Removal efficiency and mass balance of polycyclic aromatic hydrocarbons, phthalates, ethoxylated alkylphenols and alkylphenols in a mixed textile-domestic wastewater treatment plant

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Highlights

- PAHs, PAEs, AP₁₋₂EOs and APs are studied in a mixed textile-domestic WWTP
- Micropollutants in the effluent meet the European environmental quality standards
- Mass balance of pollutants was calculated and its accuracy checked using Pb and Cd
- The whole WWTP provides a quasi-quantitative mass loss of targeted micropollutants
- Biotransformation is the most important removal process followed by sludge sorption

1 Abstract

2 In this work the occurrence and fate of polycyclic aromatic hydrocarbons (PAHs), phthalic acid 3 esters (PAEs), mono and diethoxylate alkylphenols (AP₁₋₂EOs) and alkylphenols (APs) have been 4 investigated during a two-weeks period in a facility treating mixed textile-domestic wastewater 5 (Prato, Italy). The wastewater treatment plant (WWTP) consists of primary sedimentation, activated 6 sludge biological oxidation, secondary sedimentation, clariflocculation and ozonation. The sludge is 7 treated within the facility by thickening, dewatering and final incineration, thus providing the 8 almost quantitative removal of the adsorbed micropollutants. Naphthalene (50%), di(2-ethylhexyl) 9 phthalate (74%) and branched 4-nonylphenols (59%) were the individual main representative 10 compounds of each class in the influent wastewater, which showed concentration ranges of 5.6-66, 11 85-290 and 21-133 µg/L for PAHs, PAEs and APs+AP₁₋₂EOs, respectively. The WWTP efficiently 12 removed PAHs, PAEs and APs+AP₁₋₂EOs, providing effluent concentrations of 0.075-0.16 ng/L 13 0.38-9.9 µg/L and 0.53-1.4 µg/L. All targeted priority and priority-hazardous micropollutants 14 showed effluent concentrations in line with the European environmental quality standards (EQS), 15 even though for di(2-ethylhexyl) phthalate and benzo(a)pyrene after correction for the dilution 16 factor of the recipient. The WWTP performance were evaluated by mass balance, verifying its accuracy by monitoring Pb and Cd as conservative species. The biological treatment sections 17 18 provided mass losses of 85.5%, 74.5% and 56.8% for APs+AP1-2EOs, PAEs and PAHs, 19 highlighting efficient biotransformation performances of the activated sludge process. However, for 20 the more volatile PAHs (e.g. naphthalene), a significant contribution of stripping cannot be excluded. A remarkable mass loss was also determined in the ozonation stage for PAEs (72.9%) 21 22 and especially PAHs (97.0%), whereas a lower efficiency was observed for APs+AP₁₋₂EOs 23 (41.3%). The whole plant allowed for obtaining an almost quantitative removal (96.7-98.4%) for all 24 targeted compounds.

Keywords: organic micropollutants; activated sludge; ozonation; environmental quality standard;
biotransformation; sludge sorption

1

27 **1 Introduction**

Wastewater treatment plants (WWTPs) have an important role in the control of environmental 28 29 pollution, especially towards organic micropollutants, but may represent at the same time a point-30 source of contaminants in watercourses. Accordingly, the accurate monitoring of organic 31 micropollutants within the various treatment stages of WWTPs is required in order to obtain an 32 effluent with the lowest possible contamination, through the optimization of the treatment 33 conditions. This aspect is particularly relevant since organic compounds may undergo different 34 processes in WWTPs (i.e. degradation, partition between liquid and solid phases, aerosolization and evaporation). The fate of organic compounds during wastewater treatment may be carefully 35 36 investigated by the mass-balance approach, which consists in the simultaneous evaluation of mass 37 flows of target contaminants in the various WWTP treatment stages. This approach allows for 38 obtaining a number of relevant information on organic compounds in wastewater, such as (i) mass 39 loading, (ii) partitioning behaviour, (iii) degradability, (iv) overall persistence under real-world 40 conditions, as well as (v) mass loading to surface water via effluent discharge (Heidler and Halden, 41 2008). This approach has been adopted for evaluating the fate of different organic pollutants, 42 including polycyclic aromatic hydrocarbons (PAHs) (Manoli and Samara, 1999; Qiao et al., 2014; 43 Sun et al., 2018; Yao et al., 2012; Zhao et al., 2018), phthalic acid esters (PAEs) (Clara et al., 2010; 44 Gao et al., 2014; Martínez-Alcalá et al., 2017; Roslev et al., 2007; Tan et al., 2007), as well as 45 ethoxylate alkylphenols (AP_nEOs) and alkylphenols (APs) (Loyo-Rosales et al., 2007; McAdam et 46 al., 2010; Tan et al., 2007). These micropollutant classes have been frequently detected in 47 wastewater and their occurrence at µg/L levels has been associated with various toxicological 48 effects, such as endocrine disruption in marine organisms, neurotoxicity and alterations at the 49 ecosystem level (Sánchez-Avila et al., 2009).

PAHs are ubiquitous pollutants formed mainly by incomplete combustion processes (Deblonde et
al., 2011; Peng et al., 2015). Natural processes such as forest fires or volcanic eruptions contribute
to PAH emissions, as well. Being ubiquitous, PAHs have been often detected in several wastewater

treatment plants (Blanchard et al., 2004). In humans, the metabolization of PAHs with four or more rings leads to products able to interact with enzymatic systems, such as cytochrome P450, inducing the development of tumours (Apostoli P. Cassano F., 1997). For these reasons most PAHs have been listed as priority (naphthalene and fluoranthene) or priority hazardous substances (benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(g,h,i)perylene, indeno (1,2,3c,d)pyrene and anthracene) (Directive 2013/39/EU).

59 PAEs are widely used in various industrial and household applications such as adhesives, paints, 60 inks and rubbers. However, about 90% of PAEs are used as plasticizers for polymers, mainly 61 polyvinyl chloride and other resins, because of the stability and fluidity of high molecular weight 62 congeners (Bergé et al., 2014; Julinová and Slavík, 2012). Since phthalates are not chemically 63 bound to the polymeric matrix, they can enter the environment by losses during manufacturing processes and by leaching and/or evaporation. Accordingly, also PAEs can be considered 64 65 ubiquitous pollutants and may reach WWTPs through both domestic and industrial wastewater, as well as urban runoff and atmospheric depositions (Clara et al., 2010; Dargnat et al., 2009). 66 67 Although these compounds are excreted from the organism quite rapidly, they may accumulate in 68 fatty tissues and biological fluids (Del Bubba et al., 2018), thus negatively affecting hormonal and 69 reproduction systems due to their estrogenic activity (Julinová and Slavík, 2012). The most widely 70 used PAE is the di-2-ethylhexyl phthalate (DEHP) which has been listed as a priority substance in 71 the Directive 2013/39/EU.

AP_nEOs with a branched alkyl chain of 8-9 carbon atoms are non-ionic surfactants largely used as detergents, emulsifiers, wetting and solubilizing agents (Salgueiro-González et al., 2017). Their main degradation metabolites are AP_nEOs oligomers with 1-2 EO units (AP₁₋₂EO) and APs, the latter being the final stage of the ethoxylate chain breakdown (Sacco et al., 2006). Furthermore, branched octylphenols (OPs) and nonylphenols (NPs) are highly used as such, for plastic manufacture as well as for production of textiles and pesticide formulations (Sánchez-Avila et al., 2009). These metabolites are known to be more toxic and more estrogenic than the parent 79 substances. In particular, branched OPs and NPs can mimic natural hormones by interacting with 80 the estrogenic receptors causing serious damages to the endocrine system (Ying et al., 2002). 81 Therefore, they are included in the group of endocrine disrupting chemicals and their occurrence in 82 the water cycle is considered a relevant environmental issue. The potential adverse effects of APs 83 and AP_nEOs to humans and other organisms led to the inclusion of 4-tert-octylphenol (4-t-OP) and 84 4-nonylphenol (4-NP, i.e a mixture of more than two hundred isomers) in the list of chemicals of 85 concern in European waters as priority and priority hazardous substances, respectively (European 86 Parliament and Council of the European Union, 2008). Moreover, the Directive 2013/39/EU set 87 annual environmental quality standards (EQS) in water for 4-t-OP and 4-NP (European Parliament 88 and Council of the European Union, 2013).

