Antineoplastic drugs in urban wastewater: Occurrence, nanofiltration treatment and toxicity screening☆

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ABSTRACT

Antineoplastic drugs are pharmaceuticals that have been raising concerns among the scientific community due to: (i) their increasing prescription in the fight against the disease of the twentieth century (cancer); (ii) their recalcitrance to conventional wastewater treatments; (iii) their poor environmental biodegradability; and (iv) their potential risk to any eukaryotic organism. This emerges the urgency in finding solutions to mitigate the entrance and accumulation of these hazardous chemicals in the environment. Advanced oxidation processes (AOPs) have been taken into consideration to improve the degradation of antineoplastic drugs in wastewater treatment plants (WWTPs), but the formation of by-products that are more toxic or exhibit a different toxicity profile than the parent drug is frequently reported. This work evaluates the performance of a nanofiltration pilot unit, equipped with a Desal 5DK membrane, in the treatment of real WWTP effluents contaminated (without spiking) with eleven pharmaceuticals, five of which were never studied before. Average removals of $68 \pm 23\%$ were achieved for the eleven compounds, with decreasing risks from feed to permeate for aquatic organisms from receiving waterbodies (with the exception of cyclophosphamide, for which a high risk was estimated in the permeate). Additionally, no significative impact on the growth and germination of three different seeds (*Lepidium sativum, Sinapis alba,* and *Sorghum saccharatum*) were determined for permeate matrix in comparison to the control.

1. Introduction

In 2020, about 2.68 million new cancer cases were diagnosed in European citizens (ECIS, 2022). This number has been rising and until 2040 it is estimated an increase of about 21% in cancer incidence. Among the several cancer treatments, chemotherapy is the most used one, consisting in the administration of pharmaceuticals called antineoplastic drugs. They can interfere with cell division in different ways: by disturbing the metabolism, and by damaging the DNA of a cell, among others (ASCO, 2022; Kischkel, 2016). Although being used to fight cancer, antineoplastic drugs have been reported to cause long-term side effects, such as secondary cancers (ASCO, 2019). Thus, contact with these drugs should be restricted to patients who need it, avoiding the exposure of healthy lives to antineoplastic drugs.

As every other pharmaceutical, after being administered, antineoplastic drugs are excreted (in their original form or metabolized) and

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released into the sewage system (Gouveia et al., 2023). Due to their poor degradation by conventional treatments used in wastewater treatment plants (WWTPs) (Ioannou-Ttofa and Fatta-Kassinos, 2020), their presence in environmental waters has been widely reported (Franquet-Griell et al., 2017; Gouveia et al., 2022) and some studies have already revealed that there is a risk for aquatic organisms associated with antineoplastic drugs' presence in surface waters (Gouveia et al., 2019; Moermond et al., 2018). Since most of the times these compounds are administered to outpatients (HAS, 2005) (Cristóvão et al., 2020), domestic effluents are the major route of environmental contamination; therefore, the implementation of efficient removal technologies for the elimination of antineoplastic drugs and other pollutants in WWTP effluents is of utmost importance, avoiding their release to the environment. Among the several available treatment technologies employed in WWTPs (*e.g.*, chlorination, UV radiation, ozonation), membrane-based processes are other alternatives. Pressure-driven membrane processes have the advantages of: (i) achieving high removal rates for low molecular-weight (MW) organic pollutants (if nanofiltration or reverse osmosis are applied); (ii) being able to be easily integrated with other treatment technologies in WWTPs; (iii) being able to remove some compounds (*e.g.*, cyclophosphamide), which were proven not to be efficiently removed by other technologies such as advanced oxidation processes; and (iv) not needing additional chemicals. Since the MW of antineoplastic drugs usually ranges from ~100 to ~900 Da, reverse osmosis and nanofiltration using membranes with molecular weight cut offs (MWCO) varying from <100 to 1000 Da, are the most adequate to be applied for their removal (EPA U.S, 2005). In a previous study performed by the authors, the application of membrane-based processes on antineoplastic drugs removal from waters was reviewed; removals varied from 35% to >95–100% for cyclophosphamide, paclitaxel, capecitabine, fluorouracil and cytarabine (Cristóvão et al., 2022; Cristóvão et al., 2019; Kazner et al., 2008; Verliefde et al., 2009; Verliefde et al., 2007; Wang et al., 2009). Although these processes have proved to be promising technologies to be applied in WWTPs for antineoplastic drugs' removal, information is inexistent for some of the highly consumed and riskier antineoplastic drugs (*e.g.*, mycophenolic acid, mycophenolate mofetil, bicalutamide). Furthermore, pilot and large-scale experiments, using realistic concentrations (ng/L range), are also very limited; up to the authors' best knowledge, there is only one study which evaluated the performance of a pilot-scale nanofiltration unit in the removal of six antineoplastic drugs (capecitabine, cyclophosphamide, etoposide, ifosfamide, paclitaxel, and tamoxifen) present in wastewaters effluents at realistic concentrations (Cristóvão et al., 2022). In that study, experimental rejections were only obtained for capecitabine, cyclophosphamide and ifosfamide, since the other pharmaceuticals (etoposide, paclitaxel and tamoxifen) were not detected in the feed wastewater (Cristóvão et al., 2022).

In the present work, the performance of a pilot-scale nanofiltration unit was studied for the removal of eleven pharmaceuticals from a WWTP secondary effluent, at environmentally relevant concentrations (without spiking/fortification), in triplicate experiments, performed in three different days. For some of the selected antineoplastic drugs, no data is available yet (*e.g.*, mycophenolic acid, bicalutamide, mycophenolate mofetil, megestrol, and tamoxifen), despite being highly consumed and frequently associated to potential aquatic organisms risks and human health effects (*e.g.*, tamoxifen is classified as carcinogenic to humans by the International Agency for Research on Cancer).

The reuse of wastewater effluents is becoming increasingly considered for many applications, especially in developing countries. Its reuse for agriculture and aquaculture purposes has already been practiced in many countries for some decades. Although there are guidelines carefully defined by the World Health Organization (WHO, 1989), where microbiological and chemical parameters are considered, others (*e.g.*, the presence of toxic compounds such as antineoplastic drugs) should also be carefully evaluated, when considering reusing of wastewater for several applications. Thus, the impact of the three unspiked

nanofiltration matrices (feed, permeate and retentate) on plants was estimated by phytotoxicity tests (thinking on the reuse of wastewaters for land and crops irrigation). Other studies have been using the same approach (Bakopoulou et al., 2011; Yotova et al., 2019; Yotova et al., 2021). On the other hand, the impact of the discharge of the final effluents into water bodies was also assessed through the estimation of risk quotients (*RQ*) for aquatic organisms, according to the guidelines for environmental risk assessment of pharmaceuticals proposed by the European Medicines Agency (EMEA, 2006). Therefore, this study provides the first results on the estimation of the risk of permeate stream to aquatic organisms from receiving bodies, as well as to plants (phytotoxicity), if the reuse of the water for irrigation purposes is envisaged.

For all above-mentioned reasons, this study entails several novelty aspects, representing an insight into the evaluation of the removal efficiencies of pharmaceuticals, particularly antineoplastic drugs which have not been widely studied, by nanofiltration technology. It also contributes to the future knowledge on removal programs of pharmaceuticals of environmental concern from wastewaters to be designed worldwide.

