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# A thorough analysis of the occurrence, removal and environmental risks of organic micropollutants in a full-scale hybrid membrane bioreactor fed by hospital wastewater

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## HIGHLIGHTS

- A 1-year investigation on micropollutant removal was carried out on a full scale MBR.
- 232 target and 90 non-target organic micropollutants were monitored.
- 0.1 g/L and 0.2 g/L of PAC added in the MBR acting as an hybrid MBR
- Key organic micropollutants selected by means of three new approaches
- Removal significantly enhanced for antibiotics and psychiatric drugs at 0.2 g/L of PAC.

## GRAPHICAL ABSTRACT
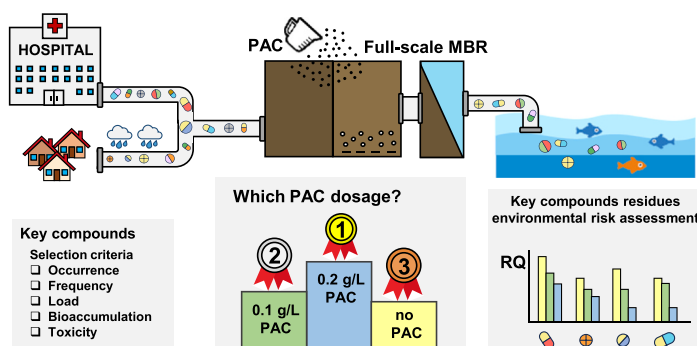


## ARTICLE INFO

## ABSTRACT

The Urban Wastewater Treatment Directive recent draft issued last October 2022 pays attention to contaminants of emerging concern including organic micropollutants (OMPs) and requires the removal of some of them at large urban wastewater treatment plants (WWTPs) calling for their upgrading. Many investigations to date have reported the occurrence of a vast group of OMPs in the influent and many technologies have been tested for their removal at a lab- or pilot-scale. Moreover, it is well-known that hospital wastewater (HWW) contains specific OMPs at high concentration and therefore its management and treatment deserves attention. In this study, a 1-year investigation was carried out at a full-scale membrane bioreactor (MBR) treating mainly HWW. To promote the removal of OMPs, powdered activated carbon (PAC) was added to the bioreactor at 0.1 g/L and 0.2 g/L which resulted in the MBR operating as a hybrid MBR. Its performance was tested for 232 target and 90 non-target OMPs, analyzed by UHPLC-QTOF-MS using a direct injection method. A new methodology was defined to select the key compounds in order to evaluate the performance of the treatments. It was based on their frequency, occurrence, persistence to removal, bioaccumulation and toxicity. Finally, an environmental risk assessment of

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the OMP residues was conducted by means of the risk quotient approach. The results indicate that PAC addition increased the removal of most of the key OMPs (e.g., sulfamethoxazole, diclofenac, lidocaine) and OMP classes (e.g., antibiotics, psychiatric drugs and stimulants) with the highest loads in the WWTP influent. The hybrid MBR also reduced the risk in the receiving water as the PAC dosage increased mainly for spiramycin, lorazepam, oleandomycin. Finally, uncertainties and issues related to the investigation being carried out at full-scale under real conditions are discussed.

## Abbreviations

| | |
|---|---|
| **0.1PAC** | experimental campaign MBR with PAC added at 0.1 g/L to the bioreactor |
| **0.2PAC** | experimental campaign MBR with PAC added at 0.2 g/L to the bioreactor |
| **AOP** | advanced oxidation process |
| **B** | bioaccumulation |
| **BET** | Brunauer-Emmett-Teller |
| **BOD$_5$** | biological oxygen demand |
| *c* | concentration |
| **CAS** | conventional activated sludge |
| **CEC** | contaminant of emerging concern |
| **COD** | chemical oxygen demand |
| **DI** | direct injection |
| **DOC** | dissolved organic carbon |
| **DOM** | dissolved organic matter |
| **EFF** | WWTP effluent |
| **F** | frequency (of occurrence) |
| **FO$_{av}$BT** | frequency, average occurrence, bioaccumulation and toxicity |
| **FO$_{max}$BT** | frequency, maximum occurrence, bioaccumulation and toxicity |
| **FLBT** | frequency, load, bioaccumulation and toxicity |
| **FOPBT** | frequency, occurrence, persistence, bioaccumulation and toxicity |
| **FPBT** | frequency, occurrence, persistence, bioaccumulation and toxicity |
| *Freq* | frequency of detection |
| **GAC** | granular activated carbon |
| **HRMS** | high-resolution mass spectrometer |
| **HRT** | hydraulic retention time |
| *i* | a generic target OMP ($i = 1, 2, ..., 232$) |
| **INF** | WWTP influent |
| **IQR** | interquartile range |
| **ISTD** | isotopically labelled internal standards |
| *j* | experimental campaign ($j = 1, 2, 3$) |
| *k* | generic day in a sampling campaign |
| **L** | load |
| **LOD** | limit of detection |
| **LOQ** | limit of quantification |
| **log$K_{ow}$** | octanol-water partition coefficient |
| **log$D_{ow}$** | octanol-water distribution coefficient |
| **HWW** | hospital wastewater |
| *m* | number of values considered to calculate the removal efficiency |
| **MBR** | membrane bioreactor |
| **MBRperm** | MBR permeate |
| **ME** | matrix effect |
| *n* | number of samples/sampling days |
| **na** | data not available |
| **nd** | not detected |
| **NITRO** | nitrification tank |
| **noPAC** | experimental campaign referred to the MBR |
| **NTS** | non-target screening |
| **N$_{tot}$** | total nitrogen |
| **O** | occurrence |
| **OMP** | organic micropollutant |
| **P** | persistence |
| **PAC** | powdered activated carbon |
| **PE** | person equivalent |
| **p$K_a$** | acid dissociation constant |
| **PNEC** | predicted no-effect concentration |
| **P$_{tot}$** | total phosphorous |
| **Q** | flow rate |
| *q* | number of OMPs in a mixture |
| **R** | removal efficiency |
| **Signal Resp** | OMP signal response in the INF |
| **RQ** | risk quotient |
| **$\overline{RQ}$** | average risk quotient |
| **RQ$_{mix}$** | risk quotient of a mixture of OMPs |
| **RQ$_{sp}$** | specific risk quotient |
| **S** | final score |
| **SD** | standard deviation |
| **SPE** | solid phase extraction |
| **SRT** | sludge retention time |
| **T** | toxicity |
| **TSS** | total suspended solids |
| **UHPLC-QTOF-MS** | ultra-high performance liquid chromatography system coupled to hybrid quadruple time-of-flight mass spectrometry |
| **UV** | ultraviolet radiation |
| **UV$_{254}$** | ultraviolet absorbance at 254 nm |
| **UWW** | urban wastewater |
| **UWWTD** | Urban Wastewater Treatment Directive |
| **VSS** | volatile suspended solids |
| **WWTP** | wastewater treatment plant |

*Subscripts*

| | |
|---|---|
| INF | WWTP influent |
| perm | MBR permeate |

## 1. Introduction

Hospital wastewater (HWW) has been a topic of interest for many years, with an increasing number of investigations aiming to treat it in many countries worldwide (Khan et al., 2020; Kumari et al., 2020; Mousel et al., 2021; Verlicchi, 2021; Verlicchi et al., 2015). The

numerous activities taking place in hospital facilities (including in- and out-patient care, administration of pharmaceuticals, surgeries, research and diagnosis) lead to the release of conventional contaminants and contaminants of emerging concern (CECs) into wastewater, which requires an accurate management and adequate treatments. Concerns are usually related to the high concentration of organic micropollutants (OMPs) such as pharmaceuticals, disinfectants and X-ray contrast media (Oliveira et al., 2018); the presence of microplastics (Tuvo et al., 2023);

and the microbiological load, also related to antibiotic resistance bacteria and genes (ARB and ARGs, respectively) (Paulus et al., 2019), which can lead to the spread of persistent infections and exert pressure on the development of new antibiotics to deal with them (Miethke et al., 2021). In this regard, HWW has been identified as one of the main sources of antibiotic release into the environment (Chonova et al., 2018). In addition to this, cytostatic agents, radioactive markers, solvents and heavy metals present in preservatives and diagnostic agents (e.g., gadolinium, mercury and zinc) are also frequently discharged from hospitals (Wiest et al., 2018). In short, HWW may contain a higher proportion of toxic (Casas et al., 2015), persistent (Weissbrodt et al., 2009) and infectious pollutants (Paulus et al., 2019) than domestic wastewater. The unique composition of HWW, together with its observed seasonal variations and the occurrence of disease outbreaks, calls for the adoption of further and/or dedicated treatments of this type of wastewater (Verlicchi et al., 2013, 2015). While in a few countries HWW is pre-treated or treated on-site, as reported in Deliverables 4.1 and 4.3 of the Interreg Project Less is More (https://gfw.pl/projekty/less-is-more/) and in (Casas et al., 2015; Wiest et al., 2018), in most countries it is still considered as having the same polluting nature as domestic wastewater, and is thus directly discharged into municipal sewers and co-treated at urban wastewater treatment plants (WWTPs) (Kumari et al., 2020; Verlicchi et al., 2015). As is widely known, conventional treatments have proven to be inefficient in removing many CECs (Verlicchi et al., 2012b) and, therefore, enhanced and hybrid technologies, as well as novelty solutions, have been tested in both developed and developing countries (Bui et al., 2016) and some of them have been suggested in the upgrading of existing WWTPs (Pistocchi et al., 2022a). Many studies have focused on the use of advanced biological treatments such as membrane bioreactors (MBR) in combination with other biological, chemical and physical technologies, in the so-called enhanced or hybrid MBRs (among them, Goswami et al. (2018)) to treat HWW as well as domestic wastewater. The most frequently adopted options are MBRs coupled with "polishing" treatments to treat the MBR permeate with advanced oxidation processes (AOPs) (preferably $O_3$) and/or activated carbon (Bourgin et al., 2018). For instance, an MBR coupled with powdered activated carbon (PAC) as a post-treatment (with or without AOPs) (Kovalova et al., 2013; Mailler et al., 2016; Margot et al., 2013; McArdell et al., 2011), an MBR with $O_3$/UV/reverse osmosis/$H_2O_2$ (Köhler et al., 2012) and an MBR with $O_3$ and granular activated carbon (GAC) (STOWA, 2021) or $O_3$/GAC/UV (DHI, 2016; Paulus et al., 2019). In addition to these, other novel treatments, including sponge MBRs (Vo et al., 2019), moving bed biofilm MBRs (Casas et al., 2015), or the integration of novel membranes in MBRs (Zhao et al., 2022), such as the anti-biofouling polyvinyl chloride/zinc oxide membrane (Alsalhy et al., 2018), have recently been tested for treating HWW, for which more details and examples can be found in the review by Verlicchi (2021).

In Europe, the recently published (26th October 2022) proposal for a revision of the Urban Wastewater Treatment Directive (UWWTD) (European Commission, 2022a) has addressed growing evidence of the issue that OMPs from urban sources (including hospitals) are present in European water bodies. New articles in the UWWTD introduce the obligation to apply a quaternary treatment in existing WWTPs and to monitor its performance for a limited set of representative OMPs, following an approach similar to that defined by the current Swiss regulation discussed in Bourgin et al. (2018). According to the draft, a quaternary treatment consists in an additional treatment able to remove the "broadest spectrum of micropollutants". Furthermore, environmental risk assessments for WWTP effluents aim to be implemented to identify the areas where the concentration or accumulation of OMPs pose a risk to human health or the environment, for which additional measures should be taken (including, the removal of OMPs not present in the list of representative OMPs).

In this context, the identification and selection of the substances that may be of the highest concern in wastewater is key in order to assess the performance of the specific treatment and the toxicity of the final

effluent that is discharged into the receiving water body. When determining OMPs in wastewater samples, most of the developed methods are limited to <100 compounds, despite the fact that the samples may contain a much larger number of them. In this regard, high-resolution mass spectrometers (HRMS) have contributed to the development of true broad-spectrum multi-residue screening methods (Hernández et al., 2012b). Several examples of targeted multi-residue screening methods using HRMS can be found in the literature, where between 300 and 600 compounds are analyzed in water samples using quadruple time-of-flight mass spectrometry (QTOF) instruments (Gago-Ferrero et al., 2020; Robles-Molina et al., 2014). Moreover, the possibility of direct injection without the need for sample preparation is increasingly being used for the analysis of water samples in which a large number of OMPs are expected. With the aforementioned devices, it is also possible to analyze samples with limits of detection (LOD) down to ng/L, which definitely eliminates the need for sample preparation. This reduces the contamination of samples caused by human handling, as well as sample losses and the required sample volume (approximately 100 μL per sample rather than 10 mL or 100 mL), allowing a higher time resolution in the archives. Direct injection method is suitable for the detection of compounds for which only low recoveries are obtained during sample preparation, but not for other compounds such as fatty acids, characterized by high LODs due to background contamination, thus requiring a preconcentration step before analysis (King et al., 2019; Simarro-Gimeno et al., 2023).

In addition, new methods based on the non-target screening (NTS) of suspect substances lead to the identification of up to 1000 compounds in principle, for which standards can be used to confirm their presence (Gago-Ferrero et al., 2018; Hernández et al., 2015). The application of NTS allows the identification of "non-target OMPs" for which it is possible to confirm their qualitative presence in the sample, but it is not possible to quantify it. This technique allows to include in the experimental investigation a potential new CECs, as well as to look for unknown sources of toxicity as remarked in Schollée et al. (2021). In view of the high number of substances that may be identified in HWW with up-to-date analytical methods, it is necessary to select key compounds whose abatement improves WWTP performance and reduces the overall toxicity of the final effluent. An approach is outlined in (Castaño-Trias et al., 2023).