89 Based on the previous considerations, the aim of this work was to give an accurate picture of the 90 concentrations of PAHs, PAEs, 4-t-OP₁₋₂EOs and 4-NP₁₋₂EOs, as well as 4-t-OP and 4-NPs, both 91 within wastewater and sludge treatment lines of the main WWTP of the industrial textile district in 92 the city of Prato (Italy), which is one of the most important in Europe. This facility consists of a 93 complex wastewater treatment train (i.e. preliminary treatments, primary settling, oxic biological 94 treatment, secondary sedimentation, clariflocculation and ozonation), together with a sludge section 95 including thickening, dewatering and a multiple-hearth waste-to-energy plant for sludge 96 incineration. As far as we know, such a complex configuration has not been previously considered 97 for mass balance of organic micropollutants. The WWTP object of this research was previously 98 investigated for the presence of the aforementioned organic compound classes (Lepri et al., 1997). 99 However, in the previous work, only the wastewater treatment line was considered, which at that 100 time was devoid of the ozonation phase. The evaluation of the WWTP output in the receiving water 101 body in relation to the environmental quality standards set by the European Directives was also 102 given. Furthermore, the calculation of the mass balance of these analytes was performed in various 103 treatment sections of the WWTP, as well as in the whole plant. Accordingly, the aforementioned 104 analytes were determined in wastewater and sludge within all treatment stages, as well as in ashes produced during sludge incineration. The heavy metals Cd and Pb, which are listed as priority substances by the Directive 2013/39/EU, were also included in the study and monitored in the WWTP matrixes mentioned above, as species not subjected to degradation processes, as well as to any significant evaporation/aerosolization phenomena, in order to check the correctness of the mass balance calculation. In this regard, it should be highlighted that in only one study the analysis of conservative species was performed in order to assess the reliability of mass balance calculation of organic micropollutants in WWTPs (Clara et al., 2010).

112 **2** Experimental

113 2.1 Standards, reagents and materials

All labelled and unlabelled reference standards of PAHs, PAEs, AP₁₋₂EOs and APs were purchased by Sigma-Aldrich (St. Louis, MO, USA) and LGC (Teddington, UK). All solvents and reagents were supplied by Sigma-Aldrich. Solid-phase extraction cartridges were purchased from Agilent (Santa Clara, CA, USA). Glass fibre filters were obtained from Whatman (Maidstone, UK). For full details, see section *S1.1* of *Supplementary Material*.

119 Chemical name of target analytes, abbreviations, logarithms of the octanol-water partition 120 coefficients (log K_{OW}) and attributes of priority or priority hazardous substance (European 121 Parliament and Council of the European Union, 2013), are reported in **Table 1**.

122 2.2 WWTP description

123 The scheme of the centralized WWTP "Baciacavallo", which is based on a conventional activated 124 sludge process (CASP), followed by an ozonation treatment, is reported in **Fig. 1**. This facility, 125 managed by GIDA S.p.A., is located in Prato (Italy) and processes daily about 50,000-130,000 m³ 126 (depending on the working or non-working days) of industrial-domestic mixed wastewater from the 127 textile district and the municipality of Prato. For further details of the WWTP, see section *S1.2* of 128 *Supplementary Material*.

129 2.3 Sample collection

130 For each sampling point shown in Fig. 1, two series (i.e. one for the analysis of Pb and Cd and the 131 other for the determination of organic compounds) of 7 samples were collected during a period of 132 two weeks in the summer 2011 (range of daily mean temperatures of wastewater in the aeration 133 tank 24.4-31.6°C; concentration of the mixed liquor suspended solids, MLSS = 5.5 g/L), using 134 bottles pre-treated as specified in the section S1.3 of the Supplementary Material. More in detail, 135 the sampling was performed taking into account the hydraulic residence time (HRT) of the various 136 treatment sections and of the whole plant, applying a proper delay time in the collection of the effluents from the various sections, with respect the sampling time of the corresponding influents. 137 138 Full details of the sampling procedure are provided in Section S1.4 of the Supplementary Material.

139 2.4 Analysis of organic micropollutants

For each sampling point, wastewater and sludge samples, as well as the coagulant/flocculant reagents R1, R2, and R3, underwent the pretreatment protocols reported in the *Section 1.5.1* of the *Supplementary Material*. The resulting filtered wastewater, sludge supernatant samples and Milli-Q water diluted (1/10 v/v) coagulant/flocculant reagents were then extracted by means of the EPA 3535A SPE method (U.S. EPA, 2007a).

145 Particulate matter and solid phase of sludge deriving from the aforementioned sample pretreatment 146 protocols were extracted by means of the EPA 3550C ultrasound assisted method (U.S. EPA, 147 2007c). Ashes were extracted according to the EPA 3546 microwave assisted method (U.S. EPA, 148 2007b). The extraction methods were properly optimized as illustrated with full details in the 149 Section S1.5.2 of the Supplementary Material. The extracts were fractionated according to a 150 procedure reported elsewhere (Lepri et al., 1997), optimized as described in the Section S1.5.3 of 151 the Supplementary Material. The fractionated extracts were analysed by gas chromatography 152 coupled with mass spectrometry (GC-MS), under the experimental conditions reported in the Section S1.5.4 of the Supplementary Material. The determination coefficients (\mathbb{R}^2) of the organic 153 micropollutant calibration curves in matrix were in all cases greater than 0.99. Apparent recovery 154

155 percentages (AR%) and method quantification limits (MQL), calculated by the procedure described 156 in the Section S1.5.2 of the Supplementary Material, are reported in the Section S1.5.5. AR% in wastewater samples were in the ranges of 63-83% and 52-75% for liquid and particulate phases, 157 158 respectively. As regards sludge, AR% ranged from 53 to 84% in the supernatant and from 47% to 159 78% in the solid phase, whereas for ashes they were included in between 41% and 85%. MQLs 160 strongly varied depending on the matrix and the compound considered. As a general consideration, 161 PAHs showed much lower MQLs (up to two orders of magnitude) than PAEs, APs and $AP_{1,2}EOs$. 162 Moreover, for liquid samples, much lower MQLs were achieved in sampling points C, D and E (0.07-11 ng/L) than the others (1.4-269 ng/L). For solid samples the lowest MQLs were found in 163 164 the ash samples (0.05-3.6 ng/g). As regards the solid matrixes deriving from wastewater and sludge, 165 MQLs determined in sampling stations C, D and E (5.7-862 ng/g) were about one order of 166 magnitude lower than the others (29-3231 ng/g).

167 2.5 Analysis of Cd and Pb

168 determination of cadmium and lead in water samples, sludge supernatant and The 169 coagulant/flocculant reagents (after dilution 1/10 v/v) was performed by acidic-oxidant microwave 170 digestion (65% HNO₃ and 30% H₂O₂ mixture 1/7 v/v) followed by ICP-OES analysis. Solid phases 171 of sludge samples were digested using a 65% HNO₃ and 45% HClO₄ acidic-oxidant mixture 7/1 v/vand then analysed by ICP-OES. Ash samples were mineralized with 7 mL of 65% HNO₃, 1 mL of 172 173 37% HCl and 1.5 mL of HF at 40% before the ICP-OES analysis. For full details of the 174 mineralization and instrumental analysis protocols, see the section S1.6 of the Supplementary 175 Material.

176 2.6 Data analysis

Data plots were performed by Microsoft® Office Excel 2016 (Microsoft Corporation, Redmond,
WA, USA). In order to evaluate the significance of the differences between mean values, the oneway ANOVA coupled with the Games-Howell nonparametric contrast test were carried out using
the statistical package Minitab® 17.1.0 (Minitab Inc., State College, PA, USA).

181 2.7 Mass balance calculation

In order to evaluate the mass balance of metals and organic compounds within the WWTP, the overall treatment has been schematically divided into five sections that identify technologically different depuration stages to which the wastewater is subjected. The whole plant has been also considered for the mass balance evaluation.

186 Within each treatment section, as well as for the whole plant, the total mass $(M_{X,S})$ of each targeted 187 chemical species "X" passing through the different sampling points "S" of the WWTP (see **Fig. 1**) 188 in the 24 hours, has been calculated.