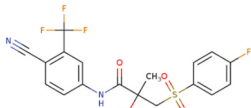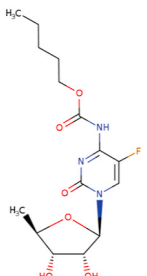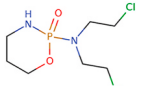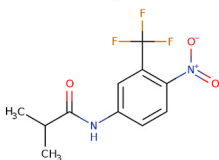## 2. Materials and methods

2.1. Chemicals and reagents
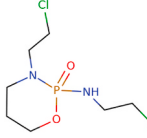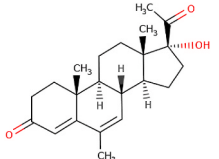
Bicalutamide, capecitabine, cyclophosphamide, flutamide, ifosfamide, megestrol, mycophenolate mofetil, mycophenolic acid, paclitaxel, prednisone, and tamoxifen analytical standards of 98–99% purity, used in the calibration curve and validation experiments, were acquired from Sigma-Aldrich (St. Louis, USA) and Cayman Chemical Company (Ann Arbor, USA). The chemical structure of the target compounds is represented in Table 1. Besides their high consumption and occurrence in wastewater effluents, the target compounds present a wide range of chemical structures, functional groups, molecular weight, and properties and are thus good target drugs to evaluate: (i) the performance of the nanofiltration process and (ii) their toxicological risks. Although prednisone is not considered an antineoplastic drug, it was added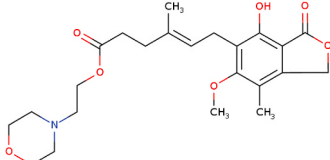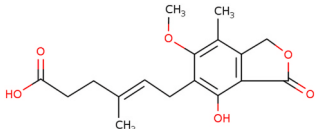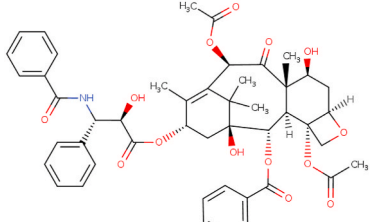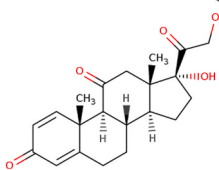 to this work since it is prescribed/administered in combination with several antineoplastic drugs during cancer treatment. Methanol (MeOH), acetonitrile (ACN), isopropanol, Milli-Q water, and ammonium acetate (NH$_4$OAc) were supplied by Merck (Darmstadt, Germany). All solvents used were of LC–MS grade. 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 were prepared at 10 mg/L in MeOH, except paclitaxel that was prepared in ACN. Formic acid (HCOOH) and HCl 1 M used for pH adjustment, were purchased from Sigma-Aldrich (St. Louis, USA). 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. Nanofiltration experiments

The nanofiltration experiments were carried out in a pilot-scale unit installed at the Oeiras Agro-Tech Campus, in Portugal. Three experiments were performed, between February and May 2022, using real effluents (without fortification/spiking of the target analytes) collected at an urban WWTP, prior to discharge in a river. The characteristics of the wastewater effluents used are compiled in Table A1.
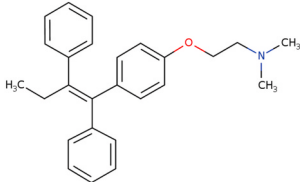
The nanofiltration membrane used during the experiments was a spiral wound Desal 5DK module (model DK4040F30, Suez membranes, Lenntech, Delfgauw, Netherlands). According to the manufacturer, this thin film composite membrane is characterized by a MWCO of 150–300 Da, a minimum MgSO$_4$ rejection of 98% and an active surface area of 7.9 m^2. More information regarding the pilot-scale nanofiltration unit is described elsewhere in detail (Cristóvão et al., 2022).

Table 1

Molecular structure, molecular weight (MW), log K_{OW}, pKa, charge at pH 7–8, hydrophobicity and molecular size of the target pharmaceuticals.

Chemical name	Molecular structure	Molecular weight (g/mol)	log K_{OW}	pKa	Charge at pH 7-8	Hydrophobicity
Bicalutamide $C_{18}H_{14}F_4N_2O_4S$		430.377	2.5	12.00	Neutral	Hydrophobic
Capecitabine $C_{15}H_{22}FN_3O_6$		359.350	0.4	0.073; 8.63	Neutral	Hydrophilic
Cyclophosphamide $C_7H_{15}Cl_2N_2O_2P$		261.083	0.8	0.02; 12.78	Neutral	Hydrophilic
Flutamide $C_{11}H_{11}F_3N_2O_3$		276.212	3.3	−3.70; 12.81	Neutral	Hydrophobic
Ifosfamide $C_7H_{15}Cl_2N_2O_2P$		261.080	0.9	<2.5; 14.64	Neutral	Hydrophilic
Megestrol $C_{22}H_{30}O_3$		342.472	3.2	−4.90; 17.83	Neutral	Hydrophobic
Mycophenolate mofetil $C_{23}H_{31}NO_7$		433.498	3.0	5.60; 8.50	Neutral	Hydrophobic
Mycophenolic acid $C_{17}H_{20}O_6$		320.339	2.6	−4.10; 3.57	Negative	Hydrophobic
Paclitaxel C47H51NO14		853.906	3.0	−1.20; 11.90	Neutral	Hydrophobic
Prednisone $C_{21}H_{26}O_5$		358.428	1.5	−3.30; 12.58	Neutral	Hydrophilic

Table 1 (*continued*)

Chemical name	Molecular structure	Molecular weight (g/mol)	log K_{OW}	pKa	Charge at pH 7-8	Hydrophobicity
Tamoxifen $C_{26}H_{29}NO$		371.521	6.5	8.76	Positive	Hydrophobic

Note: MW, log K_{OW} and pKa values were obtained from the Drug bank database (https://go.drugbank.com/), retrieved on August 2022; Molecular size was obtained from Chem3D desktop modelling program.

The performance of this nanofiltration unit using the Desal 5DK membrane was previously optimized using the same matrix, to define the best operating conditions in different assays conducted at controlled permeate flux or controlled transmembrane pressure, using different water recovery rates, defined as the volume of permeate obtained per volume of feed processed (Cristóvão et al., 2022). In the three nanofiltration assays conducted, 1000 L of wastewater effluent was processed at the constant pressure of 6 bar with a recovery of approximately 70%. The permeate was collected in a different tank and the retentate was recirculated to the feed tank. The permeate flux normalized at 20 °C varied from approximately 21 to 15 L/(h m^2) during the approximately 5 h nanofiltration assays. All experiments were conducted at a constant crossflow velocity, corresponding to an internal recirculation flow of 1000 L/h. The average permeability measured with tap water (filtered with an activated carbon filter before the assays) was 3.72 ± 0.11 L/(h m^2 bar). After each experiment, tap water and ultrasil (1%, m/m) were used to clean the membrane for approximately 30 min to ensure the same initial conditions in each experiment.

The three matrices (initial feed, final permeate and retentate) from the nanofiltration process were collected in triplicate and analyzed in quadruplicate. Sample preparation required two filtration steps: the first one with 0.8 μm nylon membrane filters and the second one with 0.45 μm nylon membrane filters (Gouveia et al., 2022). Then, samples were acidified at pH 2 with HCl 1 M. After being extracted by solid-phase extraction (SPE) and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) (more details can be found in section 2.4), the rejection of each pharmaceutical in the nanofiltration unit was calculated according to Equation (1):

$$\% \, Rejection = \left(1 - \frac{C_p}{C_f}\right) \times 100 \qquad \text{Equation 1}$$

where C_p and C_f are the concentration of the target pharmaceutical in the permeate and feed of the nanofiltration system, respectively.

2.3. Analysis of the target pharmaceuticals by SPE-LC-MS/MS

2.3.1. Sample preparation and extraction procedure

The SPE procedure was performed in quadruplicate for each sample collected (feed, permeate or retentate). The SPE conditions used in this work were based on the methodology previously developed by Gómez-Canela et al. (2014). This methodology was however extended to the extraction of other antineoplastic drugs of concern (*i.e.*, bicalutamide, flutamide, mycophenolate mofetil, and mycophenolic acid) and further validated (Gouveia et al., 2022).

SPE cartridges were conditioned using 6 mL MeOH and 6 mL of an aqueous solution of 100 mmol/L NH$_4$OAc. Then, 100 mL of sample (pH = 2) was loaded through the cartridge at a flow 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. The eluate was slowly evaporated to dryness and reconstituted in 200 μL ACN for further analysis in the LC-MS/MS system.

2.3.2. Instrumental analysis

The analyses of the extracts were carried out in a liquid chromatograph (Shimadzu Corporation, Tokyo, Japan) equipped with an Autosampler SIL-30 AC, an Oven CTO-20 AC, two Pumps LC-30AD, a Degasser DGU-20A5, a System Controller CBM-20 A, 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.

The column used in the chromatography was a Luna C18 (150 × 2.1 mm ID, particle size 5 μm; Phenomenex) and the mobile phase consisted of a binary mixture of water (A) and MeOH (B), both acidified with 0.1% HCOOH, 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 regained (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 in Table A2 of Supplementary Information). 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 (Gouveia et al., 2020).

