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Research article

Tackling water contamination by oncologic drugs: Supported ionic liquids as sustainable adsorbents for cyclophosphamide removal

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ABSTRACT

Due to the increasing incidence of cancer, the consumption of highly toxic oncological drugs is continuously growing. Given the current lack of efficient technologies to remove/treat these toxic drugs in wastewater treatment plants, the environmental quality is compromised, and aquatic organisms are at risk. To address this critical environmental burden, a new strategy based on supported ionic liquids (SILs) for the simultaneous removal of oncologic drugs and toxicity reduction of aqueous samples is here proposed. Silica-based SILs functionalized with imidazolium-based and quaternary ammonium-based ILs were designed and kinetics and isotherm adsorption studies performed. Aiming to develop an adsorbent able to reduce the toxicity of aqueous samples contaminated with oncological drugs, the toxicity reduction was appraised using the model organism *Danio rerio*. The obtained results disclose that among the studied SILs, the [Si][N_{3888}]Cl (silica functionalized with propyltrioctylammonium chloride) is the best adsorption material (maximum adsorption capacity, q_{max} = 67.64 mg g^{-1}), with a fast adsorption rate (<20 min). Furthermore, [Si][N_{3888}]Cl was able to remove the toxicity of the treated aqueous samples towards *D. rerio* embryos, as assessed by lethal and several sublethal endpoints, demonstrating that this material holds remarkable potential for oncological drugs pollution remediation.

1. Introduction

Based on the most recent data available, there were 19.3 million cancer cases globally – a number projected to suffer a 47% increment by 2040 (Sung et al., 2021). To improve the life expectancy of oncologic patients, chemotherapy, i.e., the use of anti-cancer drugs, including cytostatics, is among the standard cancer therapies. Because of the increasing incidence of cancer, the consumption of cytostatics is also expected to follow the same trend in the coming years. Although intended to attack cancer cells, most of the commercialized cytostatics are not cancer cell-specific and are predisposed to cause mutagenic, carcinogenic, and teratogenic effects toward non-target healthy cells (Grosse et al., 2009; Kar et al., 2020). Due to these deleterious effects, the disposal of cytostatics as well as contaminated materials and samples (e.g., faeces, blood, urine) is well regulated in healthcare facilities (World Health Organization, 2014). However, cancer drugs are in large part (ca. 80%) administered to outpatients rather than to hospitalized patients, being most likely to be excreted outside healthcare facilities (Besse et al., 2012).

According to these patterns of consumption, it is presumed that cytostatics (as well as their metabolites) will reach wastewater treatment plants (WWTPs), where biodegradation and adsorption to biomass act as the main removal technologies (Mukherjee et al., 2021; Zhang et al., 2013). However, these technologies are not specifically designed, struggling with the removal/treatment of cytostatics (Valente et al., 2022). As a result of this technological flaw, the contamination of surface waters takes place, producing adverse outcomes in aquatic organisms (i.e., from unicellular organisms to vertebrates) (Castellano-Hinojosa et al., 2023; Gouveia et al., 2022; Jureczko and Kalka, 2020). Data proving the occurrence of cytostatics in surface waters, e.g., rivers, are currently available, in some cases surpassing expected concentration values (i.e., >1 ng L^{-1}) (Jureczko and Kalka,

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2020). Ultimately, these contamination events negatively affect the quality of potable water, as supported by reports on the detection of cytostatics in drinking water treatment plants (Franquet-Griell et al., 2016).

Several advanced strategies can be used to upgrade cytostatics removal in WWTPs. Advanced oxidation processes, biological treatments and chemical treatments resorting to oxidizing agents are some examples (Valente et al., 2022; Zhang et al., 2013). However, these processes lead to cytostatics degradation, normally producing toxic subproducts/metabolites and are energy- and cost-intensive. Therefore, the questionable cost-effectiveness and/or eco-friendliness prevent the widespread implementation of current advanced techniques in domestic WWTPs (Valente et al., 2022; Zhang et al., 2013). Physicochemical technologies allowing the removal of cytostatic from aqueous solutions without toxic substances generation are more convenient options (Kümmerer, 2007; Quiton et al., 2021). Among these, adsorption processes based on activated carbon stand out due to low-cost, enhanced operability, and eco-friendliness (Guillossou et al., 2019). Although deemed effective for the removal of a multitude of pharmaceuticals, activated carbon is not specifically designed to remove cytostatics and may be of limited selectivity when treating complex aqueous samples.

By taking full advantage of the designer solvent character of ionic liquids (ILs) (Freemantel, 1998) and supported ionic liquids (SILs), as well as of the low-cost and biocompatible nature of silica solid supports (Albrecht et al., 2006; Asefa and Tao, 2012), SILs, i.e. IL moieties covalently attached to the silica surface, can be considered as an alternative class of adsorbents for the removal of pharmaceuticals from aqueous samples (Almeida et al., 2020; Bernardo et al., 2020). Despite remarkable removal efficiencies of 63.5% for acetylsalicylic acid (Bernardo et al., 2020) and 100% for sodium diclofenac (Almeida et al., 2020), to the best of our knowledge, SILs have not been designed and applied for the removal of highly toxic cytostatic or oncologic drugs, such as cyclophosphamide (Santos et al., 2017), from aqueous samples.

Herein, we show the enhanced adsorption capacity of designed SILs to address the ever-growing challenge of cytostatics in the environment. In this work, cyclophosphamide (CYP – cf. Fig. 1 with chemical structure) was used. It is an alkylating agent widely used in chemotherapy, that is ranked third in the priority anticancer drugs in Portugal and is considered to pose a moderate risk to aquatic biota (Santos et al., 2017). Cyclophosphamide levels in hospital wastewater samples as high as 687 μg L^{-1} have been reported (Hamon et al., 2018). This highly toxic cytostatic has been widely detected in river waters in the μg·L^{-1} to ng·L^{-1} range (Usawanuwat et al., 2014; Valente et al., 2022), being also detected in drinking water from China at concentrations up to 4 ng L^{-1} (Gu et al., 2019). To accomplish our goal, silica-based SILs functionalized with imidazolium-based and quaternary ammonium-based ILs were prepared and evaluated for their removal efficiency through adsorption kinetics and isotherms, as well as toxicity reduction using the model test organism *Danio rerio*.

