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Research article

Nanofiltration combined with ozone-based processes for the removal of antineoplastic drugs from wastewater effluents

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ABSTRACT

Over the past years, there has been an increasing concern about the occurrence of antineoplastic drugs in water bodies. The incomplete removal of these pharmaceuticals from wastewaters has been confirmed by several scientists, making it urgent to find a reliable technique or a combination of techniques capable to produce clean and safe water. In this work, the combination of nanofiltration and ozone (O_3)-based processes (NF + O_3, NF + O_3/H_2O_2 and NF + O_3/H_2O_2/UVA) was studied aiming to produce clean water from wastewater treatment plant (WWTP) secondary effluents to be safely discharged into water bodies, reused in daily practices such as aquaculture activities or for recharging aquifers used as abstraction sources for drinking water production. Nanofiltration was performed in a pilot-scale unit and O_3-based processes in a continuous-flow column. The peroxone process (O_3/H_2O_2) was considered the most promising technology to be coupled to nanofiltration, all the target pharmaceuticals being removed at an extent higher than 98% from WWTP secondary effluents, with a DOC reduction up to 92%. The applicability of the clean water stream for recharging aquifers used as abstraction sources for drinking water production was supported by a risk assessment approach, regarding the final concentrations of the target pharmaceuticals. Moreover, the toxicity of the nanofiltration retentate, a polluted stream generated from the nanofiltration system, was greatly decreased after the application of the peroxone process, which evidences the positive impact on the environment of implementing a NF + O_3/H_2O_2 process.

1. Introduction

Antineoplastic drugs are pharmaceuticals used during chemotherapy, in the treatment of cancer disease. However, due to their high toxicity and non-selectivity for tumor cells, these compounds and/or derivates may cause health problems to exposed life beings (Izar et al., 2015). As with every other pharmaceutical, a fraction of antineoplastic medications that are administered to patients is excreted in its unchanged form (*e.g.*, up to 96% of capecitabine is recovered in urine) (National Library of Medicine, 2021), being continuously released into

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the sewage systems. Since conventional wastewater treatment plants (WWTPs) are not designed to remove these hazardous pharmaceuticals, they have been detected in WWTP effluents at concentrations up to a few μg/L (Gouveia et al., 2019; Ternes, 1998), which are then released into surface waters, posing serious risks to environmental integrity and sustainability. In addition, the great need of reusing treated wastewater or surface waters in daily practices (*e.g.*, irrigation, agriculture, aquaculture, potable water supply) increases the concern related to human health safety (Galindo-Miranda et al., 2019; Gouveia et al., 2022; Wilkinson et al., 2017). Therefore, effective treatment solutions targeting antineoplastic pollution at the source (mainly wastewaters) are needed to prevent the health risks resulting from the exposure to these hazardous chemical agents.

UV disinfection and chlorination are the most common treatment strategies currently applied in WWTPs as tertiary treatments, but efficiencies regarding the elimination of antineoplastic drugs are not enough (Gouveia et al., 2019). Indeed, some co-authors of this work found out that bicalutamide, capecitabine, cyclophosphamide, ifosfamide, and mycophenolic acid were not effectively degraded in a Portuguese WWTP using UV as tertiary treatment (Gouveia et al., 2020). Concerning chlorination, the by-products that chlorine originates are reason for prudence due to their possible teratogenicity, carcinogenicity, and genotoxicity (Srivastav et al., 2020). Recently, the use of membrane-based systems such as nanofiltration (NF) or reverse osmosis has gained attention as promising options for the removal of pharmaceuticals from wastewater because of their capability of removing low molecular weight compounds, being easily integrated with other treatment technologies (Cristóvão et al., 2022; Gouveia et al., 2023a). However, an additional treatment of the NF permeate is recommended to be coupled if complete elimination of toxic compounds is targeted. Moreover, special attention should be taken to the retentate stream, attending to the hazardous substances that are concentrated on it (Gouveia et al., 2023a).

This work aims to investigate, for the first time, the performance of an integrated strategy of treatment (nanofiltration followed by O_3-based treatment processes) for the effective elimination of risky antineoplastic drugs and prednisone in wastewaters. This coupling treatment strategy was already successfully investigated for the abatement of other emerging pollutants, but it was never addressed for this class of contaminants (Miralles-Cuevas et al., 2014; Ouali et al., 2022) considered one of the most threatening to the aquatic environment (Heath et al., 2016; Li et al., 2021). The removal of ten risky antineoplastic drugs (bicalutamide, capecitabine, cyclophosphamide, flutamide, ifosfamide, megestrol, mycophenolate mofetil, mycophenolic acid, paclitaxel, and tamoxifen) and prednisone (frequently administered in combination with antineoplastic drugs during cancer therapy) (Chemocare, 2002) from wastewaters by NF was previously studied in a pilot-scale system (Gouveia et al., 2023a). Since NF is neither enough for the complete elimination of the target pharmaceuticals nor for toxicity abatement to levels considered safe for aquatic biota (Gouveia et al., 2023a), the integration of NF with O_3-based processes arises as a potentially effective treatment approach. Therefore, in this study, a NF permeate stream produced by the same NF pilot-scale system was used as feed for O_3-based treatments (O_3, O_3/H_2O_2 and O_3/H_2O_2/UVA) aiming at process intensification.

This study also incorporates two distinct risk assessment approaches that, up to author's knowledge, have never been addressed to final streams resulting from an integrated treatment strategy. This assessment goes beyond solely evaluating the removal efficiency of both techniques (isolated and combined) and considers the potential impacts on aquatic biota (considering the applications of the final streams of each individual technique, NF or O_3-based processes, in aquaculture practices and discharge to the environment). Moreover, it considers the possible impact on human health of the effluent treated by the combined technology (thinking on the potential of the clean water for recharging aquifers used as abstraction sources for drinking water production), by comparing the human exposure by ingestion during a lifetime with the respective permitted daily doses (*PDE*), according to a previously published work (Gouveia et al., 2022). By integrating this risk assessment approach, it is provided a more comprehensive understanding of the effectiveness and implications of the NF and O_3-based process in addressing the risks associated with antineoplastic drugs' presence in final streams.

Knowing that the NF retentate is a very polluted stream generated during the treatment of wastewater by NF, the decrease in its toxicity was also studied applying the same O_3-based treatments, aiming for its safe discharge into the water bodies. The impact of this final stream (NF retentate treated by O_3-based processes) on aquatic biota was assessed by estimating risk quotients (*RQ*).

