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Occurrence and fate of OMPs and ARGs in innovative full-scale WWTPs

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Abstract: The presence of antibiotic resistance determinants in wastewater treatment plants (WWTPs) presents a potential risk for human health and for the environment (Manaia et al., 2018). The scientific knowledge related to this topic is still limited, while new technologies are being implemented to reduce the environmental impact associated to conventional WWTPs. Besides removing conventional pollutants; innovative technologies should deal with contaminants of emerging concern (CECs). CECs are typically divided into chemicals (for example pharmaceuticals and other organic micropollutants, OMPs) and biological pollutants, such as pathogens or antibiotic resistant microbes (ARMs). The aim of this study is to evaluate the occurrence and fate of some CECs in two full-scale urban WWTPs based on advanced technologies. The selected CECs were 8 OMPs (antibiotics) and 35 antibiotic resistance genes (ARGs). Two sampling campaigns were performed to determine the presence and the removal efficiencies of the selected CECs in each treatment step. The results of the first sampling campaign showed moderate removal efficiencies for OMPs, between 20 and 80 %; and a higher distribution of ARGs in the sludge line, compared to the water line, where a removal of 2-3 log_{10} units was achieved. A second campaign was already completed and the results will be available in coming weeks.

Keywords: Real sewage, innovative technologies, organic micropollutants, antibiotic resistances.

1. Introduction

The current wastewater treatment main objectives go further than removing the pollution from wastewater: to achieve an integrated protection of the environment and move towards circular economy (EEA, 2022). One of the limits when applying such strategies is the presence of CECs, since conventional treatment processes are not designed to remove them efficiently (Mousel et al., 2017).

Among CECs, organic micropollutants (OMPs) have been the focus of different European initiatives related to water quality. Within the Water Framework Directive, successive Watch Lists were elaborated (2015, 2018, 2020 and 2022) to monitor some concerning compounds, with a remarkable attention focused on antibiotics. These pharmaceuticals are related to the current human health concern about ARMs.).

WWTPs are suspected to be hotspots for ARMs development. During biological treatment some conditions, such as availability of nutrients, presence of antibiotic residues, or close cell-to-cell interactions, can promote the horizontal transfer of ARGs between the microbiome (Manaia et al., 2018), and promote its release to the environment.

The main objectives of this study are to analyze the occurrence and removal of antibiotics and the distribution and fate of ARGs along the water and sludge treatment lines of two full-scale innovative urban WWTPs.

2. Materials and Methods

2.1. Plant characteristics

Two full-scale urban WWTPs located in Galicia, in NW Spain were selected for sampling of OMPs and ARGs: WWTP A (800,000 pe) and WWTP B (355,000 pe). The flow scheme of both plants is presented in Figure 1. They both include four main stages in water line: pretreatment, primary treatment, secondary treatment, and tertiary treatment. In the sludge treatment line, there are also four main stages: centrifuge thickening, thermal hydrolysis, anaerobic digestion, and stabilized sludge dewatering. Finally, the centrate from anaerobic digestion is treated in autotrophic reactors (anammox) to remove TN before recycling it to the inlet. The main differences among the plants are the biological and supernatant treatment technologies.

WWTP A secondary treatment is performed in a two-step biofilter (anoxic/aerobic) to remove both COD and TN. The excess sludge from backwashing is sent to primary settling. WWTP B biological treatment is performed in different sequential biological reactors (SBRs) alternating cycles of aeration to achieve COD and TN removal as well.

2.2. Sampling strategy

To assess the behavior and fate of CECs, the inlet and outlet of the main treatment units were sampled, in both water and sludge line (Figure 1). Grab sampling strategy was used, starting in the influent at the pollution load peak of the day and considering the hydraulic retention time (HRT) of each unit in the water line for the following samples. Since the sludge line consist of technologies operating with higher HRT, integration was considered sufficient to collect grab samples without considering HRT.

2.3. OMPs selection and analysis

Two sulfonamides, sulfamethoxazole (SMX) and trimethoprim (TMP); four macrolides, erythromycin (ERY), roxithromycin (ROX), clarithromycin (CLA) and azithromycin (AZI); one beta-lactam, cefalexin (CFX) and one quinolone, ciprofloxacin (CIP) were selected for this study. The selection criteria were the presence of the compounds in the EU Watch List and the concern about beta-lactam resistances in WWTPs. Since according to the physical properties of the selected compounds sorption is negligible, the analysis in the water line was restricted to the liquid phase. Samples were collected in aluminum bottles, prefiltered by 0.45 µm, stored at 4 ºC, and analyzed by LC-MS-MS after a preconcentration step (Alvarino et al., 2015).

2.4. ARGs selection and analysis

The selection of ARGs was based on previous sampling campaigns in a WWTP in Galicia (Li et al., 2021; Quintela-Baluja et al., 2019), since the expected antibiotic consumption habits should be similar. The selected genes are gathered in Table 1. Besides the ARGs linked to the selected OMPs selection, different genetic elements as integrons and transposons that participate in the horizontal transfer of antibiotic resistances were included, as well as the 16S rRNA.