2. Experimental

2.1. Materials

This work is divided in three major sections: (i) preparation of the supported ionic liquids (SILs); (ii) adsorption studies; and (iii) toxicity studies resorting to assays with *Danio rerio* embryos. To perform such tasks, the materials used are listed in Table S1 in the Supplementary Material, which includes their purity, supplier, and CAS number. The chemical structure of the cytostatic drug studied, i.e., cyclophosphamide (CYP) is given in Fig. 1. For high performance liquid chromatography coupled to diode-array detector (HPLC-DAD) analyses, ultra-pure water, which was double distilled and subsequently treated with a Milli-Q plus 185 water purification apparatus, was used.

2.2. Safety precautions

Given the toxicity of cyclophosphamide, all laboratory procedures

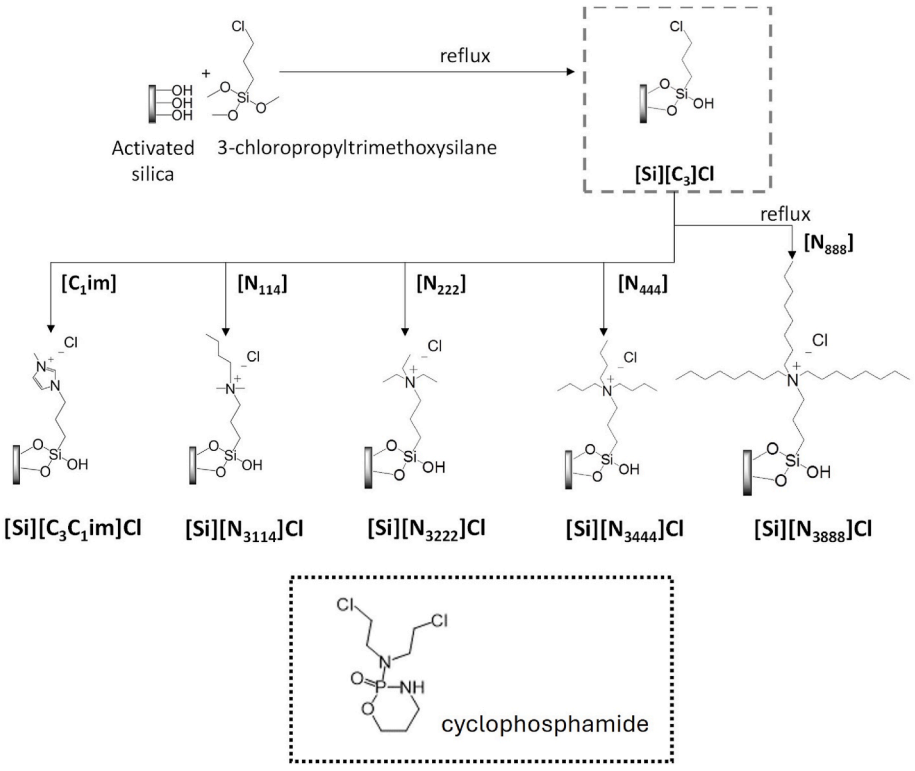

Fig. 1. Synthetic pathway of the supported ionic liquids investigated, along with their chemical structures and abbreviations, and chemical structure of the cytostatic drug cyclophosphamide.

were carried out with as tight security measures as possible, according to the current recommendations (Eitel, A., Scherrer, M., Kümmerer, 1999; Queruau Lamerie et al., 2012; World Health Organization/Pan American Health Organization, 2013). The cytostatic aqueous solutions were prepared inside a Laminar Flow Cabinet (type II) and all experiments were performed on a cytostatic preparation mat (absorbent material made of 3 sheets of polyethylene). Throughout the experiments dischargeable material was used and treated as hazardous waste (Type IV).

2.3. Preparation of the supported ionic liquids

The five SILs studied were: silica functionalized with propylmethylimidazolium chloride ([Si][C_3C_1im]Cl), silica functionalized with dimethylpropyltributylammonium chloride ([Si][N_{3114}]Cl), silica functionalized with propyltriethylammonium chloride ([Si][N_{3222}]Cl), silica functionalized with propyltributylammonium chloride ([Si][N_{3444}]Cl), and silica functionalized with propyltrioctylammonium chloride ([Si][N_{3888}]Cl). The synthesis of the SILs was based on the use of distinct cation sources, namely *N*-methylimidazole ([C_1im]), *N,N*-dimethylbutylamine ([N_{114}]), triethylamine ([N_{222}]), tributylamine ([N_{444}]) and trioctylamine ([N_{888}]). SILs were prepared through silica functionalization following the method initially described by Qiu et al. (Qiu et al., 2006) for *N*-methylimidazole, and more recently adapted and validated by us for the remaining tertiary amines (Bernardo et al., 2020; Francisco et al., 2022). In brief, 50 mL of toluene were added to 5 g of silica (preactivated in hydrochloric acid for 24 h) and 5 mL of 3-chloropropyltrimethoxysilane, yielding a suspension that was refluxed at 105 °C under magnetic agitation over 24 h. The suspension was filtered immediately after room temperature was reached, using a vacuum glass filter and washed with the following solvents (by order): toluene (100 mL), ethanol:water at 1:1, v/v (200 mL), distilled water (500 mL) and methanol (100 mL). Once dried at 50 °C for 24 h, chloropropyl silica ([Si][C_3]Cl) – the SIL precursor – was produced. To the [Si][C_3]Cl, 5 mL of [C_1im], [N_{114}], [N_{222}], [N_{444}] or [N_{888}] were added in toluene (50 mL). To achieve the final SILs, the resultant suspension was refluxed under constant magnetic stirring (105 °C, 24 h), filtered and washed with the following solvents: toluene (100 mL), methanol (350 mL), distilled water (300 mL) and methanol (150 mL). Then, the materials produced were dried at 50 °C for 24 h. SILs' synthetic pathway, chemical structures and abbreviations are summarized in Fig. 1.

The structure and characterization of the SILs studied are elsewhere reported (Bernardo et al., 2020; Francisco et al., 2022), namely by elemental analysis, specific surface area (S_{BET}), solid-state ^{13}C nuclear magnetic resonance (NMR) and FTIR spectroscopy, Point of Zero Charge (PZC), and/or Scanning Electron Microscopy (SEM). Analysis carried out in this work agree with previous results (Bernardo et al., 2020; Francisco et al., 2022).