2. Materials and methods

2.1. Chemicals and reagents

Bicalutamide, capecitabine, cyclophosphamide, flutamide, ifosfamide, megestrol, mycophenolate mofetil, mycophenolic acid, paclitaxel, prednisone, and tamoxifen analytical standards of 98–99% purity, used in the calibration curve and validation experiments, were acquired from Sigma-Aldrich (St. Louis, USA) and Cayman Chemical Company (Ann Arbor, USA). The chemical structures of the target pharmaceuticals are represented in Figure A.1 from Supplementary Information. Methanol (MeOH), acetonitrile (ACN), isopropanol, Milli-Q water, hydrogen peroxide (H_2O_2) (30% w/v) and ammonium acetate (NH_4OAc) were supplied by Merck (Darmstadt, Germany). All solvents used were of LC–MS grade. Mycophenolic acid-d3 (MPA-d3) and cyclophosphamide-d4 (CYC-d4) were used as internal standards; both were acquired from Sigma-Aldrich (St. Louis, USA). Stock standard solutions were prepared at a concentration of 1000 mg/L in MeOH, except paclitaxel that was prepared in ACN. Working solutions with a concentration of 1 mg/L in Milli-Q water, used to spike the matrices, were prepared in the same day of the experiments. Formic acid (HCOOH) used for LC-MS/MS mobile phase and HCl 1 M used for pH adjustment, were purchased from Sigma-Aldrich (St. Louis, USA), as well as ascorbic acid, used to quench O_3 experiments. SPE cartridges Oasis HLB (6 cc, 200 mg) were purchased from Waters (Milford, USA). Nylon membrane filters (Whatman 0.8 and 0.45 μm), used for sample filtration, were acquired from Sigma-Aldrich (St. Louis, USA).

2.2. Safety considerations on antineoplastic drugs handling

Safety regulations were adhered during antineoplastic drugs manipulation, with a rigorous control on handling techniques and storage circumstances. These chemicals' handling procedures were carried out in a safety hood with vertical laminar airflow. The surfaces in touch with antineoplastic drugs were cleaned with isopropanol, and an absorbent paper made of polyethylene was used to protect the workbenches. The materials that should be discharged were treated as hazardous waste. UVA radiation was applied in the safety hood for 15 min following each procedure.

2.3. Nanofiltration experiments

Prior to ozonation experiments, pilot-scale NF experiments were carried out in triplicate, using a real effluent from the secondary treatment of an urban WWTP as collected. The NF system is equipped with a spiral wound Desal 5DK module (model DK4040F30, Suez membranes, Lenntech, Delfgauw, Netherlands). This thin film composite membrane has a MWCO of 150–300 Da, a minimum $MgSO_4$ rejection of 98% and an active surface area of 7.9 m^2. The average tap water permeability (tap water filtered with an activated carbon filter before the assays) was 3.72 $\pm$ 0.11 L/(h m^2 bar). More information regarding the pilot-scale NF unit and trials is described in detail elsewhere (Cristóvão et al., 2022;

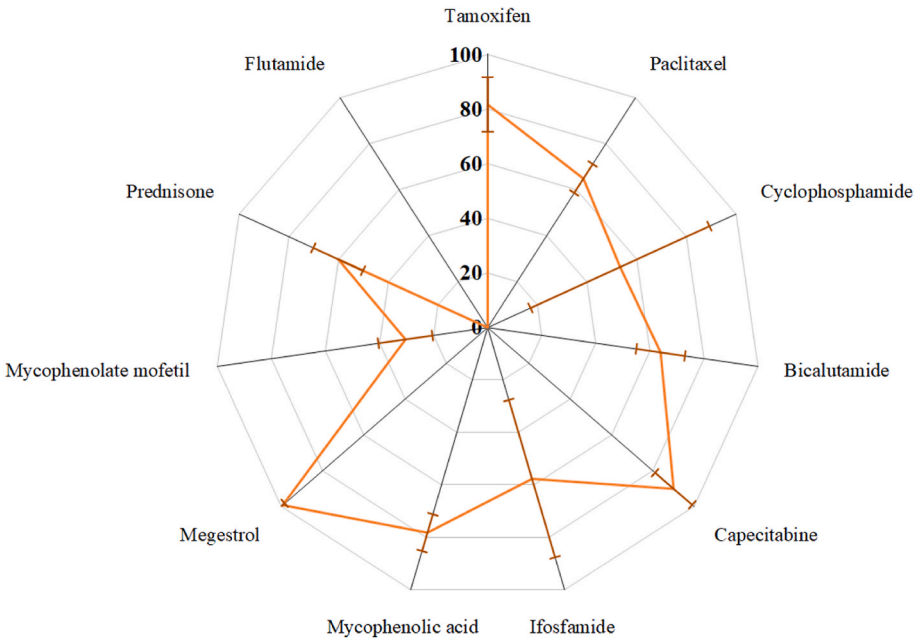

Fig. 1. Removal (%) of the target pharmaceuticals by a pilot-scale nanofiltration system equipped with a spiral wound Desal 5DK module (adapted from Gouveia et al. (2023a)).

Gouveia et al., 2023a). In the NF experiments, 1000 L of a WWTP secondary effluent was processed at the constant pressure of 6 bar with a recovery of approximately 70%. The permeate flux normalized at 20 °C varied from approximately 21 to 15 L/(h m^2) during 5 h, the approximate duration of each experiment. After experiments, tap water and ultrasil solution (1%, m/m) were used to clean the membrane for approximately 30 min to ensure the same initial conditions for further experiments. After NF experiments, NF permeate and NF retentate streams were collected and analyzed before treatment by O_3-based processes. Samples of WWTP effluent feeding the NF system were also analyzed to determine the initial concentrations of antineoplastic drugs.

2.4. Ozone-based degradation experiments

O_3-based degradation experiments (O_3, O_3/H_2O_2 and O_3/H_2O_2/UVA) were performed in a flow-through glass bubble column reactor (3 cm internal diameter × 70 cm height) operating in continuous flow mode. Before starting the experiments, it was assured that the target compounds were not retained in the setup material by the measurement of their concentrations before and after a simulated ozonation experiment using O_2 instead of O_3. The permeate and retentate streams from NF experiments (1.5 L), as well as the WWTP effluent (1.5 L), were spiked with 300 μL of a 1 mg/L aqueous standard solution containing all pharmaceuticals to mimic a real effluent contaminated by all targets at an initial concentration level (200 ng/L) that allows precisely and accurately following their degradation along the process (at least up to 98% degradation, taking into consideration the method detection limits). The spiked matrices were fed at the bottom of the reactor by using a peristaltic pump (Watson-Marlow, UK) being collected after treatment at the outlet stream located at the top of the reactor. A detailed representation of this system can be found in a previous work of the team (Gorito et al., 2021).

