mixture of 5 spot samples taken every 30 minutes; each outlet spot sample was taken 30 minutes later than the inlet considering the hydraulic retention time of the pilot plant.

5.2.6 Chemicals and reagents

High purity micropollutant standards, deuterated standard and reagents used for PMF analysis in water samples have been listed previously (Tasselli and Guzzella, 2020).

5.2.7 Selection of PMFs and analytical method

Table V-3 shows the micropollutants analyzed in this study and some of their properties.

Table V-3 Characteristics of polycyclic musk fragrances analyzed in this study. Values taken from Chase et al., (2012).

| Compound | Formula | Molecular weight (g mol⁻¹) | Water Solubility (mg L⁻¹) | Vapor pressure (Pa) | Henry's Law Constant (Pa m³ mol⁻¹) | Log Kow |
|-------------------------------------|--|--|---|----------------------------|---|----------------|
| <i>Celestolide</i> ADBI | C ₁₇ H ₂₄ O | 244 | 0.015 | 0.020 | 1801 | 6.6 |
| <i>Phantolide</i> AHDl | C ₁₇ H ₂₄ O | 244 | 0.027 | 0.024 | 646 | 6.7 |
| <i>Galaxolide</i> HHCb | C ₁₈ H ₂₆ O | 258 | 1.75 | 0.073 | 11.3 | 5.9 |
| <i>Galaxolidone</i> HHCb-lactone | C ₁₈ H ₂₄ O ₂ | 272 | N/A | N/A | N/A | 5.3 |
| <i>Tonalide</i> AHTN | C ₁₈ H ₂₆ O | 258 | 1.25 | 0.068 | 12.5 | 5.7 |

All composite water samples were immediately filtered with 0.7 µm glass fiber filters, stored at 4°C in amber glass bottles and analyzed within one week from sampling according to (Tasselli and Guzzella, 2020). Briefly, water samples were spiked with Tonalide D3 internal standard and extracted with solid phase extraction (SPE). After the elution, extracts were concentrated using N₂ flux and transferred to GC microvials for GC-MS/MS analysis. This was performed using a Trace 1310 (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatograph equipped with an automatic injector and a TSQ 8000 Evo triple-quadrupole mass spectrometer using the analytical method previously validated by our group (Tasselli and Guzzella, 2020). Laboratory contamination by PMFs was limited by avoiding the use of cleaning products with fragrances and using dedicated acetone-washed glassware. The use of personal care products by laboratory researchers was limited and ambient contamination was constantly monitored by analyzing blank samples of Milli-Q water and subtracting their concentrations to the simultaneously analyzed wastewater samples. Limits of detection (LOD) for each PMF were calculated, according to Shrivastava and Gupta, (2011), considering mean (µ) and standard deviation (σ) of all blank samples analyzed during the entire study. Table V-4 summarizes detection limits for every PMF in water samples. Since laboratory contamination can vary daily, LODs built on blank samples can also vary within a short period. For this reason, the LODs of the two advanced treatments are different, based on the blanks analyzed for each test set.

Table V-4 Limits of detection (LOD) of each PMF in water samples.

| PMFs | LOD O₃ (ng L⁻¹) | LOD PAC (ng L⁻¹) |
|---------------------|--|------------------------------------|
| <i>ADBI</i> | 8 | 2 |
| <i>AHDl</i> | 7 | 1 |
| <i>AHTN</i> | 64 | 34 |
| <i>HHCb</i> | 227 | 136 |
| <i>HHCb-lactone</i> | 226 | 28 |

5.2.8 Other analysis

Regarding ozonation pilot plant, instantaneous samples were collected during each experimental campaign to analyze pH, conductivity (SEC), alkalinity, UV absorbance at 254 nm, residual dissolved ozone and COD, to better characterize the pilot plant influent. In the present work average and standard deviation for each campaign are reported.

5.3 Results and discussion

5.3.1 Effect of ozone on PMF removal

PMFs were measured at the inlet of the pilot plant to assess the variability of the wastewater in terms of micropollutant concentrations and see if samples collected during the campaigns were comparable. Results showed that PMFs occurred at the inlet of the ozonation pilot plant with a mean concentration higher than the LOD, except for AHDI, with a significant presence of HHCB and its biological metabolite HHCB-lactone at levels higher than $1 \mu\text{g L}^{-1}$. AHTN registered concentrations from 207 to 616 ng L^{-1} while ADBI was detected only at trace levels (Table V-5). Given the similar values of each PMF detected in the wastewater entering the ozonation pilot plant of each campaign, a mean sample composition can be calculated: HHCB represented the 68% of the total amount of fragrances detected in the wastewater entering the ozonation pilot plant while HHCB-lactone 28% and AHTN only 4%. In Table V-5 principal characteristics of the wastewater entering the ozone pilot plant are listed.

Table V-5. Ozone pilot plant influent characteristics and concentrations of PMFs detected during the four sampling campaigns.

| <i>Ozone pilot plant influent characteristics</i> | | | | | |
|---|----------------------------------|------------------|---------------------|---------------------|------------------|
| <i>Parameter</i> | <i>Units</i> | <i>July 2019</i> | <i>October 2019</i> | <i>January 2020</i> | <i>July 2020</i> |
| <i>Temperature</i> | °C | 27.31±0.00 | 20.53±0.02 | 15.02±0.54 | 25.42±0.87 |
| <i>pH</i> | Unit | 7.14±0.04 | 7.19±0.02 | 7.28±0.02 | 7.46±0.10 |
| <i>Conductivity</i> | $\mu\text{S cm}^{-1}$ | 971.68±188.88 | 934.54±99.82 | 777.71±11.36 | 1170.33±78.50 |
| <i>Transmittance</i> | UV ₂₅₄ | 0.069±0.01 | 0.084±0.01 | 0.077±0.01 | 0.072±0.01 |
| <i>COD in</i> | $\text{mgO}_2 \text{ L}^{-1}$ | 9.27±0.66 | 18.20±2.51 | 14.97±2.27 | 12.63±0.46 |
| <i>Alkalinity</i> | $\text{mgCaCO}_3 \text{ L}^{-1}$ | 211.33±15.01 | 140.00±0.00 | 192.33±5.69 | 186.00±4.00 |
| <i>Polycyclic Musk Fragrances</i> | | | | | |
| <i>ADBI</i> | ng L^{-1} | 22±2 | 33±6 | 13±1 | 11±5 |
| <i>AHDI</i> | ng L^{-1} | <LOD | <LOD | <LOD | <LOD |
| <i>HHCB</i> | ng L^{-1} | 5902±315 | 9557±1616 | 4236±356 | 5781±2060 |
| <i>HHCB-lactone</i> | ng L^{-1} | 1882±307 | 2486±425 | 2657±444 | 3362±1074 |
| <i>AHTN</i> | ng L^{-1} | 372±21 | 616±112 | 207±8 | 255±86 |

The concentration of PMFs in water and the respective ozone dose consumed was measured at the outlet of both columns of the pilot plant. In this way it was possible to calculate the effect of the ozone dosage on the removal of the three main PMFs detected in the wastewater, HHCB, HHCB-lactone and AHTN. Results are summarized in Fig. V-4.

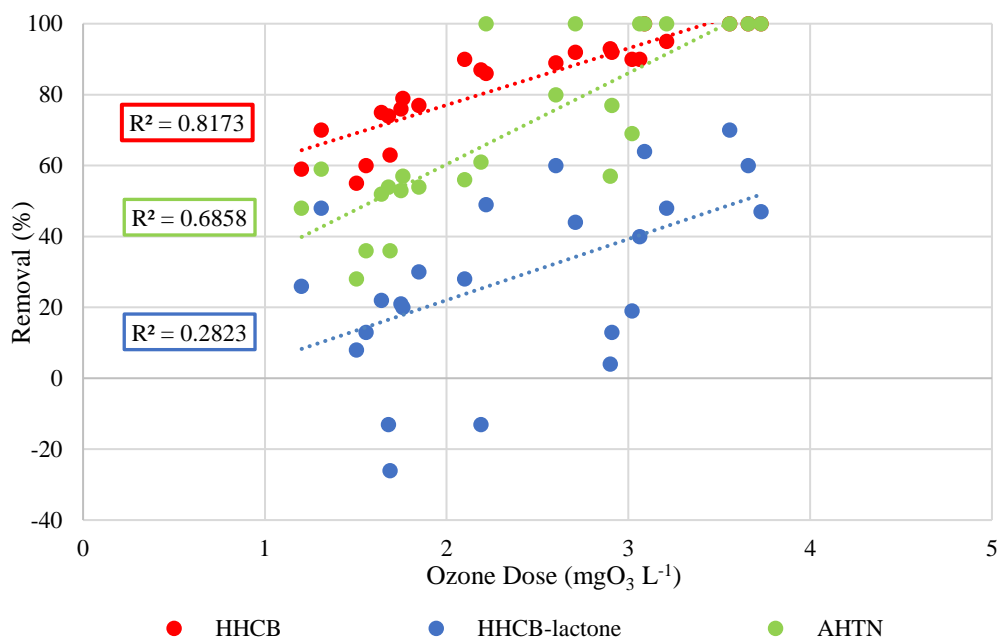


Fig. V-4 Relationship between PMF removals and ozone dose applied to wastewater.