2.4. Adsorption studies

2.4.1. Initial screening

Before proceeding with kinetics and isotherm studies, an initial screening aimed to identify the best SILs for cyclophosphamide removal was performed. All SILs prepared (viz., [Si][C_3C_1im]Cl, [Si][N_{3222}]Cl, [Si][N_{3114}]Cl, [Si][N_{3444}]Cl, [Si][N_{3888}]Cl) were benchmarked against the intermediary product of the reaction ([Si][C_3]Cl) and the activated silica. 10 mL of a solution containing cyclophosphamide at a concentration of 118 mg L^{-1} were added to 50 mg of each SIL and maintained under agitation in an orbital shaker during 60 min (25 °C, 150 rpm). Following this contact period, the samples were centrifuged for 10 min at 12,000 rpm and the supernatant was analysed by HPLC-DAD for cyclophosphamide quantification.

The adsorption efficiency of cyclophosphamide onto SILs (% *AE*) was calculated by Equation (1), while the determination of its equilibrium concentration in the solid phase (q_e, mg·g^{-1}) was carried out by Equation (2).

$$\%\,AE = \frac{C_0 - C_e}{C_0} \times 100 \tag{1}$$

$$q_e = \frac{(C_0 - C_e) \times V}{w} \tag{2}$$

where C_0 and C_e correspond to the equilibrium concentrations of cyclophosphamide, respectively before and after adsorption onto the materials (mg·L^{-1}), w represents the weight of the material (g), and V represents the volume of the cyclophosphamide aqueous solution (L).

Further experiments were conducted resorting to [Si][N_{3222}]Cl, [Si][N_{3444}]Cl, [Si][N_{3888}]Cl – the SILs displaying the best adsorption efficiencies.

2.4.2. Adsorption kinetics

Adsorption kinetics studies were performed with either of the previously identified SILs. 10 mL of an aqueous solution of cyclophosphamide at a concentration of 90 mg L^{-1} were placed in contact with a fixed amount of SIL of 50 mg. Under constant agitation in an orbital shaker (25 °C, 150 rpm), samples were taken from different flasks at different times, from 1 to 180 min and centrifuged for 10 min at 12,000 rpm for supernatant collection and subsequent quantification by HPLC-DAD (see section 1.4).

The modelling of the adsorption mechanism was done by fitting the experimental results to the Lagergren pseudo-first-order model (Lagergren, 1898) (Equation (3)) and the Pseudo-second-order model by Ho and Mckay (Ho and McKay, 1999) (Equation (4)).

$$\frac{dq_t}{dt} = k_1 \times (q_e - q_t) \tag{3}$$

$$\frac{dq_t}{dt} = k_2 \times (q_e - q_t)^2 \tag{4}$$

where t is the time (min), q_e is the equilibrium concentration of cyclophosphamide in the solid phase (mg·g^{-1}), and q_t is the concentration of cyclophosphamide in the solid phase at the time t (mg·g^{-1}). k_1 (min^{-1}) and k_2 (g·mg^{-1}·min^{-1}) are the sorption rate constants of first and second orders, respectively.

2.4.3. Adsorption isotherms

For the adsorption isotherms, the mass of the SIL and time were kept constant at 50 mg and 120 min, respectively, while the concentration of cyclophosphamide was varied from 50 to 1500 mg L^{-1}. Following a protocol similar to the adsorption kinetics studies, 10 mL of each test solution were placed in contact with each SIL in separate flasks and accordingly processed for cyclophosphamide quantification by HPLC-DAD.

The experimentally obtained curves for the adsorption isotherms were fitted by the Langmuir (1918), the Freundlich (1907), and the Sips (1948) adsorption models, which are described by Equations (5)–(7), respectively:

$$q_e = \frac{q_{max} \times B \times C_e}{1 + B \times C_e} \tag{5}$$

$$q_e = K_f \times C_e^{\,1/n} \tag{6}$$

$$q_e = \frac{q_{max} \times K_S \times C_e^{\,1/n}}{1 + K_S \times C_e^{\,1/n}} \tag{7}$$

where q_{max} (mg·g^{-1}) represents the maximum adsorption capacity, B (L·mg^{-1}) is the Langmuir isotherm constant, K_f is the Freundlich equilibrium constant (mg·g^{-1}), n (dimensionless) is the Freundlich/Sips exponent, and K_S is the Sips equilibrium constant (L·mg^{-1}).

GraphpadPrism7 software (San Diego, CA, USA) was used to carry out all fittings of the experimental data corresponding to the adsorption kinetics and isotherms.

2.5. Quantification of cyclophosphamide by HPLC-DAD

For the quantification of cyclophosphamide in aqueous solutions, HPLC-DAD was used. The liquid chromatograph used was a Shimadzu, Prominence Modular HPLC, equipped with a Reprosil C_{18} analytical column (250 × 4.6 mm) bearing porous spherical silica with a pore size of 25 μm and pore diameter of 100 Å from GmbH. The temperature of the column was set at 25 °C. The mobile phase consisted of a mixture of acetonitrile and water in a ratio of 20:80 (v/v) under isocratic elution mode (flow rate = 1.0 mL min^{-1}), and the injection volume was 20 μL. The quantification was carried out at 197 nm using calibration curves previously determined and each sample was analysed at least twice.

2.6. Toxicity studies

The performance of the best SIL, [Si][N_{3888}]Cl, in the removal or reduction of the toxicity of a cyclophosphamide solution was evaluated through an ecotoxicity assay with *D. rerio* embryos. The concentration of cyclophosphamide used in the toxicity assay (1306 mg L^{-1}) was chosen based on preliminary assays that indicated beforehand significant effects on the survival and development of zebrafish embryos (Monteiro, 2021).

2.6.1. Zebrafish maintenance

Danio rerio eggs were obtained from an in-house laboratory culture, in which adult fish, with no externally visible diseases, were kept in a ZebTEC recirculating system (Tecniplast) under controlled conditions (temperature: 27 ± 1 °C; conductivity: 794 ± 50 μS cm^{-1}, pH: 7.5 ± 0.5, dissolved oxygen: equal or above 95% saturation, photoperiod cycle: 14h:10 h light/dark). The water used in the system was tap water filtered with activated charcoal and reverse osmosis, supplemented with "Instant Ocean Synthetic Sea Salt" (Spectrum Brands, USA). Fish were fed once a day with commercially available artificial diet (Gemma Micro 500, Skretting®, Spain).

