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Research article

Removal of contaminants from wastewater using the bivalve *Corbicula fluminea* - Comparative assessment of biofiltration and biosorption

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ABSTRACT

Bivalves, such as *Corbicula fluminea*, and their milled shells have been shown to efficiently remove some compounds from the water, but their ability to remove contaminants of emerging concern, namely pharmaceuticals and stimulants, remains largely unknown. Hence, this study aimed to compare the efficiency of *C. fluminea* and the corresponding milled shells for removal of 9 common wastewater contaminants at concentrations of 0.5 and 1.0 mg.L^{-1}, further appraising the entailed ecotoxicity variation. After 24 h, clams removed mainly fluoxetine (≥91 %) and, to a moderate extent, paracetamol (≥26 %). Milled shells removed mainly caffeine (≥49 %), fluoxetine (≥42 %) and naproxen (≥35 % at 0.5 mg.L^{-1}), after 24 h of contact. Clams were more effective than shells in removing fluoxetine, paracetamol, carbamazepine, metformin and diclofenac whereas the opposite was observed for caffeine and naproxen. Despite this effectiveness, clams and shells had minor effects on ecotoxicity abatement to the microalgae *Raphidocelis subcapitata* and the bacteria *Aliivibrio fischeri*, except for fluoxetine. Indeed, the remarkable toxicity reduction to the microalgae exposed to the biofiltered fluoxetine sample matches the pronounced removal %, confirming the beneficial effect of *C. fluminea* on the quality of water contaminated with this compound. Although biofiltration outperformed biosorption in general, the requirements for clams' maintenance and the risk of spreading this invasive species might constitute a drawback for the use of this species for bioremediation of contaminated wastewaters, highlighting the importance of analyzing the pros and cons of these approaches for each specific application.

1. Introduction

In recent decades, many contaminants have been identified in aquatic systems, which constitutes an issue of increasing concern. Recent focus has been placed in the so-called contaminants of emerging concern (CECs), which are organic compounds of anthropogenic or natural origin, commonly occurring in the range of ng.L^{-1} to μg.L^{-1} (Rout et al., 2021), most having no regulatory standards but potentially causing adverse toxicological effects in the environment (Khan et al., 2023). Despite occurring at low concentrations, their constant release to the environment, allied to their persistent nature in some cases, and bioaccumulation potential, can cause deleterious biological effects both to the environment and human health (Rout et al., 2021).

Within the most common CECs in surface water are stimulants, pharmaceuticals and personal care products, which originate mainly from Wastewater Treatment Plants (WWTPs) (e.g., Rout et al., 2021) owing to their insufficient ability to fully remove most CECs from wastewaters (Ahmed et al., 2021; Rout et al., 2021). Aiming to improve the quality of WWTP effluents, a wide diversity of physical, chemical, biological and hybrid processes have been suggested and applied (Ahmed et al., 2021). Among these, bioremediation is a process using biological systems that has been proposed as a promising strategy for treating wastewater contaminated with CECs (Ahmed et al., 2021). This process relies on the removal of contaminants from water, mainly