As regards the contributions deriving from each sampling point of the water line and the sludge line, with the sole exception of ash, the calculation was carried out using the following equation:

191
$$M_{X,S} = \frac{Q_{V,S} \cdot C_{X,S} \cdot 1000}{1 \cdot 10^6}$$
(1)

- 192 where:
- 193 M_{X,S} is the mass of the chemical species "X" passing through a sampling point "S" during the
 194 24-h time span, expressed in g/d;
- 195 Q_{V,S} is the volumetric flow rate at the different sampling points "S", expressed as m³/d (see
 196 Table *S1* of the *Supplementary Material*).
- 197 C_{X,S} is the concentration of the chemical species "X" at the different sampling points "S",
 198 expressed as μg/L;
- 199 the factor 1000 represents the conversion factor from m^3 to L;
- 200 $1 \cdot 10^6$ represents the conversion factor from μg to g.
- 201 As regards the ash produced by the incinerator, the total mass $(M_{X,K})$ of each targeted chemical
- 202 species "X" was calculated by considering the quantity of ash produced by the incinerator (sampling
- 203 point K, see Fig. 1) within one day, according to the following equation:

204
$$M_{X,K} = \frac{Q_{K} \cdot C_{X,K}}{1000}$$
 (2)

where:

- 206 $M_{X,K}$ is the mass of the chemical species "X" in the ash, expressed as g/d;
- 207 Q_K is the quantity of ash produced daily, expressed as kg/d (see Table *S1* of the *Supplementary*208 *Material*).
- 209 $C_{X,K}$ is the concentration of the chemical species "X" in the ash, expressed as mg/kg;
- 210 the factor 1000 represents the conversion factor from mg to g.
- The mass of target contaminants eventually introduced into the WWTP due to the dosage of the coagulant/flocculant reagents (dosage points R1, R2 and R3, see **Fig. 1**) was calculated using the following equation:

$$M_{X,R} = \frac{Q_{V,R} \cdot C_{X,R}}{1000}$$
(3)

215 where:

214

- 216 $M_{X,R}$ is the mass of the chemical species "X" dosed during the 24-h time span, owing to the input 217 of the reagents, expressed as g/d;
- 218 $Q_{V,R}$ is volumetric flow of the reagents expressed as L/d;
- 219 $C_{X,R}$ is the concentration of the chemical species "X" in the reagent, expressed as mgL;
- 220 1000 represents the conversion factor from mg to g.
- The following equations can be identified for the calculation of the mass balance related to the different sections of the treatment system and for the whole WWTP (see Fig. 1).
- 223 Preliminary treatment and primary sedimentation (HRT≈7 h for working days; HRT≈16 h for
- non-working days)

225
$$M_{X,A} - M_{X,B} - M_{X,F} + M_{X,G} + M_{X,H} + M_{X,R1}$$
(4)

- Biological treatment and secondary sedimentation (HRT≈11 h for working days; HRT≈24 h for
 non-working days)
- 228 $M_{X,B} M_{X,C} M_{X,G}$ (5)
 - Final clariflocculation (HRT≈6.7 h for working days; HRT≈15 h for non-working days)

230
$$M_{X,C} - M_{X,D} - M_{X,H} + M_{X,L} + M_{X,R2}$$
 (6)

- Ozonation (HRT≈3 h for working days; HRT≈6.3 h for non-working days)

232
$$M_{X,D} - M_{X,E}$$
 (7)

233 - Sludge treatment

$$M_{X,F} - M_{X,I} - M_{X,K} - M_{X,L} + M_{X,R3}$$
(8)

- 235 Whole plant
- 236

234

$$M_{X,A} - M_{X,E} - M_{X,K} - M_{X,I} + M_{X,R1} + M_{X,R2} + M_{X,R3}$$
(9)

237 **3 Results and Discussion**

Concentrations of the investigated micropollutants (i.e. PAHs, PAEs, AP₁₋₂EOs and APs) were monitored in the sampling points reported in **Fig. 1**, which involved both the water and the sludge treatment lines.

241 3.1 Overall removal performances of the WWTP

242 3.1.1 PAHs

In this study 16 different PAHs have been investigated and their relative percentages in the WWTP
influent and effluent are shown in Fig. 2A.

Among investigated PAHs, Nap resulted by far the most abundant compound in inlet samples, 245 246 accounting for about 50% of the whole PAH concentration. This finding might be due to the fact 247 that this PAH is the only one used for the production of dyes and moth-killer products, which are 248 widely applied in textile industry (Azpíroz et al., 2008; Rogers, 1996). Interestingly, Nap was the 249 prevalent PAH in the effluent wastewater, as well. The predominance of Nap in the WWTP outlet 250 could be explained by its lower tendency to be partitioned into sludge. Furthermore, it should be 251 remarked that an increased resistance to degradation by ozone has been reported for PAHs, with 252 decreasing the ring number (Butkovic et al., 1983; Kulik et al., 2006; Trapido et al., 1995). In this 253 regard, it should also be noted that a prevalence of Nap in effluent wastewater is a finding 254 highlighted also in other studies regarding either the same WWTP herein investigated (Lepri et al., 1997) or other facilities (Mezzanotte et al., 2016; Vogelsang et al., 2006). 255

Inlet concentrations (water and particulate matter) of total PAHs ranged from 5.6 to $66 \mu g/L$ (see **Table 2**), which were in quite good agreement with data obtained about twenty years ago in the influent of the same WWTP (Lepri et al., 1997). The lowest concentrations were determined in samples 4 and 7, which represented public holidays. However, even considering only the working days, a high concentration variability was observed, probably due to the fact that PAHs are ubiquitous pollutants deriving from a wide range of sources, including any incomplete combustion process of organic substances (Peng et al., 2015).

As illustrated by data reported in **Table 2**, the largest decreases of PAH concentrations were observed after the biological treatment and secondary sedimentation (sampling point C) and following ozonation (sampling point E), as well. It is particularly remarkable that this latter treatment provided removal efficiencies in respect to the previous stage, mostly included between 94% and 98%, with the only exception of sample 7, which showed a quite lower performance (82%). As a result, in all the investigated samples the effluent wastewater showed total PAH concentrations \leq 220 ng/L.

270 In agreement with data reported in a previous study (Lepri et al., 1997), a strong enrichment of total 271 PAHs was generally observed in the particulate phase of WWTP inlet (sampling point A) and 272 effluents from primary and secondary sedimentation (sampling points B and C), where mg/kg to 273 tens of mg/kg were determined, depending on the sampling day considered. The enrichment tended 274 to decrease going from the inlet to the secondary settling. PAH concentrations separately 275 determined in the aqueous and particulate phases were conversely not available in samples taken at the clariflocculation outlet (sampling point D) and in the WWTP effluent (sampling point E). In 276 277 fact, in these sampling stations, the concentrations of suspended solids were always lower than 25 278 mg/L and the extraction was therefore performed on the whole sample (see paragraph S1.5.1 of the 279 Supplementary Material section).

280 Concentrations of priority hazardous PAHs found in the influent ranged from 0.6 to 18 μ g/L and 281 accounted on average for about 17% of total PAHs, highlighting their significant occurrence in the inlet of the sewage facility under study. The whole treatment reduced the concentrations of thesePAHs at tens of ng/L, which is an overall remarkable result.

284 Table 3 illustrates the concentrations of priority and priority-hazardous PAHs found in the WWTP 285 effluent (unfiltered water samples), together with the values corrected for the dilution factor 286 calculated on the basis of flow rates of WWTP effluent and receiving water body. Moreover, in 287 **Table 3**, these concentration values are compared with environmental quality standards set by the 288 Directive 2013/39/EU as maximum acceptable (MAC-EQS) and annual average (AA-EQS) 289 concentrations in inland surface waters. All targeted priority and priority-hazardous PAHs exhibited 290 effluent concentrations much lower than MAC-EQS. Nap and Ant were also found at lower 291 concentrations than AA-EQS. Conversely, the outlet average value of Flu was equal to its AA-EQS, 292 whereas for B(a)Pyr a mean effluent concentration (5.9 ng/L) approximately one magnitude order 293 higher than AA-EQS was highlighted (see **Table 3**). In this latter regard, it should be however noted 294 that this EQS was set at 0.17 ng/L, which is a concentration level that, to the best of our knowledge, 295 is not obtained in the effluents of WWTPs, even adopting the best available technologies for 296 wastewater treatment (Busetti et al., 2006; Wu et al., 2013; Zhang et al., 2012), including ozonation 297 (Mezzanotte et al., 2016). More in detail, effluent B(a)Pyr concentrations have been reported in the 298 very wide range of 1-280 ng/L, depending on the kind of treated wastewater and adopted treatment 299 technology. It must also be remarked that the Baciacavallo WWTP effluent drains into a river with 300 a dilution factor of about 20, corresponding to a two-week mean B(a)Pyr concentration in the 301 receiving water body of 0.30 ng/L, which is close to the AA-EQS value.

Since PAHs are congeners characterized by very different environmental toxicity, as a further estimation of the impact on the receiving water body of PAHs outflowing the investigated WWTP, it is interesting to express their total concentration as B(a)Pyr_{eq}, calculated on the basis of their toxic equivalency factors (TEFs) (Nisbet and LaGoy, 1992) (**Table 1**). Effluent B(a)Pyr_{eq} concentrations ranged from 8.3 and 20.2 ng/L, depending on the sampling date, with a mean value of 12.8 ng/L. This value was similar to the ones reported by Liu et al. (4.6 ng/L) (Liu et al., 2017) and Mezzanotte et al. (7.8 ng/L) (Mezzanotte et al., 2016) and much lower than those found by other authors (166-591 ng/L) (Busetti et al., 2006; Zhang et al., 2012).

When inlet daily concentrations of total PAHs were plotted as a function of the outlet values within each section described in the paragraph 2.2 and for the whole plant, significant linear correlations $(R^2 > 0.66; P<0.05)$ with positive slopes were observed, indicating a quite homogeneous operation of the various WWTP treatment stages in the examined period.