The embryos for the toxicity assays were obtained by housing adult zebrafish males and females in breeding aquaria. In the next morning, the eggs, shielded from predation from adult individuals (Spence et al., 2007), were collected, gently rinsed in water from the culture system, and screened using a stereomicroscope (Stereoscopic Zoom Microscope-SMZ 1500, Nikon) (Organisation for Economic Co-operation and Development (OECD) 2013), to discard any coagulated, unfertilized, or injured eggs with obvious irregularities during cleavage.

2.6.2. 96-h fish embryo toxicity assay with D. rerio

Assays with embryos of *D. rerio* were performed according to the Organisation for Economic Co-operation and Development (OECD) guideline 236 on Fish Embryo Acute Toxicity (FET) Test (Organisation for Economic Co-operation and Development (OECD) 2013), with small adaptations. Five different treatments were considered for this assay: (i) a control group, that consisted solely of the water used in the maintenance of adult fishes; (ii) a treatment to assess the toxicity of the silica, consisting of maintenance water after being in contact with the silica for 1.5 h; (iii) a treatment to evaluate the toxicity of the material itself, consisting of maintenance water after being in contact with [Si][N_{3888}]Cl for 1.5 h; (iv) a treatment with an aqueous solution of cyclophosphamide at 1306 mg L^{-1} (known beforehand to cause effects on *D. rerio* embryos); and (v) a treatment with the same cyclophosphamide solution after being in contact [Si][N_{3888}]Cl for 1.5 h (in which the adsorption of cyclophosphamide was expected).

Apart from the control group which had 60 eggs, 30 eggs per treatment were transferred to 24-well plates, in which a single egg was placed in each well with 1 mL of the respective test solution. Zebrafish embryos were exposed for 96 h, at a temperature of 26 ± 1 °C and a 16:8 h light/dark photoperiod, and observations were made at each 24 h (i.e., 24, 48, 72, and 96 h) with the help of a stereomicroscope (Zoom-SMZ 1500, Nikon Corporation).

Besides the mortality endpoint (that included coagulated eggs, arrested development, or lack of heartbeat), the sublethal endpoints concerning the hatching rate, final body length, and malformations (such as tail and skeletal malformations, oedemas, and delayed development) were also evaluated (Lammer et al., 2009). The evaluation of sub-lethal endpoints is particularly relevant since cyclophosphamide has teratogenic properties. Mortality and hatching rates were expressed considering the total number of embryos exposed to the different treatments, while the endpoints concerning the percentage of abnormalities and final body length were evaluated concerning the number of alive larvae.

The results from the toxicity assays with *D. rerio* embryos were analysed using the software SigmaPlot 14.0 (Systat Software, Inc., SigmaPlot for Windows). Firstly, the results were checked for normality and homoscedasticity, but since the data sets failed in at least one of the assumptions, a non-parametric ANOVA was carried (Kruskal-Wallis), followed by the multicomparison Dunn's test. The significance level was set at an alpha value of 0.05.

3. Results and discussion

3.1. Material synthesis and adsorption screening

Using chloride as the common anion, five ammonium-based SILs were studied, which contained the imidazolium cation or quaternary ammonium cations bearing alkyl chains of variable length. The SILs studied comprised silica functionalized with the following ILs: propylmethylimidazolium chloride ([Si][C_3C_1im]Cl), dimethylpropylbutylammonium chloride ([Si][N_{3114}]Cl), propyltriethylammonium chloride ([Si][N_{3222}]Cl), propyltributylammonium chloride ([Si][N_{3444}]Cl) and propyltrioctylammonium chloride ([Si][N_{3888}]Cl). Given the chemical structure of the studied SILs, only with the cation covalently attached, from an environmental and health point of views it is safer to use SILs with chloride instead of SILs comprising organic and more toxic anions. The use of chloride avoids also the need of an extra step of anion exchange, decreasing the cost and environmental impact in producing SILs. Overall, these SILs are known for being cheap, easy to prepare, poorly to no cytotoxic, and not prone to leaching in aqueous medium (Bernardo et al., 2020). Furthermore, by considering the cyclophosphamide extreme pKa's (2.31 and 11.11) (Mioduszewska et al., 2017), at working and even real conditions of application, electrostatic interactions are not prone to occur. Accordingly, we here address SILs that allow different types of SIL-cyclophosphamide interactions by changing the cation alkyl chain size and the cation family. SILs were characterized by different techniques to guarantee its successful synthesis, being in agreement with previous results (Bernardo et al., 2020).

A preliminary evaluation of adsorption efficiencies for cyclophosphamide was carried out to identify the most promising SILs. Silica and the intermediary product of the synthesis reaction – chloropropyl silica, [Si][C_3]Cl – were used as benchmarks. 50 mg of each SIL were placed in contact with 10 mL of a cyclophosphamide solution (118.0 mg L^{-1}) for 60 min. The obtained adsorption efficiency (*% AE*) and equilibrium concentration in the solid phase (q_e, mg·g^{-1}) are given in Table 1. Under the conditions tested, the adsorption efficiency is ranked as follows: [Si][N_{3888}]Cl (46.8%) > [Si][N_{3444}]Cl (25.7%) > [Si][C_3]Cl (19.0%) > [Si][N_{3222}]Cl (14.2%) > [Si][N_{3114}]Cl (10.6%) > [Si][C_3C_1im]Cl (0%) = silica (0%).

Previously, imidazolium-based SILs have been identified as highly efficient IL-based functionalized materials for the removal of contaminants from water samples, overcoming the performance of tetraalkylammonium-based SILs (Almeida et al. 2020; Almeida et al., 2024). In the current study, and according with Table 1, a different trend was verified, with [Si][C_3C_1im]Cl completely failing at adsorbing

Table 1
Adsorption efficiency of cyclophosphamide onto SILs (% AE) and equilibrium concentration in the solid phase (q_e, mg·g^{-1}) obtained during the initial screening.