The application of the above-mentioned treatment solutions aims to improve the quality of the final effluents in terms of a vast group of CECs (including OMPs). This is achievable not only by means of the cited end-of-pipe treatments, but also by upgrading or modifying the usual operation of the existing treatment. In this regard, the addition of PAC to the biological reactor of an MBR is a suitable option to upgrade an existing bioreactor (i.e., conventional activated sludge (CAS) or MBR) or as a temporary solution should an additional reduction in OMP load be needed. The presence of PAC in the bioreactor promotes adsorption and biodegradation processes in particular with regard to OMPs. To that end, activated carbon (and in this case, PAC) can remove a broad spectrum of OMPs (Gutiérrez et al., 2021) and reduce the toxicity of the final effluent (Pistocchi et al., 2022a). In this way, the resulting hybrid MBR acts as a quaternary treatment. PAC concentrations ranging from 0.05 to 2 g/L (corresponding on average to 5–200 g/gDOC assuming a DOC concentration of 10 mg/L in the bioreactor) have been extensively tested and proven to be sufficient for the removal of several OMPs as discussed, among other studies, in (Alvarino et al., 2016, 2017, 2020; Echevarría et al., 2019; Li et al., 2011; L. N. Nguyen et al., 2013a; Remy et al., 2011; Serrano et al., 2011; Yang et al., 2010; Yu et al., 2014). In the current study, an existing full-scale MBR treating mainly HWW is upgraded to a hybrid MBR with the addition of PAC to the biological reactor. Two PAC dosages (0.1 g/L and 0.2 g/L) were tested based on the conclusions drawn in Gutiérrez et al. (2021), where a comparison of the average removal efficiencies of 48 OMPs from the literature concluded that a PAC dosage of 0.1 g/L is sufficient to achieve an 80 % removal for most of the tested compounds, aligned with the requirements set by the

UWWTD draft.

The main aim of this study was to investigate the removal of a wide set of target (232) and non-target (90) OMPs in a full-scale MBR and in the hybrid MBR (full-scale MBR coupled with PAC) when PAC is added at two dosages (0.1 g/L and 0.2 g/L). The influent consists of HWW (75 %) and urban wastewater (UWW, 25 %). The selection of the key target OMPs was carried out on the basis of their frequency and occurrence in the wastewater arriving to the plant as well as their bioaccumulation and toxicity. Moreover, to better evaluate the performance of the treatment another criterium was added: the persistence to their removal. Regarding the non-target OMPs, the performance analysis was carried out on the basis of their frequency, persistence to removal, bioaccumulation and toxicity. The study also includes an environmental risk assessment based on the risk quotient (*RQ*) approach and concludes with a discussion of the main uncertainties related to the investigation on real wastewater and the full-scale WWTP.

## 2. Material and methods

### 2.1. WWTP under investigation

The investigation was carried out in an indoor full-scale wastewater treatment plant (WWTP) in operation since July 2011, located in the outskirts of the town of Ferrara, north east Italy. It was sized for 4000 person equivalent (PE), with an average dry weather flow rate equal to 700 m³/d. The plant feed mainly comes from the large hospital nearby (on average 75 %) and the remaining part from the small urban settlement located around the hospital (approximately 2000 inhabitants). An in depth description of the plant is available in the Supplementary material (Text S1). Fig. 1 reports the treatment train and shows the four points sampled during the experimental campaigns: HWW, the mixture fed to the preliminary treatments representing the influent to the plant (INF), the MBR permeate (MBRperm), and the disinfected effluent (EFF).

### 2.2. Experimental investigations

Three operation periods were considered. In the first one (March – August 2021), the MBR worked as it was designed, without the addition of PAC (noPAC experimental campaign); in the second (September – November 2021), a concentration of 0.1 g/L of PAC was maintained constant inside the bioreactor (0.1PAC campaign), and in the third (April – May 2022), the concentration of PAC in the bioreactor was increased to and maintained at 0.2 g/L (0.2PAC campaign). Both PAC dosages were selected in accordance with previous studies dealing with the removal of OMPs in hybrid MBRs coupled to PAC, which in most cases ranges between 0.05 and 2 g/L as remarked in Gutiérrez et al.

(2021). The PAC concentration was maintained stable by periodical and controlled additions of fresh PAC, following the procedure reported in the Supplementary material (Text S2).

The commercial PAC used was purchased from Comelt S.p.A (Cernusco sul Naviglio, Milan, Italy). The PAC properties (iodine number 750 mg/g, Brunauer-Emmett-Teller (BET) specific surface area 850 m²/d, bulk density 430 kg/m³, ash content 10 % and humidity 5 %) were provided by the manufacturer. The adsorbent was selected following its physicochemical characteristics, in accordance with literature data (BET 238–1363 m²/g, bulk density 250–420 kg/m³, iodine number 850–1250 mg/g) (Alves et al., 2018; Gutiérrez et al., 2021).

### 2.3. Organic micropollutants investigated

A total set of 232 target and 90 non-target compounds were selected for the investigation campaigns on the basis of: (*i*) annual consumption, (*ii*) potential environmental risk posed by their residues, (*iii*) compounds included in the current European watch list (European Commission, 2022b), and (*iv*) availability of the analytical methods. Target OMPs pertain to 20 different classes: analgesics/anti-inflammatories (39), antiarrhythmic agents (5), antibiotics (41), antifungals (3), antihistamines (2), antihypertensives (1), antiparasitics (6), antiseptics (1), beta-blockers (3), calcium channel blockers (1), diuretics (1), hormones (9), illicit drugs (13), plastic additives (2), psychiatric drugs (76), receptor antagonists (2), stimulants (3), UV filters (1), veterinary drugs (22) and X-ray contrast media (1). The compounds are grouped according to the listed classes together with their physicochemical properties (octanol-water partition coefficient (log$K_{ow}$), octanol-water distribution coefficient (log$D_{ow}$), acid dissociation constants (p$K_a$) and charge), and ecotoxicity data (Predicted No-Effect Concentration (PNEC)) and are reported in Table S1 of the Supplementary Material.

The 90 OMPs determined by non-targeted screening (NTS) pertain to 27 different classes: analgesics/anti-inflammatories (12), antiacids (3), antiarrhythmic agents (1), antibiotics (12), anti-cancer drugs (4), anti-diabetic drugs (2), antiemetics (1), antigout preparations (1), antihistamines (1), antihypertensives (8), antiparasitics (1), antiseptics (2), antithrombotics (2), beta-blockers (2), bronchodilators (2), food additives (2), hormones (2), illicit drugs (1), lipid regulators (2), pesticides (1), plastic additives (1), psychiatric drugs (8), stimulants (4), synthetic musks (2), UV filters (1), X-ray contrast media (3) and 9 additional compounds classified as "Other". Their physicochemical properties and PNEC values are reported in Table S2.

The charge in Table S1 and Table S2 represents the prevalent ionic form of the compound at pH = 7. The charge calculation can be found in Text S3 and the calculated percentage of the ionic forms for both target and non-target OMPs can be found in Table S3 and Table S4,
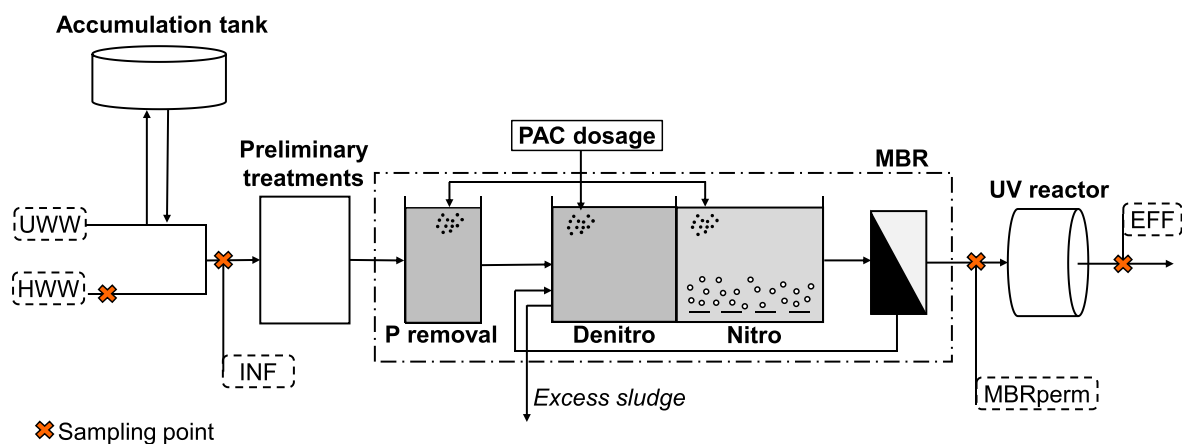


**Fig. 1.** WWTP schematic diagram and sampling points (red crosses) for the analysis of OMPs. HWW (hospital wastewater), UWW (urban wastewater), INF (influent to the plant), MBRperm (MBR permeate) and EFF (effluent).

respectively.

### 2.3.1. Selection of key OMPs

Three approaches were applied to both HWW and INF to identify which target OMPs can be considered of greater concern. The selection is based on the following criteria: frequency (F), occurrence (O) or load (L), bioaccumulation (B) and toxicity (T). Each criterion has a surrogate (Pavan and Worth, 2008) as explained below, which corresponds to a measurable attribute characterized by a different interval of variability. For each attribute thresholds are set defining the ranges to which a score must be assigned from 1 to 5.

The surrogate used for the frequency (F) criterion is the frequency of detection (*Freq*) of the target OMP, which measures how often the compound is present above the LOD during a sampling campaign. The corresponding thresholds were defined in the range of variability (0-100 %): 20 %, 40 %, 60 %, and 80 % and a score was assigned to each interval as shown in Table 1: the higher the score, the higher the frequency of occurrence of the compound in the water stream.

The occurrence (O) is related to the measured average or maximum concentration (*c*) of the OMP. On the basis of the considerations reported in Gutiérrez et al. (2021), the variability range was assumed between 0.01 μg/L and 1 μg/L and the thresholds set at 0.01, 0.1, 0.5 and 1 μg/L are reported in Table 1: the higher the concentration, the higher the score. If the compound concentration was found below the corresponding LOD, the score of 1 is assumed. It should be noted that in all cases the LOD of each of the target compounds is lower than 0.01 μg/L.

Regarding the load (L) criterion, corresponding to the amount of a substance occurring in a defined interval of time, expressed in g/d, the thresholds were arbitrarily set on the basis of the observed daily load at the WWTP under study. As for most of the investigated compounds, the daily load is <50 mg/d and is >1000 mg/d for only a few, the thresholds were set at: 50, 100, 500, 1000 mg/d. The higher the load, the higher the score (Table 1).

The parameter $\log K_{ow}$, which is defined as a partition coefficient for a two-phase system consisting of *n*-octanol and water, is the surrogate for the bioaccumulation (B) criterion, since it indicates the tendency of a substance to be solubilized in the adipose tissue of living organisms. The higher the value of $\log K_{ow}$, the higher the expected bioaccumulation of the OMP. Predicted $\log K_{ow}$ values of target OMPs were obtained from CAS SciFinder (https://scifinder.cas.org/) and are reported in (Table S1). According to Daouk et al. (2015), the thresholds were set at 1, 2, 3 and 4.5. The higher the $\log K_{ow}$, the higher the score (Table 1).

The surrogate for the toxicity (T) criterion was the OMP PNEC value in freshwater obtained from the NORMAN database (https://www.norman-network.com/) (Table S1). The PNEC is defined as the concentration of a compound below which adverse effects are most unlikely to occur. They represent the lowest values obtained, either experimentally or predicted by QSAR models, from the most sensitive freshwater species analyzed in long-term exposure (among them: bacteria, algae, invertebrates, and fish). In this way, lower PNEC values indicate higher toxicity. In accordance with Daouk et al. (2015), the thresholds for this

surrogate were set at: 0.1, 1, 10, 100 μg/L. The lower the PNEC, the higher the score.

A safety score of 5 was given to OMPs for which there were no available PNEC values, assuming the inherent toxicity of the compound (Table 1).

That being said, three different approaches, combining the above criteria, were followed in order to identify the key compounds among the whole set of target OMPs, on which basis the hybrid MBR removal capacity will be assessed in detail.

- The first approach combines frequency (F), average OMP concentration for the occurrence ($O_{av}$), bioaccumulation (B) and toxicity (T) criteria and is called $FO_{av}BT$;
- the second considers, rather than the average OMP concentration, its maximum value ($O_{max}$) for the occurrence criterion ($FO_{max}BT$);
- the third approach does not use the occurrence criterion, but the average load (L) of the compound and is called FLBT.

Once the thresholds are defined and the corresponding scores assigned (Table 1), the final score *S* of each OMP is the result of the sum of the assigned score for each criterion (Verlicchi et al., 2023). In this way, OMPs can obtain a maximum score of 20 and a minimum of 4. A total score of 14, corresponding to 70 % of the maximum score (20), was selected as the threshold to select the OMPs in accordance with (Verlicchi et al., 2023).

The three approaches were also applied to both HWW and INF, since the OMP frequency of detection and concentration may vary once the urban wastewater is mixed with the HWW. This results in a total of 6 analyses (=3 approaches × 2 sampled wastewaters) for the selection of OMPs applied simultaneously, which discerns and ultimately includes the key compounds that are of particular concern in HWW and UWW.