314 As regards the sludge treatment line, **Table 4** illustrates the concentrations of total and priority 315 hazardous PAHs determined in sampling points F-L. For samples F, G and H results regarding the 316 solid fraction of sludge and the corresponding aqueous supernatant are separately reported. Data 317 reported in **Table 4** clearly evidenced in all sludge samples the high enrichment of the solid phase. 318 The enrichment factors (i.e. the ratio between the concentrations found in the solid fraction and in 319 the supernatant) were higher for priority hazardous PAHs (range 9.4-144) than total ones (range 10-320 101), in agreement with the higher tendency of the former group to be partitioned into the solid 321 phase, as measured by log K_{OW} values (**Table 1**). Moreover, a significant decline of the enrichment 322 factor was observed in the sampling stations, following the order: F > G > H. This trend is in 323 agreement with the efficient PAH removal along the various treatment stages, as well as with the 324 recirculation of secondary and tertiary sludge upstream the primary sedimentation (Fig. 1).

325 Total PAH concentration found in water from the sludge dewatering process (sampling point I) was 326 included in the wide range of 7.3-144 µg/L, with an occurrence of priority hazardous compounds of 327 about 20% (1.2-25 µg/L). These concentrations should be considered significant, since they are 328 comparable with those found in the Baciacavallo influent and represented on average 6% of the 329 whole PAH concentration of sludge (694 µg/L). Much lower concentrations of total and priority 330 hazardous PAHs (0.58-7.9 and 0.13-0.66 µg/L, respectively) were found in the water from fume 331 scrubber (sampling point L). These values were much lower than total PAH concentrations (15 332 µg/L) determined in the wet scrubber effluent from a fluidized bed incinerator treating biological 333 sludge containing PAH concentrations similar to those herein determined (Wang et al., 2002).

In fly ash (sampling point K), total PAHs were included from 0.19 to 0.37 mg/kg, which is a concentration range fully included in the very wide group of data reported in literature for various kind of incineration facilities treating sewage sludge (0.011-43 mg/kg) (Deng et al., 2009; Park et al., 2009; Wang et al., 2002).

338 *3.1.2 PAEs*

For this class of micropollutants, seven compounds were investigated, including DEHP, which is
listed as hazardous priority substance (European Parliament and Council of the European Union,
2013).

Mean relative percentages of PAEs determined in the Baciacavallo WWTP influent and effluent are shown in **Fig. 2B**. DEHP was the main PAE determined in both sampling points, accounting on average for 74% and 87% of total PAEs in the influent and effluent, respectively. This finding was observed in previous studies performed in various European countries (Bergé et al., 2014; Clara et al., 2010; Sánchez-Avila et al., 2009) and can be explained considering its large use as plasticizers (Lassen et al., 2009), as well as the higher recalcitrance to biological degradation compared to other PAEs with shorter alkyl chain (Gao and Wen, 2016).

Inlet concentration values of total PAEs and DEHP (water and particulate matter) were in the ranges of 85-290 and 75-235 μ g/L, respectively (**Table 2**). A strong enrichment of the particulate phase was observed for total PAEs, with total PAEs concentrations in the particulate matter from few thousands to few hundreds of mg/kg moving from the WWTP inlet to the effluent of secondary sedimentation.

No particular weekly trend of total and DEHP concentrations was observed as a function of the industrial contribution to the whole WWTP hydraulic loading. More in detail, the sample 4 taken on a no-working day showed higher concentrations than those collected on working days (sample 3 and 6). Accordingly, phthalates did not seem strictly dependent on the industrial activity of Prato textile district. This finding is in agreement with other studies that reported comparable concentrations of total PAEs and DEHP in runoff and inlet WWTPs, highlighting the strong diffuse character of phthalate contamination, compared to other pollutant classes, more related to industrial
point-source origins (Clara et al., 2010; Sánchez-Avila et al., 2009).

362 PAE concentrations herein found in the WWTP inlet were similar to those determined twenty years 363 ago in the influent of the same WWTP (Lepri et al., 1997), but also to the inlet concentrations more 364 recently observed in various WWTPs in Europe, irrespective of the origin of wastewater (i.e. 365 industrial, domestic or mixed), evidencing the high and ubiquitous presence of PAEs (Bergé et al., 366 2014; Clara et al., 2010; Sánchez-Avila et al., 2009).

As illustrated in **Table 2**, and similarly to what was observed for PAHs, also for total PAEs the greatest concentration reductions were obtained thanks to the biological treatment followed by secondary sedimentation (sampling point C), as well as to ozonation (sampling point E). More in detail, the biological stage allowed to obtain a very high PAE removal, with percentage reductions ranging approximately from 61% to 90%. The ozone treatment provided a further strong PAE reduction, with removal percentages in the range of 53-96%, leading to a mean effluent concentration of about 4.3 μ g/L.

374 As regards DEHP, the whole WWTP provided a mean removal of about 96%, with an effluent 375 concentration on average equal to 3.9 µg/L, which was included in the range of values determined 376 in various European facilities (1.6-5.0 µg/L) (Clara et al., 2010; Dargnat et al., 2009; Deblonde et 377 al., 2011). For DEHP the Directive 2013/39/EU/ provides an AA-EQS of 1300 ng/L (Table 3), 378 which is lower than the mean effluent DEHP concentration determined herein. However, as already 379 specified for PAHs, a dilution factor of about 20 must be considered, thus leading to a DEHP 380 concentration in the receiving water body of 195 ng/L, which fully satisfies the aforementioned 381 AA-EQS.

Based on TEFs reported in **Table 1** and considering that DEHP represents by far the largest contribution to total effluent PAEs, effluent DEHP_{eq} concentrations (0.4-9.4 μ g/L) were very similar to those referring to the DEHP only and therefore the same aforementioned considerations apply.

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As previously observed for PAHs, also for PAEs the sedimentation and clariflocculation stages (A-B and C-D, see **Fig. 1**) showed homogeneous removal efficiencies, as evaluated for each treatment stage by plotting inlet concentrations vs outlet ones (\mathbb{R}^2 of regression lines = 0.94-0.95, *P*<0.05). Conversely, the B-C and D-E stages showed erratic treatment efficiencies as a function of the monitoring day.

390 Data regarding the sludge treatment line (Table 4) highlighted decreasing total PAE and DEHP 391 enrichments in the solid fraction, following the order F > G > H. This trend was the same 392 previously found and discussed for PAHs. Interestingly, as observed for suspended particulate 393 matter in the WWTP influent and effluents from primary and secondary sedimentation, the PAE 394 enrichment factors were in all cases two-three times higher than those observed for PAHs. This 395 finding was consistent with the higher log K_{OW} values of phthalates found in the analysed samples, 396 compared to the partition constants of most occurring PAHs (see Table 1). Total PAE and DEHP 397 concentrations determined in water from the sludge dewatering process (sampling point I) were in 398 the ranges 12-347 and 8.4-298 µg/L, respectively. When these concentrations were compared to 399 those found in primary sludge, it was clear that they accounted for a much lower percentage (about 400 2.6%) compared to PAH data, in agreement with the higher PAE hydrophobicity.

The presence of PAE in the water from the fume scrubber and especially in the fly ash was much lower than that found for PAHs (**Table 4**), in agreement with the fact that they, unlike PAHs, can only undergo processes of destruction during combustion.

404 *3.1.3 AP*₁₋₂*EOs and APs*

For this group of micropollutants, 4-*t*-OP (listed as priority substance), 4-n-NP and branched 4-NPs (listed as priority hazardous compounds), as well as 4-t-OP₁₋₂EO and 4-NP₁₋₂EOs, were herein investigated (see **Table 1**).

Data provided by this study (related to wastewater sampled in the year 2011), together with those regarding the occurrence of the same pollutants in samples collected in the same WWTP in 1995 (Lepri et al., 1997) and 2014-2015 (Ciofi et al., 2014; Ciofi et al., 2016) allow for obtaining a 411 picture of the evolution of their presence in wastewater from the Prato textile district during the last 412 20 years. Fig. 3 shows the trend of the sum of target $AP_{1,2}EOs$ and APs (expressed as nmol/L) 413 found within this study in the Baciacavallo WWTP inlet and outlet, in comparison with previously 414 published data. Concentrations slightly lower than 4500 nmol/L were determined in the mid-1990s 415 at the inlet of the facility, which was at that time without the tertiary ozonation treatment. The total 416 removal was in fact equal to about 75% with concentrations of about 1200 nmol/L in the WWTP effluent. The values determined in this study at the WWTP inlet (332 nmol/L) were extremely 417 418 lower than those found in 1995. This strong decrease can be ascribed to the restrictions of the 419 commercialization and use of NPnEOs and NPs introduced by the European Community in 2003 420 (European Parliament and Council of the European Union, 2003). It is also worth noting the sharp 421 removal increase (up to about 99%), which is obtained thanks to the introduction of the ozonation 422 stage. The data regarding the years 2014-2015 confirm the decreasing trend of the use of these 423 compounds in the textile district of Prato. However, effluent concentrations did not show any further decrease, suggesting that it is very difficult to reach the "zero" emission target requested by 424 425 the European regulation.