SIL	% AE (%)	q_e (mg·g^{-1})
Silica	0	0
[Si][C_3]Cl	19.0	4.51
[Si][C_3C_1im]Cl	0	0
[Si][N_{3114}]Cl	10.6	2.50
[Si][N_{3222}]Cl	14.2	3.36
[Si][N_{3444}]Cl	25.7	6.08
[Si][N_{3888}]Cl	46.8	11.07

cyclophosphamide, while all remaining (quaternary ammonium-based) SILs outperform silica. Since the correct silica functionalization is necessary for cyclophosphamide adsorption to occur, these results highlight the tailoring ability of SILs in the development of adsorption processes. Furthermore, although [Si][C_3]Cl is ranked third, the most efficient SIL, [Si][N_{3888}]Cl, is 2.5 times more efficient in the adsorption process, reinforcing the need for silica modification. The adsorption efficiency of the SILs correlates well with the size of the alkyl chains of the quaternary ammonium cation and with the polarity/hydrophilicity of the cation core. Cyclophosphamide has a logarithmic octanol-water partition coefficient (log$K_{o/w}$) of 0.63, which is indicative of cyclophosphamide's lipophilic nature (ChemSpider - Search and Share Chemistry.). Consequently, SILs with longer alkyl side chains, mainly [Si][N_{3888}]Cl, but also [Si][N_{3444}]Cl and [Si][N_{3222}]Cl, are the most appropriate by allowing dispersive interactions to occur with cyclophosphamide. Adsorption of cyclophosphamide onto these more hydrophobic SILs seems thus to be ruled by dispersive interactions, which is in accordance with the extreme pKa's (2.31 and 11.11) (Mioduszewska et al., 2017) of the drug and its presence as a neutral molecule at the working conditions, in which electrostatic interactions are not prone to occur. The extreme pKa's of the target drug allow to have the target drug in its neutral state in a large pH range, with the provided results ultimately not depending strongly on the pH of the samples. On the other hand, having an adsorption phenomenon mainly ruled by dispersive interactions further brings one additional advantage: results will not strongly vary with temperature as well, contributing to the design of a reproducible process. Dispersive interactions (London dispersion forces or van der Waals forces), arising from temporary fluctuations in electron density, depend weakly on temperature. Furthermore, since the imidazolium-based SIL was not able to adsorb cyclophosphamide, hydrogen-bonding interactions between the drug and the SIL are not predominant. In summary, based on the disucssed results, the SILs with higher performance to adsorb cyclophosphamide, i.e. [Si][N_{3888}]Cl, [Si][N_{3444}]Cl and [Si][N_{3222}]Cl, were selected to proceed with the following adsorption kinetic and isotherm assays.

3.2. Adsorption kinetics and isotherms

Adsorption kinetic studies were performed to gather information on the time needed to reach the equilibrium. 10 mL of aqueous solutions of cyclophosphamide at 90 mg L^{-1} were used, while changing the contact time with [Si][N_{3888}]Cl, [Si][N_{3444}]Cl, and [Si][N_{3222}]Cl (50 mg) from 1 to 180 min. Experimental data were fitted using two models, namely the Lagergren pseudo-first-order model (Lagergren, 1898) and the pseudo-second-order model by Ho and Mckay (Ho and McKay, 1999).

Fig. 2 provides the adsorption kinetic curves and respective fittings, showing that the adsorption of cyclophosphamide onto the three SILs is a fast process. Cyclophosphamide adsorption took 10–20 min to reach equilibrium, which was further maintained for at least 180 min. Since no desorption was observed as a function of time, adsorption isotherms determination was carried out at 120 min to guarantee that equilibrium was reached.

Table 2 shows the kinetic models' parameters. Under the conditions adopted, the model better describing the cyclophosphamide adsorption kinetics, i.e. the one displaying higher correlation coefficients (R^2), is the pseudo-second-order model. Therefore, the adsorption of cyclophosphamide onto all SILs is governed by chemisorption at the solid-liquid interface in the SIL rather than a diffusion-controlled process (typical for systems better described by the pseudo-first-order model) (Simonin, 2016). This behaviour is in agreement with the previously discussed interactions occurring between SILs and cyclophosphamide, and the different results obtained using different IL ligands. Moreover, the experimental q_e values obtained (i.e., 14.63 mg g^{-1} for [Si][N_{3222}]Cl, 14.69 mg g^{-1} for [Si][N_{3444}]Cl and 14.70 mg g^{-1} for [Si][N_{3888}]Cl) closely approach the values estimated by the models, further reinforcing the accurate fittings. From the three SILs, [Si][N_{3888}]Cl provides the fastest adsorption process, followed by [Si][N_{3444}]Cl, and finally by [Si][N_{3222}]Cl, according to the sorption rate constants of first and second orders k_1 and k_2 (cf. Table 2), obeying to the ranking of the SILs' hydrophobicity and performance in terms of adsorption efficiency (Table 1).

Adsorption isotherms portray the relationship between the

Table 2
Parameters of the kinetic models and correlation coefficients (R^2): q_e – equilibrium concentration in the solid phase; k_1 and k_2 – sorption rate constants.

	[Si][N_{3222}]Cl	[Si][N_{3444}]Cl	[Si][N_{3888}]Cl
$q_{e,\,exp}$ (mg·g^{-1})	14.63	14.69	14.70
Pseudo-first-order model			
q_e (mg·g^{-1})	14.46 ± 0.04	14.55 ± 0.04	14.49 ± 0.05
k_1 (min^{-1})	3.393 ± 0.241	3.115 ± 0.191	3.017 ± 0.024
R^2	0.9990	0.9989	0.9981
Pseudo-second-order model			
q_e (mg·g^{-1})	14.53 ± 0.03	14.63 ± 0.03	14.59 ± 0.04
k_2 (g·mg^{-1}·min^{-1})	1.594 ± 0.214	1.265 ± 0.132	1.094 ± 0.157
R^2	0.9997	0.9998	0.9995

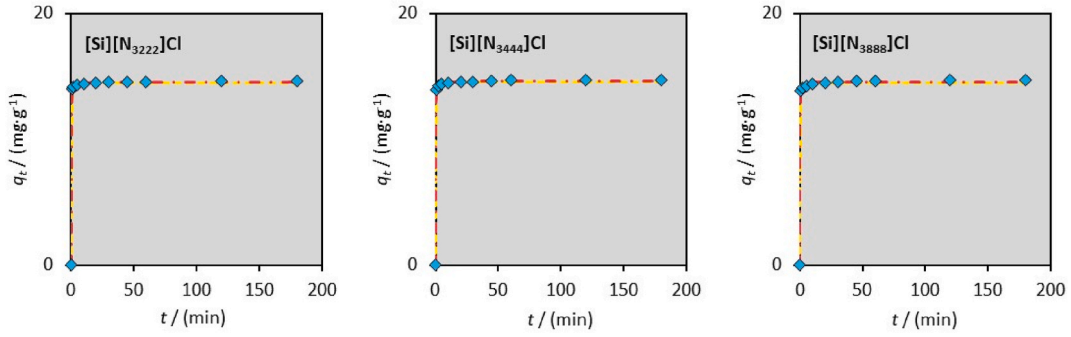

Fig. 2. Adsorption kinetic curves for cyclophosphamide using the best three identified SILs at 25 °C. The blue diamonds are the experimental data, the dashed yellow line is the fitting with the pseudo-first-order model and the red dotted-dashed line is the fitting with the pseudo-second-order model.