### 2.4. Sampling and chemical analysis

Water samples were collected on dry days at four sampling points as shown in Fig. 1: hospital wastewater (HWW); WWTP influent (INF), after the mixing between urban and HWW; MBR permeate (MBRperm) and final effluent after the UV treatment (EFF). A set of conventional parameters was periodically monitored, namely chemical oxygen demand (COD), biological oxygen demand ($BOD_5$), total suspended solids (TSS), volatile suspended solids (VSS), nitrates ($NO_3^-$), nitrites ($NO_2^-$), ammonium ion ($NH_4^+$), total nitrogen ($N_{tot}$), and total phosphorous ($P_{tot}$).

Samples for conventional parameters were taken in the HWW, INF and EFF once every week during the 0.1PAC campaign, whereas anionic, cationic and total surfactants concentration, together with *E. coli* concentration and *D. magna* ecotoxicity measurements, were carried out once every month to evaluate the water quality in the HWW, INF and EFF. Samples for TSS, dissolved organic carbon (DOC) and $UV_{254}$ absorbance were monitored at least once a week during the 0.1PAC campaign and once every two weeks during 0.2PAC in HWW, INF and EFF as well as in the mixed liquor of the nitrification tank (NITRO). 24-h

**Table 1**
Scores and thresholds of the criteria for the selection of key OMPs.

| Criteria | Frequency (F) | Occurrence (O) | Load (L) | Bioaccumulation (B) | Toxicity (T) | Persistence (P) |
|---|---|---|---|---|---|---|
| Surrogates→ Score ↓ | Frequency of detection (%) | Average or maximum concentration (μg/L) | Average load (mg/d) | $\log K_{ow}$ | PNEC (μg/L) | Average removal efficiency (%) |
| 1 | *Freq* < 20 | *c* < 0.01 | $L \leq 50$ | $\log K_{ow} < 1$ | PNEC >100 | *R* > 80 % |
| 2 | 20 ≤ Freq <40 | $0.01 \leq c < 0.1$ | $50 < L \leq 100$ | $1 \leq \log K_{ow} < 2$ | 10 < PNEC ≤100 | 60 < *R* ≤ 80 |
| 3 | 40 ≤ Freq <60 | $0.1 \leq c < 0.5$ | $100 < L \leq 500$ | $2 \leq \log K_{ow} < 3$ | 1 < PNEC ≤10 | 40 < *R* ≤ 60 |
| 4 | 60 ≤ Freq <80 | $0.5 \leq c < 1$ | $500 < L \leq 1000$ | $3 \leq \log K_{ow} < 4.5$ | 0.1 < PNEC ≤1 | 20 < *R* ≤ 40 |
| 5 | Freq ≥80 | $c \geq 1$ | $L > 1000$ | $\log K_{ow} \geq 4.5$ | PNEC ≤0.1 | $R \leq 20$ |

time proportional composite samples were used for the analysis of conventional wastewater parameters, while grab samples were taken of the mixed liquor. Table S5 shows the analytical methods used for the analysis of conventional parameters in water, mixed liquor and sludge.

Regarding OMPs, a wide spectrum of compounds (322) was analyzed in 24-h time proportional composite water samples of HWW, INF, MBRperm and EFF by means of automatic samplers. The sampling strategy went as follows: during the noPAC campaign, samples were taken once every two months ($n = 3$), whereas for the PAC campaigns samples were taken every week (for 0.1PAC $n = 9$, for 0.2PAC $n = 6$). Samples of the HWW, INF and EFF were taken on the same day of the week, while the MBRperm was sampled 24 h later in order to account for the MBR hydraulic retention time (HRT). Water samples were taken at least 1 day after the addition of fresh PAC to guarantee a homogeneous distribution of the adsorbent inside the tanks.

Water samples were collected in polycarbonate bottles (500 mL), frozen immediately after sampling and stored at $-20$ °C until analysis. Refrigeration between 0 and 4 °C (only a few days) and freezing at $-20$ °C (longer periods) are the most common preservation methods for organic samples that cannot be analyzed immediately after sampling. Before analysis, the water samples were thawed and then filtered through 0.2 μm PTFE filters.

### 2.4.1. Organic micropollutants

A total set of 232 target (Table S1) and 90 non-target (Table S2) OMPs were analyzed in the investigation.

Briefly, OMPs were determined by an ultra-high performance liquid chromatography system (1290 UHPLC, Agilent Technologies, USA) coupled to hybrid quadrupole time-of-flight mass spectrometry (6550 i-Funnel Q-TOF–LC/MS, Agilent Technologies, USA) using the direct injection method. The UHPLC analyses were performed using an Agilent Series 1290 UHPLC system (Santa Clara, CA, USA) equipped with a Waters RP column ACQUITY UPLC, HSS T3 (150 mm × 2.1 mm, 1.8 μm). The analytical method developed is based on a previously-described method (Stipaničev et al., 2017). Out of the 232 targeted analytes covered by the method, 135 components were mentioned in (Stipaničev et al., 2017), but due to the addition of 97 new components, it was necessary to perform an optimization that resulted in the modification of the mobile phase in terms of solvent A, column temperature and flow, and all the operating conditions in ESI(+) mode (Stipaničev et al., 2015). Ionization in the positive mode may be performed and is consistent with the fact that a much larger number of compounds are detected in the positive mode than in the negative one or detected in both modes (Ferrer and Thurman, 2005; Hernández et al., 2015; Hollender et al., 2018; Robles-Molina et al., 2014). This resulted in slightly higher LODs and limits of quantification (LOQs) for most components of the previous method, all intending to obtain the optimal method for the analysis of the 232 components. The mobile phase consisted of both 10 mM ammonium formate in water (solvent A) and methanol (solvent B). Elution began with a 17-min gradient from 95 % A to 95 % B, which was maintained for 6 min, followed by a 0.1 min linear gradient back to 95 % A. In all cases, after gradient elution, the column was equilibrated for 3 min before another injection. The analytes were separated at a temperature of 40 °C. The flow rate was 0.35 mL/min with an injection volume of 100 μL for all analyses. The analytes were detected using a 6550 i- Funnel Q-TOF–LC/MS (Agilent Technologies, USA) at a 4 GHz detector rate. The resolution power for ESI(+) was 52,296 at 922.009798 *m/z* and 21,801 at 118.086255 m/z, with 2 ppm accuracy. Ions were generated using a dual AJS ESI (Agilent Jet Stream) ion source. The operating conditions in ESI(+) mode were as follows: sheath gas temperature 350 °C, gas temperature 160 °C, heat gas 12 L $N_2$/min, drying gas 14 L $N_2$/min, capillary voltages 4000 V, fragmentor 250 V, and nebulizer 30 psi. Correction during measuring for any possible drift in the mass axis was done automatically with lock 2 mass ion software. Analyses were performed using MS and MS/MS (All ions mode) with fixed collision energy (0, 20, 40 V) and in a mass range of 50–1200 m/z.

Data were further processed with Agilent MassHunter Workstation software (Quantitative Analysis version B.10.00/Build 10.0.707.0 for QTOF, Agilent Technologies, USA). The calibration curve was obtained by direct injecting, in triplicate standard solutions at 7 concentration levels ranging from 1 to 1000 ng/L. Correlation coefficients >0.999 were used as linearity acceptance criterion. To cope with the matrix effects, the method described in (Stipaničev et al., 2017), and aligned with (Diaz et al., 2013; Hernández et al., 2012a), was adopted and it was applied to surface, ground, sea and drinking water. These matrices have an almost unchanged composition unlike the wastewater matrix, which may greatly vary. For this reason, no wastewater matrix was used in this step. The same approach was adopted in analyzing pharmaceuticals in raw influent and treated effluent of a Croatian WWTP (Topić Popović et al., 2015) and in surface water from Danube River (Stipaničev et al., 2015). A similar approach was followed to determine LOD and LOQ for all compounds, according to (Stipaničev et al., 2015, 2017).

Acceptance criteria for accuracy were recoveries between 70 % and 110 % and for repeatability a relative standard deviation lower than 25 %.

The limit of detection (LOD) for all compounds ranged from 0.26 to 5.07 ng/L and the limit of quantification (LOQ) ranged from 1.64 to 29.92 ng/L. LOD and LOQ values for each OMP are listed in Table S6, as well as the MS/MS specifications.

For this study, NTS was used as a preliminary assessment for the presence of non-target OMPs and to assess their removal efficiency achieved by the hybrid MBR.

NTS was performed employing the same analytical instrument, for which the MS/MS specifications are listed in Table S7. Ultrapure laboratory water samples were always processed in parallel with the real water samples and all samples were analyzed in triplicate with the main goal to gain additional information. A data-independent acquisition technique that enables fragmentation can be obtained for all substances rather than selected precursor masses and a better integration of retention information. It is interesting to point out that the detection and identification were performed by using full MS acquisition data. Therefore, data can be reprocessed at any time in the future and re-evaluated using new or modified databases to search for other interesting compounds, by including their empirical formulae into the database. Retrospective analysis using HRMS allowed the identification of metabolites of pharmaceuticals and drugs of abuse without additional injection of the sample extract (Hernández et al., 2011). Sample analyses were performed without using isotopically labelled internal standards (ISTDs). Only peaks that occurred in three repeated analytical runs were considered.

### 2.5. Removal efficiency of target and non-target OMPs

Target OMP daily removal efficiencies $R$ were obtained on the basis of their daily influent and permeate loads (mg/d). To calculate the loads, the OMP concentration ($c$) at the sampling point is multiplied by the corresponding flow rate ($Q$) on the sampling day. Given that the flow rate in the INF and MBRperm can be assumed the same during sampling, it can be neglected, resulting in Eq. (1) for the removal efficiency evaluation as follows,

$$Removal\ efficiency\ (\%) = R = \frac{c_{INF} - c_{perm}}{c_{INF}} \times 100 \qquad (1)$$

where $c_{INF}$ and $c_{PERM}$ are the OMP concentrations (μg/L) in the INF and MBRperm, respectively.

If the OMP concentration ($c_{INF}$ or $c_{PERM}$) was below the corresponding LOD of the instrument, 1/2 LOD was assumed, and the removal efficiency could be calculated. Otherwise, if both $c_{INF}$ and $c_{PERM}$ were below the LOD, the removal efficiency was not calculated. Negative removal efficiencies arose when the concentration of the MBRperm was greater than the corresponding INF concentration. Average OMP

removal efficiencies per campaign (i.e., noPAC, 0.1PAC and 0.2PAC) were calculated by discarding the negative daily removal efficiencies and performing the arithmetic average solely with regard to the positive removal efficiencies according to Bourgin et al. (2018). Negative removal efficiencies are addressed in the Discussion (Section 4.2).

To calculate the daily removal efficiency for each of the OMP classes reported in Table S1 (first column), concentrations $c_{INF}$ and $c_{PERM}$ of Eq. (1) correspond to the sum of the concentrations of each OMP belonging to that class. As detailed above, the average removal efficiency per campaign was obtained as the arithmetic average of the daily removal efficiencies.

For non-target OMPs, the removal efficiencies were calculated by substituting the term $c_{INF}$ and $c_{PERM}$ of Eq. (1) by the signal response of the OMP in the INF (INF) and MBRperm (*perm*) (Eq. (2)) in accordance with Li et al. (2018),

$$Removal\ efficiency\ (\%) = R = \frac{Signal\ Resp_{INF} - Signal\ Resp_{perm}}{Signal\ Resp_{INF}} \quad (2)$$

If no signal was detected in the MBRperm, a removal of 99.99 % was assumed. Instead, if INF or both INF and MBRperm had no signal response, the removal was not calculated. As for target OMPs, negative values were discarded to calculate the average removal efficiency.

It should be noted that, in this study, the term removal efficiency refers to the concentration (Eq. (1)) or the signal response (Eq. (2)) of a given OMP, regardless of whether it is mineralized, transformed, adsorbed or even formed in the system similarly to Kovalova et al. (2012).

### 2.5.1. FOPBT and FPBT analysis

Once selected the key OMPs, in order to compare their behavior in the different treatment scenarios (the three campaigns), and evaluate the quality of the permeate, other two approaches were defined and adopted.

In this context, a new parameter is considered: the Persistence P, defined, according to Verlicchi et al. (2023), as the resistance of an OMP to be removed by means of the studied treatment (MBR alone or coupled with PAC). It is calculated as P = (100 − R), where R derives from Eqs. (1) and (2). The thresholds were set at R equal to 20 %, 40 %, 60 % and 80 % according to Verlicchi et al. (2023) and the assigned scores (from 1 to 5) are reported in Table 1. It is evident that the lower the removal, the higher the persistence, and, correspondingly, the higher the score.

The first approach was defined for the target key OMPs and was based on OMP frequency (F) and occurrence (O, in terms of average concentration) in the permeate, persistence (P) to the removal, bio-accumulation (B), and toxicity (T). It is called FOPBT approach. According to it, each OMP is characterized by a final score S, obtained as the sum of the scores assigned to each criterion. As for F, O, B, and T the scores are those already discussed in Section 2.3.1, the final score S may vary between 5 and 25.

For each non-target compound, another approach was defined, based on OMP frequency F, persistence P, bioaccumulation B and toxicity T. It was called FPBT. The occurrence O is not considered as it is not a measurable parameter in NTS. In this case, the final score S characterizing each non target compound may vary between 4 and 20.

### 2.6. Ecotoxicological issues

An environmental risk assessment due to the residues of target OMPs discharged into the surface water was carried out by means of the risk quotient (*RQ*) approach (European Commission, 2003).

The RQ of a compound is evaluated as the ratio between its concentration in the water sample (measured environmental concentration, MEC) and its PNEC in freshwater.