426 As regards the mean relative composition of APs and AP₁₋₂EOs in the WWTP inlet, in all samples, 427 4-n-NP was never detected, in agreement with findings reported in other European studies, which 428 assessed its very low or null presence in wastewater (Ciofi et al., 2016; Gatidou et al., 2007), 429 because of its much lower use, compared to branched isomers (Salgueiro-González et al., 2017). 430 Branched 4-NP isomers resulted by far the most abundant compounds among those herein investigated and showed an abundance about ten times higher than the corresponding 4-tert-octyl 431 432 derivative (Fig. 2C). Percentage abundance of NP₁₋₂EOs was also higher than that of $OP_{1-2}EOs$ 433 (about 21% vs 15%). These findings, observed also in influent water samples collected in the same 434 WWTP, both before (Lepri et al., 1997) and after (Ciofi et al., 2014; Ciofi et al., 2016) the sampling 435 period of this study, confirm the higher utilization of branched nonyl derivatives compared to the 436 homologues with 4-t-octyl chain in the textile district of Prato, in agreement with the high

437 worldwide production and utilization of NP and NP_nEO mixtures (Sharma et al., 2009). The very 438 high abundance of 4-NPs suggests a significant use of these compounds as such, rather than their 439 exclusive origin from NP_nEO degradation through progressive shortening of the ethoxylate chain. 440 The trend of the sum of AP₁₋₂EO and AP inlet concentrations clearly evidenced lower values in 441 samples taken on public holidays compared to working days, in accordance with the origin from the 442 industrial textile district of these micropollutants (**Table 2**).

443 The biological oxidation and secondary settling provided the largest removal (mean 90.6% and 444 range 88.6-99.7%), compared to sedimentation, clariflocculation and ozonation stages (A-B, C-D 445 and D-E stages, see Fig. 1). These latter depuration stages showed very similar removal efficiencies 446 (i.e. 40-45%). Furthermore, they exhibited a homogeneous behaviour, as evaluated by plotting for each treatment stage daily inlet vs outlet concentrations (R^2 of regression lines = 0.67-0.91, 447 P < 0.05). A much lower determination coefficient ($R^2 = 0.31$) was conversely found for the 448 449 biological stage (B-C, see Fig. 1), thus evidencing a higher between-days variation of the treatment efficiency. 450

As a whole, the WWTP was very effective in $AP_{1-2}EO$ and AP removal, allowing for obtaining depuration efficiencies in all cases higher than 97%. Similar removal percentages (97-99%) were achieved also for the priority hazardous 4-NPs. For this compound mixture, effluent concentrations (mean 280 ng/L; range 170-460 ng/L) satisfied the AA-EQS (300 ng/L) and were well-below MAC-EQS (2000 ng/L), in most cases also without considering the dilution factor of the receiving water body (**Table 3**). Mean outlet concentrations in accordance with the guidelines of Directive 2013/39/EU/ were also observed for the priority 4-*t*-OP.

458 TEFs of $AP_{1-2}EOs$ and APs reported in **Table 1**, which were provided by the Canadian 459 Environmental Agency (Environment Canada, 2002) were 0.5 and 1.0, respectively. According to 460 these values, the total $AP_{1-2}EO$ and AP output was, on average, about 650 ng as 4-NP_{eq}/L, which is 461 much lower than the AA-EQS when the dilution factor of the receiving water body is considered.

High enrichments were highlighted also for AP₁₋₂EOs+APs in suspended particulate matter of 462 WWTP inlet, effluents from primary and secondary sedimentation (from hundreds to tens of 463 464 mg/kg), as well as in sludge. Data concerning the sludge treatment line (Table 4) evidenced decreasing enrichments in the solid fraction, following the order F > G > H, in accordance with 465 466 considerations previously reported for PAHs and PAEs. The enrichment factors observed for AP₁. 467 ₂EOs+APs (i.e. 201, 70 and 26 in F, G and H sampling points, respectively) were in-between those 468 of PAHs and PAEs, consistently with log K_{OW} values of compounds actually determined in the 469 samples. The higher hydrophobicity of branched 4-NP isomers, compared to the other APs and AP₁. 470 ₂EOs investigated, was also responsible of the higher enrichment factors observed for this 471 compound (i.e 224, 92 and 34 in F, G and H sampling points, respectively) in all the three sampling 472 points of sludge.

473 Concentrations of APs and $AP_{1-2}EOs$ in the fume scrubber and fly ash were very similar to those 474 previously shown and discussed for PAEs (**Table 4**), consistently with the fact that they can only 475 undergo processes of destruction during combustion.

476 *3.2 Mass balance*

Table 5 shows the mass of heavy-metals and organic compound classes herein investigated, passing through the various sampling points of **Fig. 1**. **Table 6** illustrates the mass balances (MB) deriving from equations 4-9, together with the percentage imbalances (Δ), obtained as the percentage ratio between the mass balance and the total positive contributions to a given treatment section. Hence, positive values of MB and Δ represent net removal within each WWTP section and whole facility.

A correct approach to the evaluation of the results of a mass balance calculation for a given substance, must take into account its physicochemical properties, which determine its fate during the various stages of wastewater and sludge treatment. Among them, it should be considered the tendency of the substance to undergo transfer from the liquid phase to the atmosphere or to be degraded by the activated sludge biomass. Furthermore, it is required the achievement of a dynamic equilibrium between all the positive and negative contributions to the mass balance itself. In this 488 regard, it should be remarked that, even within the same depuration section, such an equilibrium is 489 difficult to be obtained because of the many processes and treatments included in each section. 490 Another limitation in the evaluation of the mass balance of P and PH substances derives from their 491 low concentrations (i.e. ppt-ppb). Hence, even small measuring inaccuracies, which are intrinsic in 492 the determination of so low concentrations, may have a significant impact on the correctness of the 493 mass balance calculations, due to the error propagation related to the multiplying factors of volumetric flow rates of WWTP and coagulant/flocculant reagents, as well as ash amounts 494 495 produced by the incinerator (see equations 1-3).

As regards the contribution of the addition of coagulant/flocculant reagents to the mass balance, the analysis performed on these reagents did not reveal concentrations above method detection limits with the only exception of Cd and Pb in FeCl₃, which is dosed at the R1 dosage point (see **Fig. 1**). More in detail, for these metals, concentrations of 15.1 ± 3.2 and 92.4 ± 3.9 mg/L were determined based on five replicated analyses in the FeCl₃ reagent.

501 *3.2.1 Cd and Pb*

Among the chemical species analysed herein, metals can be considered reference substances for mass balance calculation, since they are not subject to degradation and have a negligible vapour pressure, thus exhibiting an insignificant tendency to transfer in the aerosol/gaseous phase even during the aeration process in biological oxidation tanks.

Data reported in **Table 6** showed that a mass balance equal to zero was not obtained in any of the investigated treatment sections. However, the absolute values of the imbalances were always quite small when compared to the total masses of the metals entering the sections, thus generating relatively small percentage imbalances (i.e Pb between -1.5% and 13.9% and Cd between -9.1% and 17.1%). The presence of these imbalances should not be surprising since even higher inaccuracies in the evaluation of the mass balance of WWTPs have been reported through the determination of conservative species (Clara et al., 2010).

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513 As regards the section of sludge treatment (equation 8), it should be noted that the analysed metals 514 are more or less transferred to the incinerator fumes, depending on their greater or lesser volatility (vapour pressure at 25°C equal to $3.12 \cdot 10^{-9}$ and $4.26 \cdot 10^{-24}$ Pa, for Cd and Pb, respectively), and may 515 therefore escape from the chimney. Even though the analysis of the fumes flowing out the chimney 516 517 plume was not included in our study design, GIDA S.p.A. provided weekly data regarding the 518 concentrations of Cd and Pb in the plume within the period under investigation. Cd and especially 519 Pb concentrations were found in wide ranges of concentrations (0.6-2.8 and 0.2-10.5 μ g/m³, 520 respectively), evidencing the high variability of this contribution. These concentrations can be 521 converted in masses per day by multiplying them by the daily air mass flowing out from the smokestack (about 380,000 m³/d), thus obtaining average values of about 0.5 and 2.6 g/d, for Cd 522 523 and Pb, respectively, which account for only 1.2% and 1.6% of the mass balances reported in Table 524 6, being they therefore negligible.