Dosing ozone up to 2 mg L⁻¹ resulted in constant removal values for all the PMFs analyzed. In details, HHCB removals were always higher than 60% while as regards AHTN removal values were around 50%. Similar behaviours were already observed by Li et al. (2016) in which the degradation of polycyclic musk fragrances in wastewater by ozone was examined. Individual PMFs showed different patterns of degradation, with HHCB and ADBI being more easily removed rather than AHTN and ADHI. Ozone was dosed from 0.25 up to 5 mgO₃ L⁻¹ into secondary wastewater previously spiked with the same concentration (5 µg L⁻¹) of all PMFs. Already at 3 mgO₃ L⁻¹ HHCB was efficiently removed while AHTN reached removal of 60% only after the dosage of 5 mgO₃ L⁻¹. In our study, the same removal values were achieved with lower ozone dosage values, but this was related to the lower concentrations of the compounds initially present in the wastewater to be treated. Nöthe et al., (2007) studied the effect of ozonation on wastewater samples to which a high dose of ozone (10 mg O₃ L⁻¹) was applied for 10 minutes. Even in this study, HHCB was more effectively removed than AHTN, which was found to be poorly reactive. In our study, by increasing the ozone dosage from 2 to 3.7 mgO₃ L⁻¹ both HHCB and AHTN showed removal values above 80% with AHTN reaching complete removal values after an ozone dosage of 2.7 mgO₃ L⁻¹. The fact that AHTN was completely removed with lower O₃ dosages respect to HHCB is related to the initial concentration of AHTN in the wastewater entering the pilot plant, one order of magnitude lower than HHCB. A general insufficient removal was instead observed at all applied O₃ doses for HHCB-lactone which registered sometimes negative values at the lower ozone doses indicating a possible new formation of this compound during the ozonation treatment. This phenomenon could be explained by the initial oxidation of HHCB by ozone into the less reactive compound HHCB-lactone as previously verified by Janzen et al. (2011) which demonstrated that the reaction between HHCB and ozone is responsible for the production of HHCB-lactone. The

reactivity of these three PMFs to ozone treatment measured in our study confirmed the second-order kinetic constants for ozonation of HHCB, AHTN and HHCB-lactone in wastewaters that were empirically estimated by Janzen et al. (2011). Author measured in wastewater values of 67, 10 and 3 $M^{-1} s^{-1}$ respectively thus considering even the effect of the wastewater matrix which have been demonstrated to influence HHCB removal rate. In fact, Nöthe et al., (2007) measured rate constants of 140 $M^{-1} s^{-1}$ and 8 $M^{-1} s^{-1}$ for HHCB and AHTN reaction with ozone in pure water. Thus, wastewater matrix reduced the reaction efficiency of ozone and HHCB but was not relevant for the efficiency of AHTN removal. Therefore, in comparison to HHCB, AHTN and HHCB-lactone are more refractory to ozone. Another parameter which must be considered is the hydraulic retention time during the ozonation treatment. Always Janzen et al., (2011) studied the changes in concentration levels of HHCB and its metabolite HHCB-lactone during ozonation of wastewater with a constant ozone input of 5 $mgO_3 L^{-1}$ evidencing a simultaneous production and oxidation of HHCB-lactone; in fact during the first 10 min, HHCB-lactone increased its concentration due to the complete oxidation of the parental HHCB. After 10 min, HHCB registered levels <LOD and HHCB-lactone started to decrease until reaching its complete oxidation at 120 min. In our experiments, the low O_3 dose applied (below 2 $mgO_3 L^{-1}$) together with the short hydraulic retention time of the first column (below 15 min) may have caused the oxidation of the 60% of HHCB into HHCB-lactone but not the complete oxidation of this metabolite thus producing a net formation of HHCB-lactone. Overall, it can be said that wastewater matrix, ozone dose applied, and hydraulic retention time drive the fate of HHCB and HHCB-lactone during ozonation so operating on these parameters is essential to obtain efficient removals. The formation of oxidation products associated with the use of ozone at real scale for the removal of micropollutants in WWTPs have been previously evidenced by several authors because technological conditions and economic aspects do not permit the complete mineralization of micropollutants to H_2O and CO_2 using this technique (Vom Eyser et al., 2013, Gunten, 2003). Concerning PMFs, besides HHCB, the formation of many stable oxidation by-products during ozonation was demonstrated also for AHTN (Fang et al., 2017; Janzen et al., 2011). Generally, transformation products are less toxic and have a higher bioavailability than the parental compounds (Vom Eyser et al., 2013) but, however, toxicological evaluation are needed in order to prove it. Concerning musk fragrances, toxicological evaluation of parental compound and transformation products were already carried out using ozonated spiked pure water in consideration of environmental concentrations (Vom Eyser et al., 2013). Neither HHCB nor HHCB-lactone showed were cytotoxic and genotoxic up to 50 $\mu g L^{-1}$ and no estrogenic effects occurred up to this concentration. Same results were also obtained for AHTN with a starting concentration of 0.1 $mg L^{-1}$. For these reasons, the application of ozone in wastewater treatment do not seem to have any negative environmental impacts.

5.3.2 Ozonation pilot plant efficiency

The simultaneous sampling of the wastewater coming out of the two columns made it possible to evaluate, in terms of PMF removal, the efficiency of both the entire pilot plant and the first column alone. Results are summarized in Fig. V-5.

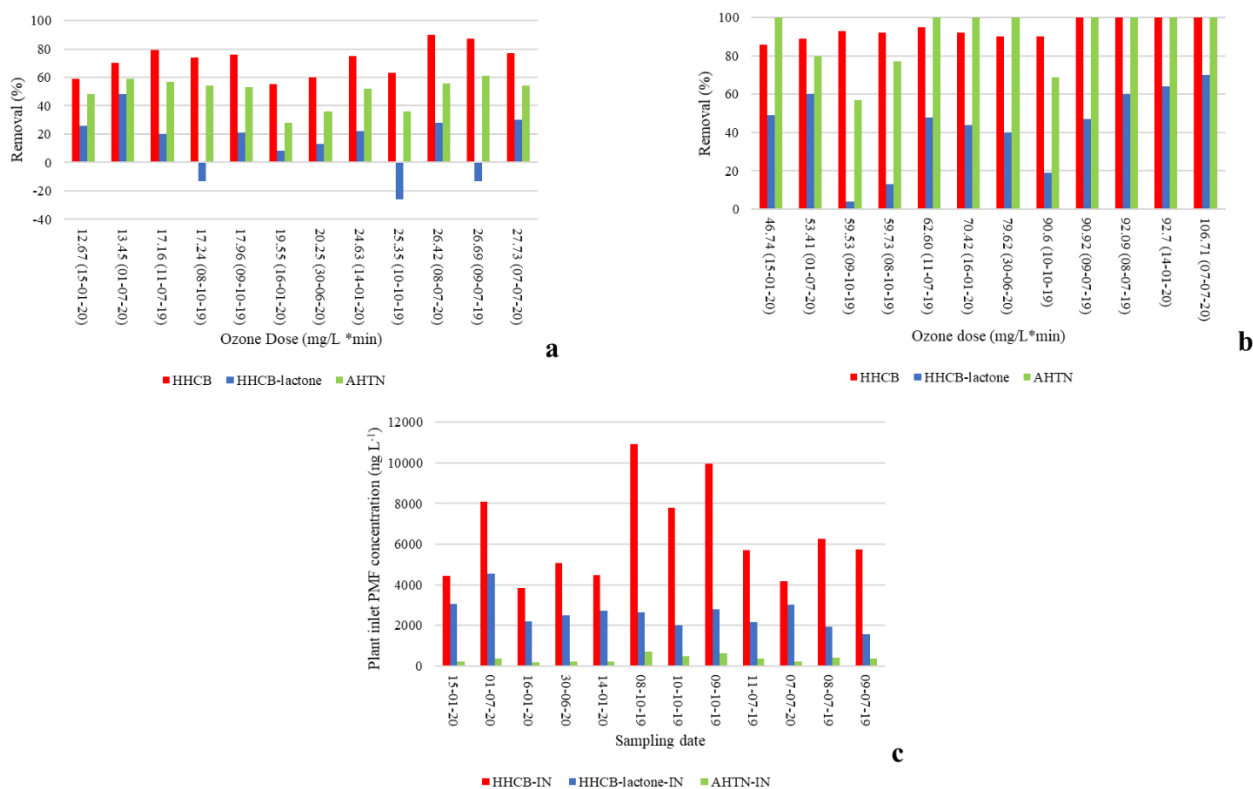


Fig. V-5 Removals (%) of PMFs according to the corresponding ozone dose and column hydraulic retention time (mg/L*min) calculated at the outlet of the first column (a) and at the outlet of the entire pilot plant system (b). Concentration of HHCB, HHCB-lactone and AHTN at the ozone pilot plant inlet during each sampling date are also reported (c).