In each campaign $j$ ($j = 1$ corresponds to noPAC, 2 corresponds to 0.1PAC, 3 corresponds to 0.2PAC), the environmental risk posed by each

OMP $i$ ($i = 1, ..., 232$) is evaluated as the average value $\overline{RQ_i}$ among the $RQ_i$ referring to the $n$ sampling days ($n = 2, 9, 6$ for the three campaigns) (Eq. (3)).

$$\overline{RQ}_{i,j} = \frac{1}{n} \sum_{k=1}^{n} RQ_{i,k} \bigg|_j \quad (3)$$

Moreover, in each campaign, the environmental risk $RQ_{mix}$ posed by a mixture of $q$ OMPs (the whole spectrum of 232 target compounds or the selected key compounds) in (waste) water was carried out by means of Eq. (4).

$$RQ_{mix,j} = \sum_{i=1}^{q} \overline{RQ_i} \bigg|_j \quad (4)$$

Another parameter here defined to evaluate the contribution by each OMP to the environmental risk of a mixture is the *specific risk quotient*, corresponding to the ideal risk posed by each OMP of the mixture, assuming that the OMPs have the same risk (Eq. (5)).

$$RQ_{sp,j} = \frac{RQ_{mix,j}}{q} \quad (5)$$

The environmental risk is assessed following the criteria often adopted in similar studies and in particular (Verlicchi et al., 2012a): (*i*) RQ < 0.1 minimal risk to aquatic organisms; (*ii*) $0.1 \leq RQ < 1$ medium risk and (*iii*) $RQ \geq 1$ high risk.

## 3. Results and literature comparison

### 3.1. HWW and WWTP influent characterization

An in-depth analysis of the daily flow rates from the two pathways is reported in Fig. S1, together with the precipitation during the three campaigns. Table S8 shows the characterization of HWW and INF during the whole experimental period in terms of conventional parameters. Samples were taken for a year at different seasons to fully characterize HWW and the influent to the treatment plant. All parameters, except for TSS and *E. coli*, were found on average at higher concentrations in HWW compared to INF. Occurrence, load, and range of variability of the target OMPs in HWW and INF are analyzed in Text S4 referring to the data reported in Fig. S2 (OMP load distribution in HWW and INF during the campaigns), Table S9 (average load of each class of target OMPs and the corresponding contribution to the overall load in HWW and in INF), Tables S10 (frequency of detection, range of variability and average concentrations in HWW and INF) and Table S11 (range of variability and average load of the target OMPs in HWW and INF).

### 3.1.1. Key OMPs

The high number of compounds analyzed in the present study underlines the need to establish a method by which the most relevant results are displayed. In this regard, different analyses were performed by means of three approaches, called $FO_{av}BT$, $FO_{max}BT$, and FLBT aiming to select the key OMPs of greater concern and for which the treatment efficiency will be discussed in detail in Section 3.3.

Table S12 reports for the 232 target OMPs the scores assigned to each criterion (F, $O_{av}$, $O_{max}$, L, B and T) and the final score S in the case of the three approaches for the HWW and INF. It emerges that the OMPs with a final score $S \geq 14$ are different for the three approaches and in the two sampling points.

It emerges that a higher number of OMPs obtained the minimum score of 14 in HWW compared to the INF (namely 40 versus 34 in $FO_{av}BT$; 57 versus 47 in $FO_{max}BT$ and 32 versus 30 in FLBT), being the OMPs selected for the INF always present in the HWW selection with the exception of verapamil. Since most of the OMPs arrive at the WWTP with the HWW, it is not surprising that the scores assigned to occurrence and frequency are higher in the HWW.

Secondly, the number of compounds selected considering the maximum occurrence ($FO_{max}BT$) is higher for both HWW and INF compared to those selected considering the average occurrence ($FO_{av}BT$). When comparing the results in more detail, the application of $FO_{av}BT$ leads to more representative results than $FO_{max}BT$, since some compounds occasionally show high concentrations on certain sampling days, which are not representative for the wastewater itself. This is the case of procaine, which has a maximum concentration of 0.235 µg/L and average concentration of 0.04 µg/L in HWW. These unexpected results may be due to the hospital work flow and other factors such as the chemical and biological composition of the wastewater (see Section 4.1 for further details).

Thirdly, the use of the average load (FLBT) instead of the occurrence could be insightful when dealing with full-scale systems, since it also takes into account the wastewater flow rate and therefore the seasonal OMP consumption pattern.

The thresholds set here for score assignment lead to a lower number of compounds selected, 32 and 30 OMPs for HWW and INF, respectively, which are already included in both the $FO_{av}BT$. and $FO_{max}BT$. approaches. The use of the load may entail different kinds of uncertainties related to the flow rate which varies over the day (Verlicchi, 2018).

For the above-mentioned considerations, the preferred and adopted approach is the $FO_{av}BT$, leading to the 40 OMPs selected for HWW, including the 33 compounds also selected within the INF, and additionally verapamil, selected for INF, resulting in the identification of 41 key OMPs (Fig. 2). It comprises 8 analgesics/anti-inflammatories, 1 antiarrhythmic agent, 11 antibiotics, 1 antiparasitic, 1 calcium channel blocker, 4 illicit drugs, 1 plastic additive, 11 psychiatric drugs, 1 stimulant, 1 UV filter and 1 X-ray contrast medium.

Fig. 2 reports the key OMPs selected ranking in descending order according to their $FO_{av}BT$ final score $S$ and it shows the contribution of each criterion. Text S5 in the Supplementary material analyses and compares the different contributions for the selected substances.

### 3.2. Removal efficiencies of macroparameters, DOM and $UV_{254}$

An in depth analysis of the removal of the investigated macropollutants is reported in Text S6 in the Supplementary material: attention is here paid only to DOC and $UV_{254}$. In this context, Table 2 shows their concentrations in different sampling points for both the 0.1PAC and 0.2PAC campaigns. DOC concentration and UV254 absorbance decrease as follows: HWW, INF, NITRO and EFF. DOC and $UV_{254}$ can be considered surrogates of the organic content, in particular OMPs (Altmann et al., 2016; Zietzschmann et al., 2016). The observed decrement in their values can thus be related to the organic substance concentration trend. The total average concentration of OMPs reported in the last row of Table 2 was found to be higher in the HWW, to decrease after being

mixed with the UWW (INF), then to reduce in the bioreactor due to the metabolic processes.

It is important to note that both DOC and $UV_{254}$ present higher values in HWW and INF during the 0.2PAC campaign, despite the total average concentration of OMPs in the two sampling points being significantly lower. This results in higher values in NITRO and EFF sampling points after the treatment with 0.2 g/L of PAC. While DOC removal did not show significant differences between the two PAC dosages, UV254 absorbance exhibited better results with 0.2 g/L of PAC, by increasing its removal efficiency by 15 % (from 46 % to 61 % on average). Indeed, while the range of $UV_{254}$ removal varied greatly during the 0.1PAC campaign (27 % – 61 %), it was maintained between 54 % and 65 % for 0.2PAC.

The decrease in UV254 is directly related to the removal of recalcitrant compounds with aromatic rings and unsaturated bonds of both OMPs and DOM constituents (Altmann et al., 2016). Although DOC removal does not seem to improve with the increasing dosage of PAC, recalcitrant compounds do seem to be removed to a greater extent.

### 3.3. Removal efficiencies of target OMPs in the different scenarios

The removal efficiency was investigated for the MBR and hybrid MBR (PAC addition) and not for the whole treatment train which includes a disinfection step (UV reactor). Attention was focused on the enhancement evaluation when PAC was added to the bioreactor.

The removal efficiency was evaluated at three different levels: key OMPs, OMP classes and the whole set of OMPs. In order to express the required PAC dosage to achieve a certain OMP removal, many authors (Streicher et al., 2016; Zietzschmann et al., 2014) suggest normalizing the PAC dosage to the DOC of the water under treatment (g PAC/g DOC), commonly referred to as the specific PAC dosage. Siegrist et al. (2018), and more in general the Micropol Strategy (https://micropoll.ch), recommended a specific dosage of 2–3 g PAC/g DOC when it is added directly to the biological treatment, and 1.5 g PAC/g DOC when used as a post-treatment. In our study, the DOC concentration in the nitrification tank is 6.2 ± 1.2 mg/L for the 0.1PAC campaign and 7.9 ± 1 mg/L for 0.2PAC. In this way, the resulting PAC normalized dosage is 16 g PAC/g DOC for 0.1PAC and 25 g PAC/g DOC for 0.2PAC. Since DOC concentrations were in the range of 4.7–9.1 mg/L during the whole experimental period, the specific PAC dosages used were much higher than those suggested by the Micropol Strategy. According to it, the applied PAC dosages should have been 0.009 g/L and 0.027 g/L.

#### 3.3.1. Removal of key OMPs

Fig. 3 reports the box plots depicting the removal efficiencies of the key OMPs in the three experimental campaigns. The box plots show the median, average and daily removal efficiency in each campaign. The box
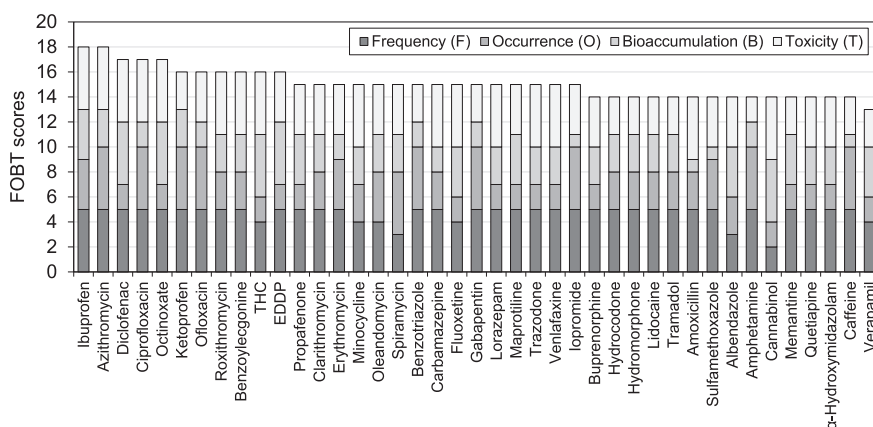


**Fig. 2.** Key OMPs selected, final scores and contribution of the different criteria.

**Table 2**
DOC concentration and UV$_{254}$ absorbance in HWW, INF, NITRO and EFF, as well as their removal efficiency (between INF and EFF) in 0.1PAC and 0.2PAC campaigns. n: number of samples. SD: standard deviation. n.a.: data not available.

| | | 0.1PAC | | | | Rem. eff. | 0.2PAC | | | | Rem. eff. |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HWW | INF | NITRO | EFF | Av (%) ± SD | HWW | INF | NITRO | EFF | Av. (%) ± SD |
| DOC (mg/L) | n | 10 | 10 | 16 | 10 | 68 ± 6 | 2 | 3 | 3 | 3 | 72 ± 13 |
| | Range | 14.7–36.2 | 12.4–22.2 | 4.7–8.8 | 3.7–5.9 | | 29.3–36.9 | 16.3–39.8 | 7.3–9.1 | 6–7.3 | |
| | Average ± SD | 22.2 ± 7.3 | 16.6 ± 3.4 | 6.2 ± 1.2 | 5.2 ± 0.6 | | 33.1 ± 5.4 | 27 ± 11.9 | 7.9 ± 1 | 6.6 ± 0.7 | |
| UV$_{254}$ (ABS/cm) | n | 10 | 10 | 18 | 10 | 46 ± 9 | 3 | 3 | 3 | 3 | 61 ± 6 |
| | Range | 0.280–0.508 | 0.262–0.427 | 0.177–0.253 | 0.114–0.258 | | 0.542–0.737 | 0.529–0.665 | 0.271–0.435 | 0.205–0.241 | |
| | Average ± SD | 0.379 ± 0.077 | 0.333 ± 0.049 | 0.21 ± 0.024 | 0.178 ± 0.036 | | 0.630 ± 0.099 | 0.584 ± 0.072 | 0.343 ± 0.084 | 0.225 ± 0.018 | |
| Target OMP concentration | n | 8 | 9 | n.a. | 9 | | 6 | 6 | n.a. | n.a. | |
| | Average ± SD | 60.2 ± 22.7 | 53.9 ± 16.0 | n.a. | 10.6 ± 2.7 | | 40.7 ± 13.4 | 31.6 ± 5.2 | n.a. | n.a. | |

represents the interquartile range (IQR) for every OMP. The whiskers extend up to 1.5 times the IQR, and any data beyond this range is considered an outlier.

The analysis which follows refers to the 41 key OMPs selected by the FO$_{av}$BT approach. An overview of the treatment efficiency for the whole set of OMPs is available in Table S13 in the Supplementary Information, where the removal efficiencies of all the target OMPs during the noPAC, 0.1PAC and 0.2PAC campaigns are displayed. Negative removal efficiencies obtained on certain sampling days were discarded for the calculation of the minimum, maximum and average removal of each treatment (Section 2.5). For that matter, they are reported separately for each campaign in Table S14. For the sake of completeness, the range of variability and average occurrence of the 232 target compounds during the noPAC (Table S15), 0.1PAC (Table S16) and 0.2PAC (Table S17) campaigns is reported, as well as the corresponding OMP loads (Table S18-S20).