525 3.2.2 Organic compounds

526 Preliminary treatment and primary sedimentation. Imbalance percentages of 8.2, 15.4 and 27.5 were determined within this section for PAHs, PAEs and APs+AP₁₋₂EOs, respectively (Table 6). For 527 528 PAHs the imbalance was of the same magnitude order of those found in the same section for metals, 529 which are considered reference species, as they are conservative. These findings are in agreement 530 with the characteristics of this type of process that should not provide any significant degradation or 531 transfer into aerosol and/or gaseous phase of the target compounds. However, it should be noted 532 that for PAEs and especially APs+AP₁₋₂EOs the percentage of imbalance assumed a higher value 533 than the upper imbalance limit observed for the metals, thus suggesting some contribution of 534 degradation to the mass balance of these compounds. The occurrence of degradation even in the 535 primary sedimentation can be explained by the presence of sludge recirculation coming from secondary sedimentation (Fig. 1). Going into detail of the distribution of the various classes within 536 537 this treatment section, the amounts of target compounds adsorbed on sludge coming out the settler

538 (sampling point F) were in between 14.4% and 17.2% of the total inlets of the section (see **Table 5**), 539 which included the sludge recirculating from the secondary sedimentation and clariflocculation 540 (sampling points G and H, see Fig. 1). Interestingly, the PAH fraction adsorbed by the solid phase 541 during the primary sedimentation (17.2%) was quite similar to that elsewhere found (23.3%) on a 542 wastewater dominated by the presence of naphthalene (Manoli and Samara, 2008). Moreover, the 543 value determined for phthalates (15.2%) is in good agreement with the one found (20%) for the 544 primary sedimentation of a wastewater containing DEHP as predominant phthalic acid ester (Fauser 545 et al., 2003). Also with regards the percentage of transfer in the primary sludge of APs+AP₁₋₂EOs, 546 the result obtained in this study (14.4%) is quite similar to some values reported in literature (19-547 21%) (Loyo-Rosales et al., 2007).

548 Biological treatment and secondary sedimentation. This section provided a very high net removal 549 for all the investigated classes of organic compounds. As illustrated by Table 6, the highest 550 efficiency was found for APs+AP₁₋₂EOs (85.5%), followed by PAEs (74.5%) and PAHs (56.8%). 551 The removal of these organic compound classes may be attributed in principle to degradation, 552 volatilisation and aerosolization processes. Based on data previously reported regarding PAHs, 553 PAEs, AP₁₋₂EOs and APs emitted by aerosolization from the Baciacavallo biological oxidation tank (Lepri et al., 2000; Masi et al., 1999), the contribution to the mass balance of this process is 554 555 negligible when compared to the mass of the targeted compounds flowing along the treatment. On 556 the other hand, removal due to the transfer in the gaseous phase occurring in the aeration tank, may 557 significantly contribute to the overall removal of the section of biological oxidation for organic 558 compounds characterized by high values of the Henry's constant, such as Nap (Manoli and Samara, 559 1999; Qiao et al., 2014), whereas it is considered negligible for high molecular weight PAEs, as 560 well as for AP₁₋₂EOs+APs (McAdam et al., 2010; Tan et al., 2007).

561 Some literature studies provided data regarding the mass balance of PAHs in biological oxidation 562 treatment sections, within CASP (Manoli and Samara, 1999; Ozaki et al., 2015), as well as 563 anoxic/anaerobic/oxic (A/A/O) (Qiao et al., 2014; Zhao et al., 2018) and anaerobic/oxic (A/O)

treatments (Qiao et al., 2014; Sun et al., 2018; Yao et al., 2012). Interestingly, the loss of PAHs 564 565 herein obtained in the biological section with HRT = 6-13 h and MLSS = 5.5 g/L (56.8%) was included in the range of values (46.9-63.0%) elsewhere obtained by CASP with HRT of 3-8 hours 566 567 and MLSS concentrations of 1.5-3.5 g/L (Manoli and Samara, 1999; Ozaki et al., 2015). 568 Conversely, mass balance of PAHs in A/A/O and A/O biological treatments evidenced much lower 569 net removals of PAHs (0-20%) (Liu et al., 2017; Qiao et al., 2014; Sun et al., 2018; Yao et al., 570 2012) and correspondingly much higher mass transfers in sludge. In fact, sorption on sludge in 571 A/A/O and A/O systems accounted for 60-65% of PAHs entering in the section (Qiao et al., 2014; 572 Sun et al., 2018; Yao et al., 2012), a much higher percentage compared to what was observed in this 573 study (17.8%, see **Table 5**) and other researches published on CASP (Manoli and Samara, 1999; 574 Ozaki et al., 2015). It should also be noted that only using HRT as high as 40 hours and 575 temperatures of 30-35°C, it was possible to achieve PAH losses of about 50% in a pilot-scale 576 A/A/O system (Zhao et al., 2018).

Lower biotransformation performances of A/A/O and especially A/O systems (i.e. less than 10%)
have been also reported for PAEs, in comparison with oxic treatments (about 60%) (Gao et al.,
2014). Interestingly, the percentage of biotransformation obtained in this study (i.e. 74.5%, see **Table 6**) was quite similar to the values elsewhere obtained within facilities adopting CASP (6288%) (Balabanic and Klemencic, 2011; Fauser et al., 2003; Roslev et al., 2007).

582 AP₁₋₂EOs+APs showed the highest mass loss in the biological section (85.5%) and a low sorption 583 percentage on sludge (8.8%). These data were comparable with the ones obtained with CASP by 584 Fauser et al. (degradation percentage 70.7% and sorption on sludge of 8.6%) and Pothitou et al. 585 (degradation percentage 90% and sorption on sludge of about 2%) (Fauser et al., 2003; Pothitou and 586 Voutsa, 2008). The latter performances, which are the highest reported in literature, were attributed 587 to the very high HRT (94 h) and wastewater temperatures during the sampling period (22–30 $^{\circ}$ C). 588 In this regard, it should be remarked that a strong correlation between degradation rate of AP_{1} . 589 ₂EOs+APs and temperature was highlighted (Loyo-Rosales et al., 2007). Accordingly, activated

sludge plants working at low temperatures (9°C and 6°C) and lower HRT (6-14 and 5.6 h), provided for lower biotransformation capabilities, with the accumulation of AP₁EO and AP₂EO, as well as their corresponding carboxylate derivatives as intermediates in the shortening process of the ethoxylate chain, which is a well assessed degradation process for AP₁₋₂EOs (Loyo-Rosales et al., 2007; McAdam et al., 2010).

595 <u>Final clariflocculation</u>. Very low percentages of imbalance (6.6, 7.4 and -4.7 for PAHs, PAEs and 596 APs + AP₁₋₂EOs, respectively) were obtained in this section, thus highlighting the absence of 597 degradation for all the classes examined. Approximately 20% of PAHs and PAEs and 40% of 598 APs+AP₁₋₂EOs entering the clariflocculation tank are adsorbed on sludge (sampling point H, see 599 **Table 5**) and are therefore eliminated from the water phase, as a consequence of the removal of 600 colloids, prior to ozonation.

601 Ozonation. The ozonation section, which simply refers to the balance between inlet and outlet of the 602 ozonation tank, provided a significant degradation for all the three classes of organic pollutants. The 603 percentage imbalance of this section was in fact equal to 97.0%, 72.9% and 41.3%, for PAHs, PAEs 604 and APs+AP₁₋₂EOs, respectively (see Table 6). The much lower removal efficiency of ozone 605 towards APs+AP₁₋₂EOs could be considered as unrealistic, since the ozone-based conversion of 606 long ethoxylate chain oligomers in oligomers with shorter ethoxylate chain, as well as APs, may 607 occur. In fact, AP_nEO transformation via advanced oxidation processes, including ozone treatment, 608 has in general been suggested to proceed preferentially via fragmentation of the ethoxylate chain 609 length due to high specific rate constants of the hydroxyl radical toward ethylene oxide subunits, 610 rather than the aromatic ring and the alkyl chain (Karci, 2014). Furthermore, the determination of 611 the overall kinetic rate constants of the degradation by ozone of selected PAHs (i.e. Fl, Chr and 612 B(a)Pyr) and PAEs (i.e. DNBP) in water, generally highlighted a faster kinetic for the former 613 compounds than the latter (Miller and Olejnik, 2004; Wen et al., 2011), thus giving a possible 614 explanation of the different removal observed for the two classes.

Sludge treatment. As expected, this section provided an almost complete mass loss of organic micropollutants investigated, in accordance with the presence of an incineration step of dewatered sludge (see **Table 6**). Within this section two outputs can be identified, namely the water from sludge thickening and dewatering (sampling point I, see **Fig. 1**) and the water from fume scrubber (sampling point L, see **Fig. 1**). Between them, only the water extracted from sludge contained appreciable amounts of the investigated micropollutants, since PAHs, PAEs and APs+AP₁₋₂EOs accounted for 7.3%, 2.9% and 2.4% of the masses entering the section (see **Table 5**).