DNA was extracted from both water and sludge samples using a commercial kit (NucleoSpin Microbial DNA). For water samples, a preconcentration step using reverse dialysis was carried out. The ARGs were analyzed in a SmartChip q-PCR (Resistomap).

Table 1. Selection of ARGs.

Class	Gene
Integrons	intI1_2
	intI1_3
	intl3
	intI3_1
MGE	tnpA_2
	tnpA_1
	tnpA_3
	tnpA_4
Sulfonamide	sul1_1
	sul2_1
MLSB	ereA
	mefA
Trimethoprim	dfrA1
	dfrA12
Beta Lactam	cfxA
	cphA_2
	blaVIM
	cphA_1
	blaAIM
	blaMOX/blaCMY
	beta_B2
16S rRNA	16S rRNA

3. Results and discussion

3.1. Fate of OMPs

The selected compounds were present at the influent of the two WWTPs at different concentration levels, as shown on Table 2. Both population areas are quite different in terms of inhabitants (number, population ageing), industrial and tertiary activities, presence of nearby rural areas, etc., which are probably the explanation for these deviations.

Table 2. Concentrations of OMPs in the raw wastewater at the inlet of the WWTPs.

Antibiotic	WWTP A (µg L^{-1})	WWTP B (µg L^{-1})
SMX	2.893±0.075	0.263±0.017
TMP	7.953±0.559	1.134±0.089
ERY	1.056±0.008	0.057±0.001
ROX	0.906±0.012	0.456±0.019
CLA	0.204±0.006	0.121±0.004
AZI	2.798±0.087	1.839±0.175
CFX	0.009±0.001	n.d.
CIP	1.122±0.001	1.092±0.329

Figure 2 presents the removal efficiencies for both plants regarding the water line. WWTP A shows a moderate removal of the selected OMPs, with removal efficiencies between 20 and 60 %, approximately while WWTP B removal efficiencies are higher, between 60 and 80 %. In both cases biological treatment was the stage with a higher contribution to the removal. This behavior was expected, since biodegradation has been indicated to be the main removal mechanism when volatilization and sorption are not significative, due to the physical properties of the selected compounds (Suárez et al., 2008). One of the factors that enhance the biodegradation of OMPs is the HRT, which was significantly different in both plants: WWTP B HRT of 260 min compared to WWTP HRT of 180 min. This could explain the differences in OMP removals in the water line of both WWTPs.

Regarding the sludge line, the initial concentrations detected in mixed sludge are gathered in Table 3. The different physicochemical properties of the compounds determine their sorption onto sludge.

Table 3. Concentrations of OMPs in the mixed sludge of the WWTPs.

Antibiotic	WWTP A (ng g^{-1})	WWTP B (ng g^{-1})
SMX	49.65±0.76	27.36±7.19
TMP	922.97±32.18	331.55±5.52
ERY	27.02±1.10	9.22±4.39
ROX	93.87±2.30	75.92±4.08
CLA	43.36±1.93	8.33±0.11
AZI	241.01±2.77	310.57±38.55
CFX	n.d.	n.d.
CIP	231.85±41.93	144.37±5.04

The removal efficiencies of OMPs in the sludge line are presented in Figure 3, showing lower values than in the water line. Both thermal hydrolysis and anaerobic digestion play an important role in antibiotic removal, a similar behavior than previously reported by Sun et al., 2019.

3.2. ARGs distribution

From the 35 ARGs selected, 19 were detected in the first sampling campaign. The number of detected genes in each treatment step in both WWTPs is presented in Figure 4. The distribution among liquid and solid phase is different, as it was previously found by Quintela-Baluja et al., 2019. Sulfonamide resistance genes (*sul1* and *sul2*) presented a recalcitrant behavior, while other like Quinolone *qnrS* were easily removed from the system.

Regarding removal efficiencies, the sludge line seems to be the most effective treatment in the removal of ARGs, achieving 3 $\log_{10}$ (cp mL^{-1}) in WWTP A and 1.9 $\log_{10}$ (cp mL^{-1}) in WWTP B. Thermal hydrolysis was the main removal stage, since cell walls are destroyed and DNA is more susceptible to be hydrolyzed (Ma et al., 2011).

4. Conclusions

The results of the first sampling campaign in two full-scale innovative WWTPs showed different removal efficiencies of OMPs and ARGs, possibly related to the different technological configurations for the biological treatment. Biological treatment and thermal hydrolysis play an important role in biodegradation of antibiotics and removal of ARGs, respectively.