Briefly, the column reactor was packed with glass Raschig rings (diameter: 3 mm and length: 3 mm; filling volume of 320 mL) to improve the O_3-water mass transfer. A BMT 802X ozone generator (BMT Messtechnik, Germany) was used to produce O_3 from pure oxygen (O_2: 99.999%) at a constant gas flow rate (150 cm^3N/min). Ozone in the gas phase was monitored by using a BMT 964 ozone analyzer (BMT Messtechnik, Germany) and O_3 in the liquid phase was measured using a Q45H/64 dissolved ozone probe (Analytical Technology, USA), calibrated through Indigo colorimetric method (Bader and Hoigne, 1982). To remove the remaining O_3 from the gas phase leaving the reactor, gas washing bottles filled with potassium iodide solution were used. In the case of UVA experiments, eight 10 W UVA high intensity light emitting diodes (LEDs, 15.5 mm × 23.0 mm) with a dominant emission wavelength at 382 nm and long service life (intensity remains above 70% after 10,000 h work) were set along the reactor with a distance of 5 cm (Gorito et al., 2021). The LEDs medium nominal irradiation was 390 W/m^2 determined with a UV–Vis spectroradiometer (USB, 2000 + OceanOptions, USA). In peroxone experiments, H_2O_2 was directly added as a single pulse to the spiked matrices under agitation, and the dosage of H_2O_2 was selected according to the stoichiometric amount with respect to their dissolved organic carbon (DOC) content (*i.e.*, 17 mg/L H_2O_2 for NF permeate and 55.5 mg/L for WWTP effluents). For all tested treatments, the spiked effluents were pumped to the reactor at 32 mL/min, corresponding to a hydraulic retention time (HRT) of 10 min. Treated samples were withdrawn after 30 min, ensuring that the steady-state had been reached (that was previously confirmed by measuring the conductivity of a 2 g/L NaCl aqueous solution in and out of the system, maintaining the remaining mechanical parameters).

In order to quench the reactions, 5 g/L of ascorbic acid was added to the collected samples (Wang et al., 2020) prior to extraction and instrumental analysis. Residual O_3 was removed from the samples used for DOC analysis by bubbling air for O_3 stripping instead of adding ascorbic acid. These quenching mechanisms were previously used by some of the group co-authors (Garcia-Costa et al., 2022).

The degradation experiments of antineoplastic drugs by ozonation-based processes (O_3, O_3/H_2O_2 and O_3/H_2O_2/UVA) were performed in triplicate for each type of matrix, each collected sample being processed in triplicate. Therefore, a total of 162 solid phase extraction (SPE) procedures were conducted, considering the sampling before (*i.e.*, non-spiked and spiked effluents) and after ozonation. Inlet O_3 concentrations of 2.4 mg/L and 6.6 mg/L in the liquid phase after stabilization were used to treat NF permeate and WWTP effluents, respectively, which corresponds to an O_3 specific dose (g_{O3}/g_{DOC}) of 0.4; this ratio is within the range applied in previous studies with wastewater matrices (Bourgin et al., 2017a; Garcia-Costa et al., 2022). Unfortunately, for the treatment of the NF retentate, it was not possible to keep the g_{O3}/g_{DOC}

ratio constant (*i.e.*, 0.4 g_{O3}/g_{DOC}) as it would require the use of extremely high amounts of oxidant in the liquid phase (>80 mg O_3/L), which is not practicable. Therefore, it was decided to use an amount of O_3 corresponding to the DOC of the WWTP secondary effluent (feed stream of the NF experiments), assuming a ratio of 0.4 g_{O3}/g_{DOC}, *i.e.*, 6.6 mg O_3/L. DOC content was always determined in a TOC-L analyzer (Shimadzu Scientific Instruments, Japan), following the procedure 5310B of the Standard Methods for Examination of Water and Wastewater (APHA, 1998).

2.5. Analysis of antineoplastic drugs by SPE-LC-MS/MS

2.5.1. Sample preparation and extraction procedure

An offline SPE followed by ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) analytical method was used to determine the concentration of the target pharmaceuticals in the wastewater samples. The SPE conditions used in this work were based on the methodology previously developed by Gómez-Canela et al. (2014) and validated elsewhere for the remaining compounds (Gouveia et al., 2022, 2023b). SPE cartridges (HLB, 200 mg) were conditioned with 6 mL MeOH and 6 mL of an aqueous solution of 100 mmol/L NH_4OAc. Then, 100 mL of sample (pH = 2) was loaded through the cartridge at a flow rate of approximately 1 mL/min. The cartridges were further dried for about 30–45 min and the elution was performed with 6 mL MeOH and 6 mL MeOH:HCOOH (95:5, v/v). The internal standards were added in this step of the process to a final concentration of 20 μg/L (MPA-d3 was used as surrogate for capecitabine, mycophenolic acid, mycophenolate mofetil, and prednisone, and CYC-d4 for the other antineoplastic drugs). The eluate was slowly evaporated to dryness and reconstituted in 200 μL ACN for further analysis in the LC-MS/MS system.

2.5.2. Instrumental analysis

The analyses of the extracts were carried out in a liquid chromatograph (Shimadzu Corporation apparatus and UHPLC Nexera) equipped with an Autosampler SIL-30 AC, an Oven CTO-20 AC, two Pumps LC-30AD, a Degasser DGU-20A5, a System Controller CBM-20A, a LC Solution Version 5.41SP1 and coupled to a triple quadrupole mass spectrometer detector Shimadzu LCMS-8040. Data were acquired and processed using the LabSolutions software package.

Luna C18 (150 × 2.1 mm ID, particle size 5 μm; Phenomenex) was used in the chromatography and a binary mixture of water (A) and MeOH (B), both acidified with 0.1% HCOOH, was used as mobile phase, at a flow rate of 0.2 mL/min. Gradient elution started at 5% B, increased to 20% B in 15 min, with a further increase up to 45% B in 15 min and up to 100% in 9 min. After 2 min at 100% B, the initial conditions were recovered (4 min) and the system was stabilized for 5 min (total running time: 50 min). The injection volume was 5 μL. An electrospray ionization source was operated in positive and negative modes. The precursor ions $[M+H]^+$/$[M-H]^-$ and the two most abundant fragments were used for the identification (transition 2) and quantification (transition 1) of the target analytes (detailed information can be found in previous works (Gouveia et al., 2022; Gouveia et al., 2023b; Gouveia et al., 2020)). Optimized parameters were cone voltage (4.5 V for positive and −3.5 V for negative ionized compounds), collision energy (from 10 to 50 eV), 3.0 dm^3/min for nebulizing gas flow, 7.5 dm^3/min for drying gas flow, 400 °C for heat block temperature and 250 °C for desolvation line temperature.