622 4 Conclusions

The mass balance approach for PAHs, PAEs and APs+AP₁₋₂EOs, validated by monitoring Pb and Cd as conservative species, allowed to assess the removal contribution of each treatment section of the Baciacavallo WWTP, as well as the very good overall performances of the facility. In fact, as illustrated in **Table 6**, the whole plant provided an almost quantitative net removal of all targeted micropollutants (Δ =96.7-98.4%). The overall removal efficiencies of the WWTP were high enough to accomplish the European EQS for all the investigated compounds.

Comparing the various mass losses found in the different sections of the facility (Table 6) with the 629 630 total masses of the investigated classes of organic micropollutants entering the WWTP (M_{X,A}, see 631 Table 5), it was clear that biotransformation occurring in the aeration tank was the most important 632 removal process. In fact, the biological section provided mass loss percentages of 50-56%, 633 depending on the class considered. As previously mentioned, primary sedimentation seems to 634 provide some biotransformation, as well. In fact, mass losses accounting for about 17% and 29% 635 were calculated for PAEs and APs+AP₁₋₂EOs, compared to the total quantities of these classes 636 inflowing the WWTP. The mass transfer from water to sludge and its incineration was a further important removal mechanism inside the plant, which contributed to overall mass losses of 15-19% 637 638 of the total amounts of inflowing micropollutants. Similar percentages of mass losses were also 639 provided by ozonation for PAHs (17.4% of the total amounts of PAHs entering the WWTP), 640 whereas a much lower contribution was observed for PAEs (7.5%) and especially APs+AP₁₋₂EOs

- 641 (0.9%). In this regard, the crucial role of ozonation for fulfilling European EQS for effluent PAH
- 642 concentrations should be emphasized.
- 643 Moreover, data provided in this research on $AP_{1-2}EOs$ and APs in the inlet of the Baciacavallo
- 644 WWTP, together with those achieved in 1995 and 2014-2015, highlighted a decreasing temporal
- trend of their occurrence in the Prato textile district during the last 20 years. This decrease can be
- 646 ascribed to the restrictions of the commercialization and use of NP_nEOs and NPs introduced by the
- 647 European Community in 2003.

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Table 1 – Chemical name of target analytes, abbreviations, logarithms of the octanol-water partition coefficients (log K_{OW})^(a), toxicity equivalence factors (TEFs) and attribute of priority (P) or priority hazardous (PH) substance, according to the Directive 2013/39/EU.

Compound	Abbreviation	Log K _{OW}	TEFs	P and PH Attribute
PAHs				
Naphthalene	Nap	3.36	0.001^{f}	Р
Acenaphthylene	Acy	3.27	$0.001^{\rm f}$	-
Acenaphthene	Ace	3.73	$0.001^{\rm f}$	-
Fluorene	Fl	4.32	0.001^{f}	-
Phenanthrene	Phe	4.55	0.001^{f}	-
Anthracene	Ant	4.55	0.01^{f}	PH
Fluoranthene	Flu	5.00	0.001^{f}	Р
Pyrene	Pyr	5.00	0.001	-
Benzo(a)anthracene	B(a)A	5.73	0.1^{f}	-
Chrysene	Chr	5.73	0.01^{f}	-
Benzo(b)fluoranthene	B(b)Flu	6.19	0.1^{f}	PH
Benzo(k)fluoranthene	B(k)Flu	6.19	0.1^{f}	PH
Benzo(a)pyrene	B(a)Pyr	6.19	$1.0^{\text{ f}}$	PH
Indeno(1,23-c,d)pyrene	IPyr	6.65	0.1^{f}	PH
Dibenzo(a,h)anthracene	D(ah)A	6.91	$1.0^{ m f}$	-
Benzo(g,h,i)perylene	BPy	6.65	0.01^{f}	PH
PAEs				
Di-n-butyl phthalate	DBP	4.75	0.9 ^g	-
Di-isobutyl phthalate	DIBP	4.44	0.9 ^g	-
Di-n-pentyl phthalate	DNPP	5.77	n.a.	-
Di-n-hexylphthalate	DNHP	6.79	n.a.	-
Benzyl butyl phthalate	BBP	4.91	1.0 ^g	-
Di(2-ethylhexyl) phthalate	DEHP	8.52	1.0 ^g	PH
Di-n-decylphthalate	DNDP	10.87	n.a.	-
APs and AP ₁₋₂ EOs				
4-tert-Octylphenol	4- <i>t</i> -OP	5.18	1.0^{h}	Р
4-tert-Octylphenol monoethoxylate	$4-t-OP_1EO$	4.99	0.5^{h}	-
4-tert-Octylphenol diethoxylate	$4-t-OP_2EO$	4.90	0.5^{h}	-
4-n-Nonylphenol	4-n-NP	6.14	1.0^{h}	-
Branched 4-Nonylphenol ^(b)	4-NP	5.79-5.94	1.0^{h}	PH
Branched 4-Nonylphenol monoethoxylate ^(c)	$4-NP_1EO$	5.60	0.5^{h}	-
Branched 4-Nonylphenol diethoxylate ^(d)	$4-NP_2EO$	5.50 ^(e)	0.5^{h}	-

^(a) Calculated using the Chemistry Development (ACD/Labs) Software V11.02 (© 1994-2015 ACD/Labs). ^{b)} The sum of branched 4-nonylphenols has been evaluated on the basis of the reference standards 4-(1,1-dimethylheptyl)-phenol (4-NP₉), 4-(1-ethyl-1-methylhexyl)-phenol (4-NP₆₅), 4-(1-ethyl-1,3-dimethylpentyl)-phenol (4-NP₁₁₁) and 4-(1-ethyl-1,4-dimethylpentyl)-phenol (4-NP₁₁₂). ^{c)} The sum of branched 4-nonylphenols monoethoxylate has been evaluated on the basis of the reference standard "technical mixture of branched 4-nonylphenol monoethoxylate". ^{d)} The sum of branched 4-nonylphenols diethoxylate has been evaluated on the basis of the reference standard "technical mixture of branched 4-nonyl phenol diethoxylate". ^{e)} This log K_{OW} value refers to the corresponding compound with linear alkyl chain, since that of the branched oligomer was not available. ^{f)} Nisbet and LaGoy, 1992. ^{g)} Del Bubba et al., 2018. ^{h)} Environment Canada, 2002.

Table 2 - Total concentrations (μ g/L) of PAHs, PAEs, APs, AP₁EOs and AP₂EOs, together with priority hazardous compounds (Ant, B(b)Flu, B(k)Flu, B(a)Pyr, IPyr, BPy, DEHP and 4-NP) found in the different WWTP collection points during the seven samplings. **A** - Influent post grilling. **B** - Biological oxidation input. **C** - Secondary settling outlet. **D** - Clariflocculation outlet. **E** – Effluent. See Table 1 for the meaning of the abbreviations of priority hazardous compounds.

Sampling	1	2	3	4	5	6	7
Total PAHs							
A	38	32	66	6.3	12	11	5.6
В	35	31	52	4.9	9.2	7.4	3.2
С	3.6	10	15	2.5	1.9	2.3	1.1
D	2.5	8.2	13	1.5	1.3	2.1	0.85
Ε	0.13	0.16	0.22	0.075	0.078	0.10	0.15
Priority haza	rdous PAH	ls					
Α	18	6.2	3.6	0.60	1.2	1.3	0.75
Ε	0.030	0.062	0.030	0.028	0.032	0.031	0.029
Total PAEs							
Α	209	290	100	146	265	128	85
В	146	181	69	127	197	96	71
С	24	37	27	12	25	11	19
D	21	26	21	9.1 20		8.5	12
Ε	9.9	4.4	8.3	3.2	3.3	0.38	0.40
Priority haza	rdous PAE	s (DEHP)					
Α	128	190	75	132	235	118	76
Ε	8.7	4.3	7.2	3.1	3.1 3.2 0.27		0.30
$APs + AP_{1-2}E$	COs						
Α	87	133	87	21	77	111	35
В	53	68	64	8.0	43	68	22
С	2.8	3.1	3.2	2.4	4.0	3.0	1.6
D	1.7	1.8	2.2	1.1	2.2	1.6	0.98
Ε	0.53	1.3	1.4	0.58	1.2	0.89	0.55
Priority haza	rdous APs	(NPs)					
Α	44	42	61	14	27	40	22
Ε	0.22	0.30	0.46	0.32	0.24	0.28	0.17

Table 3 – WWTP effluent concentrations (EC) of priority ^(a) and priority hazardous ^(b) compounds belonging to the classes of polycyclic aromatic hydrocarbons, phthalates and alkylphenols, as such and corrected for the dilution factor (EC-DF) of the receiving water body. These values are shown in comparison with annual average and maximum allowable concentration environmental quality standards (AA-EQS and MAC-EQS), as reported in the Directive 2013/39/EU. All values are expressed as ng/L; n.r. = not reported. The meaning of the compound abbreviations is reported in Table 1.