equilibrium distribution of cyclophosphamide between the aqueous phase and the SIL, being shown in Fig. 3. Experiments were performed along a fixed period of 120 min and using 10 mL of aqueous solutions of cyclophosphamide, with concentrations ranging from 50 to 1500 mg L^{-1}, and 50 mg of each SIL. Among the three models used to fit the experimental data, namely the Langmuir (1918), the Freundlich (1907) and the Sips (1948) models, the latter is the one providing the best fitting for all SILs, despite the discrepancy between the experimental and predicted q_e values observed with [Si][N_{3444}]Cl ($R^2 \geq 0.9761$, cf. Table 3). In the case of [Si][N_{3888}], although it was possible to fit the Langmuir model, the associated error is higher than the value obtained for q_{max} and B by the model. This phenomenon is observed for other materials in the fitting with different models, namely for [Si][N_{3222}] with the Freundlich model and for [Si][N_{3444}] with the Sips model. By merging the Langmuir and the Freundlich models, the Sips model considers both homogeneous and heterogeneous systems. Accordingly, while the adsorption of cyclophosphamide onto the appraised SILs approaches a monolayer adsorption process at high concentrations (characteristic of processes described by the Langmuir model), at low concentrations it adopts a non-ideal behaviour on heterogeneous surfaces with multilayer sorption (typical of processes described by the Freundlich model) (Tang et al., 2016). Overall, the experimental q_{max} values of the investigated SILs range from 55.67 to 67.64 mg of cyclophosphamide per g of material (Table 3). Thereby, and in addition to providing the fastest process (as revealed by the kinetics results), the SIL endowed with the highest hydrophobicity, i.e. [Si][N_{3888}]Cl, displays as well the best adsorption capacity.

Table 3
Parameters of the adsorption isotherm models and correlation coefficients (R^2): q_{max} – maximum adsorption capacity; B – Langmuir isotherm constant; K_f – Freundlich equilibrium constant; n – Freundlich/Sips exponent; K_S – Sips equilibrium constant.

	[Si][N_{3222}]Cl	[Si][N_{3444}]Cl	[Si][N_{3888}]Cl
$q_{max,\ exp}$ (mg·g^{-1})	55.67	57.45	67.64
Langmuir			
q_{max} (mg·g^{-1})	—[a]	—[a]	828.6 ± 2160
B (L·mg^{-1})			(0.8281 ± 2.321) × 10^{-4}
R^2			0.9588
Freundlich			
K_f (mg·g^{-1})	(1.348 ± 1.431) × 10^{-3}	(4.023 ± 2.769) × 10^{-2}	(6.615 ± 6.391) × 10^{-2}
n	0.6649 ± 0.0680	0.9783 ± 0.0967	1.005 ± 0.144
R^2	0.9752	0.9751	0.9576
Sips			
q_{max} (mg·g^{-1})	67.12 ± 4.47	213.9 ± 371.0	86.63 ± 20.28
K_s (L·mg^{-1})	(1.288 ± 0.063) × 10^{-5}	(3.495 ± 7.668) × 10^{-4}	(1.622 ± 0.418) × 10^{-3}
$1/n$	3.436 ± 0.322	1.202 ± 0.399	2.28 ± 0.70
R^2	0.9974	0.9761	0.9763

[a] Ambiguous as identified by the GraphpadPrism7 software.

3.3. Toxicity of treated aqueous samples

Aiming to develop a strategy to reduce the risk associated with the release of cyclophosphamide into the environment, a toxicity assessment of the cyclophosphamide aqueous samples after adsorption with SILs using zebrafish (*Danio rerio*) was performed. Given its better capacity to adsorb cyclophosphamide (cf. Table 3, $q_{max,\ exp}$ = 67.64 mg g^{-1}), [Si][N_{3888}]Cl was the SIL selected to proceed with the toxicity studies. These assays comprising embryos of *D. rerio* were carried out following the Organisation for Economic Co-operation and Development (OECD) guideline 236 on Fish Embryo Acute Toxicity (FET) Test (Organisation for Economic Co-operation and Development (OECD) 2013), slightly modified.

To address the toxicity removal after SIL treatment, *D. rerio* embryos were subjected to different treatments, namely only with water (control group), with silica, with [Si][N_{3888}]Cl, and with an aqueous solution of cyclophosphamide at a 1306 mg L^{-1} before and after being in contact with [Si][N_{3888}]Cl for 1.5 h. Considering the potential mode of action of the cytostatic under appraisal, besides the traditional lethal endpoints, several sublethal endpoints were studied. Mortality, hatching rates, malformations, and final body length in embryos and larvae of *D. rerio* are presented in Fig. 4; evidence of malformations is further provided in Fig. 5.

Regarding mortality, both the silica and the SIL did not cause any significant effects, whereas exposure to the cyclophosphamide solution resulted in 20% of mortality (CYP before, in Fig. 4a). Remarkably, the percentage of mortality was reduced to zero in organisms exposed to the cyclophosphamide solution that was previously in contact with the SIL (CYP after, in Fig. 4a). No significant differences were found for the hatching rates following all treatments (Fig. 4b).

As for the endpoint of malformations, silica and the [Si][N_{3888}]Cl treatments did not pose any effect on the organisms, emphasizing the nontoxic nature of the SIL material. In turn, the cyclophosphamide solution (before being in contact with [Si][N_{3888}]Cl) induced significant malformations in 52% of the zebrafish embryos/larvae (Fig. 4c; Dunn's test: $p < 0.05$). The observed malformations were mainly oedemas (pericardial and/or ventral) and tail curvatures - cf. Fig. 4a (Control) *versus* 4b. In the treatment where the embryos were exposed to the cyclophosphamide solution after being in contact with [Si][N_{3888}]Cl this value reached residual levels (around 3%, which according to the guideline OECD (2013) is not statistically significant) (Figs. 4c and 5c).