2.5.3. Validation parameters

The calibration curves were performed within a concentration range of 1–500 μg/L (depending on the pharmaceutical – Table A.1 from Supplementary Information), using ten calibration points and the internal standard quantification method. Good linearity was achieved for all compounds in ACN, the same solvent used for extracts reconstitution, with correlation coefficients higher than 0.998 (Table A.1). The instrumental detection limits (IDLs) were determined for a signal-to-noise ratio of 3, considering the average of the values obtained for all calibration points. The method detection limits (MDLs) were further obtained from IDLs, considering the concentration factor of the extraction process (500 ×). MDL values obtained were relatively low, varying from 0.03 ng/L for bicalutamide to 3.65 ng/L for prednisone. The accuracy of the method was previously assessed through the determination of the percentage of recovery of all analytes in standard addition assays. An average recovery of all the target pharmaceuticals of 59 ± 25% for NF retentate, 65 ± 18% for WWTP effluents and 77 ± 20% for NF permeate was obtained, as fully described in Gouveia et al. (2023a) (Table A.1). Intra-day and inter-day precisions were obtained by measuring the analytical response for three analytical standards (5 μg/L, 50 μg/L and 250 μg/L) in six consecutive injections over six different days. A relative standard deviation ranging from 2% (cyclophosphamide) to 11% (megestrol, mycophenolic acid and tamoxifen) was obtained for intra-day precision, and from 1% (cyclophosphamide) to 19% (ifosfamide) for inter-day precision (Table A.1). More details regarding method validation are available in Gouveia et al. (2023a).

2.6. Risk assessment

2.6.1. Risk for humans from long-life drinking of clean water

Since the main application of the clean water from NF and O_3-based systems is for the production of a high-quality water stream, possibly for recharging aquifers used as abstraction sources for drinking water production, human exposure to the target pharmaceuticals was assessed considering a long-life consumption of this drinking water source. Regarding the assessment of the exposure of humans to the target pharmaceuticals under this context, the concentrations measured in clean water produced from the most promising treatment approach were considered. In the case of a target pharmaceutical that was not detected in clean water, a concentration equal to the respective MDL was used to assess the exposure and to estimate the risk – worst-case scenario approach. The calculations were performed for children (7–10 years old and 11–14 years old) and for adults (men and women), according to Equation (1). More details about human exposure and risk assessment are fully described in a previous work of the team (Gouveia et al., 2022).

$$ADD = \frac{C_{medium} \times IngR}{BW} \times 10^9 \quad (1)$$

where *ADD* is the average daily potential dose (pg/kg-day), C_{medium} corresponds to the concentration of each pharmaceutical predicted in the clean water (mg/L), *IngR* the water ingestion rate (1.43 L/day for men, 1.31 L/day for women, 0.77 L/day for older children and 0.63 L/day for younger children) (ATSDR, 2021; EPA, U.S, 2011) and *BW* the body weight (71.8 kg for men, 65.4 kg for women, 48.2 kg for older children and 30.2 kg for younger children) (EPA, U.S, 2011).

Then, exposure values were compared to the respective permitted daily doses (*PDE*, pg/day), to check if there is any potential risk associated. *ADD* values were multiplied by the *BW* , thus allowing to compare the daily exposure masses for each pharmaceutical with the *PDE*. If $PDE > ADD$, then no risk is foreseen; if $PDE < ADD$, human health may be at risk. The *PDE* was calculated according to Equation (2) (EMA, 2010).

$$PDE = \frac{NOAEC \times weigth\ adjustment}{F1 \times F2 \times F3 \times F4 \times F5} \quad (2)$$

where *NOAEC* is the No Observed Adverse Effect Concentration (mg/kg/day), the *weight adjustment* is an arbitrary human body weight of 50 kg for adults and 10 kg for children (EMA, 2018), and $F1, F2, F3, F4$ and $F5$ are modifying factors, which depend on the toxicological data used for each pharmaceutical (full details can be found in Gouveia et al. (2022)).

2.6.2. Risk for aquatic biota from direct reuse of treated wastewater in aquaculture practices or from discharge of treated wastewater into water bodies

The risk for aquatic organisms from the exposure to the target pharmaceuticals was estimated through Equation (3), considering two potential exposure contexts: (i) direct reuse of treated wastewater in aquaculture practices; and (ii) discharge of treated wastewater into water bodies. In this case, treated wastewater corresponds to any stream treated by NF, the best performing O_3 treatment technology, or both.

$$RQ = \frac{MEC\ or\ PEC}{PNEC} \quad (3)$$

where MEC is the concentration of each pharmaceutical measured in the treated water, and PEC is the concentration of each pharmaceutical in surface waters, predicted from MEC by applying a standard dilution factor of 10-fold from wastewater treatment facility to surface waters, according to the Guidelines on the Environmental Risk Assessment of Medicinal Products for Human Use (EMEA, 2006). For the pharmaceuticals not detected in treated wastewater, a concentration equal to their MDL was used for risk estimation, to gather information for the worst-case exposure scenario.

PNEC values are related to the toxicity of the drug, determined through the ratio between the ecotoxicity and an Assessment Factor (*AF*). An *AF* of 10 was used when information regarding 3 trophic levels was present, 50 for 2 trophic levels, and 100 for just 1 trophic level. Chronic ecotoxicity (NOEC) was preferred over acute ecotoxicity (which was used with an AF of 1000 when chronic ecotoxicity could not be found) – Table A.2.

The risk for aquatic organisms was estimated attending on the following criteria: $RQ \geq 1$ indicates high risk, $0.1 \leq RQ < 1$ suggests moderate risk, $0.01 \leq RQ < 0.1$ anticipates low risk, and $RQ < 0.01$ denotes no risk to aquatic biota (Sánchez-Bayo et al., 2002).

3. Results and discussion

3.1. Nanofiltration experiments

NF experiments were previously conducted in triplicate, in a pilot-scale equipment, using a real WWTP secondary effluent (non-spiked) as feed of the system as described elsewhere (Gouveia et al., 2023a). Fig. 1 represents the removals achieved for each pharmaceutical from the three nanofiltration experiments. An average removal of 68 ± 23% was achieved, megestrol being the one for which a higher removal was attained (98.3 ± 0.4%) and flutamide the one for which the removal was negligible. DOC values before (16.5 ± 0.3 mg/L) and after (6.0 ± 0.1 mg/L) NF experiments also confirmed a decrease of about 64% in the dissolved organic matter present in the WWTP effluent. The risks that NF permeate could pose to aquatic life were also previously estimated (Gouveia et al., 2023a). If permeate stream is thought to be reused in aquaculture practices, a high risk to aquatic organisms is estimated from the exposure to cyclophosphamide (classified as carcinogenic to humans by the International Agency for Research on Cancer (IARC, 2022)), and a low risk from the exposure to capecitabine, flutamide and mycophenolic acid (Gouveia et al., 2023a). If permeate stream is thought to be discharged to water bodies, a moderate risk is still estimated from the exposure of aquatic organisms to cyclophosphamide.