Concerning the first column (Fig. V-5a) ozone was dosed from 13 to 28 mg/L*min, corresponding to an ozone dose from 1.20 to 2.2 mg L⁻¹ and a hydraulic retention time from 9 to 15 min. HHCB was removed at values around 60% already at low O₃ dosages and slightly increased its removal values up to 80% at a dosage of 28 mg/L*min. AHTN registered constant removals of 50% within the same O₃ dosage range. On the contrary, HHCB-lactone showed the best removals at values around 30% while sometimes negative values were registered indicating a new formation of this compounds as explained before. By crossing the removal efficiency data together with the applied ozone dose, the hydraulic retention time in the column and, above all, the concentration of PMFs in the wastewater entering the pilot plant (Fig. V-5c), it can be highlighted that the new formation of HHCB-lactone occurs in correspondence of high HHCB concentrations at the pilot plant inlet (above 8 µg L⁻¹), with low ozone dosages (about 1.70 mg L⁻¹) and with relatively short contact times (maximum 15 min). Hence the importance of these three different parameters to obtain a satisfactory removal effectiveness. These results evidenced the necessity to improve this advanced wastewater treatment operating on the parameters discussed above. The configuration of two columns in series can be a valid implementation solution as it allows to evaluate different dosages and

contact times in the same test. Moreover, the second column could also be used not only to add ozone but even only to extend contact times to facilitate the completion of the oxidation reactions with residual ozone. Results about the removals calculated at the outlet of the entire ozonation pilot plant are plotted in Fig. 5b. Here, the ozone dose ranged from 2.22 to 3.73 mg L⁻¹ while the total hydraulic retention time from 19 to 30 min. In this case, HHCB was effectively removed. As for AHTN, even if recalcitrant to ozone oxidation, values lower than the LOD thus translated into a complete removal were already measured at ozone doses of about 45 mg/L*min (2.22 mgO₃ L⁻¹ and hydraulic retention time of 21 min) but these results must be related to the lower AHTN concentration originally in the wastewater than the one of HHCB and its metabolite (Fig. V-5c). HHCB-lactone, on the other hand, was never enough removed from the wastewater, not even at the highest tested dose of ozone (3.73 mgO₃ L⁻¹) reaching here a removal of only about 60%. Our results can be comparable to Hernández-Leal et al. (2011) who showed removal from wastewaters of about 70% for HHCB and of about 50% for AHTN with an ozone dose of 5 mg L⁻¹ even if in this case the initial HHCB concentration was one order of magnitude lower than ours. Ternes et al. (2003) have shown on pilot-scale that an ozone dosage between 5 and 15 mg L⁻¹ is appropriate for reducing concentrations of pharmaceuticals and musk fragrances under LOQ level. Regarding HHCB-lactone, even Hollender et al., (2009) in a WWTP with ozonation system measured a removal value of only 63% using an ozone dose of 3.24 mg O₃ L⁻¹. For this reason, our results can confirm the refractoriness of HHCB-lactone towards this type of advanced wastewater treatment. Considering a typical range of O₃ dosage between 3 and 8 mgO₃ L⁻¹ in WWTPs (Lee et al., 2012; Nakada et al., 2007; Rosal et al., 2010), slightly higher doses can be further tested in our pilot plant while maintaining operationally realistic concentrations in order to efficiently remove all the studied micropollutants.

5.3.3 Powdered activated carbon choice and isotherm experiments

Since laboratory experiments for the evaluation of the three different PAC performances were carried out using real wastewaters samples taken from the outlet of the secondary sedimentation tank of MS2, variability between samples was evaluated to establish whether the removals observed with the use of PACs were comparable or not. Results are summarized in Figure V-6. ADBI and AHDI were always below the limit of detection while HHCB registered the highest variation, from 1740 to 3750 ng L⁻¹; however, since all the compounds registered concentrations in the same order of magnitude, comparisons between different PACs can be carried out. All the three tested PACs removed all the analysed compounds below the limit of detection after 30 min (data not showed) so no differences regarding their removal efficiency were registered. PAC 1 and PAC 2 were finally chosen to be tested on the pilot plant.

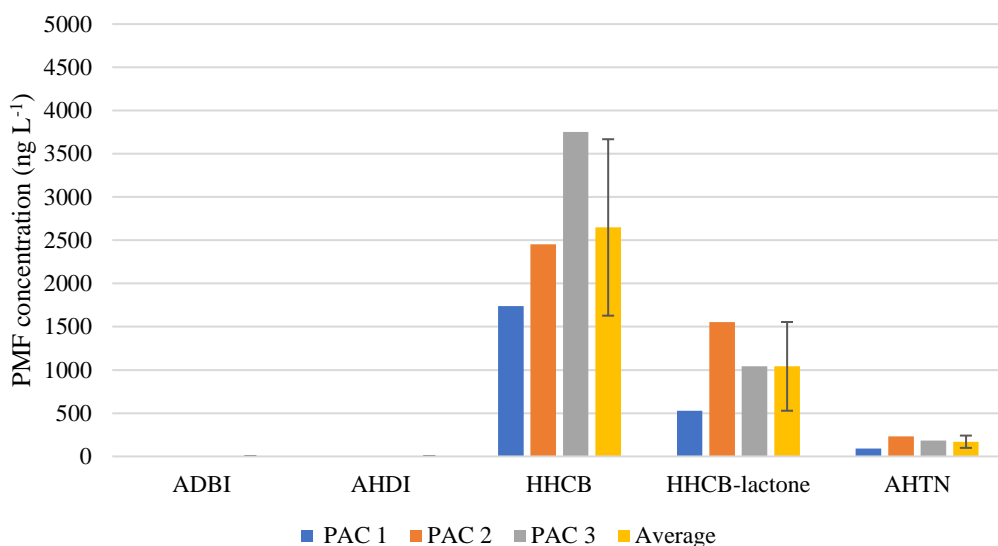


Fig. V-6 Concentrations (ng L⁻¹) of PMFs detected in wastewater samples employed for laboratory jar test with PAC 1, PAC 2 and PAC 3. Average of the three different wastewater samples is also reported.

5.3.4 Powdered activated carbon pilot plant setups

Since experiments carried out at the pilot plant scale involved the use of the wastewater coming from the water line of MS2 WWTP, it is important to evaluate the variability of this matrix over time to verify that comparisons between the performances of the activated carbon of the different configurations could be carried out (Fig. V-7).

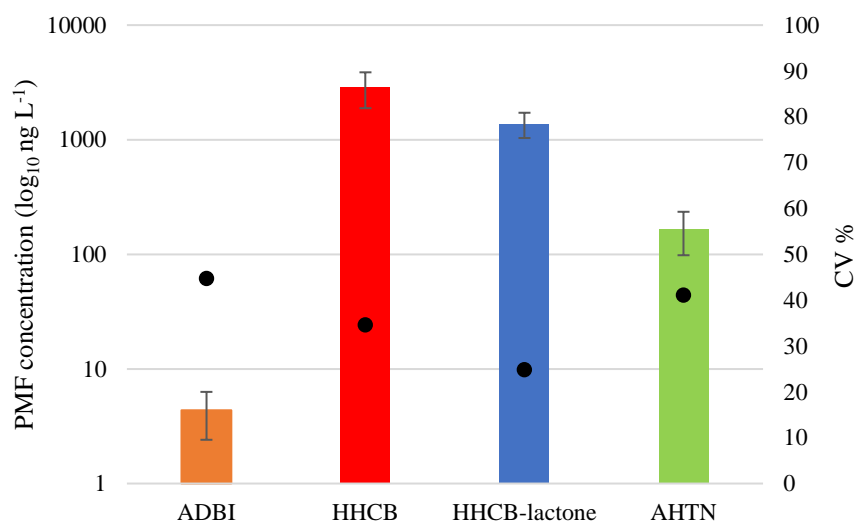


Fig. V-7 Mean concentrations (log₁₀ ng L⁻¹) of PMFs detected in wastewater samples at the inlet of the powdered activated carbon pilot plant. Bars represent standard deviations while points, on the secondary y-axis, represent coefficient of variation.