2.3.3. Validation parameters

The calibration curves were performed in a concentration range of 1–500 μg/L (depending on the pharmaceutical – Table A3), corresponding to 2–1000 ng/L in the samples before extraction, using ten calibration points and the internal standard quantification was accomplished using MPA-d3 as surrogate for capecitabine, mycophenolic acid, mycophenolate mofetil, and prednisone, and CYC-d4 for the other pharmaceuticals. Both were added before the evaporation, which is the extraction step more prone to losses.

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.

Recovery assays were performed in triplicate for the three matrices studied (feed, permeate and retentate) spiked at a concentration level of 100 ng/L of each pharmaceutical. The spiked and non-spiked matrices were processed and extracted by SPE. Afterwards, the final extracts of both spiked and non-spiked samples were analyzed by LC-MS/MS. The difference between the mass of each compound in the SPE extracts from the spiked matrices (*Ms*) and non-spiked matrices (*M0*) was compared to the mass of pharmaceutical added (*Ss*). The *Ss* consisted in a standard prepared in ACN, with the same spike concentration used to fortify the

matrices, 100 ng/L, and the same spike of internal standards added to the SPE extracts, and by direct injecting it in the LC-MS/MS. Recoveries were then calculated according to Equation (2):

$$\%R = \frac{(Ms - M0)}{Ss} \times 100 \qquad \text{Equation 2}$$

where *Ms* is the mass of pharmaceuticals in the extract from the spiked wastewater, *M*0 the mass of pharmaceuticals in the extract from the original wastewater (without spike) and *Ss* the mass of pharmaceuticals in the spike.

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 through six different days.

2.4. Phytotoxicity tests

Phytotoxicity tests were performed with the aim of estimating the potential impact of the feed, retentate and permeate streams on the environment if another application of the produced water is envisaged (agriculture practices; land and crops irrigation). This approach was considered, attending on the high-quality of the permeate stream, but without disregarding the most probable fate of the treated water, *i.e.*, discharge into the water bodies. The PHYTOTOXKIT for liquid samples, from MicroBioTests Inc., is a standardized test kit that is designed for the evaluation of potential toxicity in liquid samples, specifically on plant germination and growth. These analyses were performed according to the procedure recommended by MicroBioTests Inc., evaluating the percentage decrease of the seed germination and the growth of the plant roots and shoots in the studied matrices in comparison to the control (distilled water). PHYTOTOXKIT utilizes a limit test and features three plant species by default: *Lepidium sativum*, *Sinapis alba* and *Sorghum saccharatum*. The protocol involves the incubation of 10 seeds of each plant in a single test plate. However, to ensure that each replicate of each species and also each of the 3 species is subjected to the exact same matrix content and conditions, a quadruplicate analysis with three seeds of each plant is applied in each toxicity plate. This results in a total of 12 seeds of each plant per matrix studied, in addition to control samples where distilled water is used, which totalizes 144 seeds incubated.

The procedure started with the germination of the seeds by adding 20 mL of each matrix, in quadruplicate assays with three seeds of each species, and letting them grow for 72 h at 25 °C. Then, both the number of germinated seeds and the length of the roots and shoots were measured using ImageJ software. Equation (3) was applied for the determination of the percentage effect, PE (%).

$$\% PE = \frac{(A - B)}{A} \times 100 \qquad \text{Equation 3}$$

being *A* the number of germinated seeds or the length of the roots/shoots in the control sample and *B* the number of germinated seeds or the length of the roots/shoots in the studied matrices (feed, permeate and retentate).

The germination index (GI) is a commonly used parameter in phytotoxicity tests to assess the impact of a matrix on plant growth and development. In addition to following the standard operating procedure of PHYTOTOXKIT, the germination index of each seed was determined using Equation (4).

$$\% GI = \left(\frac{Gt}{Gc}\right) \times \left(\frac{It}{Ic}\right) \times 100 \qquad \text{Equation 4}$$

being *Gt* the number of germinated seed in the treated sample, *Gc* the number of germinated seeds in the control sample, *It* the average length of shoots in the treated sample and *Ic* the average length of shoots in the control sample.

2.5. Estimation of the risk for aquatic organisms

To evaluate if the concentrations measured for each pharmaceutical in each matrix (feed, permeate and retentate) represent a threat to aquatic biota, especially if used for aquaculture purposes, the risk quotient (*RQ*) was estimated.

Among the reasons considered in this study for the *RQ* estimation over experimental assays with aquatic organisms, are:

(i) Not all aquatic species have equal sensitivity to a certain toxicant (de García et al., 2016); using the *RQ* approach, the risk each pharmaceutical may pose to aquatic lives can be determined considering toxicity data of up to three trophic levels, available in the literature. Also, the existence of toxicity information for more aquatic species, allows the selection of the one associated to the worst case-scenario.

(ii) there is a need to focus on long-term exposure assessment to better judge on the effects of pharmaceutical residues in aquatic systems (Fent et al., 2006). However, long-term assays can be more resource-intensive, requiring more maintenance and monitoring of the test organisms over a longer period. Furthermore, long-term assays may also require specialized equipment, and facilities to maintain and support the test organisms.

(iii) for most pharmaceuticals, acute effects on aquatic organisms are unlikely, especially if the tests are conducted at low concentrations (Fent et al., 2006). As an example, toxicological experiments were performed for tamoxifen (concentrations below 5.26 μg/L) on D. pulex, and the size and reproduction effects were not significantly different from controls (Borgatta et al., 2016).

RQ was then calculated from the quotient between the average concentration of each compound measured in the matrix (*MEC*) and the *PNEC* (Predicted no effect concentration), a value obtained from published toxicological data by applying an assessment factor (AF) – Equation (5).

$$RQ = \frac{MEC}{PNEC} \qquad \text{Equation 5}$$

Depending on the toxicological dose descriptor, the nature of the toxicity value and the number of known trophic toxicological levels, the selected AF values were assumed, as previously described (Gouveia et al., 2019). Whenever available, long-term toxicity values were used instead of short-term values, even though short-term values may result in lower *PNEC* values. Then, an acknowledged criterion for risk quotient clarification was applied (Sánchez-Bayo et al., 2002), where RQ ≥ 1 indicates high risk, 0.1 ≤ RQ < 1 indicates moderate risk, and 0.01 ≤ RQ < 0.1 indicates low risk for aquatic biota. If RQ < 0.01, no risk is associated to that compound at the concentrations measured in the matrix. The toxicological information and the *PNEC* values used to estimate the risks are compiled in the Supplementary Information (Table A4).

3. Results and discussion

3.1. Validation of the SPE-LC-MS/MS method for the analysis of the target pharmaceuticals in the different wastewaters

Good linearity was achieved for all compounds in ultrapure water using the optimized parameters for LC-MS/MS analysis, in a range of 1–500 μg/L (depending on the pharmaceutical considered - Table A3), which corresponds to 2–1000 ng/L in the samples before extraction by SPE, with correlation coefficients higher than 0.996 (Table A3). The MDL values obtained were relatively low, varying from 0.03 ng/L for bicalutamide to 3.65 ng/L for prednisone. These limits are suitable for the evaluation of the target pharmaceuticals' concentrations in wastewater effluents (reported concentrations ranged from low ng/L to μg/L; *i.e.*, 0.19 ng/L for cyclophosphamide – 2.9 μg/L for ifosfamide)

(Llewellyn et al., 2011; Ternes, 1998).

The results of intra and inter-day precisions were satisfactory, varying between 2% for cyclophosphamide and 11% for megestrol, mycophenolic acid and tamoxifen (intra-day precision), and between 1% for cyclophosphamide and 19% for ifosfamide (inter-day precision) - Table A3. Good recoveries were achieved for most compounds, in average being $77 \pm 20\%$ for the permeate, $65 \pm 18\%$ for the feed and $59 \pm 25\%$ for the retentate.