Although NF had an evident positive impact on the removal of most target pharmaceuticals, all of them were still detected in the NF permeate (in concentrations varying between 0.1 ± 0.1 ng/L for tamoxifen and 29 ± 13 ng/L for prednisone) (Gouveia et al., 2023a). Having this observation in consideration, coupling another treatment process would be a good option to enhance the removal of antineoplastic drugs in both permeate and retentate, as well as to reduce the risks to aquatic organisms (and the overall environment) and humans.

3.2. Ozone-based experiments

Aiming to check if O_3-based processes would be able to significantly degrade the target pharmaceuticals from the same WWTP secondary effluent, this technology was applied to this matrix by spiking it with 200 ng/L of each compound. Table A.3 and Fig. 2 show the average degradations achieved for the target pharmaceuticals. The results show that O_3 alone was only able to efficiently degrade paclitaxel, megestrol, cyclophosphamide, capecitabine, prednisone, and mycophenolate mofetil (removals >98%). The addition of H_2O_2 and H_2O_2/UVA to the process, respectively represented by O_3/H_2O_2 and O_3/H_2O_2/UVA in Fig. 2, improved the degradation of 4 of the 5 pharmaceuticals that were still detected after O_3 alone (tamoxifen, ifosfamide, flutamide, and bicalutamide), to levels below detection. The increase in the efficiency of the advanced oxidation process was expected since ozone is a very selective oxidant whereas OH radicals are unselective and react fast with many dissolved compounds and the water matrix (von Gunten, 2003). It is important to mention that there were no considerable differences between O_3/H_2O_2 and O_3/H_2O_2/UVA processes, which can be justified by the use of UVA (rather than UVC) radiation (Gorito et al., 2021). Mycophenolic acid was the only antineoplastic drug that was detected in the final effluent for all tested treatments, a removal of 63.4 ± 0.2% being achieved by O_3, 59.2 ± 0.2% by O_3/H_2O_2 and 62 ± 1% by O_3/H_2O_2/UVA. Up to the authors' knowledge, this is the first-time that O_3-based processes are applied for paclitaxel and tamoxifen elimination in real wastewaters. The results achieved for the remaining pharmaceuticals can be corroborated by considering other studies (Azuma et al., 2019; Fernández et al., 2010; Ferre-Aracil et al., 2016; Garcia-Costa et al., 2021, 2022), except for bicalutamide, megestrol, mycophenolic acid, and flutamide (Garcia-Costa et al., 2021, 2022). Despite high removals for several antineoplastic drugs were achieved in a study performed in the same ozonation equipment (Garcia-Costa et al., 2022), other pharmaceuticals were not completely degraded, even after O_3/H_2O_2/UVA process, as is the case of megestrol, bicalutamide and flutamide (Garcia-Costa et al., 2022). The slightly lower removals by O_3/H_2O_2/UVA for these antineoplastic drugs (bicalutamide was degraded up to 90%, megestrol 81% and flutamide 95%) might be due to the use of lower H_2O_2 doses, *i.e.*, 7.5 mg/L of H_2O_2 (Garcia-Costa et al., 2022) *vs.* 55.5 mg/L in the present work. Furthermore, a complete elimination of mycophenolic acid from wastewaters by single ozonation was previously reported (Garcia-Costa et al., 2021). The differences in pH, concentrations of inorganic ions (carbonate, nitrate, chloride, sulfate, among others) and organic matter between matrices, as well as the possible presence of ozone scavenger species in the matrix of the current study may have significantly affected the degradation of mycophenolic acid (Calderara et al., 2002; Miklos et al., 2018; Munoz et al., 2018).

If the treated stream is thought to be reused in aquaculture practices, a low risk to aquatic organisms is estimated from the exposure to cyclophosphamide and mycophenolic acid (being cyclophosphamide an extremely toxic compound, when *RQ* is calculated using the MDL value, a low risk can be still foreseen for aquatic organisms). No evident risk was attained for aquatic organisms after O_3-based processes if the treated stream was discharged to water bodies. However, the *ALARA* (as low as reasonably achievable) principle is the best standard, if human consumption of the final stream is considered. Thus, the incomplete degradation reported by other authors for some of these compounds and the reasonable removal verified for mycophenolic acid, classified by several studies as a potentially carcinogenic, mutagenic and teratogenic compound (Katiboina and Jacob, 2022; Khan et al., 2022; Straub et al., 2019) in the final stream, prevents the reuse of the treated WWTP secondary effluent by O_3-based processes for drinking purposes, highlighting the need of a combined technique.

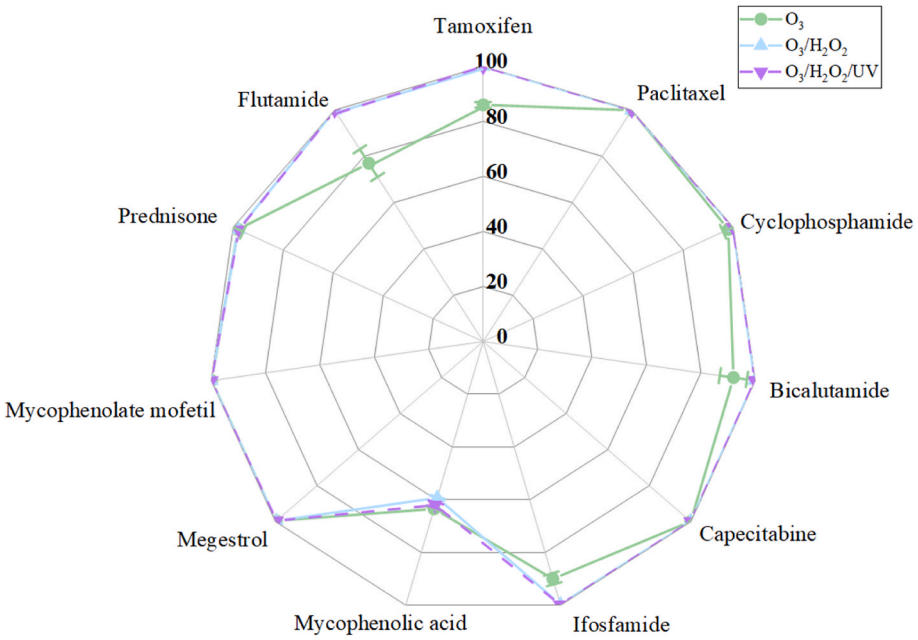

Fig. 2. Degradation (%) of the target pharmaceuticals by ozone-based processes (O_3, O_3/H_2O_2 and O_3/H_2O_2/UVA) applied to a WWTP secondary effluent spiked with 200 ng/L of each target pharmaceutical: $[O_3]$ = 6.6 mg/L; $[H_2O_2]$ = 55.5 mg/L; UV = eight 10 W UVA high-intensity light emitting diodes; HRT = 10 min.