*3.3.1.1. Analgesics/anti-inflammatories.* The 8 analgesics/anti-inflammatories selected showed a good overall increase in their removal with the addition of PAC. Only the two compounds, hydrocodone and hydromorphone, did not show any substantial change in their removal efficiencies due to the addition of PAC, since the removals in MBR were already high ($\geq$ 97 %). For them, less than a 2 % difference in the average removal efficiency was observed between the campaigns. PAC addition had a positive effect on four compounds. Buprenorphine showed a slight increase in its removal efficiencies even though it was already removed to a great extent in the MBR (80 % on average). In the case of tramadol, lidocaine and diclofenac, the increasing dosage of PAC contributed to narrowing the range of variability in their removal efficiencies. In particular, tramadol and diclofenac increased their average removal efficiency from low in noPAC (i.e., 4 % in the case of tramadol, 15 % for diclofenac) to medium (42 % and 50 % on average) in the presence of PAC and lidocaine, which showed a negative removal in noPAC, was removed to 57 % with PAC. Diclofenac is a hydrophobic recalcitrant compound (Radjenović et al., 2009; Verlicchi et al., 2012b), with low removal efficiency in MBR (Alvarino et al., 2017; Wijekoon et al., 2013). Nguyen et al. (2013b) found that the removal in MBR with 0.1 g/L of added PAC was approximately 15 %, whereas an increment up to 70 % in the removal efficiency was observed with 0.5 g/L of PAC. Diclofenac removal seems to be influenced not only by the PAC dosage, but also by the presence of fresh PAC since high removal efficiencies have only been observed for a limited period without any new addition of fresh PAC in similar studies (Alvarino et al., 2017; Serrano et al., 2011). It is worth highlighting that the high and variable diclofenac loads (33–14,905 mg/d) arriving with the INF would probably be the reason for the high variability in the removal efficiencies observed during the 0.1PAC campaign.

Ibuprofen and ketoprofen did not show any improvement in their removal efficiencies following the addition of PAC inside the bioreactor. Ketoprofen showed very high removal efficiencies ($\geq$ 98 %) during the noPAC and 0.2PAC campaigns, whereas a slight decrease was observed during 0.1PAC (74 % – 99.99 %). Regarding ibuprofen, a decrease in its removal efficiency was unexpectedly observed during the PAC treatment, with removal efficiencies that plummeted to 23 % and 38 % on certain sampling days of the 0.1PAC and 0.2PAC campaigns. However, the average removal remained high (76 % – 95 %) in all the campaigns. According to previous studies, ibuprofen has a low affinity to solids and its main removal mechanism is biodegradation (Alvarino et al., 2016; Serrano et al., 2011). In these studies, no differences in the removal were observed between the biological treatment and the addition of PAC, indicating that the decrease observed during the PAC campaigns may not be attributed to the presence of the adsorbent.

*3.3.1.2. Antibiotics.* The antibiotics azithromycin, erythromycin and roxithromycin are compounds frequently studied in the literature (Luo
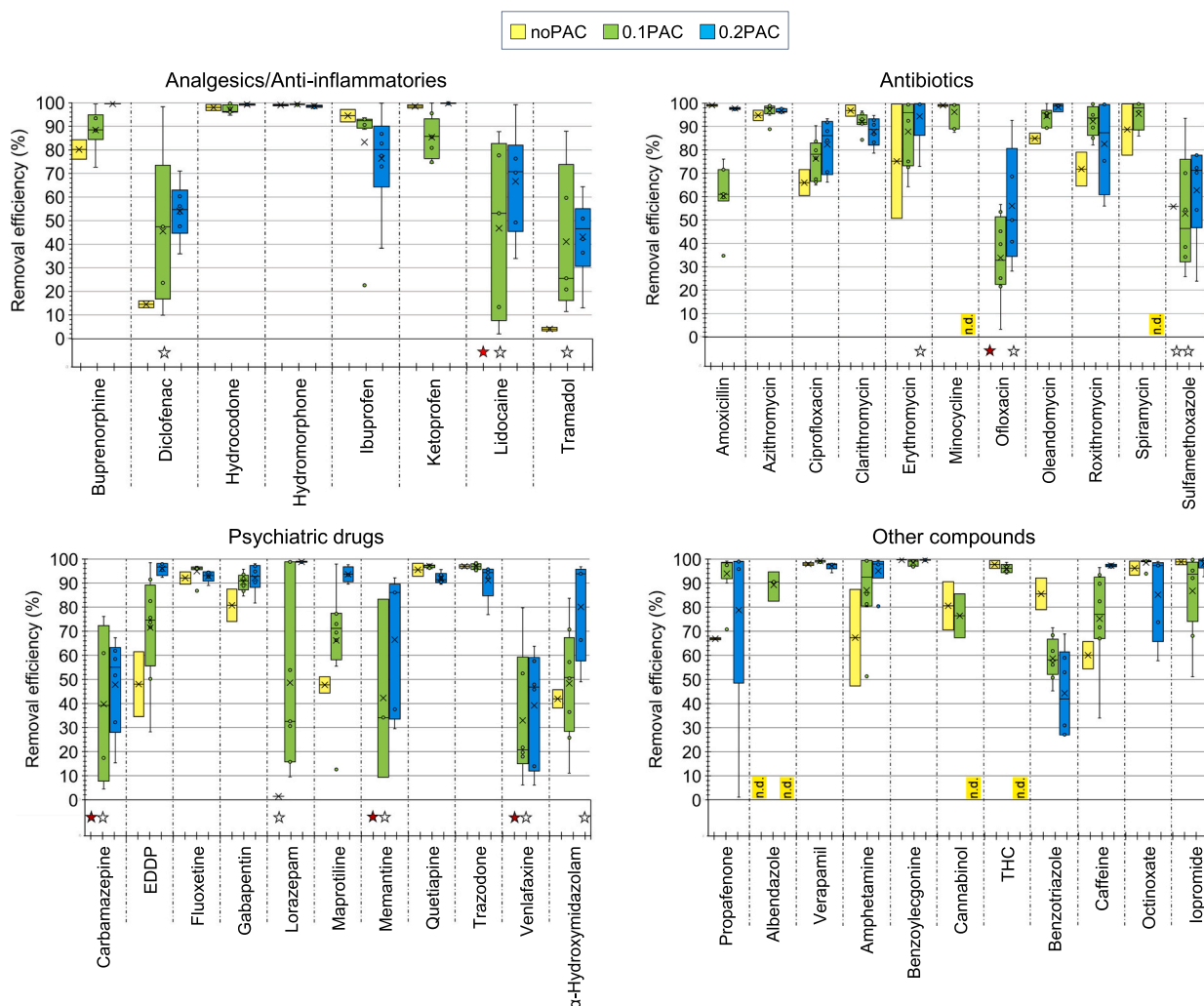
**Fig. 3.** Box plots representing the removal efficiencies of the key OMPs in each of the three campaigns. For each OMP, the experimental campaigns are depicted in the following order, from left to right: noPAC (yellow), 0.1PAC (green) and 0.2PAC (blue). n.d. indicates the compound was not detected in the INF and in MBRperm during that campaign. White stars (☆) indicate the presence of negative values not included in the chart. Red stars (★) indicate that all the removal efficiencies calculated were found to be negative during the corresponding campaign. To check the corresponding negative removal efficiencies, refer to Table S14. The circle (°) represents the daily removal efficiency, the cross (x) corresponds to the average, and the dash (–) represents the median.

et al., 2014; Rizzo et al., 2019; Verlicchi et al., 2012b). They present a complex structure with high molecular weight, and they have been removed from a moderate to high range in biological treatments (Alvarino et al., 2018; Asif et al., 2020; Echevarría et al., 2019).

The removal of azithromycin was shown to be consistent among the three campaigns, with average removal efficiencies between 95 % and 97 % regardless of the addition of PAC and its dosage. Despite the fact that the loads in the INF did not significantly change during the whole experimental period, roxithromycin showed no clear tendency when the PAC dosage was increased, with lower removals obtained on certain sampling days during the 0.2PAC campaign compared to 0.1PAC. However, the average removal increased from 72 % (noPAC) to 92 % and 83 % during 0.1PAC and 0.2PAC campaigns, respectively.

The average removal of erythromycin went from 75 % in noPAC to 88 % in 0.1PAC and 94 % in the 0.2PAC campaigns. Additionally, minimum removals were increased with the increasing dosage of PAC while reducing the range of variability of the results obtained. Erythromycin has shown a better removal in MBR compared to CAS systems, probably due to the higher sludge retention times SRTs (Echevarría et al., 2019). When PAC is added, its removal may be enhanced since the adsorption onto PAC increases its retention inside the bioreactor, and subsequently, its biodegradation (Echevarría et al., 2019). Additionally,

the removal has been shown to improve with the increase in the PAC dosage, in agreement with the literature (Alvarino et al., 2017; Echevarría et al., 2019; Serrano et al., 2011). In this way, it seems that azithromycin, erythromycin and roxithromycin are subjected to both adsorption onto PAC and biodegradation inside the MBR reactor.

A decrease in the treatment efficiency of amoxicillin is observed during the 0.1PAC campaign, with removal efficiencies that ranged between 35 % and 76 %, despite the high removal (≥ 97 %) maintained during both noPAC and 0.2PAC campaigns. The reduced removal observed during the 0.1PAC period could not be explained from the water quality but, the fact that the addition of 0.2 g/L of PAC did not reduce its average removal in the MBR indicates that the addition of the adsorbent does not hamper the removal of this compound. Indeed, amoxicillin has shown to be removed from water and wastewater by many porous adsorbents including activated carbon (Anastopoulos et al., 2020).

An average removal of 60 % was observed for ciprofloxacin during the noPAC campaign, which increased up to 76 % with the addition of 0.1 g/L PAC and 82 % with 0.2 g/L. Similar removals were observed from the biological treatment in previous studies (Kovalova et al., 2012; Margot et al., 2013), for which the WWTP effluent load of ciprofloxacin was further reduced by 63 % (Margot et al., 2013) and > 99 % (Kovalova

et al., 2013) by PAC used as a post treatment. In these studies, ciprofloxacin was considered susceptible to both adsorption and biodegradation in the hybrid treatments.

Although simultaneous removal mechanisms may take place in hybrid systems to foster the removal of OMPs, the governing mechanism may depend on the operational conditions applied. This is the case of sulfamethoxazole, for which a concentration of 0.2 g/L was needed to see a clear impact on its removal. Negative removal efficiencies were observed during the noPAC and 0.1PAC campaigns (Fig. 3). In the literature, the removal of this compound is ascribed mainly to biodegradation processes, with no further improvement with the addition of PAC (Alvarino et al., 2016, 2017). It seems that at lower PAC concentrations (0.1 g/L) the governing removal mechanism for this compound is mainly biodegradation, whereas at higher dosages the mode of degradation changes (i.e., adsorption combined with biodegradation) and sulfamethoxazole is degraded on the PAC surface, due to the interaction between the PAC and the mixed liquor (Li et al., 2011). However, it is worth highlighting that high variability was observed in any case during the addition of PAC into the bioreactor.

*3.3.1.3. Psychiatric drugs.* When it comes to the eleven psychiatric drugs selected, four compounds, namely fluoxetine, gabapentin, quetiapine and trazodone were well removed by the MBR ($\geq$ 80 %), and little effect of the addition of PAC was observed (Fig. 3). Among them, gabapentin was the most hydrophilic compound (log$D_{ow}$ = -1.42, Table S1) with the highest loads in the INF (2958 mg/d on average, Table S11) for which the addition of PAC is not expected to increase its removal (Margot et al., 2013). The remaining seven compounds still showed a great range of variability in the two experimental campaigns of the hybrid MBR although their removals were increased with the addition of PAC. For instance, the difference between the minimum and maximum removal efficiency of these compounds ranged between 70 % and 90 % during the 0.1PAC campaign. This variability was reduced when increasing the dosage of PAC for all the selected psychiatric drugs and, in particular, for lorazepam, maprotiline and the transformation product EDDP. EDDP is a metabolite of methadone, which was detected only on 33 % of the INF samples (Table S10), and solely during the 0.1PAC campaign (Table S16). During the noPAC campaign, four compounds, namely carbamazepine, venlafaxine, lorazepam and memantine, were found at higher concentrations in the MBRperm compared to the INF. The negative removal observed for carbamazepine during the noPAC campaign (Table S14) is consistent with the values previously reported in the literature (Arola et al., 2017; Bendz et al., 2005; Li et al., 2015; Moslah et al., 2018; Vieno et al., 2007), and the most likely explanation is the conversion of carbamazepine conjugated metabolites into the parent compound (Castiglioni et al., 2006; Moslah et al., 2018) due to the glucuronidase activity of the activated sludge (Moslah et al., 2018; Ternes et al., 1999). Carbamazepine and venlafaxine are compounds poorly removed in a biological reactor (Kovalova et al. (2012) (Margot et al., 2013; Radjenović et al., 2009). For these compounds, moderate average removal efficiencies (33 % and 40 %, respectively) were achieved with a PAC concentration of 0.1 g/L that slightly increased to 40 % and 48 % when doubling the dosage of PAC. In previous studies, moderate removal (approx. 50 %) (Nguyen et al., 2013b) and high removal (87 %) (Li et al., 2011) were obtained for carbamazepine with 0.1 g/L PAC. Experiments testing 1 g/L of PAC achieved up to 92 % of their removal efficiencies (Li et al., 2011; Serrano et al., 2011). However, all these studies were conducted at laboratory scale MBRs with synthetic wastewater, causing a decrease in the DOM competition effect. Indeed, this compound has been suggested as an indicator of PAC saturation of the active sites for adsorption (Echevarría et al., 2019), and it has proved to be greatly dependent on the presence of fresh PAC (Alvarino et al., 2017). In this regard, it is of great importance to consider the sampling day with respect to the addition of fresh PAC, so as not to disturb the quantification of the contaminant. In this investigation, sampling days were at least 1 day after the addition of PAC.