3.2. Presence of the target pharmaceuticals in the feed, permeate and retentate

All the eleven pharmaceuticals studied were detected in the feed of at least two of the three nanofiltration experiments. Table A5 details all the concentrations measured in the feed, permeate and retentate, which are represented schematically in Fig. 1 to facilitate interpretation. A general assessment of the results indicates that bicalutamide, megestrol, mycophenolic acid and prednisone were the pharmaceuticals found at higher concentrations in the feed of the nanofiltration unit (*i.e.*, in the effluents of the WWTP), all these four compounds being detected at concentrations equal or above 40 ± 6 ng/L in the feed of at least two experiments. This minimum value was registered for megestrol in the first experiment, whereas the highest one recorded was 127 ± 20 ng/L for mycophenolic acid in the third experiment. Although megestrol and prednisone were detected at relatively high concentrations, they were only found in two of the three feeds (Fig. 1). This highlights the importance of evaluating the nanofiltration performance in different days corresponding to different feed compositions. The compounds found at lower concentrations in the feed of the system were flutamide, mycophenolate mofetil, paclitaxel and tamoxifen, which were detected at levels below 5.2 ± 0.2 ng/L (for paclitaxel in the third experiment) (Fig. 1). Comparing these concentrations to those reported by other studies on the presence of antineoplastic drugs in wastewaters, it can be concluded that the concentrations obtained in the present study are within the ranges reported in the literature: a previous study done by some of the coauthors of the present work confirmed the presence of five antineoplastic drugs in secondary effluents of a Portuguese WWTP, at concentrations varying from 22 ± 1 ng/L for capecitabine and 74 ± 23 ng/L for mycophenolic acid (Gouveia et al., 2020). In a worldwide perspective, concentrations found in WWTP effluents are very inconstant, varying from non-detected to a maximum of 24.8 µg/L reported for epirubicin, in Spain (Gómez-Canela et al., 2012).

According to Figs. 1 and 2, the nanofiltration system was effective in the removal of all the compounds, in a greater or lesser extent. However,

all the compounds were detected in the permeate at least once, despite being at relatively lower concentrations than in the feed. The compounds found at higher concentrations in the permeate matrix were mycophenolic acid (16 ± 4 ng/L – 24.9 ± 0.2 ng/L), prednisone (20 ± 13 ng/L – 38 ± 2 ng/L) and bicalutamide (14 ± 3 ng/L – 24 ± 7 ng/L); ifosfamide was also found at a relatively high concentration (19 ± 2 ng/L) in the permeate of the third experiment. As expected, the concentrations found in the retentate generated by the system were much higher than in the corresponding feeds, since the permeate was collected during the assay in another tank.

3.3. Rejection of the target pharmaceuticals in the nanofiltration pilot unit

The rejection of a compound by a nanofiltration membrane can occur through different mechanisms: size exclusion (sieving, steric effects), electrostatic interactions, and hydrophobic interactions. In addition to membranes' properties, compounds' hydrophobicity (obtained through the distribution of octanol-water coefficient, log K_{OW}), their charge at working pH, and their MW/molecular size are contributors for the compounds' rejection mechanism. Feed chemistry, membrane fouling, and other parameters may also interfere with final rejections.

Among the studied compounds, capecitabine, cyclophosphamide, ifosfamide and prednisone are hydrophilic (log $K_{OW} < 2$), being the other ones hydrophobic. Regarding their charge at neutral pH, bicalutamide, capecitabine, cyclophosphamide, flutamide, ifosfamide, megestrol, and mycophenolate mofetil are neutral, mycophenolic acid being the only negatively charged antineoplastic drug, and tamoxifen being the only positively charged compound. These differences in compounds' hydrophobicity and charge may influence compound-membrane interaction, leading to different removals from the expected ones (*e.g.*, a positively charged compound may be more attracted to a negatively charged membrane than a neutral compound, if the other characteristics are similar).

Some compounds (flutamide, megestrol, mycophenolate mofetil, paclitaxel, and prednisone) were only detected in two out of the three nanofiltration experiments (Fig. 1). Fig. 2 shows the rejection of the studied compounds in each experiment and Fig. 3 shows the average rejection in the experiments where the compounds were quantified in the feed. Excluding flutamide, for which negligible rejections were achieved, the average rejection for all the other target pharmaceuticals was relatively good: $68 \pm 23\%$, being the lowest one obtained for mycophenolate mofetil, $30 \pm 10\%$, and the highest one was $98.3 \pm 0.4\%$ for megestrol. Up to the authors' best knowledge, there are no studies published in the literature describing the removal of mycophenolate
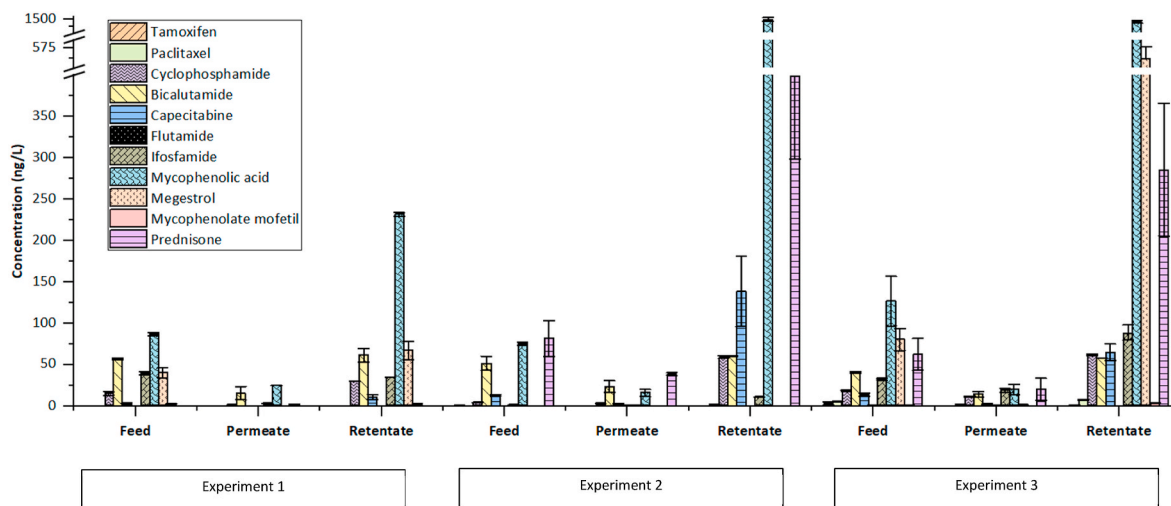

Fig. 1. Concentrations found for each pharmaceutical in the three studied matrices (feed, retentate and permeate) for the three nanofiltration experiments.
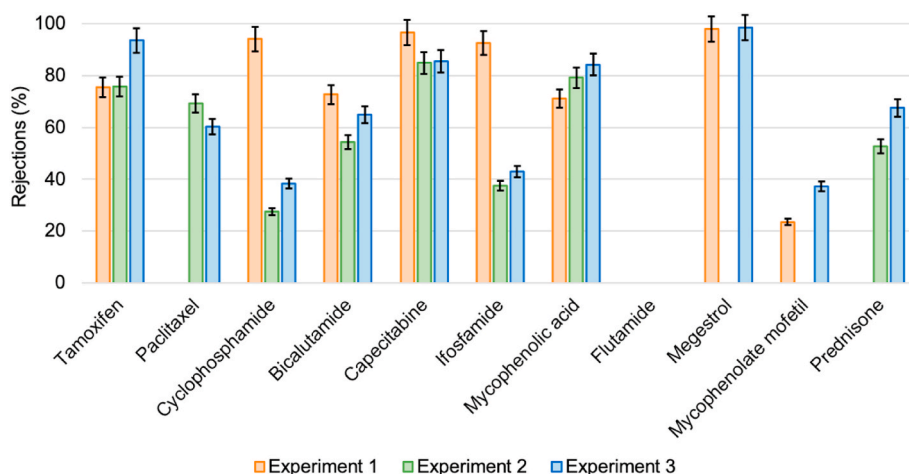
Fig. 2. Rejection of each pharmaceutical for the three nanofiltration experiments.
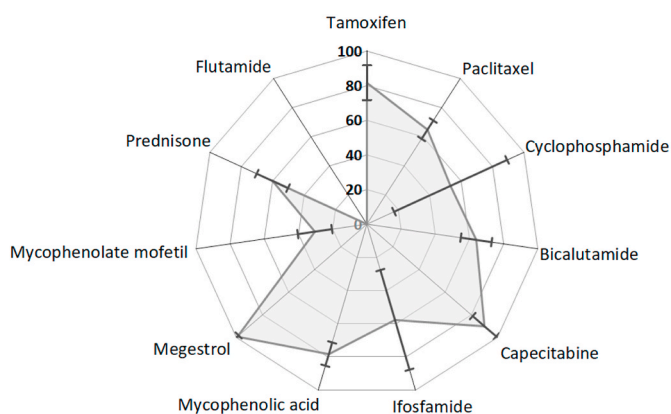

Fig. 3. Removal efficiencies/rejections of the target pharmaceuticals ± SD (%) for three nanofiltration pilot-scale experiments using Desal 5DK membrane.

mofetil, megestrol, bicalutamide, mycophenolic acid, or tamoxifen from liquid matrices by nanofiltration.