3.3. Coupling ozone-based processes to nanofiltration

3.3.1. Treatment of nanofiltration permeate to produce a high-quality water stream

Considering that: (i) NF is not completely efficient neither to remove these types of compounds nor to reduce the toxicity of the effluents (Gouveia et al., 2023a), (ii) O_3-based processes applied to WWTP secondary effluents were promising to generate a final stream that could possibly be safely discharged into the environment (section 3.2); (iii) mycophenolic acid was recalcitrant to the three O_3-based treatments studied; (iv) the main application defined for the clean water from NF systems is to have a high-quality water stream, possibly for recharging aquifers used as abstraction sources for drinking water production; and (v) following the *ALARA* principle; the complete removal of antineoplastic drugs from water would be mandatory. In this section, the application of O_3-based processes to the permeate of a pilot-scale NF system was tested. The degradation of the target pharmaceuticals in continuous-flow mode ozonation was investigated by applying an O_3 concentration of 2.4 mg/L to the spiked NF permeate. Table A.3 and Fig. 3 represent the removals (%) obtained for each compound.

The results demonstrate that bicalutamide, flutamide, ifosfamide, and tamoxifen were not completely eliminated by single ozonation (NF + O_3), despite being all of them removed above 77 ± 6% (flutamide) – Fig. 3. The addition of H_2O_2 (peroxone process) enhanced the

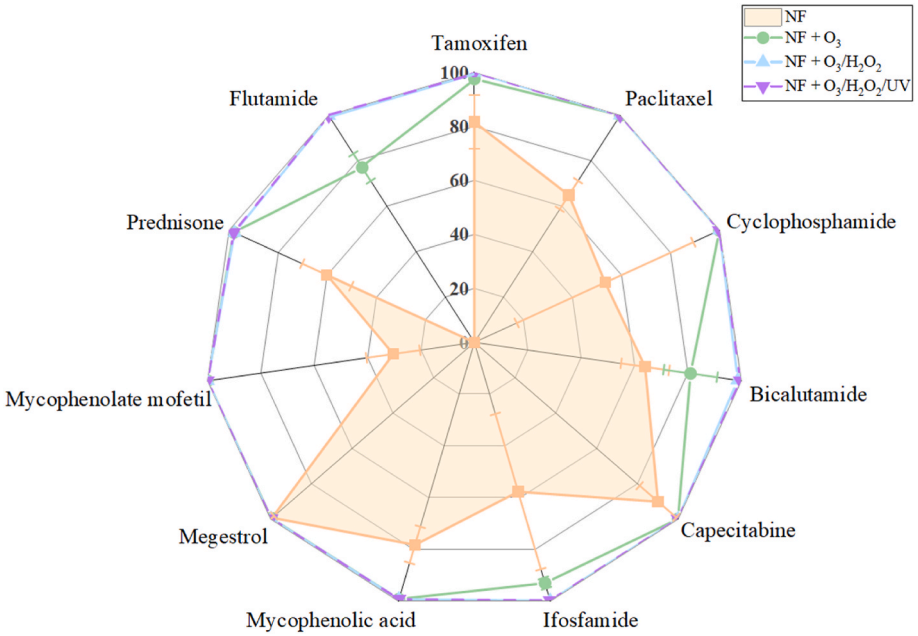

Fig. 3. Degradation (%) of the target pharmaceuticals by coupling NF and O_3-based processes (O_3, O_3/H_2O_2 and O_3/H_2O_2/UVA): $[O_3]$ = 2.4 mg/L; $[H_2O_2]$ = 17 mg/L; UV = eight 10 W UVA high intensity light emitting diodes; HRT = 10 min) and comparison with NF itself.

degradation of these pharmaceuticals (flutamide, ifosfamide, bicalutamide, and tamoxifen), being therefore not detected in the clean water. This may be explained by (i) a possible acceleration in the decomposition of ozone into highly reactive species, able to degrade antineoplastic drugs (and intermediates formed during the reaction) faster and more efficiently (Bourgin et al., 2017b; Chen and Wang, 2021; Gorito et al., 2021; von Gunten, 2003), and (ii) the generation of more hydroxyl radicals (or other reactive species), by the presence of an additional co-oxidizer, that can attack organic matter (specifically the target pharmaceuticals) present in the effluent.

DOC values for WWTP secondary effluent, NF permeate, and clean water (NF permeate after treatment by ozone-based processes) are compiled in Fig. 4. As shown, DOC abatement in the NF permeate is significant, regardless of the O_3-based process applied – 76 ± 1%. It can also be seen that the addition of a co-oxidizer (H_2O_2) and the UVA light does not have any effect on the DOC. DOC abatement from the WWTP effluent to the final clean water is approximately 92%, which confirms the high efficiency of the combination of both techniques in the removal of the organic matter present in the wastewater (Miao et al., 2021). Although there are no straight limits for total/dissolved organic carbon (TOC/DOC) in drinking water in Europe or even worldwide, 2 mg/L was found to be considered as the maximum permitted DOC concentration in Portuguese legislation (ERSAR, 2012). As can be seen in Fig. 4, the NF permeate had a DOC of 6.0 ± 0.1 mg/L, meaning that it may not be suitable for human drinking use. However, the DOC of clean water is below the threshold value (2 mg/L), and therefore, it is considered acceptable for drinking water purposes. While it was not specifically evaluated the degradation by-products of the target pharmaceuticals in this study, the substantial DOC abatement achieved (92%) suggests that the NF–O_3-based technology may have also contributed to the degradation of organic compounds, including potential by-products of ozonation.

An overall view of these results indicates that, given the treatment processes tested and the conditions applied, O_3/H_2O_2 seems to be the most promising process to be coupled to NF. With the combination of NF and O_3/H_2O_2 a complete removal (>98%) can be achieved for all the studied pharmaceuticals.

Having into account that one of the main purposes of clean water is to have a high-quality water stream, possibly for direct human consumption, a human exposure to the target pharmaceuticals was assessed considering a long-life ingestion of drinking water, in an extreme scenario where this water is produced from WWTP secondary effluents treated by the integrated technology (NF + O_3/H_2O_2). Since none of the target pharmaceuticals were detected after treatment, it means that, in the worst-case scenario, their concentrations in the clean water would correspond to the respective MDLs. Therefore, the risk was estimated under this worst-case scenario condition. The obtained *ADD* (average daily potential doses) varied between 0.6 pg/kg-day for mycophenolate mofetil in children 11–14 years old to a maximum of 39.7 pg/kg-day for bicalutamide and mycophenolic acid in children 7–10 years old. Table A.5 represents all the *ADD* and *PDE* values obtained. When the *PDE* values are compared with *ADD* values, no risk is anticipated from long-life ingestion of clean water by either children or adults (*ADD* < *PDE*), meaning that the proposed treatment technology (NF + O_3/H_2O_2) is suitable to produce drinking water from WWTP secondary effluents. However, it is important to mention that other chemical pollutants and by-products that can be generated in the O_3-based treatments may be present in the water and were not considered in the risk assessment studies. Further investigation into the identification and analysis of ozonation by-products, including their potential risks to human health, would be a valuable area for future research. Assessing the fate and toxicity of by-products resulting from the degradation of pharmaceuticals in wastewater is crucial in understanding the overall effectiveness and potential impacts of the treatment process.