*3.3.1.4. Other classes of key OMPs.* The remaining 11 key OMPs pertaining to different classes responded to the PAC addition in a variety of ways. Propafenone (antiarrhythmic agent) showed a consistent increase in its removal efficiency during the treatment with 0.1 g/L of PAC to subsequently be apparently reduced during the 0.2PAC campaign. The fact remains that removal efficiencies ranged from 96 % to 99 % with the addition of 0.2 g/L of PAC, except from one sampling day on which only 1 % of removal was observed (Table S13), which corresponds with the minimum load arriving to the WWTP (INF) during both the 0.2PAC campaign (Table S20) and the whole experimental period (Table S11).

Albendazole (antiparasitic), which was selected due to its high concentration in HWW, was only detected during the 0.1PAC campaign with a frequency of detection of 33 % in the INF. During this campaign, removal efficiencies ranged from 83 % to 95 %.

Verapamil, benzoylecgonine and THC showed removal efficiencies of over 95 % in all three campaigns. Among the four key illicit drugs, benzoylecgonine, which was selected due to its high frequency of detection, was removed by over 97 % in the three campaigns. Other transformation products and OMPs related to this substance (i.e., cocaethylene, cocaine, ecgonine methyl ester) were also well removed in the MBR ($\geq$ 78 %) and their removal was increased by up to 99 % during the 0.2PAC campaign (Table S13).

Amphetamine was one of the compounds that best responded to the addition of PAC, from moderate removals in the MBR (average of 67 %) to very high during the 0.1PAC (87 %) and 0.2PAC (95 %) campaigns. However, the results must be taken with caution, since the frequency of detection and occurrence of this compound in the INF reduced over time (Tables S15 – S17).

Caffeine, a compound that has shown to be removed biologically (Remy et al., 2012), significantly improved its average removal when increasing the PAC dosage. In contrast to amphetamine, its concentration varied between 1.65 µg/L and 6 µg/L and was consistent between the experimental campaigns.

Benzotriazole (plastic additive) has been found in the literature (Kovalova et al., 2013; Löwenberg et al., 2014; Margot et al., 2013) among the highest concentrations in WWTP effluent among the tested compounds showing low biodegradation. During the experiments, the average removal of 86 % was found in the MBR, which later decreased to 59 % and 44 % in the 0.1PAC and 0.2PAC campaigns. Benzotriazole seems to show good adsorption to activated carbon, but the literature data found only refer to PAC used as a post-treatment after the biological reactor (Boehler et al., 2012; Löwenberg et al., 2014; Margot et al., 2013). Although the water matrix was different in previous studies, the results obtained were not expected, and thus there is no explanation for the decrement in removal during the PAC campaigns. The adsorption of OMPs onto PAC may be influenced by the water matrix as shown in (Gutiérrez et al., 2023).

Finally, iopromide, pertaining to the X-ray contrast media class, showed very high removal in both the noPAC and PAC treatments. This compound is known for not being degraded in biological systems and for its low tendency to adsorption (log$D_{ow}$ = −2.66, Table S1) (Carballa et al., 2004; McArdell et al., 2011). Indeed, contrast media are designed to be highly stable in the human body to serve as markers for radiology examinations (Ternes and Hirsch, 2000). Since the radiology department conducts these tests mainly during weekdays, this compound is irregularly discharged into the sewer, leading to a highly variable concentration in the HWW and INF (Tables S15 – S17) (Ort et al., 2010a, 2010b; Weissbrodt et al., 2009). In the current study, high and moderate removals were achieved in all the campaigns, with a slight reduction in the average removal efficiency in the 0.1PAC campaign (87 %) compared to noPAC (99 %) and 0.2PAC (98 %). The reduction in removal during the first campaign with PAC may be related to the high

loads of this compound arriving at the WWTP (Table S19), likely due to an increase in pulmonary scanning during the Covid-19 pandemic. Besides, the results obtained during noPAC treatment are unexpectedly high and constant in comparison with other studies (Joss et al., 2005; Margot et al., 2013). Other X-ray contrast media (i.e., diatrizoate and iopramidol) analyzed in the NTS show much lower removal efficiencies ($\leq$ 25 %) or even negative removal (Table S22), whereas the compound iohexol showed removal efficiencies in the same trend as iopromide.

Joss et al. (2005) found unexplained great removal variations for this compound in both CAS and MBR. On the other side, PAC has shown to increase the removal of iopromide from 29 % (CAS) to 47 % (as a post-treatment, 1–2 g/L of PAC) (Margot et al., 2013). In our case, since the removal of this compound is already very high in the biological treatment, no further improvement was observed with the addition of PAC.

*3.3.1.5. Considerations.* When evaluating the effectiveness of a treatment as well as the operational conditions tested (in this case, two PAC dosages), removal efficiencies may not provide the full picture of the potential improvement of the water quality. For this reason, a FOPBT analysis (Section 2.5.1) was conducted referring to the key OMPs, in order to analyses their average removal with their frequency of detection and occurrence in the MBRperm, keeping in mind their tendency for bioaccumulation and inherent toxicity. The final scores of the key compounds and the contributions of each criterion are reported in Fig. 4 for the three campaigns.

Lower final scores are to be interpreted as satisfactory results as they mostly contribute to a better overall quality of the permeate and a lower potential impact to the receiving water body.

In this regard, the results shown in Fig. 4 indicate that, out of the 41 key OMPs, 22 and 27 compounds reduced their score in the 0.1PAC and 0.2PAC campaigns, respectively, in comparison with the total final scores obtained in noPAC. This indicates that the addition of PAC may

improve the quality of the effluent for 53 % (0.1PAC) and 65 % (0.2PAC) of the key OMPs analyzed in detail.

When taking a closer look at the contributions of the different criteria, it can be seen that the scores of the frequency F criterion were reduced in 10 and 17 OMPs from noPAC to the 0.1PAC and 0.2PAC campaigns, respectively. When the PAC dosage was increased (from 0.1 to 0.2 g/L), the frequency reduction was observed for 18 OMPs. For 9 compounds, no further reduction in the F scores could be made since their frequencies of detection were already below 20 % (score = 1).

Compounds with the highest frequency of detection and occurrence in the INF (among them, diclofenac, azithromycin, caffeine, gabapentin and ofloxacin) did not significantly decrease their frequency of detection in the MBRperm, when increasing the PAC dosage. However, the increased removal efficiencies observed for many key OMPs (including some of the above-mentioned compounds) led to a lower occurrence and therefore to a lower final score (e.g., diclofenac, caffeine, ofloxacin, sulfamethoxazole and amphetamine). A higher final score in the treatment with added PAC (e.g., iopromide, clarithromycin and benzoylecgonine) is mostly due to a higher frequency of detection of the compound in the MBRperm, rather than to a reduction in their removal or a higher occurrence in the MBRperm.

Bioaccumulation and toxicity scores, which are inherent values of the OMPs under study, do not change among the experimental campaigns. Their inclusion in Fig. 4 intends to quickly verify the compounds for which the reduction of the frequency, occurrence and persistence scores should be of utmost importance. In this regard, it is fundamental that the treatment and/or applied operational conditions focus on the reduction of the final score $S$ of these compounds. In this regard, while for some compounds (e.g., diclofenac and venlafaxine) the final score $S$ was still among the highest even after the addition of 0.2 g/L PAC, for other compounds such as lorazepam, maprotiline and memantine, it significantly reduces when increasing the dosage of the adsorbent even
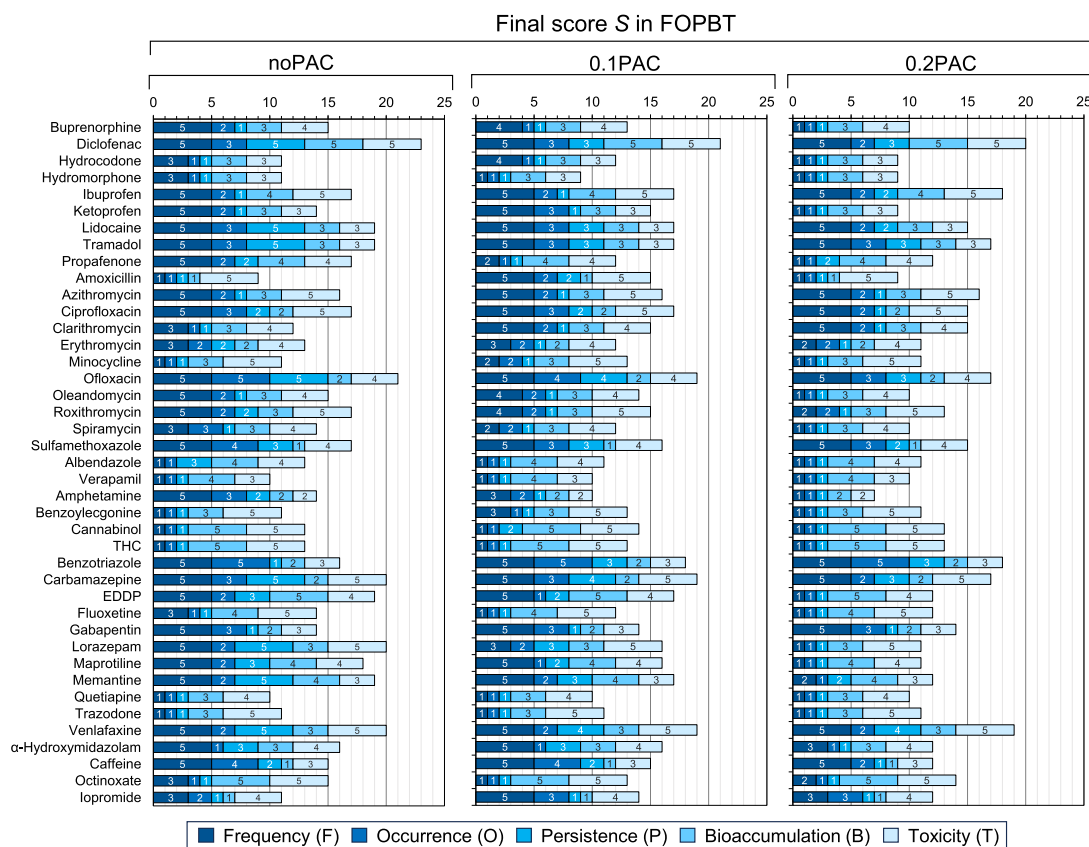


**Fig. 4.** Final FOPBT scores $S$ for the key OMPs and contributions of the different criteria during the noPAC, 0.1PAC and 0.2PAC sampling campaigns.

though bioaccumulation and toxicity criteria had scores ≥3.

### 3.3.2. Other removal analyses

Text S7 analyses the removal of the key compounds according to their physico-chemical properties (log$D_{ow}$ and charge) reported in Fig. S3. The analysis of the removal achieved during the campaigns completes with a discussion of the average removal efficiencies for the different classes (Text S8, Table S21 and Fig. S4) and for the whole set of compounds (Text S9, Fig. S5).

### 3.4. Removal efficiency of non-target OMPs

Ninety non-target OMPs were monitored in the three experimental campaigns for the four sampling points. The details of their frequency of detection and persistence are reported in Table S22. Briefly, their frequency of detection was on average 90 % for both HWW and INF. Instead, in the MBRperm it was 81 % (noPAC), 80 % (0.1PAC) and 57 % (0.2PAC). Roughly speaking, it seems that the addition of activated carbon to the MBR promotes the removal for most of these OMPs. In order to better analyze the performance of the treatments, scores were assigned for frequency, persistence, bioaccumulation and toxicity to each non-target compound according to Table 1 as shown in Table S23, thus defining a final FPBT score *S* for each of them. Following the same approach adopted for the target compounds (Section 2.5.1), a list of 40 non-target OMPs was identified including the substances with a final FPBT score ≥ 14 in the MBRperm of the noPAC campaign.

Fig. 5 reports the final scores for each compound in the three experimental campaign together with the contributions of each criterion. It emerges that a score equal to 5 was assigned to the Frequency of all the 40 OMPs in the noPAC and 0.1PAC treatments, whereas a higher variability was found in 0.2PAC, with there being 9 compounds with a score = 1 and one with score = 3. These results must be taken with caution since a low frequency of detection in the MBRperm not only implies that the compound is removed to a higher extent, but also that the OMP could have a low frequency of detection in the influent during that sampling campaign. This is the case of celestolide (Table S22) which

exhibited a frequency of detection of 33 % in the INF during the 0.2PAC campaign, and it was not detected in the MBRperm.

It is also evident that the addition of 0.1 g/L of PAC improves the removal of many compounds and subsequently persistence is reduced (for 26 out of 40 compounds, the score assigned to persistence reduces). This phenomenon is slightly enhanced by the addition of 0.2 g/L of PAC (for 30 out of 40 compounds).

Since the concentrations of the non-target OMPs are not available, the analysis of the performance of the treatment must be limited to the four abovementioned criteria. This confirms that the data of occurrence allows a complete and rigorous analysis of the performance of the treatment efficacy. At this step it can be deduced that the trend found for the target OMPs (i.e., the higher the dosage, the higher the treatment efficiency) can also be expected for the non-target compounds.

### 3.5. Environmental risk assessment

An assessment of the environmental risk posed by the residues of the OMPs in each experimental campaign was carried out by means of the risk quotient (*RQ*), that is the ratio between the average OMP (measured) concentration and its corresponding PNEC. The *RQ* was calculated referring to the MBRperm and the common ranking criterion (Hernando et al., 2006) was first applied to the key 41 OMPs, except benzoylecgonine for which the PNEC is not available. On this basis, the OMPs were classified into three environmental risk levels: high, medium and low.