Regarding the effect of the different treatments on the final body length of zebrafish larvae, only the organisms exposed to the cyclophosphamide solution before being in contact with the materials exhibited significantly smaller lengths than the larvae from the control (an average length of 4.4 ± 0.4 mm versus 5.4 ± 0.2 mm average from the control treatment) (Fig. 4d; Dunn's test: $p < 0.05$). Such results

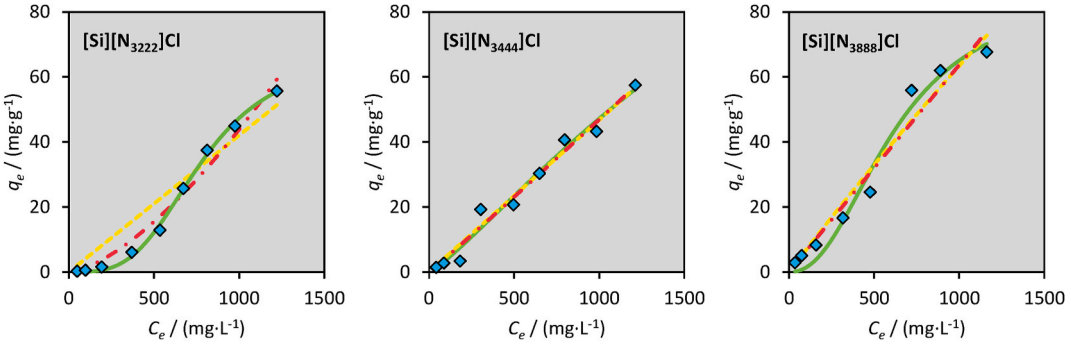

Fig. 3. Adsorption isotherm curves for cyclophosphamide using the best three SILs at 25 °C. The blue diamonds are the experimental data, the dashed yellow line is the fitting with the Langmuir model, the red dotted-dashed line is the fitting with the Freundlich model and the full green line is the fitting with the Sips model.

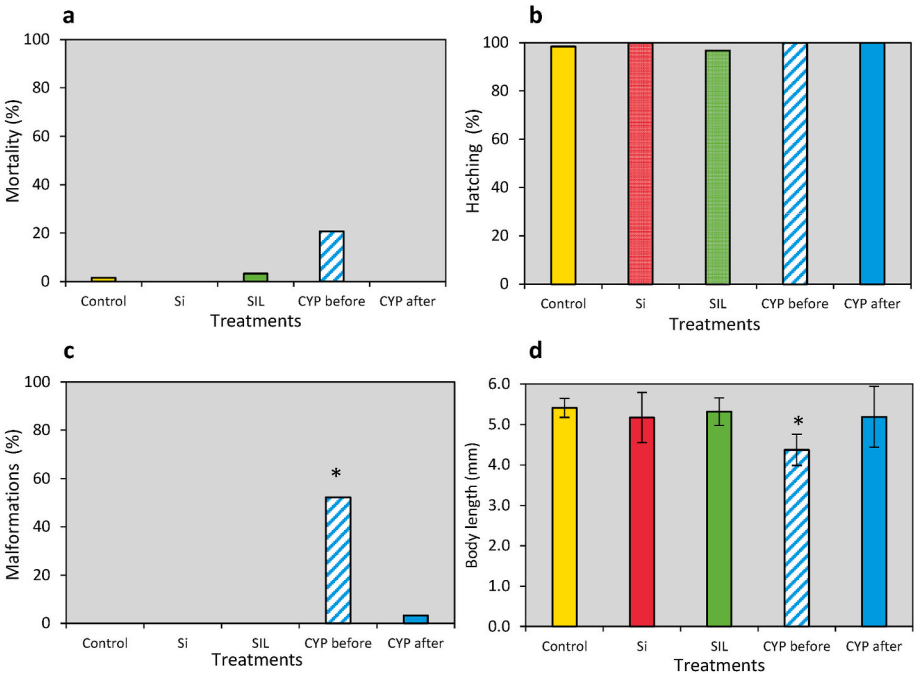

Fig. 4. Mortality (a), hatching rates (b), malformations (c) and body length (d) in *Danio rerio* larvae after a 96-h exposure to five different treatments: maintenance water (Control; yellow bars), silica (Si; red bars), [Si][N_{3888}]Cl (SIL; green bars), cyclophosphamide solution of 1306 mg L^{-1} (CYP before; dashed blue bars), and a cyclophosphamide solution of 1306 mg L^{-1} after being in contact with [Si][N_{3888}]Cl (CYP after; blue bars). * indicates a significant statistical difference in relation to control conditions (Dunn's test: $p < 0.05$).

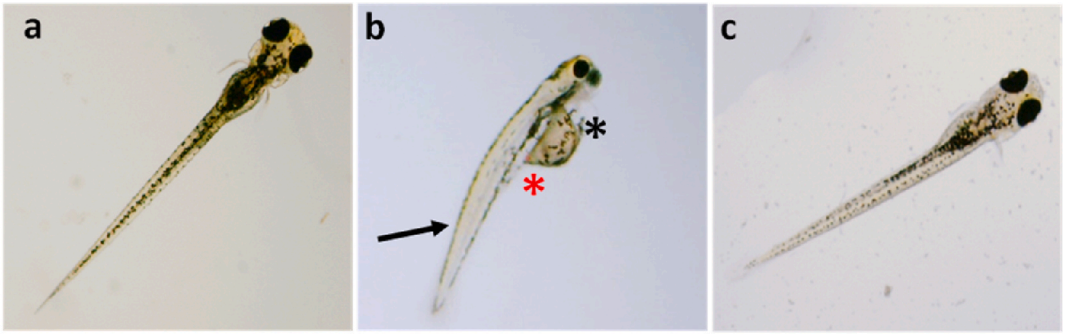

Fig. 5. Malformations in *Danio rerio* larvae after a 96-h exposure. Larvae from the control treatment (a), larvae exposed to a cyclophosphamide solution of 1306 mg L^{-1} (b), and larvae exposed to a cyclophosphamide solution of 1306 mg L^{-1} after being in contact with [Si][N_{3888}]Cl (c). Black arrow shows tail curvature, black asterisk indicates pericardial oedema and red asterisk indicates ventral oedema. (Magnification of 2x; Pictures are not related in size).

clearly demonstrate that our proposed strategy allows to significantly reduce the toxicity of the contaminated aqueous samples to a negligible value.