Megestrol (hydrophobic and neutral at working pH) has a MW of 342.472 g/mol, slightly higher than the MWCO of Desal 5DK (150–300 g/mol). Thus, moderate to high rejections were expected for this compound due to hydrophobic interactions between the compound and the membrane surface. Indeed, very high rejections were achieved for megestrol in the two experiments, when it was detected (average rejection of 98.3 ± 0.4%). Similar results were achieved by Cristóvão et al. (2019), for another compound with similar chemical properties (paclitaxel) (Cristóvão et al., 2019). If not rejected by size exclusion, after a certain time, its adsorption to the membrane reaches an equilibrium, and the compounds might start to breakthrough into the permeate side (Cristóvão et al., 2022).

Regarding mycophenolate mofetil (hydrophobic and neutrally charged at working pH), which MW is 433.498 g/mol (higher than the MWCO of the membrane used), low to moderate rejections were expected due to hydrophobic interactions between the compound and the membrane (Verliefde et al., 2007). Mycophenolate mofetil's hydrophobicity may contribute to bring solute and membrane together, increasing its adsorption on the surface of the membrane. This, consequently, increases mycophenolate mofetil's permeation through the membrane, leading to lower rejections. This was verified experimentally since relatively low rejections were obtained (20–40%).

Focusing on cyclophosphamide and ifosfamide, these compounds experienced two scenarios: rejections >92% were achieved for both of them in the first experiment, but rejections <43% were obtained for the

second and third nanofiltration experiments, resulting in an average rejection of 53 ± 36% for cyclophosphamide and 58 ± 30% for ifosfamide. This variability was already observed in other studies (Cristóvão et al., 2022; Cristóvão et al., 2019; Verliefde et al., 2009; Verliefde et al., 2007; Wang et al., 2009). Cristóvão and co-workers have studied the removal of cyclophosphamide and ifosfamide from wastewaters in a pilot nanofiltration system and they have always achieved high rejections (>86% for cyclophosphamide and >85% for ifosfamide, using a Desal 5DK membrane) (Cristóvão et al., 2022). Verliefde et al. have also obtained high rejection percentages for cyclophosphamide (>85%) by using Trisep TS-80 (MWCO = 200 g/mol) and Desal HL (MWCO = 150–300 g/mol) (Verliefde et al., 2009; Verliefde et al., 2007). However, low to moderate rejections for cyclophosphamide (20%–60%) have also been reported using the same membranes (Wang et al., 2009). Having in consideration that cyclophosphamide' and ifosfamide' MW (261.083 g/mol and 261.080 g/mol) are within membranes MWCO, this might be a possible reason for a high variability in the results, leading to an average moderate removal.

Similar to cyclophosphamide and ifosfamide, prednisone is a hydrophilic compound and a neutrally charged molecule at working pH. Since prednisone has a MW (358.428 g/mol) above the MWCO of the membrane, size exclusion is the driven rejection mechanism, conferring moderate to high removals. In this work, according to the expectations, moderate removals were achieved with an average rejection of 60 ± 10% for the 2nd and 3rd experiments, where prednisone was found in the feed. Another work reported removals above 84.1% using a GE Osmonics DK membrane (MWCO of 150–300 Da), being prednisone not detected in the permeate (MDL of 7.2 ng/L) (Foureaux et al., 2019). Differences in membrane properties (pore size, surface charge, and hydrophobicity), operating conditions (feed flow rate, pressure, and pH), feed water quality, as well as analytical variability and experimental errors, may justify differences between different studies' results. Also, phenomena such as membrane fouling, membrane degradation, concentration polarization, pH and ionic strength effects, and membrane selectivity may highly influence final results (Al Aani et al., 2020; Ashfaq and Al-Ghouti, 2022; Zhang et al., 2020).

In the same line, capecitabine is hydrophilic, neutral and has a MW > MWCO; thus, moderate to high removals are predicted for this compound. Expected rejections were confirmed for capecitabine in the three experiments, averaging 89 ± 7%. Up to the authors' knowledge, capecitabine's removal by nanofiltration with Desal 5DK membrane was tested in only one study coauthored by some of the authors of the present work, which actually used the same pilot-scale membrane system (Cristóvão et al., 2022). In that study, capecitabine was removed in an extent of >96%, since it was not detected in the permeate matrix (MDL of 0.05 ng/L).

Regarding bicalutamide and paclitaxel that are both neutral at working pH, hydrophobic and with a MW > MWCO, moderate to high rejections were expected (Bellona et al., 2004). Bicalutamide was detected in the three nanofiltration experiments, being moderately removed (average of $64 \pm 9\%$), as expected. Up to the authors' best knowledge, there are no published studies regarding nanofiltration experiments for bicalutamide. Regarding paclitaxel, moderate removals were achieved in this work ($65 \pm 6\%$ rejection) as it would be expected for a molecule with a MW above the MWCO of the membrane, under a rejection mechanism governed by hydrophobic interactions. Another work, which used the same nanofiltration membrane, achieved much higher rejections, above 95%, for this compound (Cristóvão et al., 2019). Differences in both works may be related to the different experimental conditions and fluid dynamics in the systems used – while Cristóvão et al. (2019) used spiked concentrated matrices in a dead-end system (Met cell), in this work a real unspiked wastewater effluent was filtered at pilot scale using a spiral wound membrane module. Flutamide (neutral at working pH, hydrophobic and with a MW within the membrane MWCO) was detected at low concentrations in the feed of system (0.2 ± 0.2 ng/L in the 2nd experiment, and 0.8 ± 0.2 ng/L in the 3rd experiment), and similar concentrations were found in the permeate (0.5 ± 0.1 ng/L in the 2nd experiment and 1.2 ± 0.1 ng/L in the 3rd experiment), leading to a negligible rejection. Although adsorption may contribute to a high initial rejection of the compound, since the MW is within the MWCO, breakthrough of the compound may occur after reaching the adsorption equilibrium.

The only charged compounds from this study are mycophenolic acid (negatively charged) and tamoxifen (positively charged). Considering tamoxifen, relatively high rejections were achieved for the three experiments ($82 \pm 10\%$). Tamoxifen's positively charged structure is easily attracted to the negatively charged surface of the membrane, facilitating compounds' diffusion (Bellona et al., 2004). The fact that tamoxifen is very hydrophobic is also contributing to bring solute and the membrane together, and to increase the adsorption of tamoxifen on the surface of the membrane. This thus gives tamoxifen the chance of permeation through the membrane, leading to lower rejections. However, the fact that tamoxifen has a MW higher than the MWCO of the membrane, as well as its lack of strong functional groups, might contribute for the retention of tamoxifen (Kiso, 1986). Up to the authors' knowledge, there are no other studies regarding tamoxifen's removal from liquid matrices using nanofiltration.

Mycophenolic acid is negatively charged and hydrophobic. In this sense, charge repulsion is expected to be the main rejection mechanism, leading to high removals. Looking at mycophenolic acid, relatively high removals were achieved for the three experiments, with an average of $78 \pm 7\%$. Mycophenolic acid's negatively charged structure is repelled from the negative surface of the membranes, increasing the compound rejection. Up to the authors' best knowledge, there are no studies regarding mycophenolic acid removal by nanofiltration process.