Results showed that AHDI was never detected at the inlet of the pilot plant. A slight variability between samples was also measured, especially regarding HHCB, which registered the highest standard deviation. Despite this, the coefficient of variation is relatively low for compounds detected at concentrations far from

the respective LOD while, as for ADBI and AHTN, the coefficient of variation is higher due to compound concentrations next to LOD values. Due to these results, comparisons between different setups could be performed. This analysis is necessary since the pilot plant worked with real wastewater samples, subjected to a natural variability given by the type of wastewater entering the WWTP. On the contrary, this evaluation is not required when, for example, experiments are conducted on a laboratory scale where a spike of micropollutants can be carried out in the samples that are subjected to the treatment with PAC in order to have always the same starting concentrations of compounds. PAC 1 and PAC 2 were tested in the activated carbon pilot plant installed in MS2 WWTP and different operating conditions were performed in order to achieve the best environmental and economic efficiency (Table V-6).

Table V-6 Operating conditions of powdered activated carbon pilot plant tested in the experimental work.

| <i>Setup</i> | <i>Q IN^a m³ h⁻¹</i> | <i>Fresh PAC ppm</i> | <i>Pilot plant PAC g L⁻¹</i> | <i>HRT^b min</i> | <i>SRT^c h</i> |
|--------------|--|--------------------------|---|--------------------------------|------------------------------|
| 1 | 8.6 | 10 | 1.186 | 23.57 | 39.27 |
| 2 | 6 | 10 | 0.858 | 33.78 | 56.29 |
| 3 | 8.6 | 20 | 0.606 | 23.57 | 19.63 |
| 4 | 6 | 20 | 0.568 | 33.78 | 28.14 |
| 5 | 8.6 | 10 | 0.734 | 23.57 | 33.38 |
| 6 | 6 | 10 | 0.792 | 33.78 | 47.84 |
| 7 | 8.6 | 10 | 0.513 | 23.57 | 19.64 |
| 8 | 6 | 10 | 0.56 | 33.78 | 28.14 |
| 9 | 6 | 20 | 0.288 | 33.78 | 7.03 |
| 10 | 8.6 | 20 | 0.293 | 23.57 | 4.91 |
| 11 | 8.6 | 10 | 0.275 | 23.57 | 9.82 |
| 12 | 6 | 10 | 0.295 | 33.78 | 14.07 |
| 13 | 8.6 | 5 | 0.284 | 23.57 | 19.64 |

^a = inlet wastewater flow, ^b = hydraulic retention time, ^c = solid retention time.

5.3.4.1 Powdered activated carbon pilot plant setups with PAC 1

Regarding PAC 1, 13 different setups were carried out and the respective mean efficiency in terms of PMF removal between the two replicates for each setup was assessed (Table V-5). In detail, two different water flows and three different doses of fresh virgin PAC were evaluated. PAC concentration in the pilot plant decreased from setup 1 to 13. ADBI was always removed below the limit of detection, since it was always detected at trace levels in the wastewater entering the pilot plant (data not showed). AHTN registered removal <100% only in setup 13, in which a lower PAC concentration (0.284 g L⁻¹) and the lowest fresh PAC dosage (5 ppm) were applied in the pilot plant (Fig. V-8). HHCB and HHCB-lactone registered satisfactory removals from setup 1 to 10, with HHCB-lactone showing slight fluctuations but, in any case, always above 90%. From setup 10 to 13 a clear decrease in removal values was observed for both HHCB and its metabolite. Since PAC concentration in the pilot plant remained constant, a possible influence of the

quantity of virgin PAC dosed in the pilot plant together with the hydraulic flow value can be hypothesized. In fact, by decreasing the quantity of fresh PAC with the same hydraulic flow rate (10→11; 11→13) and vice versa (11→12), a decrease in the percentage of removal of the two compounds was recorded.

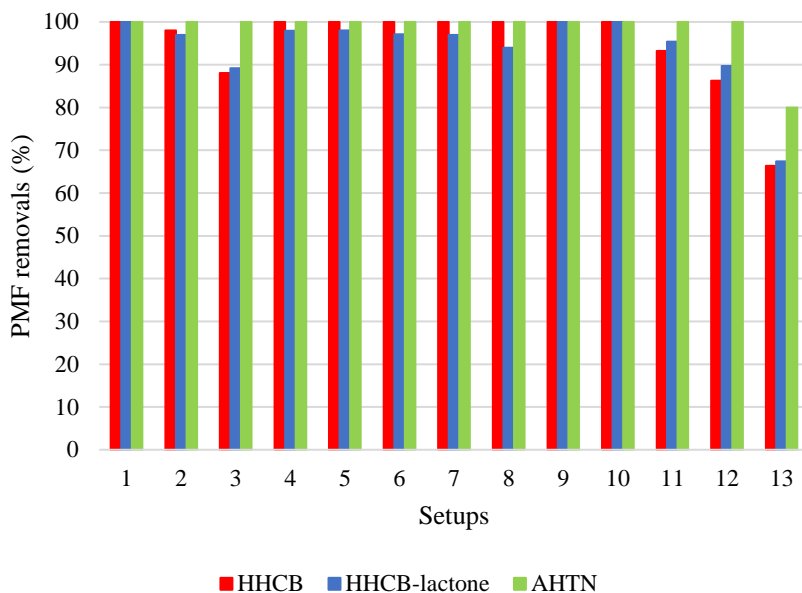


Fig. V-8 Mean removals (%) of HHCB, HHCB-lactone and AHTN registered with PAC 1 application on pilot plant scale during setups 1-13.

The efficiency of activated carbon powder in removing PMFs from WWTP secondary effluents was previously demonstrated by Hernández-Leal et al., (2011) at laboratory scale using milliQ water spiked with HHCB and AHTN in a concentration range of 20-1600 $\mu\text{g L}^{-1}$ and 0.1 g of PAC. Authors measured a removal efficiency higher than 94% for both compounds after only 5 min of contact time. However, matrix complexity of wastewater can influence PAC efficiency so pilot plant studies are needed to confirm these results. César Rego Ferreira et al., (2017) studied PAC efficiency in removing PMFs at laboratory scale using, in this case, real wastewater samples. Authors used fixed amounts of Norit Sae Super PAC in order to evaluate the removal efficiency of, HHCB, HHCB-lactone and AHTN from secondary effluent samples of a WWTP located in Germany. Considerable removal values ($\approx 60\%$) of HHCB-lactone and AHTN were obtained in the removal of these PMFs even at 5 mg L^{-1} of PAC, the lowest dose tested while for HHCB only the 10 mg L^{-1} dose led to an almost 80% removal. Regarding the use of activated carbon, the removal efficiency of contaminants is strictly correlated not only to the availability of adsorption sites but also to micropollutant initial concentrations in the matrix to be treated. In fact, even in our study, HHCB was the compound that recorded the lowest removal values; this is probably linked to its higher concentrations in the wastewater entering the pilot plant respect to other compounds since their Log K_{ow} values are almost the same. In addition to the intrinsic effectiveness of this technique, higher removal values can be obtained by recirculating the PAC within the pilot plant as in our case study in which removals always higher than 60% were obtained even with the lowest PAC dosage tested. Usually, PAC is simply injected into the wastewater to remove, for example, pesticides or taste and odour causing compounds, but this is performed usually with a short contact time and the adsorption capacity of the carbon is not fully evaluated. By recirculating the

PAC in the pilot plant, it becomes possible to maximize the full use of its adsorption capacity. In fact, several authors (Boehler et al., 2012; César Rego Ferreira et al., 2017; Meinel et al., 2016) have observed an increase in the removal efficiency of some classes of organic micropollutants of 20-40% compared to the values obtained without recirculation. According to our knowledge, there is scarce availability of data regarding the use of powdered activated carbon for the removal of PMFs on pilot plant scale. Only (Margot et al., 2013) evaluated the removal of HHCB-lactone using a PAC pilot plant equipped with an ultrafiltration system for the subsequent PAC removal installed as an advanced treatment in the Lausanne WWTP. They carried out two analyses of a 7-day composite sample treated with 12 mg L⁻¹ of PAC measuring a removal of 77% (from 335 to 220 ng L⁻¹).