As seen in Fig. 6, there were nine compounds in the MBRperm that posed a high risk (RQ ≥1) during noPAC treatment, and eight and six in the 0.1PAC and 0.2PAC campaigns, respectively. Five key OMPs exhibited the highest risk in all three campaigns (ofloxacin, carbamazepine, azithromycin, ibuprofen and diclofenac). It is important to remark that their PNECs were among the lowest of the analyzed OMPs (Table S1). In certain sampling campaigns, a major use of some substances in the hospital (e.g., iopromide) and/or a lower achieved removal efficiency (e.g., ketoprofen) caused a higher concentration in the MBRperm leading to a higher risk for the environment.
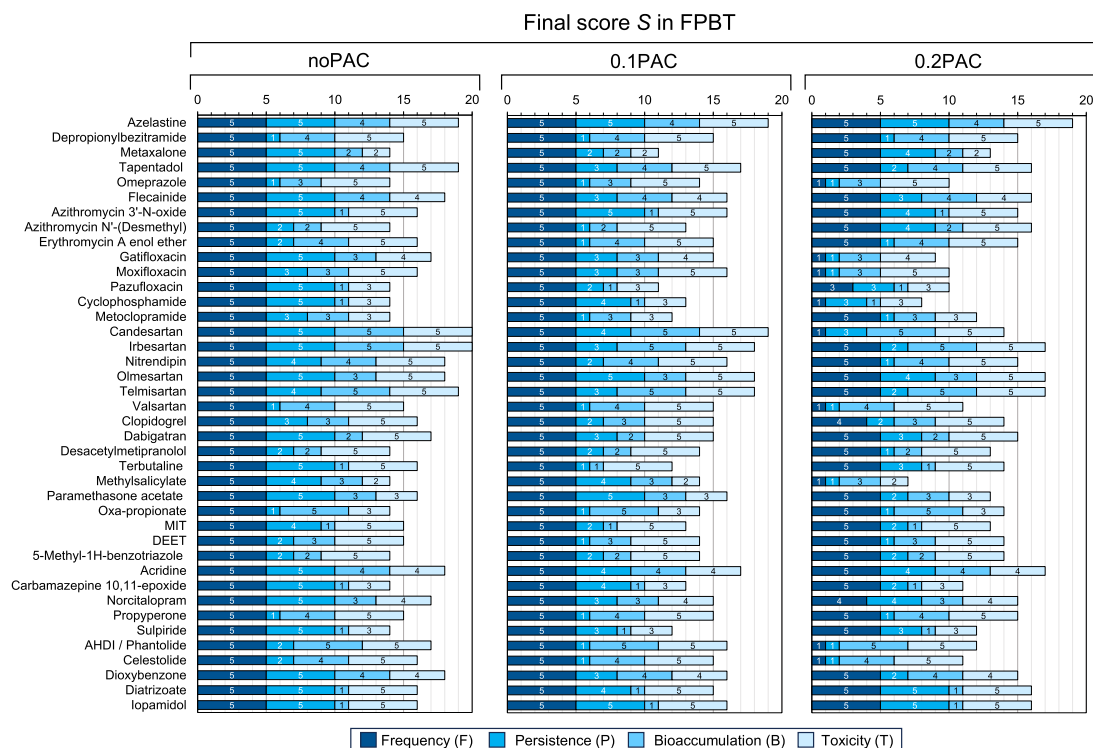


**Fig. 5.** Final FPBT scores *S* for the selected non-target OMPs and contributions by the different criteria during the noPAC, 0.1PAC and 0.2PAC campaigns.
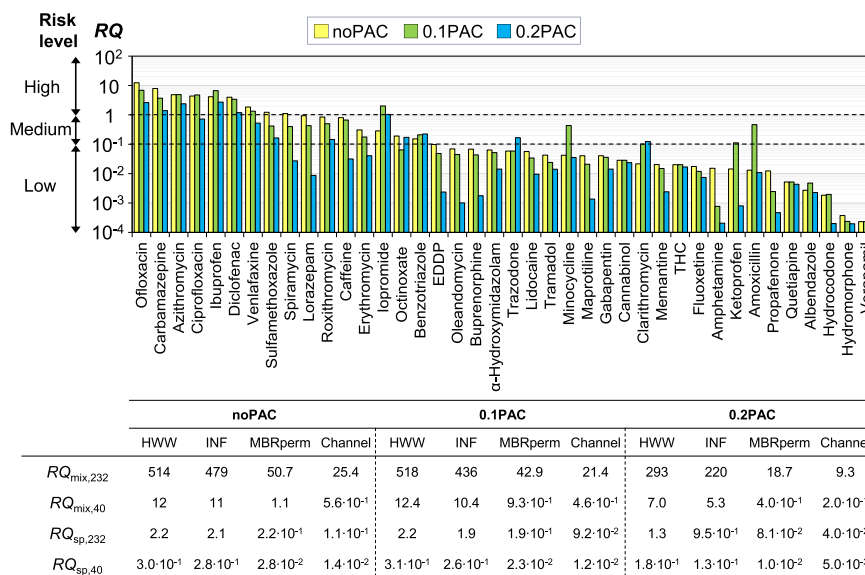
**Fig. 6.** Average risk quotients ($\overline{RQ}$) for 40 OMPs in the MBRperm during the three experimental campaigns (the 41 key OMPs except for benzoylecgonine), together with (in the table below) the $RQ_{mix}$ and $RQ_{sp.}$ Referring to the 232 target OMPs and the 40 key OMPs in HWW, INF, MBRperm and the water channel (1/2 dilution of the MBRperm).

| | noPAC | | | | 0.1PAC | | | | 0.2PAC | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HWW | INF | MBRperm | Channel | HWW | INF | MBRperm | Channel | HWW | INF | MBRperm | Channel |
| $RQ_{mix,232}$ | 514 | 479 | 50.7 | 25.4 | 518 | 436 | 42.9 | 21.4 | 293 | 220 | 18.7 | 9.3 |
| $RQ_{mix,40}$ | 12 | 11 | 1.1 | $5.6 \cdot 10^{-1}$ | 12.4 | 10.4 | $9.3 \cdot 10^{-1}$ | $4.6 \cdot 10^{-1}$ | 7.0 | 5.3 | $4.0 \cdot 10^{-1}$ | $2.0 \cdot 10^{-1}$ |
| $RQ_{sp,232}$ | 2.2 | 2.1 | $2.2 \cdot 10^{-1}$ | $1.1 \cdot 10^{-1}$ | 2.2 | 1.9 | $1.9 \cdot 10^{-1}$ | $9.2 \cdot 10^{-2}$ | 1.3 | $9.5 \cdot 10^{-1}$ | $8.1 \cdot 10^{-2}$ | $4.0 \cdot 10^{-2}$ |
| $RQ_{sp,40}$ | $3.0 \cdot 10^{-1}$ | $2.8 \cdot 10^{-1}$ | $2.8 \cdot 10^{-2}$ | $1.4 \cdot 10^{-2}$ | $3.1 \cdot 10^{-1}$ | $2.6 \cdot 10^{-1}$ | $2.3 \cdot 10^{-2}$ | $1.2 \cdot 10^{-2}$ | $1.8 \cdot 10^{-1}$ | $1.3 \cdot 10^{-1}$ | $1.0 \cdot 10^{-2}$ | $5.0 \cdot 10^{-3}$ |

In other cases, higher $RQ$ values were found in the 0.1PAC campaign with respect to the other campaigns. They correspond to key OMPs with very low PNEC values that were only detected in the MBRperm during that period. This is the case of amoxicillin (PNEC = 0.078 μg/L) and minocycline (0.041 μg/L). In any case, it emerges that the $RQ$ value for most of the key OMPs reduced when the PAC dosage was increased. In particular, during the 0.2PAC campaign, the $RQ$ values were significantly reduced for 36 out of the 40 OMPs with respect to the noPAC and 0.1PAC campaigns.

Moreover, an analysis was carried out for the risk posed by the mixture $RQ_{mix}$ of the key OMPs and the whole set (232 OMPs), according to Eq. (4), for HWW, INF and MBRperm as well as for the receiving water channel, assuming that the permeate is released (Fig. 6). As expected, higher $RQ_{mix}$ values were consistently found in HWW with respect to the INF among campaigns, since, in general, higher concentrations of OMPs are found in HWW, in agreement with past studies (Castaño-Trias et al., 2023; Santos et al., 2013; Verlicchi et al., 2013).

Secondly, when taking a closer look at the differences between experimental campaigns, it emerges that the overall risk in both HWW and INF ($RQ_{mix, 232}$) during the noPAC and 0.1PAC campaigns is approximately twice the value found in the 0.2PAC campaign. This finding is related to the fact that the overall use of (certain) pharmaceuticals in the hospital was reduced after the Covid-19 pandemic. Similarly, when considering $RQ_{mix,40}$ in the MBRperm, it emerges that its value decreases from 1.1 in noPAC and 0.93 in 0.1PAC to 0.4 in 0.2PAC. Moreover, in the MBRperm $RQ_{mix,232}$ and $RQ_{mix,40}$ reduced by at least one order of magnitude compared to the INF, showing the effectiveness of the treatment.

Assuming that the permeate is directly released into the surface water, a small channel with a flow rate (0.01 m³/d) similar to that of the WWTP (0.011 m³/d), the risk, after the release of the treated effluent, is reduced to 50 % of the value found in the permeate. The $RQ_{mix\ 232}$ in the channel amounts to 25.4, 21 and 9.3 in the three campaigns and $RQ_{mix,40}$ is equal to 0.56, 0.46 and 0.20.

Considering instead the average concentrations found for the disinfected effluent EFF during the noPAC and 0.1PAC campaigns (Table S24), $RQ_{mix,\ 232}$ becomes 47 and 46 for EFF, respectively, and 23.5 and 23 for the channel. Moreover $RQ_{mix\ 40}$ in the EFF was 0.5 in both the campaigns and in the channel 0.25.

As clearly reported in Section 2.2, for technical reasons, the

disinfected effluent was sampled on the same days as the INF, without accounting for the 24-h-HRT of the WWTP. For this reason, the measured concentrations for the effluent were not considered in the evaluation of the reduction of the concentrations along the whole treatment train during the different sampling days.

In addition to $RQ_{mix}$, the specific risk quotient ($RQ_{sp}$) for the OMPs was evaluated by Eq. (5) (Fig. 6). This value represents the $RQ$ a hypothetical OMP would have in each of the sampling points and campaigns, assuming that all the OMPs of the mixture (of 232 or 40 key OMPs) equally contribute to the risk. It emerges that $RQ_{sp,40}$ is generally one order of magnitude lower than $RQ_{sp,232}$, for each sampling points and the lowest values were found in the 0.2PAC campaign.

Although on the basis of $RQ_{mix}$ and $RQ_{sp}$ it may seem that there is not a great difference in the environmental risk reduction from the influent and permeate in the three experimental campaigns, it must be noted from Fig. 6 that the risk associated with the individual key OMPs was significantly reduced with the addition of activated carbon inside the MBR. This is the case of lorazepam and spiramycin, to cite only a couple. The environmental risk assessment of the mixture is here evaluated by means of Eq. (4), and does not consider the synergic and antagonist effects due to the presence of many OMPs (Vasquez et al., 2014). A more complex approach able to account for different effects would lead to a more accurate evaluation of the environmental risk due to a discharge of the WWTP effluent into the surface water body.

## 4. Discussion

### 4.1. Hospital load and influent load

The WWTP under study is mostly fed by HWW, the flow rate of which represents around 75 % of the total influent (Fig. S1). The OMP load due to the hospital contributes to around 80 % referring to the three campaigns (Fig. S2A). Some exceptions occurred and they are discussed here (Tables S11, S18-S20).

OMP concentration in the INF results from the mixture of HWW and UWW. As described above, only HWW and INF were sampled, and thus information on the composition of OMPs in UWW was lost. Although UWW contributes solely to 25 % of the INF flow rate, this fraction can considerably change the composition of the wastewater matrix, since the observed numerical differences among OMP concentrations do not

provide a glimpse about what actually happens when HWW and UWW get mixed.

First of all, their mixing may have two different effects when considering only the components that are already in the HWW: the dilution and the concentration effect. The dilution effect occurs for the components that are present in HWW but are not present in UWW, where an increase in volume of 25 % reduces the initial concentration of the OMP in the HWW (e.g., alfentanil, hydrocodone, naproxen, erythromycin and many others). However, it must also be considered that UWW may contain some compounds largely consumed by the local population that is conveyed to the WWTP through the public sewer, increasing the initial concentration of these components in the INF (e.g., acetaminophen, acetylsalicylic acid, diclofenac, ibuprofen and roxithromycin). In addition, the mixing of HWW and UWW leads to the introduction of substances that were not originally present in HWW (e. g., sulfaclozine). The interaction among these substances can lead to degradation processes resulting in transformation products that are concurrently reacting with the OMPs present in the stream. At the same time, complexes between the organic components and metals (which may also be present) may be formed, OMPs may be deposited in the sewer surface, and so on. According to (Martínez-Alcalá et al., 2021), all these possible processes that occur in such a complex water matrix, together with the OMP physicochemical properties that may greatly vary among compounds, can directly affect the "availability" of the compound in the analytical procedure. This leads to unexpected loads that are difficult to explain completely since they are never the result of a single process. Differences in the chemical and biological composition of the wastewater can explain the results obtained to a certain extent (Martínez-Alcalá et al., 2021).