In summary, the high performance of SILs as adsorbents for cytostatics has been demonstrated using cyclophosphamide. SILs did not simply remove cyclophosphamide from aqueous samples, but they succeeded in performing such a task reducing the toxicity of contaminated aqueous samples. This represents an advantageous feature as compared to currently used methods, such as ozonation or advanced oxidation processes, where the formation of toxic and resistant byproducts occurs (Lin et al., 2015). Furthermore, the best SIL has a high affinity towards the target cytostatic drug (cyclophosphamide), which may also be an advantage when compared with the adsorption treatments using activated carbon, which presents limited selectivity (Garcia-Costa et al., 2023; Guillossou et al., 2019). The studied silica-based SILs also offer advantages over the most recent adsorption methods, that include the use of carbonaceous materials such as carbon nanotubes (CNTs) (Garcia-Costa et al., 2023). Despite being a fast (20 min) and efficient process to remove cyclophosphamide from aqueous samples, the small dimensions of NCTs preclude their application due to difficulties associated with their separation from the aqueous phase (Toński et al., 2018). Fe3O4@SiO2@CTAB–SiO_2 has been used as well to remove cyclophosphamide from water samples, with a maximum adsorption capacity of 342.8 mg g^{-1} (by the Brouers–Sotolongo isotherm model), within 30 min, at pH 7.0, adsorbent dose of 0.01 g (for a cyclophosphamide concentration of 10 mg.L^{-1}) and at 25 °C (Zandipak et al., 2020). This material proved to be efficient towards real samples as well, where tap water, river water and medical waste-water spiked with different contents of cyclophosphamide were tested. These materials correspond to nanoparticles, sharing the same concerns of CNTs. On the other hand, Graphene Oxide (GO) was used as a platform for drug release (Sheikh and Goudarzian, 2023). The equilibrium adsorption data obtained at pH 7.4 and 40 °C fitted better to the Freundlich isotherm model, with a K_f of 3.853 mg g^{-1} (Sheikh and Goudarzian, 2023).

Adsorption processes for highly toxic drugs such as

cyclophosphamide are indeed the best approach, i.e. to immobilize this highly toxic drug into a solid matrix. Such matrix can then be easily discharged as type IV hazardous waste, precluding a possible future (re) entrance into the environment. It should be mentioned that it is possible to reuse these SILs, as previously demonstrated (Almeida et al., 2020), but given the highly toxic nature of cyclophosphamide this reuse is not advisable. Carrying out elution/regeneration studies with the studied SILs will result on having the drug dissolved in a solvent again, which is unadvisable from an environmental point of view. Furthermore, the SILs used are of low cost, with solvents used to regenerate these materials having a higher cost (Almeida et al., 2020). Therefore, elution/regeneration assays are also unadvisable from an economic point of view.

Considering the potential sources and pathways of cytostatic contamination in the aquatic environment, SILs can be envisaged as a versatile class of adsorbents. A myriad of aqueous matrices can be potentially treated using SILs. Taking into account the experimental maximum adsorption capacity of 67.64 mg of cyclophosphamide per g of [Si][N_{3888}]Cl, in an ideal scenario, 1 g of [Si][N_{3888}]Cl is expected to be sufficient to treat: (i) over 1,000,000 L of water containing cyclophosphamide at environmentally relevant concentrations (e.g., 64.8 ng L^{-1} in surface waters) (Moldovan, 2006); (ii) from over 2300 to 5100 L of highly contaminated aqueous samples, such as hospital wastewaters with concentrations of circa 29,100 ng L^{-1} and urban effluents with concentrations around 13,100 ng L^{-1}, respectively (Gómez-Canela et al., 2012; de Oliveira et al., 2021); and (iii) over 1 L of urine of oncologic patients, as a patient takes 500 mg of cyclophosphamide per day, of which 25 % is excreted in the urine, and has a daily urinary output of 2 L (Global RPh, 2022). However, it should be mentioned that these estimations were carried out considering the results obtained here with water samples and at a low scale. Increasing the complexity of the sample and increasing the volume of water to treat will expectably lead to a lower performance of the studied SILs. Even so, considering the variety of potential applications of SILs, i.e., from remediation to point of source removal, this work uncovers the potential of SILs as a new class of tailored adsorbents to tackle the emergence of cytostatic drugs in the aquatic environment.

4. Conclusions

Due to the absence of effective technologies for removing or treating highly toxic oncologic drugs in wastewater treatment facilities, water quality is compromised, posing a significant risk to aquatic organisms. To overcome this concern, several SILs were tested in cyclophosphamide adsorption, being [Si][N_{3888}]Cl the most efficient SIL identified. Taking into account the experimental maximum adsorption capacity of 67.64 mg of cyclophosphamide per g of [Si][N_{3888}]Cl, in an ideal scenario, 1 g of [Si][N_{3888}]Cl is expected to be sufficient to treat over 1,000,000 L of water containing cyclophosphamide at environmentally relevant concentrations, from over 2300 to 5100 L of highly contaminated aqueous samples, such as hospital wastewaters, and over 1 L of urine of oncologic patients, highlighting their potential for point of source applications. The toxicity of treated aqueous samples studies was addressed using *D. rerio* embryos. SILs do not cause any significant effects, whereas exposure to the cyclophosphamide solution resulted in 20% of mortality. Moreover, this percentage was reduced to zero in organisms exposed to the cyclophosphamide solution previously treated with the best identified SIL. SILs do not simply remove cyclophosphamide from aqueous samples, but they succeeded in performing such a task reducing the toxicity of contaminated aqueous samples. The best identified SIL exhibits significant promise for water remediation, particularly in point-of-source applications. For instance, they could be utilized in a device designed to directly capture drugs from the urine of oncologic patients.

CRediT authorship contribution statement

Rafael Francisco: Writing – original draft, Investigation, Formal analysis. **Bruna Monteiro:** Investigation, Formal analysis. **Maria J. Santos:** Investigation. **Francisca A. e Silva:** Writing – review & editing, Visualization. **Catia Venancio:** Writing – review & editing, Methodology. **Marcia C. Neves:** Writing – review & editing, Supervision, Methodology. **Isabel Lopes:** Writing – review & editing, Supervision, Project administration. **Ana C.A. Sousa:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Mara G. Freire:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2024.122995.

Data availability

Data will be made available on request.

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