For this reason, our study represents a fundamental step in the analysis of the effectiveness of this advanced wastewater treatment regarding PMFs, operating on a pilot plant scale and with environmentally relevant concentrations of micropollutants. This study shows a clear correlation between the removal rate of the analysed micropollutants and the concentration of PAC used in the plant. This result is very important as it highlights the non-refractoriness of these compounds to the treatment with activated carbon and the possibility of their effective removal despite the different concentrations in the matrix which can be in competition with other micropollutants for adsorption sites. Finally, it is necessary to highlight that the removals measured in this study were obtained using economically sustainable doses of adsorbent material for WWTPs and therefore can be taken as a real reference for a potential full-scale application of this advanced treatment. Further analyses will be carried in order to investigate in more depth the influence of the operating parameters under control such as the hydraulic flow rate and the quantity of virgin PAC dosed; operating on these parameters can be in fact a useful alternative to excessive dosages of activated carbon which are economically unpracticable.

5.3.4.2 Powdered activated carbon pilot plant setups with PAC 2

In a subsequent experiment also AquaSorb AFP 25, PAC 2, was tested in the pilot plant in order to highlight possible differences in PMF removal efficiency compared to PAC 1. For this purpose, the most significant configurations obtained with PAC 1 were repeated, from setup 9 to 12. Preliminary analysis (Fig. V-9) shows a comparison between the concentrations of PMFs in the wastewater entering the pilot sampled during the corresponding configurations with PAC 1 and PAC 2. This analysis allowed to verify if the concentrations of micropollutants that must be treated with both PACs were almost constant, and therefore the efficiency of the two PACs was comparable, or not. No differences were detected for all the analysed compounds and, consequently, comparisons about the removal efficiency of the two different PACs under the same experimental conditions could be carried out.

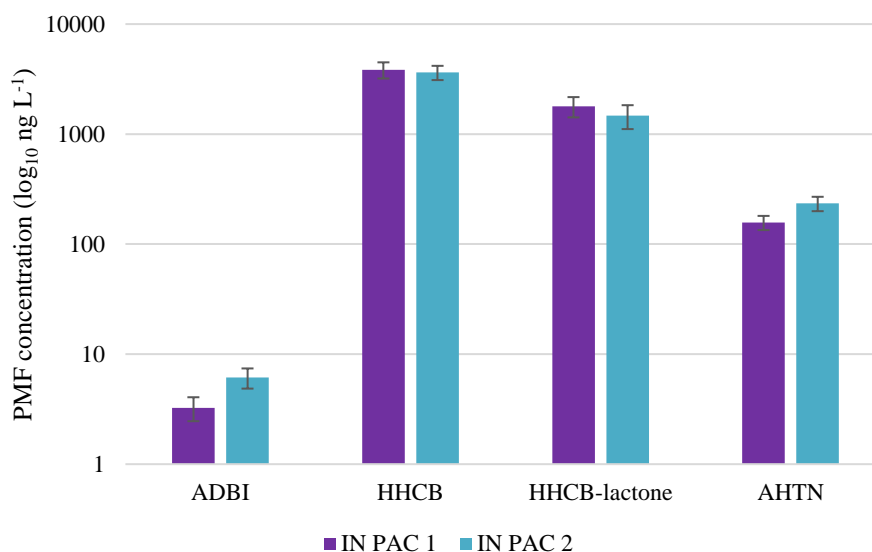


Fig. V-9 Mean concentrations ($\log_{10} \text{ ng L}^{-1}$) of PMFs in the wastewaters entering the activated carbon pilot plant during setups from 9 to 12 with PAC 1 (purple bars) and PAC 2 (grey bars).

Regarding differences between PAC 1 and PAC 2 in removing PMFs, results only of HHCB and HHCB-lactone are showed (Fig. V-10) because the other PMFs were removed below the limit of detection in all the setups. For all the considered setups, no differences were recorder between PAC 1 and PAC 2 in terms of PMF removal efficiency. Setups 9 and 10 efficiently removed all PMFs. In these two configurations the highest quantity of fresh PAC (20 ppm) was used, resulting in an efficient removal of PMFs even at low concentration values of PAC in the pilot plant. By decreasing the quantity of PAC at 10 ppm in setup 11 and 12, as with PAC 1, the removal of HHCB and HHCB-lactone slightly decreased close to 90% values. As during the experimentation with PAC 1, HHCB was the compound that registered the lowest removals, probably due to its initial concentrations one order of magnitude higher than the other PMFs. Based on the production characteristics of the two different activated carbons, PAC 2 has, respect to PAC 1, a higher iodine number which determines a higher degree of activity and therefore of absorbing capacity together with a greater total surface area, a higher apparent density, a smaller average particle size and an economic cost greater than 50% than PAC 1. Due to these characteristics, PAC 2 should be more effective than PAC 1 in removing the micropollutants in the wastewater, but our preliminary study demonstrated effective removal values (<80%) for all the analysed compounds in all the tested configurations using both types of PAC. From this emerges the importance of associating the choice of the activated carbon type to be used also with the class of compounds to be removed. This type of analysis, combined with a correct calibration of the plant operating parameters, can bring to a greater economic savings to the managers of the WWTP.

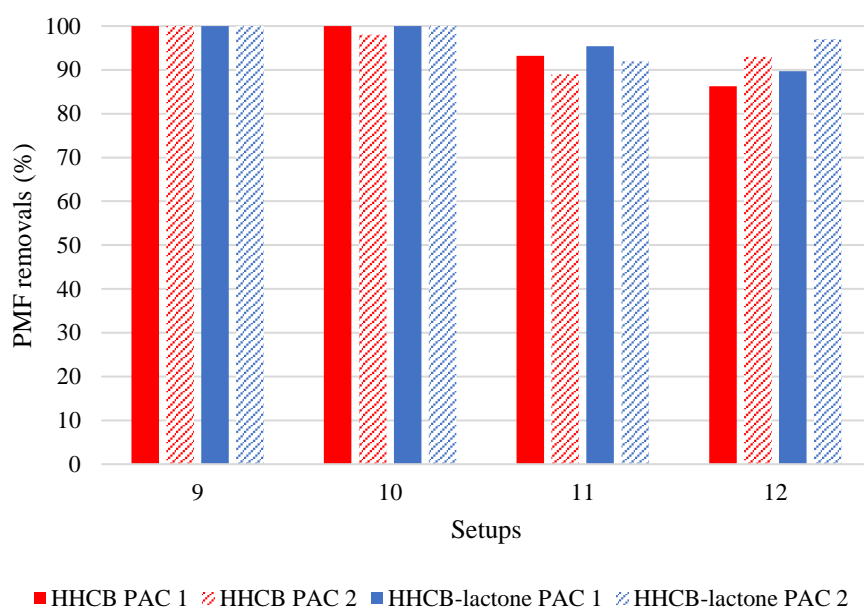


Fig. V-10 Mean removals (%) of HHCB and HHCB-lactone registered with PAC 1 (solid bars) and PAC 2 (dashed bars) application on pilot plant scale during setups 9-12.

5.4 Conclusions

In this study, two different and widely used technique of advanced wastewater treatment were tested on pilot plant scale in order to assess their efficiency in removing polycyclic musk fragrances. Operating on important pilot plant parameters, different setups were carried out for both techniques in order to reach the best compromise between plant management costs and removal efficiency of this group of organic micropollutants. Obtained results were particularly relevant to HHCB, HHCB-lactone and AHTN since ADBI was measured only at trace levels and AHDI was never detected. Ozonation was efficient in removing HHCB even at the lower tested dosages at levels of 80% while AHTN and in particular HHCB-lactone were less reactive to ozone, thus resulting, in the case of HHCB-lactone, in a non-efficient removal (max. 60%) even after the treatment in all the two column of the pilot plant which allowed higher ozone dosages and hydraulic contact times. Since the ozone was dosed up to 3.73 mg L^{-1} , higher dosages can be investigated always maintaining operating conditions that are applicable in WWTPs. However, regarding ozonation, the possible formation of oxidation by-products must always be considered. On the contrary, adsorption on powdered activated carbon efficiently removed all the PMFs up to the lower limit of 0.3 g L^{-1} of PAC and 10 ppm of fresh PAC addition. The possibility of recirculating PAC inside the plant certainly represents an important advantage for this technique, allowing to further increase the removal yields by reducing some management costs. In this study, the comparison between two different types of PAC in removing the investigated compounds highlighted the importance of associating the choice of the PAC to be used with the compounds to be removed, together with the setting of the main operating parameters of the plant such as flow rate, activated carbon concentration and contact time in order to achieve the highest removal efficiencies along with the lowest operating costs.