Additionally, it is important to emphasize that specific standards for a high-quality water may vary depending on the intended use or regulatory guidelines of a particular region. In this study, we confirmed a 92% abatement in the DOC content from the WWTP effluents to the NF–O_3 treated water, and a complete elimination of the target compounds. However, for future studies more information on long-term performance and stability of the NF–O_3-based processes, removal efficiency of a wider range of pharmaceuticals and other relevant contaminants, as well as long-term toxicity tests of the final streams, can help to support the effectiveness of NF–O_3 based processes, since the whole matrix and not the target pharmaceuticals specifically is considered.

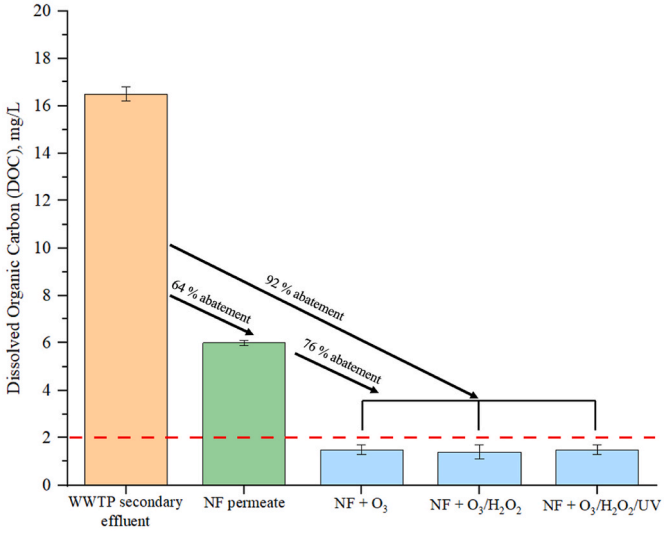

Fig. 4. DOC abatement in the WWTP secondary effluent and NF permeate, before and after O_3, O_3/H_2O_2, and O_3/H_2O_2/UVA: [O_3] = 2.4 mg/L for NF permeate, 6.6 mg/L for WWTP effluents; [H_2O_2] = 17 mg/L for NF permeate, 55.5 mg/L for WWTP effluents; UV = eight 10 W UVA high intensity light emitting diodes; HRT = 10 min.

3.3.2. *Treatment of retentate stream to safe discharge in water bodies*

The NF treatment generates a retentate stream, a complex and polluted matrix that should be treated before discharge into the environment. In this section, the O_3-based processes were applied to the retentate resulting from the experiments carried out in the pilot-scale NF system fed with WWTP effluents. The degradation of the target pharmaceuticals in continuous-flow mode ozonation was investigated by applying an O_3 concentration of 6.6 mg/L to the NF retentate. Table A.3 and Fig. 5 represent the degradations (%) obtained for each pharmaceutical. The results show that O_3 led to a complete removal of paclitaxel, megestrol, and mycophenolate mofetil in the NF retentate, lowest removals being registered for mycophenolic acid, flutamide, and tamoxifen (62 ± 5%, 52 ± 8%, and 80.3 ± 0.1%, respectively). The addition of a non-selective oxidant (H_2O_2) further led to the elimination of tamoxifen, ifosfamide, cyclophosphamide, capecitabine, prednisone, flutamide, and bicalutamide, these substances being not detected after the O_3/H_2O_2 process. As expected, the same results were verified for the O_3/H_2O_2/UVA treatment.

As previously seen in the treatment of a WWTP secondary effluent (*i.e.*, studied matrix with characteristics most similar to NF retentate), mycophenolic acid remains the only antineoplastic drug detected after the treatment of NF retentate by O_3-based processes: O_3 (62 ± 5%), O_3/H_2O_2 (57 ± 8%), and O_3/H_2O_2/UVA (63 ± 6%). These results also indicate that the addition of H_2O_2 (O_3/H_2O_2) and UVA (O_3/H_2O_2/UVA) to the process does not have a major impact on mycophenolic acid's degradation, the O_3 process alone being enough for this purpose. This corroborates that mycophenolic acid is mainly degraded by the direct ozonation mechanism (O_3 molecular attack) (Garcia-Costa et al., 2021). Experiments with a slightly higher g_{O3}/g_{DOC} ratio would possibly

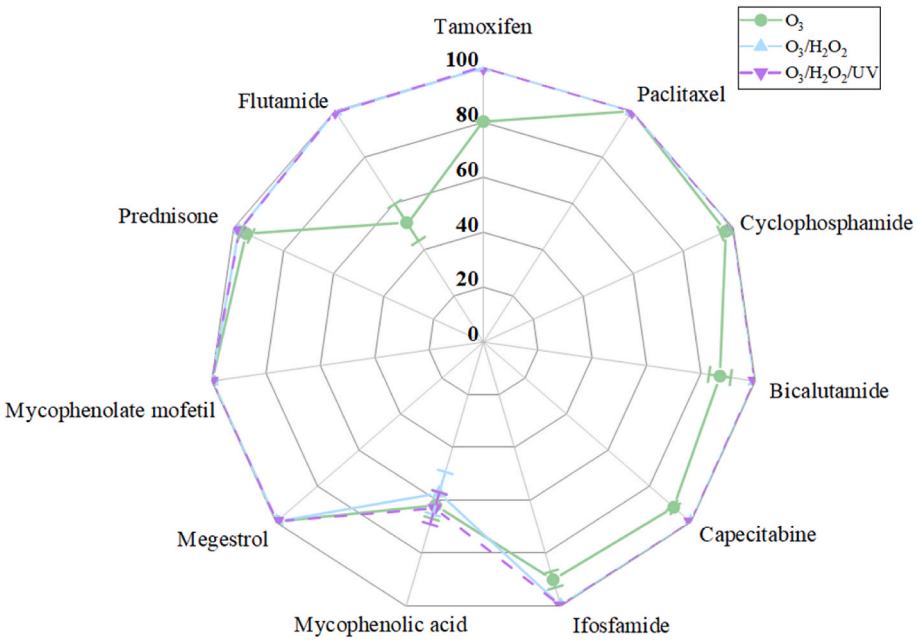

Fig. 5. Degradation (%) of the target pharmaceuticals in NF retentate by ozone-based processes (O_3, O_3/H_2O_2 and O_3/H_2O_2/UVA): $[O_3] = 6.6$ mg/L; $[H_2O_2] = 55.5$ mg/L; UV = eight 10 W UVA high intensity light emitting diodes; HRT = 10 min.

conduct to complete elimination of mycophenolic acid, and those are therefore recommended, after a proper technical and economic analysis regarding the implementation of this technology.