In general, nanofiltration showed promising results for the removal of the target compounds from wastewaters. However, if complete removal is required, this technique alone may not be enough, particularly if some of the considered pharmaceuticals (*e.g.*, flutamide, paclitaxel, bicalutamide, mycophenolic acid, mycophenolate mofetil, cyclophosphamide, ifosfamide and prednisone) are present in the feed. Other treatment processes were already exploited for the removal of the target antineoplastic drugs, including reverse osmosis (RO), forward osmosis (FO) and membrane bioreactors (MBR). These techniques were studied for the removal of cyclophosphamide, ifosfamide, flutamide and tamoxifen, present in different synthetic and real matrices (Delgado et al., 2011; Köhler et al., 2012; Kovalova et al., 2012; Luo et al., 2014; Seira et al., 2016; Wang et al., 2009; Wang et al., 2018; Zaviska et al., 2013). RO was capable of completely removing cyclophosphamide from ultrapure water and effluents of MBR (Wang et al., 2009). On the other side, variable removals (starting from 15% for cyclophosphamide) were reported for the target compounds using MBR processes (Köhler et al.,

2012). The study of Wang and co-workers combined a FO membrane coupled to an anaerobic MBR (AnOMBR) for the removal of cyclophosphamide, flutamide and tamoxifen (among other antineoplastic drugs), from wastewaters (Wang et al., 2018). They concluded compounds' adsorption on sludge flocs and biodegradation were among the main rejection mechanisms for the target antineoplastic drugs. The fact that there is only one study that uses FO membranes for antineoplastic drugs' removal, as well as RO membranes, demonstrates the lack of studies on this topic and the need for more research.

3.4. Phytotoxicity tests

The phytotoxicity of the studied matrices was evaluated through the germination and growth of three different seeds (*Lepidium sativum*, *Sinapis alba,* and *Sorghum saccharatum*), by calculating the (i) Percentage effect (PE) and (ii) Germination Index (GI), as described in section 2.4.

The results of the PE and GI (Figures A1 and A.2 of the Supplementary Information), suggest that the feed is the matrix that most affects the development of the seeds; however, in general, the variability of the results is high, and thus most of the results for the three matrices end up being similar. Still, some exceptions were verified as will be further explained.

The lengths of roots and shoots of the species in the samples were statistically compared to the control ($p = 0.05$), and it was concluded that the root growth of *Sinapis alba* was significantly affected by the feed matrix, developing significantly lower than controls ($\sim 45\%$ PE). The GI results also support that the development of *Sinapis alba* species might have been impacted by the feed matrix (average GI of $64 \pm 11\%$), when compared to retentate (average GI of $98 \pm 8\%$) and permeate (average GI of $106 \pm 9\%$). One possibility for the lower development of *Sinapis alba* species, when feed is used as a source of water and nutrients, might be the presence of a combination of specific contaminants in the feed that are toxic to *Sinapis alba*. If partially rejected by the nanofiltration equipment, these specific contaminants are then less concentrated both in the permeate and the retentate matrices.

Looking specifically at *Lepidium sativum* results, it was verified that the root growth was the most affected parameter (up to $25 \pm 15\%$ PE for feed matrix), though no significant differences were detected between the matrices both for germination and growth of the three seeds tested (average overall PE of $4 \pm 8\%$ and average GI of $93 \pm 3\%$ for the three matrices).

In the case of *Sorghum saccharatum* species, the PE results show this was the most affected species in terms of germinated seeds (11% of the seeds did not germinate). Statistically, PE values did not differ from each other between matrices. Yet, GI results showed *Sorghum saccharatum*'s germination and growth seemed to be improved when permeate matrix (average GI of $115 \pm 8\%$) was used as a source of water and nutrients when compared to feed (average GI of $73 \pm 12\%$) and retentate matrix (average GI of $91 \pm 9\%$). The lower concentrations of pharmaceuticals (and other chemical contaminants) present in permeate matrix when compared to the remaining matrices may justify this difference.

The fact that the concentrations of the target pharmaceuticals being relatively low (in the range of ng/L) in the feed and consequently in the retentate and permeate, may be not enough to accurately distinguish between the real toxicity of the matrices in a three-day assay. Furthermore, these matrices contain numerous compounds that may influence the toxicity resulting specifically from the pharmaceuticals.

It is important to note that toxicity is a complex phenomenon that can be influenced by a variety of factors, and more research may be needed to fully understand why the feed matrix resulted in more toxic effects for *Sinapis alba'* growth.

3.5. Estimation of the risk for aquatic organisms

The ecotoxicity information was gathered for all the pharmaceuticals detected. Toxicological data for aquatic organisms, as well as the AF,

Fig. 4. Risk evaluation for the studied pharmaceuticals and matrices.

and calculated *PNECs* are summarized in Table A4 from Supplementary Information. A schematic representation of the *RQs* obtained is depicted in Fig. 4.

Analyzing the results obtained, it can be concluded that the average concentration of capecitabine, flutamide, megestrol, mycophenolic acid, and tamoxifen, determined in the feed of the system, may be inducing risk to aquatic organisms. This emphasizes the need for further treatments after the conventional ones usually used worldwide at WWTP, since the feed samples used in the nanofiltration experiments are WWTP effluents. Among the antineoplastic drugs for which some risk was found, flutamide, megestrol, mycophenolic acid, and tamoxifen were suggested to have a low risk, capecitabine a moderate risk and a high risk was predicted for cyclophosphamide.

The nanofiltration system reduced the concentration of all the studied pharmaceuticals, dropping consequently, their potential risk. However, four antineoplastic drugs still may represent some risk to aquatic organisms, considering the average concentrations found in the permeate: flutamide and mycophenolic acid kept their risk as low, capecitabine risk was reduced from moderate in the feed to low in the permeate, and cyclophosphamide kept its risk as high risk. Cyclophosphamide is already identified as a carcinogenic compound for Humans by the International Agency for Research on Cancer (IARC, 2022) and, therefore, the "*As Low As Reasonably Achievable*" principle is the best standard to decrease the risks from exposure to antineoplastic drugs. It is also important to emphasize that the long-term and combined effects of multiple chemicals might be more severe than the effects of individual chemicals alone. However, the evaluation of cocktail effects and synergistic interactions of pharmaceuticals is a complex and challenging task due to the high number of chemicals and their different modes of action. Future studies focusing on the evaluation of cocktail effects and synergistic interactions are highly recommended and could provide valuable insights into the overall toxicity of the wastewaters in the environment.

Knowing that there is an increased tendency for wastewater reuse, especially for agriculture and aquaculture practices, these results are of most importance aiming to emphasize the need for the implementation of effective removal strategies for toxic compounds from wastewaters. The implementation of photolysis, ozonation, or advanced oxidation processes as post-treatment of the nanofiltration permeate, aiming to

reduce permeate toxicity, could possibly be a good strategy (Garcia--Costa et al., 2022; Prieto-Rodríguez et al., 2013).

Although nanofiltration can diminish the toxicity of the permeate, the retentate, on contrary, is expected to have increased toxicity due to the higher pollutant concentrations. In this work, a low risk was estimated for bicalutamide, megestrol, and tamoxifen, and a moderate risk was found for mycophenolic acid. It is important to remember that bicalutamide did not show any risk in the feed matrix, and megestrol, tamoxifen and mycophenolic acid were previously classified as low risky compounds in the feed matrix.

Capecitabine and cyclophosphamide whose toxicity was respectively moderate and high in the feed, were present in the retentate of the studied samples at concentrations that may be able to induce a high risk to aquatic organisms (Fig. 4). Despite retentate is not supposed to be reused, this matrix urgently needs an effective treatment before its release to the environment. Evaporation followed by carbonization of the precipitate could be an option, as well as a post-treatment by ozonation, and/or advanced oxidation processes (Shi et al., 2021).

4. Conclusions

Bicalutamide, megestrol, mycophenolic acid, and prednisone were the pharmaceuticals found at higher concentrations ($>40 \pm 6$ ng/L) in wastewater effluent samples collected in three different days. On contrary, flutamide, mycophenolate mofetil, paclitaxel, and tamoxifen were detected at very low concentration levels ($<5.2 \pm 0.2$ ng/L).