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through biodegradation, biosorption and/or bioaccumulation (Ahmed et al., 2021). Bivalves have been proposed as a sustainable approach for bioremediation of wastewaters (e.g., Gomes et al., 2018a; Sicuro et al., 2020), through biofiltration. Biofiltration is a term used in general to define the technology that harnesses living organisms to remove contaminants from contaminated matrices. This includes several mechanisms, such as biodegradation and biotransformation, as well as physical and chemical removal, including adsorption. In the particular case of bivalves, there is an additional mechanism: sedimentation of excreted (pseudo)feces (Ismail et al., 2014). In the present study, the term is used in *sensu lato*, concordantly to its use in previous studies (e.g., Binelli et al., 2014; Gomes et al., 2018b). The use of bivalves for bioremediation is generally supported by their high filtration rate. In particular, freshwater bivalves are most useable in wastewater treatment as marine bivalves would require higher salinity levels (e.g., Binelli et al., 2014; Gomes et al., 2018b). However, they are among the most threatened biota groups in the world with 40 % of the species being near threatened or extinct (Lopes-Lima et al., 2018), which largely prevents exploitation for bioremediation purposes. The use of bivalve species that have invasive capacity in non-native areas, such as the Asian clam *Corbicula fluminea*, overcomes this issue, while presenting advantages. On one hand, this species is generally tolerant to a wide range of abiotic conditions and contaminants; on the other hand, it fits pest management approaches based on the mechanical removal of the individuals from invaded ecosystems or infested industrial settings, with the add-on of opening an avenue for the valorization of the collected biomass in wastewater decontamination. *Corbicula fluminea* is indigenous to Australia, Asia and Africa, and was introduced to America and Europe in the 20th century, becoming a ubiquitous invasive bivalve in freshwater ecosystems therein. It shows a high biofiltration and bioaccumulation capacity, as well as wide ecological competence (Rosa et al., 2014; Gomes et al., 2018a; Li et al., 2023). It is noteworthy that in native areas there are no particular concerns with the exploitation of the species, while in non-native areas, dispersion prevention should naturally be in place through e.g., UV irradiation, a control method that aligns with typical wastewater treatment routines and has been proven effective against bivalve veliger stages that could be dragged out of tanks and disperse to natural surroundings (Jenner et al., 1998; Stewart-Malone et al., 2015).

The Asian clam has been found effective in the removal of a wide variety of biological and/or chemical contaminants, such as metals from acid mine drainage (Rosa et al., 2014), CECs and the bacteria *Escherichia coli* from wastewater (Ismail et al., 2014; Gomes et al., 2018b), and in the reduction of the eutrophication status of aquatic systems when used simultaneously with other aquatic species (Li et al., 2010; Song et al., 2014). However, the removal efficiency of some common contaminants in wastewater has not been addressed under conditions reflecting common operation practice in WWTPs, namely for the stimulant caffeine (CAF), and a wide diversity of pharmaceuticals, such as the analgesic and anti-inflammatory drugs paracetamol (PCT, also known as acetaminophen), ibuprofen (IBU), naproxen (NPX) and sodium diclofenac (DIC), the antiepileptic carbamazepine (CBZ), the antidepressant fluoxetine hydrochloride (FXT), the antidiabetic metformin hydrochloride (MET) and the antibiotic sulfamethoxazole (SMX). Despite spatial and temporal variation is common, these CECs are amongst the most common in WWTPs' effluents and at the highest concentrations. For instance, PCT, IBU, CAF and NPX were reported in WWTPs effluents at concentrations reaching up to 62 $\mu g.L^{-1}$, 48 $\mu g.L^{-1}$, 37 $\mu g.L^{-1}$, and 34 $\mu g.L^{-1}$, respectively (Santos et al., 2007; Tran et al., 2018). Moreover, the selected CECs also show a high hazardous potential to aquatic ecosystems (e.g., Khasawneh and Palaniandy, 2021, Parida et al., 2021). Considering that some of these contaminants are set by the European Commission to be removed by 80 % in urban WWTPs applying quaternary treatment (CBZ and DIC; European Commission, 2024), or to be monitored (SMX and MET; European Commission, 2022b) or proposed to be added to the Priority Substance list in the framework of the EU water policy (IBU; European Commission, 2022a), effective strategies to remove these contaminants from contaminated water are particularly relevant.