The DOC of the NF retentate is 200 ± 3 mg/L and, after the application of O_3-based treatments, the DOC decreases to 144 ± 4 mg/L with the O_3 process and to 103 ± 5 mg/L with O_3/H_2O_2, which corresponds to 28% and 48% DOC abatements, respectively. Moreover, the UVA light does not have a significant contribution to the DOC reduction. This suggests that O_3/H_2O_2 is the most promising technology aiming an effective elimination of antineoplastic drugs in NF retentates and simultaneous improved mineralization degrees.

The concentrations of the target pharmaceuticals in surface waters were predicted by the application of a dilution factor of 10 from the concentrations assumed for each pharmaceutical in treated NF retentate, as explained in *Section 2.6.2*. Fig. 6 shows the *RQ* interpretation before and after the application of the most promising O_3-based treatment (O_3/H_2O_2) to the NF retentate aiming stream discharge into water bodies. The *RQ* results suggest that the discharge of NF retentate without posterior treatment would probably pose a risk to aquatic organisms from exposure to cyclophosphamide, capecitabine, and mycophenolic acid. Regarding the discharge of NF retentate after treatment by O_3/H_2O_2, the only antineoplastic drug for which a low risk is still identified is mycophenolic acid (Fig. 6). This suggests that the treatment of NF retentate by peroxone process seems to be a promising approach to diminish this NF residue toxicity.

4. Conclusions

Neither nanofiltration (NF) nor O_3-based processes alone could fully eliminate all target antineoplastic drugs (bicalutamide, capecitabine,

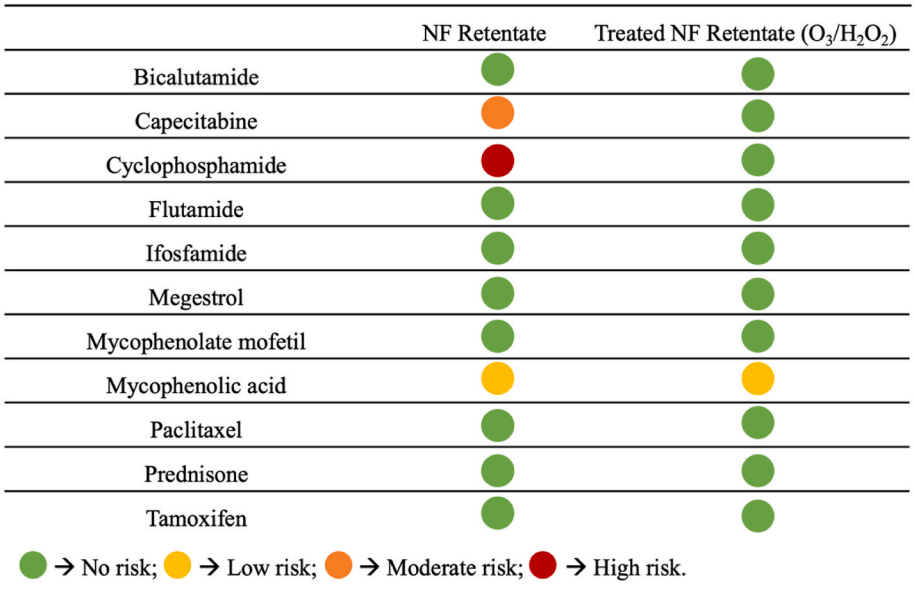

Fig. 6. Qualitative analysis of the Risk Quotients (*RQ*) calculated for NF retentate and NF retentate treated by O_3/H_2O_2 aiming stream discharge into water bodies.

cyclophosphamide, flutamide, ifosfamide, megestrol, mycophenolate mofetil, mycophenolic acid, paclitaxel, and tamoxifen) and the corticosteroid prednisone from a WWTP secondary effluent. It was demonstrated that none of these treatments alone would be good options to produce a high-quality water stream for human re-use, or at least that could be safely discharged into the water bodies. The coupling of NF with O_3 process led to a complete removal of 7 out of 11 target drugs from the permeate stream generated from WWTP secondary effluents: paclitaxel, megestrol, cyclophosphamide, capecitabine, mycophenolic acid, mycophenolate mofetil and prednisone. When H_2O_2 and UVA were simultaneously combined with ozonation (NF + O_3/H_2O_2 and NF + O_3/H_2O_2/UVA, respectively), all the compounds were removed at levels higher than 98% (prednisone) for both treatments. Therefore, O_3/H_2O_2 was identified as the most suitable process to be applied after NF, aiming at producing a high-quality water stream possibly for recharging aquifers used as abstraction sources for drinking water production. The exposure of humans to the studied pharmaceuticals by drinking the clean water for a lifetime was estimated, and no risk was predicted either for adults or for children. However, it is important to notice that this study had only in consideration the target pharmaceuticals; the existence of other hazardous compounds in the wastewaters, as well as the possible by-products that can be formed should be carefully considered.

The O_3 process led to a complete removal of paclitaxel, megestrol, and mycophenolate mofetil from the NF retentate, which is a very polluted matrix that needs prior treatment to decrease its toxicity before being discharged into the environment. The addition of H_2O_2 allowed the complete removal of the remaining compounds, mycophenolic acid being the only antineoplastic drug detected, even when UV radiation was simultaneously applied. The risk quotient of the treated NF retentate was successfully decreased after the application of the most promising technique (O_3/H_2O_2). Despite the discharge of the treated NF retentate into the water courses should only be considered after a complete elimination of hazardous substances, the implementation of NF + O_3/H_2O_2 process represents a positive net impact on the environment.

Author contributions

Sample collection, M.B.C, V.J.P.; Conceptualization, T.I.A.G. and M. S.F.S.; methodology, T.I.A.G., A.M.G.; validation of the method, A.M.G., T.I.A.G. and M.S.F.S.; formal analysis, T.I.A.G., V.J.P. and M.S.F.S.; resources, M.S.F.S., A.A., A.M.T.S. and J.G.C.; data curation, T.I.A.G.; writing—original draft preparation, T.I.A.G.; writing—review and editing, T.I.A.G., A.M.G., M.B.C., V.J.P., J.G.C., A.A., A.R.R., A.M.T.S., M.F.R.P. and M.S.F.S; visualization, T.I.A.G.; supervision, M.S.F.S. and A.A.; project administration, M.S.F.S., A.A., A.M.T.S. and J.G.C.; funding acquisition, M.S.F.S., A.A., A.M.T.S. and J.G.C. All authors have read and agreed to the published version of the manuscript.

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

The authors are unable or have chosen not to specify which data has been used.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2023.119314.

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