	EC	EC-DF	AA-EQS	MAC-EQS
Nap ^(a)	19.6	0.98	2000	130000
Ant ^(b)	5.9	0.30	100	100
Flu ^(a)	6.1	0.31	6.3	120
B(b)Flu ^(b)	7.3	0.37	n.r.	17
B(k)Flu ^(b)	5.1	0.26	n.r.	17
B(a)Pyr ^(b)	5.9	0.30	0.17	270
IPyr ^(b)	5.3	0.27	n.r.	n.r.
BPy ^(b)	5.3	0.27	n.r.	8.2
DEHP ^(b)	3900	195	1300	n.r.
$4-t-OP^{(a)}$	80	4	100	n.r.
4-NP ^(b)	280	14	300	2000

Table 4 - Mean concentrations (n=7) and ranges (in bracket) of total and priority hazardous PAHs, PAEs and APs+AP₁₋₂EOs in different WWTP sampling points. **F** - sludge from primary settling. **G** - sludge from secondary settling. **H** - sludge from clariflocculation. **I** - water from sludge dewatering. **L** - water from fume scrubber. **K** - Ash. Results are expressed in μ g/L for sampling points **F-I** and in mg/kg for sampling point **K**. ^(a) Aqueous supernatant of sludge samples. ^(b) Solid fraction of sludge samples. Bql=below quantification limit. See Table 1 for the meaning of the abbreviations.

	$\mathbf{F}^{(a)}$	$\mathbf{F}^{(\mathrm{b})}$	$\mathbf{G}^{(a)}$	$\mathbf{G}^{(b)}$	$\mathbf{H}^{(a)}$	$\mathbf{H}^{(b)}$	Ι	L	К
PAHs									
Total	6.8 (1.8-14)	687 (261-1423)	7.4 (3.6-15)	324 (147-571)	2.3 (0.98-4.6)	23 (3.5-42)	45 (7.3-144)	3.2 (0.58-7.9)	0.28 (0.19-0.37)
Priority hazardous (B(b)Flu; B(k)Flu; B(a)Pyr; IPyr; BPy)	0.79 (0.42-1.7)	114 (43-192)	0.94 (0.19-1.9)	58 (20-187)	0.32 (0.12-0.68)	3.0 (0.31-7.3)	8.20 (1.2-25)	0.47 (0.13-0.66)	0.051 (0.019-0.13)
PAEs									
Total	15 (9.9-22)	3722 (665-6994)	5.7 (3.3-7.6)	778 (283-1215)	5.2 (1.7-9.1)	113 (28-209)	97 (12-347)	1.7 (0.42-2.9)	0.052 (0.021-0.11)
Priority hazardous (DEHP)	11 (6.4-14)	3038 (564-4680)	4.6 (2.5-6.6)	681 (222-1180)	3.3 (1.5-5.9)	89 (26-193)	82 (8.4-298)	1.15 (0.36-2.44)	0.022 (Bql-0.071)
APs+AP ₁₋₂ EOs									
Total	8.7 (4.6-14)	1749 (363-4865)	5.1 (3.5-8.3)	357 (127-524)	1.6 (0.7-3.2)	42 (3.2-85)	36 (19-54)	2.9 (0.9-4.7)	0.077 (Bql-0.25)
Priority hazardous (4-NPs)	6.8 (2.9-11)	1525 (366-4309)	2.8 (1.8-3.9)	257 (100-419)	0.93 (0.46-1.8)	32 (2.5-65)	28 (13-48)	1.6 (0.43-3.7)	0.13 (Bql-0.25)

Table 5 – Mass (g/d) of lead, cadmium, polycyclic aromatic hydrocarbons (PAHs), phthalates (PAEs) and the sum of alkylphenols and ethoxylated alkylphenols (APs + AP₁₋₂EOs) at the different sampling points of the investigated wastewater treatment plant (WWTP). A - influent post grilling. B - biological oxidation input. C - secondary settling outlet. D - clariflocculation outlet. E - effluent F - sludge from primary settling. G - sludge from secondary settling. H - sludge from clariflocculation. I - water from sludge dewatering. L - water from fume scrubber. K - ashes. R1 – Point of release of FeCl₃ + anionic polyelectrolyte. R2 – Point of release of AlCl₃ + anionic polyelectrolyte.

	Lead	Cadmium	PAHs	PAEs	APs+AP ₁₋₂ EOs
$M_{X,A}$	11395	982	18549	123391	59077
$M_{X,B}$	13385	1526	16438	92396	36786
$M_{X,C}$	11412	922	4157	16491	2115
$M_{X,D}$	9825	644	3334	12652	1242
$M_{X,E}$	9323	620	98.4	3434	729
$\mathbf{M}_{\mathrm{X,F}}$	1946	413	3787	20149	9109
$M_{X,G}$	1581	386	2937	7035	3221
$M_{X,H}$	889	269	557	2628	982
$M_{\mathrm{X,I}}$	112	15	278	575	221
$M_{X,K}$	1490	355	10.7	1.9	2.9
$M_{X,L}$	177	2.1	10.0	5.2	9.7
$M_{X,R1}$	1298	212	0	0	0
$M_{X,R2}$	0	0	0	0	0
$M_{X,R3}$	0	0	0	0	0

Table 6 – Mass Balance (MB, g/d) and percentage imbalance (Δ) for lead, cadmium, polycyclic aromatic hydrocarbons (PAHs), phthalates (PAEs) and the sum of alkylphenols and ethoxylated alkylphenols (APs+AP₁₋₂EOs) in the different sections of the investigated wastewater treatment plant (WWTP). Equations in bracket represent the mass balance of each section for the generic chemical species "X". A - influent post grilling. B - biological oxidation input. C - secondary settling outlet. D - clariflocculation outlet. E - effluent F - sludge from primary settling. G - sludge from secondary settling. H - sludge from clariflocculation. I - water from sludge dewatering. L - water from fume scrubber. K - ashes. R1 – Point of release of FeCl₃ + anionic polyelectrolyte. R2 – Point of release of AlCl₃ + anionic polyelectrolyte. R3 – Point of release of cationic polyelectrolyte.

	Le	ad	Cadmium PAHs		Hs	PAEs		APs+AP ₁₋₂ EOs		
WWTP Section	MB	Δ	MB	Δ	MB	Δ	MB	Δ	MB	Δ
Preliminary treatment and primary sedimentation $(M_{X,A} - M_{X,B} - M_{X,F} + M_{X,G} + M_{X,H} + M_{X,R1})$	-168	-1.5	-90	-9.1	1818	8.2	20509	15.4	17385	27.5
Biological treatment and secondary sedimentation $(M_{X,B} - M_{X,C} - M_{X,G})$	392	2.9	217	14.2	9344	56.8	68870	74.5	31450	85.5
Final clariflocculation $(M_{X,C} - M_{X,D} - M_{X,H} + M_{X,L} + M_{X,R2})$	875	7.5	11.4	1.2	276	6.6	1216	7.4	-99	-4.7
Ozonation $(M_{X,D} - M_{X,E})$	503	5.1	23.8	3.7	3236	97.0	9218	72.9	513	41.3
Sludge treatment $(M_{X,F} - M_{X,I} - M_{X,K} - M_{X,L} + M_{X,R3})$	166	8.5	41.2	10.0	3488	92.1	19567	97.1	8875	97.4
$\label{eq:Whole plant} Whole plant \\ (M_{X,A} \text{-} M_{X,E} \text{-} M_{X,K} \text{-} M_{X,I} + M_{X,R1} + M_{X,R2} + M_{X,R3})$	1768	13.9	204	17.1	18162	97.9	119380	96.7	58124	98.4



Figure 1 – Scheme of the wastewater treatment plant investigated. Letters in bracket indicate sampling points of wastewater (A-E, I and L), sludge (F-H) and ash (K), as well as dosage points of the coagulant/flocculant reagents (R1, R2 and R3) involved in the calculation of mass balance in the various sections of the facility. Solid and dotted lines refer to the transfer within various treatment stages of wastewater and sludge, respectively.



Figure 2 – Mean relative percentages (n=7) of the investigated PAHs (A), PAEs (B), $AP_{1-2}EOs$ and APs (C) in the WWTP influent and effluent. Error bars represent the standard deviations. The meaning of the compound abbreviations is reported in Table 1.



Figure 3 – Trend of the sum of $AP_{1-2}EOs$ and APs (nmol/L) determined in the inlet (black symbols) and outlet (white symbols) of the Baciacavallo WWTP in the period 1995-2015.

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