The average rejection for the eleven pharmaceuticals by the nanofiltration system was $68 \pm 23\%$. Moderate to high rejections were confirmed for megestrol, bicalutamide and paclitaxel, whereas negligible rejection was attained for flutamide that was found to be present at extremely low concentrations in the permeate possibly due to breakthrough after initial adsorption. Capecitabine and prednisone were highly ($89 \pm 7\%$) and moderately ($60 \pm 10\%$) rejected, respectively. Variable rejections were obtained for cyclophosphamide and ifosfamide: high rejections ($>92\%$) were recorded in the first experiment and low ($<43\%$) in the remaining experiments, which may be justified by the fact that their MWs' are within the MWCO range of the membrane. Contrarily to what was expected, mycophenolate mofetil was poorly removed by nanofiltration ($30 \pm 10\%$). The fact that the mycophenolate mofetil molecule geometry is elongated, with a depth around 0.502 nm, may contribute for its enhanced permeation through the free volume of the membrane, leading to a low rejection.

Concerning the charged compounds, experimental findings are in line with predictions, being achieved high rejections: $82 \pm 10\%$ for tamoxifen and $78 \pm 7\%$ for mycophenolic acid.

Phytotoxicity experiments revealed less impact of the permeate on the growth of the roots of *Sinapis alba*'s plants, compared to the feed streams. Regarding the impact on aquatic organisms in the receiving waterbodies, an overall risk reduction was predicted after nanofiltration treatment. However, high risk was still predicted from the exposure to cyclophosphamide, an antineoplastic drug classified as carcinogenic to humans by the International Agency for Research on Cancer. These findings highlight the efficacy of the nanofiltration process in the reduction of the contamination charge and toxicity of WWTP's effluents, but if a complete reduction of the toxicity is envisaged, a post-treatment is still required.

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.; validation of the method, 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., M.B.C., V.J.P., J.G.C., A.A., A.R.R., A.M.T.S. 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.

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Declaration of competing interest

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 related to this article can be found at https://doi.org/10.1016/j.envpol.2023.121944.

References

Al Aani, S., Mustafa, T.N., Hilal, N., 2020. Ultrafiltration membranes for wastewater and water process engineering: A comprehensive statistical review over the past decade. Journal of Water Process Engineering 35, 101241. https://doi.org/10.1016/j.jwpe.2020.101241.

ASCO, 2019. Long-Term Side Effects of Cancer Treatment. https://www.cancer.net/survivorship/long-term-side-effects-cancer-treatment. 22/07/2022.

ASCO, 2022. What is Chemotheraphy? https://www.cancer.net/navigating-cancer-care/how-cancer-treated/chemotherapy/what-chemotherapy. 22/07/2022.

Ashfaq, M.Y., Al-Ghouti, M.A., 2022. In: Tseng, H.-H., Lau, W.J., Al-Ghouti, M.A., An, L. (Eds.), 60 Years of the Loeb-Sourirajan Membrane. Elsevier, pp. 533–573.

Bakopoulou, S., Emmanouil, C., Kungolos, A., 2011. Assessment of wastewater effluent quality in Thessaly region, Greece, for determining its irrigation reuse potential. Ecotoxicology and Environmental Safety 74 (2), 188–194. https://doi.org/10.1016/j.ecoenv.2010.06.022.

Bellona, C., Drewes, J.E., Xu, P., Amy, G., 2004. Factors affecting the rejection of organic solutes during NF/RO treatment—a literature review. Water Research 38 (12), 2795–2809. https://doi.org/10.1016/j.watres.2004.03.034.

Borgatta, M., Waridel, P., Decostered, L.-A., Buclin, T., Chèvre, N., 2016. Multigenerational effects of the anticancer drug tamoxifen and its metabolite 4-hydroxy-tamoxifen on Daphnia pulex. Science of The Total Environment 545-546, 21–29. https://doi.org/10.1016/j.scitotenv.2015.11.155.

Cristóvão, M.B., Bernardo, J., Bento-Silva, A., Ressureição, M., Bronze, M.R., Crespo, J.G., Pereira, V.J., 2022. Treatment of anticancer drugs in a real wastewater effluent using nanofiltration: a pilot scale study. Separation and Purification Technology, 120565. https://doi.org/10.1016/j.seppur.2022.120565.

Cristóvão, M.B., Janssens, R., Yadav, A., Pandey, S., Luis, P., Van der Bruggen, B., Dubey, K.K., Mandal, M.K., Crespo, J.G., Pereira, V.J., 2020. Predicted concentrations of anticancer drugs in the aquatic environment: What should we monitor and where should we treat? Journal of Hazardous Materials 392, 122330. https://doi.org/10.1016/j.jhazmat.2020.122330.

Cristóvão, M.B., Torrejais, J., Janssens, R., Luis, P., Van der Bruggen, B., Dubey, K.K., Mandal, M.K., Bronze, M.R., Crespo, J.G., Pereira, V.J., 2019. Treatment of anticancer drugs in hospital and wastewater effluents using nanofiltration. Separation and Purification Technology 224, 273–280. https://doi.org/10.1016/j.seppur.2019.05.016.

de García, S.O., García-Encina, P.A., Irusta-Mata, R., 2016. Dose–response behavior of the bacterium Vibrio fischeri exposed to pharmaceuticals and personal care products. Ecotoxicology 25 (1), 141–162. https://doi.org/10.1007/s10646-015-1576-8.

Delgado, L.F., Faucet-Marquis, V., Pfohl-Leszkowicz, A., Dorandeu, C., Marion, B., Schetrite, S., Albasi, C., 2011. Cytotoxicity micropollutant removal in a crossflow membrane bioreactor. Bioresource Technology 102 (6), 4395–4401. https://doi.org/10.1016/j.biortech.2010.12.107.

ECIS, 2022. Estimates of cancer incidence and mortality in 2020, for all cancer sites. https://ecis.jrc.ec.europa.eu/explorer.php?$0-0$1-AEE$2-All$4-1,2$3-All$6-0,85$5-2020,2020$7-7$CEstByCancer$X0_8-3$CEstRelativeCanc$X1_8-3$X1_9-AE27$CEstBySexByCancer$X2_8-3$X2_-1-1. 22/07/2022.

EMEA, 2006. Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-environmental-risk-assessment-medicinal-products-human-use-revision-1_en.pdf. 07/11/2022.

EPA U.S, 2005. - Membrane Filtration Guidance Manual, 815-R-06-009.

Fent, K., Weston, A.A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology 76 (2), 122–159. https://doi.org/10.1016/j.aquatox.2005.09.009.

Foureaux, A.F.S., Reis, E.O., Lebron, Y., Moreira, V., Santos, L.V., Amaral, M.S., Lange, L.C., 2019. Rejection of pharmaceutical compounds from surface water by nanofiltration and reverse osmosis. Separation and Purification Technology 212, 171–179. https://doi.org/10.1016/j.seppur.2018.11.018.

Franquet-Griell, H., Cornado, D., Caixach, J., Ventura, F., Lacorte, S., 2017. Determination of cytostatic drugs in Besos River (NE Spain) and comparison with predicted environmental concentrations. Environ. Sci. Pollut. Res. 24 (7), 1614–7499. https://doi.org/10.1007/s11356-016-8337-y (Electronic).

Garcia-Costa, A.L., Gouveia, T.I.A., Pereira, M.F.R., Silva, A.M.T., Madeira, L.M., Alves, A., Santos, M.S.F., 2022. Intensification strategies for cytostatics degradation by ozone-based processes in aqueous phase. Journal of Hazardous Materials 440, 129743. https://doi.org/10.1016/j.jhazmat.2022.129743.

Gómez-Canela, C., Cortés-Francisco, N., Oliva, X., Pujol, C., Ventura, F., Lacorte, S., Caixach, J., 2012. Occurrence of cyclophosphamide and epirubicin in wastewaters by direct injection analysis–liquid chromatography–high-resolution mass spectrometry. Environ. Sci. Pollut. Res. 19 (8), 3210–3218. https://doi.org/10.1007/s11356-012-0826-z.

Gómez-Canela, C., Ventura, F., Caixach, J., Lacorte, S., 2014. Occurrence of cytostatic compounds in hospital effluents and wastewaters, determined by liquid chromatography coupled to high-resolution mass spectrometry. Analytical and Bioanalytical Chemistry 406 (16), 3801–3814. https://doi.org/10.1007/s00216-014-7805-9.

Gouveia, T.I.A., Alves, A., Santos, M.S.F., 2019. New insights on cytostatic drug risk assessment in aquatic environments based on measured concentrations in surface waters. Environment International 133, 105236. https://doi.org/10.1016/j.envint.2019.105236.