References

- Artola-Garicano, E., Borkent, I., Hermens, J.L.M., Vaes, W.H.J., 2003. Removal of two polycyclic musks in sewage treatment plants: Freely dissolved and total concentrations. *Environ. Sci. Technol.* 37, 3111–3116. <https://doi.org/10.1021/es020226x>
- Babi, K.G., Koumenides, K.M., Nikolaou, A.D., Makri, C.A., Tzoumerkas, F.K., Lekkas, T.D., 2007. Pilot study of the removal of THMs, HAAs and DOC from drinking water by GAC adsorption. *Desalination* 210, 215–224. <https://doi.org/10.1016/j.desal.2006.05.046>
- Bester, K., 2005. Polycyclic musks in the Ruhr catchment area - Transport, discharges of waste water, and transformations of HHCB, AHTN and HHCB-lactone. *J. Environ. Monit.* 7, 43–51. <https://doi.org/10.1039/b409213a>
- Bester, K., 2004. Retention characteristics and balance assessment for two polycyclic musk fragrances (HHCB and AHTN) in a typical German sewage treatment plant. *Chemosphere* 57, 863–870. <https://doi.org/10.1016/j.chemosphere.2004.08.032>
- Boehler, M., Zwickelpflug, B., Hollender, J., Ternes, T., Joss, A., Siegrist, H., 2012. Removal of micropollutants in municipal wastewater treatment plants by powder-activated carbon. *Water Sci. Technol.* 66, 2115–2121. <https://doi.org/10.2166/wst.2012.353>
- César Rego Ferreira, J., Neuffer, D., Juliana do Amaral, K., 2017. Evaluation of the Use of Activated Carbon Powder for Removal of Emerging Micropollutants from Sewage Wastewater. *Environ. Ecol. Res.* 5, 178–183. <https://doi.org/10.13189/eer.2017.050302>
- Chase, D.A., Karnjanapiboonwong, A., Fang, Y., Cobb, G.P., Morse, A.N., Anderson, T.A., 2012. Occurrence of synthetic musk fragrances in effluent and non-effluent impacted environments. *Sci. Total Environ.* 416, 253–260. <https://doi.org/10.1016/j.scitotenv.2011.11.067>
- Clara, M., Gans, O., Windhofer, G., Krenn, U., Hartl, W., Braun, K., Scharf, S., Scheffknecht, C., 2011. Occurrence of polycyclic musks in wastewater and receiving water bodies and fate during wastewater treatment. *Chemosphere* 82, 1116–1123. <https://doi.org/10.1016/j.chemosphere.2010.11.041>
- Dodd, M.C., Kohler, H.-P.E., von Gunten, U., 2009. Oxidation of Antibacterial Compounds by Ozone and Hydroxyl Radical: Elimination of Biological Activity during Aqueous Ozonation Processes. *Environ. Sci. Technol.* 43, 2498–2504. <https://doi.org/10.1021/es8025424>
- Fang, H., Li, G., Yao, S., Liang, X., An, T., 2017. Kinetic and mechanism studies of musk tonalide reacted with hydroxyl radical and the risk assessment of degradation products. *Catal. Today* 281, 642–648. <https://doi.org/10.1016/j.cattod.2016.06.021>
- Fromme, H., Otto, T., Pilz, K., 2001. Polycyclic Musk Fragrances in the Aquatic Environment. *ACS Symp. Ser.* 773, 203–222. <https://doi.org/10.1021/bk-2001-0773.ch015>
- Heberer, T., 2003. Occurrence, fate, and assessment of polycyclic musk residues in the aquatic environment of urban areas - A review. *Acta Hydrochim. Hydrobiol.* 30, 227–243. <https://doi.org/10.1002/aheh.200390005>
- HERA, 2004. HERA Environmental Risk Assessment Polycyclic musks AHTN and HHCB 1–80.
- Hernández-Leal, L., Temmink, H., Zeeman, G., Buisman, C.J.N., 2011. Removal of micropollutants from aerobically treated grey water via ozone and activated carbon. *Water Res.* 45, 2887–2896. <https://doi.org/10.1016/j.watres.2011.03.009>
- Homem, V., Silva, J.A., Ratola, N., Santos, L., Alves, A., 2015. Long lasting perfume e A review of synthetic musks in WWTPs. *J. Environ. Manage.* 149, 168–192. <https://doi.org/10.1016/j.jenvman.2014.10.008>
- Janzen, N., Dopp, E., Hesse, J., Richards, J., Türk, J., Bester, K., 2011. Transformation products and reaction kinetics of fragrances in advanced wastewater treatment with ozone. *Chemosphere* 85, 1481–1486.

<https://doi.org/10.1016/j.chemosphere.2011.08.043>

- Lee, C.O., Howe, K.J., Thomson, B.M., 2012. Ozone and biofiltration as an alternative to reverse osmosis for removing PPCPs and micropollutants from treated wastewater. *Water Res.* 46, 1005–1014. <https://doi.org/10.1016/j.watres.2011.11.069>
- Li, W., Nanaboina, V., Chen, F., Korshin, G. V., 2016. Removal of polycyclic synthetic musks and antineoplastic drugs in ozonated wastewater: Quantitation based on the data of differential spectroscopy. *J. Hazard. Mater.* 304, 242–250. <https://doi.org/10.1016/j.jhazmat.2015.10.035>
- Marchal, M., Beltran, J., 2016. Determination of synthetic musk fragrances. *Int. J. Environ. Anal. Chem.* 96, 1213–1246. <https://doi.org/10.1080/03067319.2016.1249479>
- Margot, J., Kienle, C., Magnet, A., Weil, M., Rossi, L., de Alencastro, L.F., Abegglen, C., Thonney, D., Chèvre, N., Schärer, M., Barry, D.A., 2013. Treatment of micropollutants in municipal wastewater: Ozone or powdered activated carbon? *Sci. Total Environ.* 461–462, 480–498. <https://doi.org/10.1016/j.scitotenv.2013.05.034>
- Meinel, F., Zietzschmann, F., Ruhl, A.S., Sperlich, A., Jekel, M., 2016. The benefits of powdered activated carbon recirculation for micropollutant removal in advanced wastewater treatment. *Water Res.* 91, 97–103. <https://doi.org/10.1016/j.watres.2016.01.009>
- Molinos-Senante, M., Reif, R., Garrido-Baserba, M., Hernández-Sancho, F., Omil, F., Poch, M., Sala-Garrido, R., 2013. Economic valuation of environmental benefits of removing pharmaceutical and personal care products from WWTP effluents by ozonation. *Sci. Total Environ.* 461–462, 409–415. <https://doi.org/10.1016/j.scitotenv.2013.05.009>
- Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N., Takada, H., 2007. Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant. *Water Res.* 41, 4373–4382. <https://doi.org/10.1016/j.watres.2007.06.038>
- Nöthe, T., Hartmann, D., von Sonntag, J., von Sonntag, C., Fahlenkamp, H., 2007. Elimination of the musk fragrances galaxolide and tonalide from wastewater by ozonation and concomitant stripping. *Water Sci. Technol.* 55, 287–292. <https://doi.org/10.2166/wst.2007.422>
- Nowotny, N., Epp, B., von Sonntag, C., Fahlenkamp, H., 2007. Quantification and Modeling of the Elimination Behavior of Ecologically Problematic Wastewater Micropollutants by Adsorption on Powdered and Granulated Activated Carbon. *Environ. Sci. Technol.* 41, 2050–2055. <https://doi.org/10.1021/es0618595>
- O.S.P.A.R. Commission, 2004. Hazardous substances series, musk xylene and other musks. London, UK. <https://doi.org/ISBN 1-904426-36-0>
- Paredes, L., Alfonsin, C., Allegue, T., Omil, F., Carballa, M., 2018. Integrating granular activated carbon in the post-treatment of membrane and settler effluents to improve organic micropollutants removal. *Chem. Eng. J.* 345, 79–86. <https://doi.org/10.1016/j.cej.2018.03.120>
- Parolini, M., Magni, S., Traversi, I., Villa, S., Finizio, A., Binelli, A., 2015. Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in *Dreissena polymorpha*. *J. Hazard. Mater.* 285, 1–10. <https://doi.org/10.1016/j.jhazmat.2014.11.037>
- Peck, A., Hornbuckle, K., 2006. Synthetic musk fragrances in urban and rural air of Iowa and the Great Lakes. *Atmos. Environ.* 40, 6101–6111. <https://doi.org/10.1016/j.atmosenv.2006.05.058>
- Reiner, J.L., Berset, J.D., Kannan, K., 2007. Mass flow of polycyclic musks in two wastewater treatment plants. *Arch. Environ. Contam. Toxicol.* 52, 451–457. <https://doi.org/10.1007/s00244-006-0203-3>
- Reiner, J.L., Kannan, K., 2006. A survey of polycyclic musks in selected household commodities from the United States. *Chemosphere* 62, 867–873. <https://doi.org/10.1016/j.chemosphere.2005.10.006>