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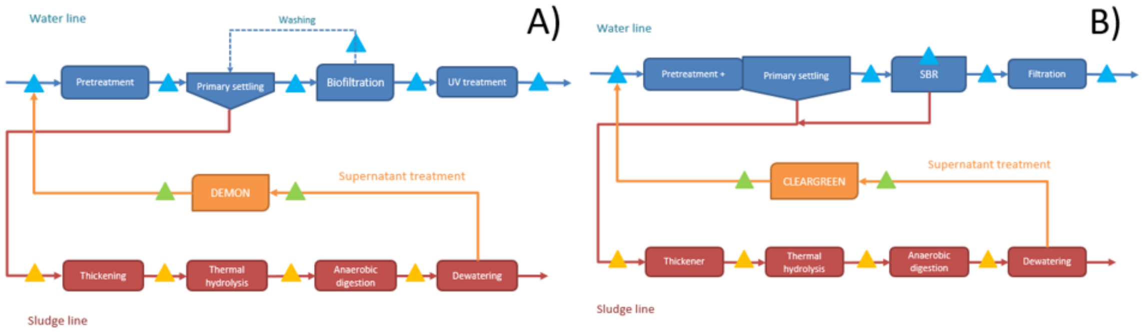

Figure 1. Flow diagram of WWTP A (left side of the figure) and WWTP B (right side of the figure). The triangle symbol is used to indicate the sampling points for OMPs and ARGs analysis.

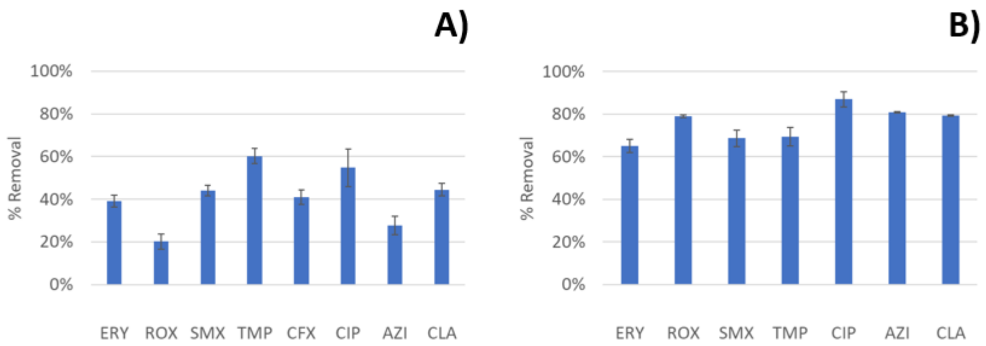

Figure 2. OMPs Removal efficiencies observed in the water line for WWTP A (left) and WWTP B (right).

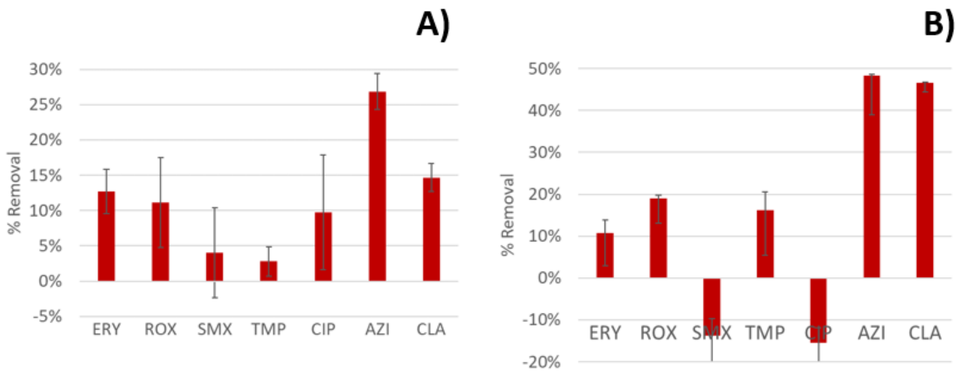

Figure 3. OMPs removal efficiencies of the sludge line for WWTP A (left) and WWTP B (right).

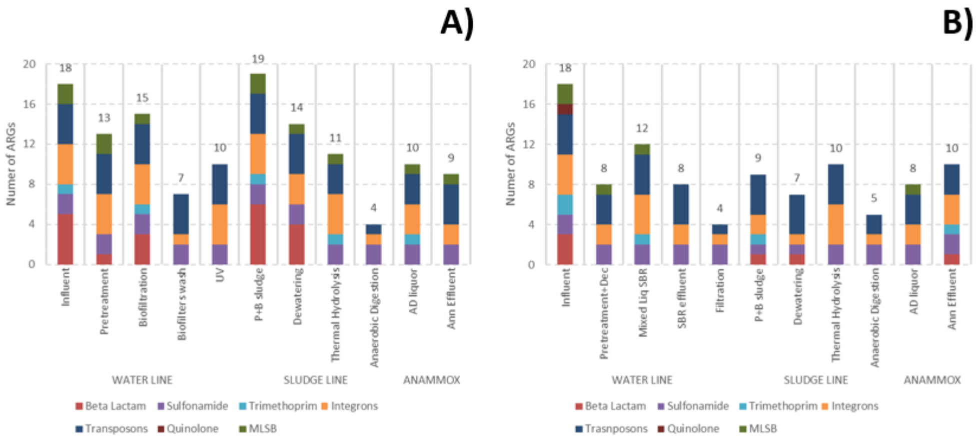

Figure 4. Number of detected genes in each treatment step of both WWTP A (left) and WWTP B (right).

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