### 4.2. Negative removal

Negative removal, corresponding to a higher OMP load in the MBRperm with respect to the INF, occurred for 62 OMPs considering all three experimental campaigns (Table S14). The results obtained are not accidental, as they have been found in the literature many times (Castiglioni et al., 2018; Gago-Ferrero et al., 2020; Margot et al., 2013; Moslah et al., 2018). Among the OMP classes studied, antibiotics, analgesics/anti-inflammatories and psychiatric drugs, exhibited the highest load in HWW and INF, suggesting a high excretion rate and/or a high resistance to biological degradation (Hapeshi et al., 2015). For this reason, it is not surprising that among the OMPs pertaining to these classes, negative removal efficiencies were observed (Table S14), i.e., erythromycin, ofloxacin, sulfamethoxazole, carbamazepine, venlafaxine and metoprolol. This can probably be attributed to (i) a number of processes occurring in the accumulation tank at the WWTP (Fig. 1); (ii) the release of compounds entrapped in fecal particles during treatment (Göbel et al., 2007); (iii) the biological cleavage during treatment of pharmaceutical conjugates (human metabolites) that re-produce the parent compound (Onesios et al., 2009); (iv) the formation of bacterial metabolites in the bioreactor; and (v) analytical uncertainties (Margot et al., 2013).

In this context, some authors have already explained the increased concentration due to deconjugation processes with reference to sulfamethoxazole. Its metabolites and conjugated forms such as N4-acetylsulfamethoxazole (Table S22) can be degraded and converted back to the parent compound as these sulfonamides occur in the wastewater (Moslah et al., 2018; Petrovic et al., 2009).

The negative removal results observed for metoprolol and venlafaxine (Table S14) are also consistent with those in the previous literature (among them (Alves et al., 2018; Aymerich et al., 2016), where these two pharmaceuticals, along with their main metabolites, desvenlafaxine (Table S14) and metoprolol acid (Table S22), were selected to evaluate the adsorption of pharmaceutical metabolites compared to their parent compounds. For this reason, it is expected that metabolites can sometimes (or often) be found at higher concentrations

in the WWTP effluent, which is the permeate in the current study (Alves et al., 2018; Aymerich et al., 2016).

All this supports the negative removal trend (Table S14) observed for 62 OMPs. However, it should be emphasized here that the feeding to the full-scale WWTP continuously changes its composition. Variations in the influent OMP concentrations can be attributed to variations in their daily consumption (Margot et al., 2013; Moslah et al., 2018), the severity of the illness, and the season. These variations underline the importance of long-term sampling campaigns lasting at least a year to capture different patterns of use of individual substances.

### 4.3. Uncertainties

Although great efforts have been made to improve the reliability of the analysis of OMPs, much remains to be done. This is partly due to the difficulty to accurately measure the flow rate, to sample within the sewer system, as well as the complex composition of the wastewater, often considered as "unique", etc. Thus, unavoidable uncertainties related to the analytical procedures may affect the measurement of the concentration and consequently the load of OMPs. The analytical procedure usually consists of five steps: sampling, sample preparation, separation, detection and data analysis. In this section, the different issues are discussed with regard to the present investigation.

#### 4.3.1. Sampling

Since most of the OMPs are currently unregulated compounds, guidelines for their sampling are not available, and defining a sampling strategy becomes fundamental to provide reliable data (Verlicchi and Ghirardini, 2019). Over the course of the experimental campaigns, 24-h time proportional composite samples were taken with an automatic sampler. This is quite a common approach since it can take into account the concentration pattern of the OMP in the wastewater (Ort et al., 2010a). However, with this method the hourly aliquots of water have the same weight. The sampling therefore does not consider that in the night some OMPs would have lower concentration at lower flow rates, and thus the corresponding loads would be over-represented, as remarked in Ort et al. (2010a, 2010b), Verlicchi and Ghirardini (2019) and Verlicchi (2018). For these reasons, the HWW and INF loads may be underestimated. Sampling has been recognized as a relevant source of uncertainty in the analysis of OMPs in wastewater (Ort et al., 2010b). In this regard, the uncertainty associated with time proportional composite sampling is clearly discussed in Verlicchi (2018) which explores the fact that compounds may show a different pattern of consumption throughout the day (e.g., analgesics/anti-inflammatories compared to contrast media), and thus a 24-h composite sample may be deemed necessary to provide representative wastewater samples (Ort et al., 2010a). In this regard, Verlicchi and Ghirardini (2019) found that flow-proportional composite sampling leads to a better measurement of the concentration and calculation of the removal of an OMP, followed by time-proportional composite sampling, in agreement with previous studies (Ort et al., 2010a, 2010b). Apart from hourly variations of OMP concentration, other weekly (weekday versus weekend) and monthly/season temporal variations may occur in the HWW and UWW (Verlicchi et al., 2013). In this investigation, samples were taken on weekdays in a two-year time lapse that covered the different seasons and the Covid-19 pandemic outbreaks that occurred in 2021 and 2022. In the hybrid MBR coupled to the PAC treatment, weekly samples reduced the uncertainty related to pattern consumption of the analyzed OMPs.

#### 4.3.2. Sample preparation

In chromatographic analysis, sample preparation is the most common cause of inadequate accuracy and poor reproducibility when determining OMPs in the range of ng/L - μg/L (Kataoka, 2003; Wardencki et al., 2007). Currently, solid phase extraction (SPE) is the most commonly used sample preparation approach in determining OMPs (Castiglioni et al., 2018; Rezaei Adaryani and Keen, 2022; Silva et al.,

2021). Although its primary purpose is to increase the analyte concentration and to obtain cleaner extracts by reducing the influence of the matrix effect (ME) on the sample (Backe and Field, 2012; Busetti et al., 2012), SPE can also lead to analyte losses and increase the analytical errors associated with sample manipulation (Busetti et al., 2012). For instance, components of the water matrix that cause the MEs may be able to coelute and concentrate together with the analytes during the SPE (Taylor, 2005; Trufelli et al., 2011).

For all these reasons, the direct injection (DI) method has been proven by many authors to be an attractive alternative especially for large and/or complex samples such as raw wastewater, since everything is simplified and accelerated, which was the reason for using it in this work (Nieto-Juárez et al., 2021; Perkons et al., 2021; Simarro-Gimeno et al., 2023; Son et al., 2021; Wilkinson et al., 2019).

In previous studies, authors have found that the analytes are more subjected to MEs with SPE methods (i.e., signal suppression during electrospray ionization), especially in complex matrix samples (Bijlsma et al., 2021; Dasenaki and Thomaidis, 2015; Gros et al., 2006; Lopez et al., 2022), while using DI reduces the ME to small or moderate. On the contrary, general enhancement of the instrument response is found for compounds as norfloxacin and venlafaxine when using DI (de la Serna Calleja et al., 2023). In summary, the DI method used in this work appears to be an excellent alternative to SPE due to its speed, lack of sample preparation (and associated lower analytical errors), and even lower MEs (Simarro-Gimeno et al., 2023).

Lastly, it is important to note that the ME in a sample can be determined by conducting the standard addition method, however this was not the case. This is because, as already mentioned, the (waste) water samples from the four sampling points may be considered of a different nature, besides the fact that the composition of the HWW and INF may change thorough the day. Furthermore, at least three times the amount of sample is required in order to properly perform the procedure, which would increase the volume required for sampling, and the space for storage and transportation. In this context, limited sample manipulation as well as the elimination of analyte losses and human errors through the use of DI limited the uncertainties related to sample preparation in the wastewater samples taken for the investigations.

### 4.3.3. Separation, detection, and data analysis

Sensitive high resolution mass spectrometers (HRMS), such as the QTOF instrument used in this study, has allowed robust data to be collected on concentrations of multiple OMPs from complex matrices such as HWW, its mixture with UWW (INF), MBRperm in the three campaigns (i.e., noPAC, 0.1PAC and 0.2PAC) and the disinfected effluent (EFF). Besides, the QTOF instrumentation used in this work has contributed to eliminate signal-related interferences (Stipaničev et al., 2017). Instrumentation, and acquisition settings and software tools utilized for the processing and analysis of mass spectral data were selected in agreement with Schymanski et al. (2015) and (2021). The confidence of a feature depends on the amount of evidence available and is usually identified by means of the level system proposed by Schymanski et al. (2014a) and Minkus et al. (2022). Target analytes are confirmed as such once they are unequivocally identified (i.e., Level 1) (Schymanski et al., 2014a) with a proposed structure that matches mass spectrometry MS, $MS^2$ and retention time RT information with a reference standard. In this study, the 232 compounds were analyzed as target OMPs with a modified analytical method proposed by Stipaničev et al. (2017). For the NTS here conducted, the identification confidence corresponds to Level 2 (i.e., probable structure) proposed by Schymanski et al. (2014a). Although the quantification is not a priority for NTS, a rough estimation of the occurrence is needed. The signal intensity measured is usually assumed to be to some extent proportional to the concentration of the compound (Schymanski et al., 2014b). However, no direct proportionality may be assumed due to different factors, among them the ME (Minkus et al., 2022). The application of isotopically labelled internal standards (ISTD) is one method used to normalize

intensities (Minkus et al., 2022). However, the size and composition of an ISTDs set for intensity normalization is difficult, as the analytes in the water sample are initially unknown to the investigation and a large structural variety may be expected.

At present, it is recognized that NTS cannot replace the conventional monitoring schemes based on target analysis, but NTS could be implemented in the regulation as a first screening step in the risk assessment chain to trigger further target analysis. For this reason, data provided in this study with regards to the NTS is limited to the frequency of detection of the analytes (absence/presence of signal) and their removal efficiency, calculated according to Eq. (2), based on the ratio between the difference in the response signal in the influent and the MBR permeate divided by the influent response signal. In this context, an uncertainty in the estimation of the signal of one order of magnitude could be considered acceptable, whereas in target analysis the uncertainty is usually accepted if it is < 20 % (Hollender et al., 2019).

Since the acceptance criteria for accuracy were recoveries between 70 % and 110 %, and samples were injected and analyzed several times with relative standard deviations lower than 25 % (Stipaničev et al., 2017), the error caused by the analytical method is reduced to an acceptable level due to all the measures taken.

### 4.3.4. Flow rate and load

In order to evaluate the OMP load, the flow rate must be known. In this study, it was assumed that for each sampling day, the hourly flow rate keeps constant and is the same in the influent and effluent. The flow rate derives from measurements of the water volumes arriving at the WWTP recorded once a day. As described in Verlicchi (2018), the flow rate may vary over the day and the assumption of a constant value introduces uncertainties also affecting the OMP load estimation, which is difficult to quantify.

## 5. Final remarks and conclusions

The results obtained in this study support the fact that the addition of PAC to the biological reactor is able to abate the loads of many OMPs at a time, reduce their concentration in the permeate and, consequently, greatly reduce the overall environmental risk posed by the residues discharged in the receiving water body.

The investigations showed that by adding PAC, the removal of most of the OMPs of greater concern improves. Referring to the classes which most contribute to the influent load of OMP, it emerged that those characterized by a high removal in the MBR, maintain a high removal in the presence of PAC (analgesics, anti-inflammatories and X-ray contrast media) and classes with a medium removal efficiency in MBR show a substantial increment in the presence of PAC (antibiotics, psychiatric drugs and stimulants). Moreover, it was found that the overall load (referring to 232 target OMPs) did not significantly reduce in the presence of PAC, but the concentrations of OMPs of greater concern were greatly reduced. This is a strength of the tested technology as it is able to improve the quality of the final effluent and thus reduce the overall risk for the environment.

It is also important to underline that the collection of data of concentrations and of the removal efficiencies here presented and discussed refers to a full-scale WWTP characterized by quali-quantitative variations in the influent, typical of the plant size (4000 PE), served population (mainly HWW) and sewer type (separate for the HWW and combined for the UWW). Variations in the WWTP performance were thus expected and, for this reason, the evaluation of the abatement capacity of the hybrid MBR was carried out taking different aspects into consideration (FOPBT and FPBT analysis for target and non-target OMPs respectively).

Non-target OMPs were also investigated in order to identify other (and emerging) compounds which could become of interest for future experimental campaigns.

The study also provides new insights for the debate regarding the

revision of the Urban Wastewater Treatment Directive (UWWTD) (European Commission, 1991). A draft proposal of the revision was published in October 2022 (European Commission, 2022a), and more recently a new document was set by the European Community (Halleux, 2023) underlining the need and the timelines of actions for WWTPs of different sizes. In particular, quaternary treatments are requested for larger WWTPs ($>$ 100,000 PE), which are able to guarantee the removal of a set of micropollutants (including OMPs) among those listed, via ozonation and/or activated carbon or advanced techniques like nanofiltration membranes (as discussed in Pistocchi et al. (2022a, 2022b)). Some of the substances selected in the draft of the Directive are included in this study: this is the case for amisulpride, carbamazepine, citalopram, clarithromycin, diclofenac, metoprolol, venlafaxine, benzotriazole, candesartan and irbesartan.

Moreover, the tested technology (MBR coupled to PAC) might represent a feasible option for existing WWTPs where the biological reactor (CAS or MBR) could be upgraded into a hybrid MBR in order to guarantee greater protection of the environment as proposed by the draft of the new directive.

Further investigations should deal with the OMP concentrations in the sludge where PAC particles are incorporated and the potential impact the (treated) sludge may have on the environment or in the case of other destinations. Lastly, the assessment of the effects of the mixture of OMPs occurring in the final effluent with regard the receiving water body still requires further research.

## CRediT authorship contribution statement

**Marina Gutierrez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation. **Dragana Mutavdžić Pavlović:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Draženka Stipaničev:** Validation, Resources, Investigation, Data curation. **Siniša Repec:** Validation, Resources, Investigation, Data curation. **Francesco Avolio:** Writing – review & editing, Resources, Investigation. **Marcello Zanella:** Writing – review & editing, Resources, Investigation. **Paola Verlicchi:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.169848.

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