Chapter V – Preliminary results regarding the application of ozonation and adsorption on activated carbon in a wastewater treatment plant: new promising advanced technologies for Polycyclic Musk Fragrances (PMFs) removal

- Rogers, H.R., 1996. Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges. *Sci. Total Environ.* 185, 3–26. [https://doi.org/10.1016/0048-9697\(96\)05039-5](https://doi.org/10.1016/0048-9697(96)05039-5)
- Rosal, R., Rodríguez, A., Perdígón-Melón, J.A., Petre, A., García-Calvo, E., Gómez, M.J., Agüera, A., Fernández-Alba, A.R., 2010. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. *Water Res.* 44, 578–588. <https://doi.org/10.1016/j.watres.2009.07.004>
- Schreurs, R.H.M.M., Legler, J., Artola-Garicano, E., Sinnige, T.L., Lanser, P.H., Seinen, W., Van Der Burg, B., 2004. In Vitro and in Vivo Antiestrogenic Effects of Polycyclic Musks in Zebrafish. *Environ. Sci. Technol.* 38, 997–1002. <https://doi.org/10.1021/es034648y>
- Shrivastava, A., Gupta, V., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles Young Sci.* 2, 21. <https://doi.org/10.4103/2229-5186.79345>
- Simonich, S.L., Federle, T.W., Eckhoff, W.S., Rottiers, A., Webb, S., Sabaliunas, D., De Wolf, W., 2002. Removal of fragrance materials during U.S. and European wastewater treatment. *Environ. Sci. Technol.* 36, 2839–2847. <https://doi.org/10.1021/es025503e>
- Tasselli, S., Guzzella, L., 2020. Polycyclic musk fragrances (PMFs) in wastewater and activated sludge: analytical protocol and application to a real case study. *Environ. Sci. Pollut. Res.* <https://doi.org/10.1007/s11356-019-06767-7>
- Ternes, T.A., Stüber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M., Teiser, B., 2003. Ozonation: A tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater? *Water Res.* 37, 1976–1982. [https://doi.org/10.1016/S0043-1354\(02\)00570-5](https://doi.org/10.1016/S0043-1354(02)00570-5)
- Tumová, J., Šauer, P., Golovko, O., Koba Učun, O., Grabic, R., Máchová, J., Kocour Kroupová, H., 2019. Effect of polycyclic musk compounds on aquatic organisms: A critical literature review supplemented by own data. *Sci. Total Environ.* 651, 2235–2246. <https://doi.org/10.1016/j.scitotenv.2018.10.028>
- Upadhyay, N., Sun, Q., Allen, J.O., Westerhoff, P., Herckes, P., 2011. Synthetic musk emissions from wastewater aeration basins. *Water Res.* 45, 1071–1078. <https://doi.org/10.1016/j.watres.2010.10.024>
- Villa, S., Assi, L., Ippolito, A., Bonfanti, P., Finizio, A., 2012. First evidences of the occurrence of polycyclic synthetic musk fragrances in surface water systems in Italy: Spatial and temporal trends in the Molgora River (Lombardia Region, Northern Italy). *Sci. Total Environ.* 416, 137–141. <https://doi.org/10.1016/j.scitotenv.2011.11.027>
- Vom Eyser, C., Börgers, A., Richard, J., Dopp, E., Janzen, N., Bester, K., Tuerk, J., 2013. Chemical and toxicological evaluation of transformation products during advanced oxidation processes. *Water Sci. Technol.* 68, 1976–1983. <https://doi.org/10.2166/wst.2013.452>
- Weinberg, I., Dreyer, A., Ebinghaus, R., 2011. Waste water treatment plants as sources of polyfluorinated compounds, polybrominated diphenyl ethers and musk fragrances to ambient air. *Environ. Pollut.* 159, 125–132. <https://doi.org/10.1016/j.envpol.2010.09.023>
- Winkler, M., Kopf, G., Hauptvogel, C., Neu, T., 1998. Fate of artificial musk fragrances associated with suspended particulate matter (SPM) from the River Elbe (Germany) in comparison to other organic contaminants. *Chemosphere* 37, 1139–1156. [https://doi.org/10.1016/S0045-6535\(98\)00110-6](https://doi.org/10.1016/S0045-6535(98)00110-6)
- Xie, Z., Ebinghaus, R., Temme, C., Heemken, O., Ruck, W., 2007. Air-sea exchange fluxes of synthetic polycyclic musks in the North Sea and the Arctic. *Environ. Sci. Technol.* 41, 5654–5659. <https://doi.org/10.1021/es0704434>
- Yang, J.J., Metcalfe, C.D., 2006. Fate of synthetic musks in a domestic wastewater treatment plant and in an agricultural field amended with biosolids. *Sci. Total Environ.* 363, 149–165. <https://doi.org/10.1016/j.scitotenv.2005.06.022>

CHAPTER VI

General conclusions

This PhD. project has been focused on the analysis of Polycyclic Musk Fragrances (PMFs), a class of synthetic organic micropollutants extensively used in different field of application and produced in large volumes. During the last years, these chemicals have been recognised worldwide as a reason of concern by the scientific community due to their ability to persist in the environment, to bioaccumulate and to cause acute and subchronic toxic effects on both aquatic and terrestrial organisms. Despite this, to our knowledge, environmental regulatory limits have not been set for these compounds.

One of the main pathways of environmental contamination from these compounds has been identified in domestic wastewater treatment plants (WWTPs) which, not being specifically designed to remove this type of pollutants, discharge them into surface aquatic environments. This problem has been highlighted in different parts of the world, however the dynamics that drive the fate of these compounds largely depend on their concentrations in wastewater, which in turn are linked to the degree of use of these compounds in the specific country. From past studies, Italy has been found to be the European country with the highest degree of use of detergents containing synthetic fragrances but, precisely in this country, the availability of data regarding the presence and environmental fate of these substances is very scarce if not almost nothing at all. In addition to this, different protocols have been developed to analyze PMFs in wastewater and sewage sludge but all of them require long analytical times combined with the use of expensive and not always available instruments and a large consumption of solvents.

In this framework fits the present PhD project, which was developed according to three sequential topics carried out during the last three years of research, the conclusions of which will be discussed here separately.

6.1 Development of the analytical protocol for the detection of Polycyclic Musk Fragrances (PMFs) in wastewater and activated sludge

In the first part of the PhD project, two novel methods for the analysis of PMFs in wastewaters and activated sludges were developed using Gas Chromatography (GC) coupled with Mass Spectrometry (MS). Regarding wastewater samples, a tripe quadrupole mass spectrometer was employed while the analysis of the activated sludge was performed using an Ion Trap. Both methods have a very limited analysis time, if compared with that of the already existing methods, but a high degree of analytical sensitivity, confirmed by the detection limits obtained for the individual compounds, much lower than the concentrations detected in the analyzed samples. These protocols were validated in terms of linearity, repeatability and recovery, allowing an evaluation of the entire analytical process thanks to the labelled internal standards and the constant monitoring of the laboratory PMF contamination levels. All this was achieved using simple and easily available instrumentation, especially regarding the preparation of this type of samples, a crucial point also as regards the risk of instrumental contamination, very high for this type of matrices. Wastewater samples were extracted using a vacuum manifold to perform solid phase extraction while freeze-dried sludge samples were extracted using an ultrasounds bath. These methods therefore represent a turning point in the analysis of this type of contaminants as they also allow the processing of many samples and the return of the respective results reliable and in a short time.

6.2 Monitoring of Polycyclic Musk Fragrance (PMFs) removal, adsorption and biodegradation in a conventional activated sludge wastewater treatment plant in Northern Italy

The second part of the project regarded the first one-year monitoring study carried out in Northern Italy which aimed to analyze the fate of polycyclic musk fragrances in wastewaters and activated sludges during the different treatments commonly applied in a conventional Wastewater Treatment Plant (WWTP). Four different stations along the wastewater treatment were considered. In this study, higher PMF concentrations respect to other countries were detected in wastewaters, as expected from the widest use of detergent registered in Italy.

Galaxolide (HHCB) and Galaxolidone (HHCB-lactone) were found in concentrations of $\mu\text{g L}^{-1}$, one order of magnitude greater than AHTN and evidenced only a slight reduction during treatments in WWTP thus being discharged in the final effluent. Adsorption processes on activated sludge during the biological treatment mainly drove the fate of all these synthetic compounds, as expected from their physicochemical characteristics; in fact, high PMF concentrations were measured in activated sludge during the entire study. HHCB-lactone registered an increase up to 70% during wastewater treatments caused by the biotransformation of the parental compound HHCB during the biological treatment. Slightly differences were recorded between seasons in terms of PMF input the WWTP and this entails a constant environmental contamination throughout the year and not concentrated, for example, over a short period of time. Overall, this study showed that current technologies employed in conventional WWTPs are not enough efficient to remove from wastewaters these organic micropollutants, which are then released from WWTP effluents thus representing a potential risk for aquatic ecosystems and human health.