Gouveia, T.I.A., Mota, I.H., Silva, A.M.T., Alves, A., Santos, M.S.F., 2022. Are cytostatic drugs in surface waters a potential threat? Science of The Total Environment 853, 158559. https://doi.org/10.1016/j.scitotenv.2022.158559.

Gouveia, T.I.A., Silva, A.M.T., Freire, M.G., Sousa, A.C.A., Alves, A., Santos, M.S.F., 2023. Multi-target analysis of cytostatics in hospital effluents over a 9-month period. Journal of Hazardous Materials 448, 130883. https://doi.org/10.1016/j.jhazmat.2023.130883.

Gouveia, T.I.A., Silva, A.M.T., Ribeiro, A.R., Alves, A., Santos, M.S.F., 2020. Liquid-liquid extraction as a simple tool to quickly quantify fourteen cytostatics in urban wastewaters and access their impact in aquatic biota. Science of The Total Environment 740, 9. https://doi.org/10.1016/j.scitotenv.2020.139995.

HAS, 2005. In-hospital and at-home cancer chemotherapy: a comparison of costs and organisation of care. https://www.has-sante.fr/upload/docs/application/pdf/chimio_eco_gb.pdf. 08/07/2022.

IARC, 2022. List of classifications. https://monographs.iarc.who.int/list-of-classifications. 20/01/2023.

Ioannou-Ttofa, L., Fatta-Kassinos, D., 2020. Fate and Effects of Anticancer Drugs in the Environment. Springer International Publishing, pp. 103–138.

Kazner, C., Lehnberg, K., Kovalova, L., Wintgens, T., Melin, T., Hollender, J., Dott, W., 2008. Removal of endocrine disruptors and cytostatics from effluent by nanofiltration in combination with adsorption on powdered activated carbon. Water Science & Technology 58 (8), 1699–1706. https://doi.org/10.2166/wst.2008.542.

Kischkel, F., 2016. Cytostatic Drugs as a Weapon against Cancer. https://www.therapyselect.de/en/blog/cytostatic-drugs-weapon-against-cancer. 04/10/2022.

Kiso, Y., 1986. Factors affecting adsorption of organic solutes on cellulose acetate in an aqueous solution system. Chromatographia 22 (1), 55–58. https://doi.org/10.1007/BF02257298.

Köhler, C., Venditti, S., Igos, E., Klepiszewski, K., Benetto, E., Cornelissen, A., 2012. Elimination of pharmaceutical residues in biologically pre-treated hospital wastewater using advanced UV irradiation technology: A comparative assessment.

Journal of Hazardous Materials 239-240, 70–77. https://doi.org/10.1016/j.jhazmat.2012.06.006.

Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., McArdell, C., 2012. Hospital Wastewater Treatment by Membrane Bioreactor: Performance and Efficiency for Organic Micropollutant Elimination. Environ. Sci. Technol. 46 (3), 1536–1545. https://doi.org/10.1021/es203495d.

Llewellyn, N., Lloyd, P., Jürgens, M.D., Johnson, A.C., 2011. Determination of cyclophosphamide and ifosfamide in sewage effluent by stable isotope-dilution liquid chromatography–tandem mass spectrometry. Journal of Chromatography A 1218 (47), 8519–8528. https://doi.org/10.1016/j.chroma.2011.09.061.

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Science of The Total Environment 473-474, 619–641. https://doi.org/10.1016/j.scitotenv.2013.12.065.

Moermond, C., Elk, M.v., Oostlander, A., Vlaardingen, P.v., Marinkovié, M., Dijk, J.v., 2018. Cytostatics in Dutch surface water: use, presence and risks to the aquatic environment. Ministry of Infrastructure and Water management.

Prieto-Rodríguez, L., Oller, I., Klamerth, N., Agüera, A., Rodríguez, E.M., Malato, S., 2013. Application of solar AOPs and ozonation for elimination of micropollutants in municipal wastewater treatment plant effluents. Water Research 47 (4), 1521–1528. https://doi.org/10.1016/j.watres.2012.11.002.

Sánchez-Bayo, F., Baskaran, S., Kennedy, I.R., 2002. Ecological relative risk (EcoRR): another approach for risk assessment of pesticides in agriculture. Agriculture, Ecosystems & Environment 91 (1), 37–57. https://doi.org/10.1016/S0167-8809(01)00258-4.

Seira, J., Sablayrolles, C., Montréjaud-Vignoles, M., Albasi, C., Joannis-Cassan, C., 2016. Elimination of an anticancer drug (cyclophosphamide) by a membrane bioreactor: Comprehensive study of mechanisms. Biochemical Engineering Journal 114, 155–163. https://doi.org/10.1016/j.bej.2016.07.001.

Shi, C., Xie, C., Zhang, Z., Rackemann, D., Wei, B., Hang, F., Lu, H., Li, K., Doherty, W.O.S., 2021. Sugar and value-added products derived from retentate concentrate of sugarcane juice. Journal of Cleaner Production 278, 123915. https://doi.org/10.1016/j.jclepro.2020.123915.

Ternes, T.A., 1998. Occurrence of drugs in German sewage treatment plants and rivers1Dedicated to Professor Dr. Klaus Haberer on the occasion of his 70th birthday.1. Water Research 32 (11), 3245–3260. https://doi.org/10.1016/S0043-1354(98)00099-2.

Verliefde, A.R.D., Cornelissen, E.R., Heijman, S.G.J., Petrinic, I., Luxbacher, T., Amy, G.L., Van der Bruggen, B., van Dijk, J.C., 2009. Influence of membrane fouling by (pretreated) surface water on rejection of pharmaceutically active compounds (PhACs) by nanofiltration membranes. Journal of Membrane Science 330 (1), 90–103. https://doi.org/10.1016/j.memsci.2008.12.039.

Verliefde, A.R.D., Heijman, S.G.J., Cornelissen, E.R., Amy, G., Van der Bruggen, B., van Dijk, J.C., 2007. Influence of electrostatic interactions on the rejection with NF and assessment of the removal efficiency during NF/GAC treatment of pharmaceutically active compounds in surface water. Water Research 41 (15), 3227–3240. https://doi.org/10.1016/j.watres.2007.05.022.

Wang, L., Albasi, C., Faucet-Marquis, V., Pfohl-Leszkowicz, A., Dorandeu, C., Marion, B., Causserand, C., 2009. Cyclophosphamide removal from water by nanofiltration and reverse osmosis membrane. Water Research 43 (17), 4115–4122. https://doi.org/10.1016/j.watres.2009.06.007.

Wang, X., Zhang, J., Chang, V.W.C., She, Q., Tang, C.Y., 2018. Removal of cytostatic drugs from wastewater by an anaerobic osmotic membrane bioreactor. Chemical Engineering Journal 339, 153–161. https://doi.org/10.1016/j.cej.2018.01.125.

Who, 1989. Health guidelines for the use of wastewater in agriculture and aquaculture : report of a WHO scientific group [meeting held in Geneva from 18 to 23 November 1987]. https://apps.who.int/iris/handle/10665/39401. (Accessed 1 September 2022).

Yotova, G., Lazarova, S., Kudłak, B., Zlateva, B., Mihaylova, V., Wieczerzak, M., Venelinov, T., Tsakovski, S., 2019. Assessment of the Bulgarian Wastewater Treatment Plants' Impact on the Receiving Water Bodies.

Yotova, G., Lazarova, S., Mihaylova, V., Venelinov, T., 2021. Water Quality Assessment of Surface Waters and Wastewaters by Traditional and Ecotoxicological Indicators in Ogosta River, Bulgaria. International Journal Bioautomotion 25, 25–40. https://doi.org/10.7546/ijba.2021.25.1.000778.

Zaviska, F., Drogui, P., Grasmick, A., Azais, A., Heran, M., 2013. Nanofiltration Membrane Bioreactor for Removing Pharmaceutical Compounds. Journal of Membrane Science 429, 121–129. https://doi.org/10.1016/j.memsci.2012.11.022.

Zhang, H., He, Q., Luo, J., Wan, Y., Darling, S.B., 2020. Sharpening Nanofiltration: Strategies for Enhanced Membrane Selectivity. ACS Applied Materials & Interfaces 12 (36), 39948–39966. https://doi.org/10.1021/acsami.0c11136.