6.3 Preliminary results regarding the application of two promising advanced wastewater treatments for Polycyclic Musk Fragrances (PMFs) removal in wastewater treatment plants.

In the third phase of the study, the evaluation of the effectiveness regarding the removal of PMFs from wastewaters of ozonation and adsorption on activated carbon was addressed. The two technologies were tested in two pilot plants installed in two WWTPs located in Northern Italy after the biological treatment. By changing the operational parameters of the two pilot plants, different conditions were tested in order to achieve the best compromise between removal efficiencies and management costs. In the ozonation pilot plant, a medium-low O_3 dosage up to $3.73 \text{ mgO}_3 \text{ L}^{-1}$ was tested. HHCB was efficiently removed (80%) even with low O_3 dosages and short hydraulic retention times while AHTN and HHCB-lactone were less reactive to oxidation. The latter compound registered insufficient removals of 60% even with the highest tested O_3 dosage and contact time also due to the formation of HHCB-lactone during HHCB oxidation. In general, ozonation was effective in removing this type of organic micropollutants from wastewater even though the formation of possible oxidation by-products may decrease its effectiveness and increase its operating costs through high O_3 doses. Adsorption on Powdered Activated Carbon (PAC) was tested in a pilot plant consisting on a series of tanks in which PAC is added directly to the wastewater together with coagulant, microsand and flocculant and can be recirculated. Even with this technology, different setups were studied by operating on the water flow, quantity of virgin PAC added to the system and concentration of PAC inside

the pilot plant. Satisfactory removals were achieved for all the studied compounds even at low concentrations of the virgin (10 ppm) and recirculated PAC (0.29 g L⁻¹). A dependence between fragrance removal efficiency and quantity of recirculated and virgin PAC was evidenced, together with an influence of the hydraulic flow rate. Lower PAC dosages can be further tested in order to obtain high removals together with lower management costs. With this study, the importance of associating the choice of the PAC type with the class of compound to be removed was stressed in order to avoid additional costs resulting from the purchase of high-performance PACs. Overall, even adsorption on activated carbon was found to be effective in removing all the compounds analyzed in this study without highlighting any criticalities.

CHAPTER VII

Supporting information

Polycyclic musk fragrance (PMF) removal, adsorption and biodegradation in a conventional activated sludge wastewater treatment plant in Northern Italy**S1- Sample preparation**

Water samples were spiked with 25 μL of AHTN-D3 20 $\text{ng } \mu\text{L}^{-1}$ and extracted with solid phase extraction (SPE) C_{18} disks (Avantor, Center Valley, PA, USA). Cartridges were conditioned with 10 mL of n-hexane, methanol and Milli-Q water. Samples (0.25L for A and B samples and 0.5L for C and D samples) were concentrated, under vacuum pressure, on C_{18} cartridges at a flow rate of 15 mL min^{-1} . After the extraction, cartridges were air dried under vacuum pressure for 1 h and eluted with 10 mL of n-hexane:dichloromethane 1:1 v/v and 10 mL of n-hexane. At the end, 1 mL of iso-octane was added to the extracts which were concentrated to 1 mL using N_2 , transferred to GC micro-vials and analyzed by GC-MS.

Sludge samples (0.2 g d.w.), spiked with 25 μL of AHTN-D3 20 $\text{ng } \mu\text{L}^{-1}$, were extracted with 20 mL of n-hexane:acetone 3:1 v/v for 30 min at 30°C in an ultrasonic water bath at 40 Hz intensity (Falc Instruments, Treviglio, IT). Extracts were centrifuged at 3000 rpm for 5 min at room temperature and the supernatant solvent was collected. Extracts were then concentrated to 5 mL using N_2 flux at 30°C, transferred in centrifuge tubes with 100 mg of Superclean ENVI-Carb 120/400 (Supelco, Bellefonte, PA, USA). After vortexing for 1 min, samples were centrifuged again as previously reported and the supernatant solvent was collected. 1 mL of iso-octane was added, and samples were concentrated to 800 μL , filtered with Acrodisc syringe filters with 1 μm glass fiber pore size (VWR International, Radnor, PA, USA) and transferred to GC micro-vials for GC-MS analysis.

Regarding water samples, GC-MS/MS analysis was performed using a Trace 1310 (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatograph equipped with an automatic injector Model AS 1310 and a triple-quadrupole mass spectrometer Model TSQ 8000 Evo operating in the selective reaction monitoring (SRM) mode. A fused silica capillary column (SLBTM-5ms, 60 m x 0.25 mm x 0.25 μm film thickness) supplied by Sigma-Aldrich (Saint Louis, MO, USA) was employed. Operating conditions were: injector port temperature starting at 50°C and raising at 1°C min^{-1} until 200°C when samples were transferred into the column; helium as gas carrier at a programmed flow-rate of 1.3 mL min^{-1} for 10 min and 1.5 mL min^{-1} for 20 min and argon as collision gas. Column temperature was maintained at 50°C for 1 min, programmed at 12°C min^{-1} until 170°C and 6°C min^{-1} until 230°C, held for 3 min, then raised at 20°C min^{-1} at 290°C and held for 3 min. Total analysis time was 30 min. Mass spectrometer operated in electron impact ionization mode (70 eV). The ion source temperature and the MS transfer line temperature were both 290°C.

For sludge samples, GC analysis were performed using a Thermo Electron TraceGC 2000 coupled with a PolarisQ Ion Trap (ThermoElectron - Austin, Texas) mass spectrometer equipped with a PTV injector and an AS 3000 auto sampler. A SLB[®]-5ms capillary column, 30 m x 0.25 mm i.d. x 0.25 μm film thickness (Supelco, Bellefonte, PA, USA) was employed for PMFs separation in the following conditions: carrier gas helium at 1.3 mL min^{-1} ; injector temperature at 50°C then ramped to 200°C at 1°C s^{-1} ; split mode with a split flow of 10 mL min^{-1} and a split ratio of 8; initial oven temperature set at 50°C (held 1 min), then ramped first

to 170°C at 12 °C min⁻¹ and then to 230°C at 6°C min⁻¹ (held 20 min). A final cleaning ramp at 310°C (25°C min⁻¹) was applied for 5 min to clean the column from sludge impurities. Samples were analyzed using EI mode with standard electron energy of 70 eV; the transfer line was maintained at 300°C, the damping gas at 1 mL min⁻¹, and the ion source at 250°C.

Table S1 Median, Min, Max concentrations (ng L⁻¹) of PMFs in water in the four sampling stations (1-4) of the WWTP.

| <i>PMF</i> | <i>Parameter</i> | <i>1</i> | <i>2</i> | <i>3</i> | <i>4</i> |
|---------------------|------------------|-------------------|-----------|-----------|-----------|
| <i>ADBI</i> | Min | <LOD ^a | <LOD | <LOD | <LOD |
| | Max | 30 | 36 | <LOD | <LOD |
| | Mean ± St.Dev | 13±7 | 18±11 | <LOD | <LOD |
| | CV% ^b | 54 | 61 | - | - |
| <i>AHDI</i> | Min | <LOD | <LOD | <LOD | <LOD |
| | Max | <LOD | <LOD | <LOD | <LOD |
| | Mean ± St.Dev | <LOD | <LOD | <LOD | <LOD |
| | CV% | - | - | - | - |
| <i>HHCB</i> | Min | 2872 | 2939 | 2719 | 3727 |
| | Max | 6854 | 9326 | 4710 | 6701 |
| | Mean ± St.Dev | 5063±1296 | 6251±2266 | 3647±592 | 5072±897 |
| | CV% | 26 | 36 | 16 | 18 |
| <i>HHCB-lactone</i> | Min | 288 | 483 | 771 | 1436 |
| | Max | 1202 | 3661 | 4026 | 4404 |
| | Mean ± St.Dev | 581±292 | 1602±920 | 2315±1070 | 2773±1085 |
| | CV% | 50 | 57 | 46 | 39 |
| <i>AHTN</i> | Min | 174 | 191 | 159 | 196 |
| | Max | 487 | 891 | 278 | 302 |
| | Mean ± St.Dev | 350±100 | 469±234 | 207±34 | 242±34 |
| | CV% | 29 | 50 | 17 | 14 |

^a= Values below the limit of detection

^b= Coefficient of variation