Besides using living bivalves to remove contaminants from water, bivalve shells can also be used as a biosorbent, profiting from an abundant material that, otherwise, would be landfilled as a biological waste from the food industry, thus promoting sustainable practices within a circular economy approach. For example, bivalve shells have been reported to efficiently remove nutrients, metals and dyes from water (Summa et al., 2022), as well as an antibiotic and an endocrine disruptor (Henrique et al. 2020, 2021). Hence, considering that *C. fluminea* shells are available in invaded ecosystems at no or low cost, or accumulated as residues in many Asian countries where the species is consumed (Yang et al., 2019), and also the environmental benefit of their removal from the ecosystems, the potential of *C. fluminea* shells for CECs removal from water should be studied. Despite bivalve shells can undergo diverse pre-treatment processes to increase their removal efficiency, such as thermal treatments (e.g., calcination or pyrolysis, Henrique et al. (2020)), these are high energy demanding processes compromising environmental sustainability. In the present study, shells were pre-treated only by milling, in face of the evidence that milled bivalve shells can efficiently remove contaminants from water, namely nutrients and metals (Abdullah et al., 2023, Summa et al., 2022; Thind et al., 2022) and of the low environmental impact of the milling process.

The present study intended to assess the efficiency of *C. fluminea* for the removal of CAF, PCT, IBU, NPX, DIC, CBZ, FXT, MET and SMX from water, further comparing the removal efficiency of the living bivalves (through biofiltration) and of their milled shells (through biosorption). Aiming to clarify whether the removal efficiency is influenced by contaminants' concentration, studies were performed at 0.5 and 1.0 $mg.L^{-1}$ of each compound. These concentrations are contextually relevant for some of the tested compounds, namely PCT, IBU and CAF, which registered concentrations above 500 $\mu g.L^{-1}$ in WWTPs influents in several countries (Parida et al., 2021) and up to several tens of $\mu g.L^{-1}$ in WWTPs effluents, as previously mentioned. Furthermore, aiming to assess whether contaminants removal translates into an effective reduction of water toxicity, an ecotoxicological assessment of the untreated and treated water samples was performed using a primary producer – the microalgae *Raphidocelis subcapitata* – and a decomposer – the bacteria *Aliivibrio fischeri*.

2. Material and methods

2.1. Collection of C. fluminea individuals and shells

Corbicula fluminea individuals were collected in mid-September 2023 in the Mondego River (Montemor-o-Velho, Portugal: 40.163147, −8.671106) and transported to the laboratory in local water. Clams were gradually acclimated to dechlorinated tap water and were quarantined under laboratorial conditions (20 ± 1 °C, 16 h light: 8 h dark photoperiod) for at least two weeks before the experiments. The cultures were fed *ad libitum* with a concentrated suspension of the microalgae *R. subcapitata* three times a week, immediately after renewal of the culture water. Clam shells were collected from Pateira do Requeixo (Aveiro, Portugal; 40.588158, −8.5301091), washed with tap water, and soaked in distilled water for 2 days, before drying at 60 °C. Dry shells were then milled using a laboratory disc mill (<0.5 mm).

2.2. Tested chemicals

Nine CECs were tested individually in the present study by dissolving high purity standards/salts in dechlorinated tap water. Caffeine (1,3,7-Trimethyl-3,7-dihydro-1H-purine-2,6-dione; CAS: 58-08-2), CBZ (5H-dibenzo[b,f]azepine-5-carboxamide; CAS: 298-46-4) and MET (N,N-dimethylimidodicarbonimidic diamide hydrochloride (1:1); CAS: 1115-70-4) were supplied by Thermo Scientific as standards with a purity of

99.7 %, 98 % and 97 %, respectively. Diclofenac was dosed from its sodium salt (sodium {-[(2,6-dichlorophenyl)amino]phenyl}acetate, CAS: 15307-79-6), 98 % pure, from Alfa Aesar. Naproxen ((2 S)-2-(6-methoxy-2-naphthyl)propanoic acid; CAS: 22204-53-1), fluoxetine hydrochloride (N-methyl-3-phenyl-3-[4-(trifluoromethyl)phenoxy]-1-propanamine hydrochloride (1:1); CAS: 56296-78-7; >98 % purity) and SMX (4-amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide; CAS: 723-46-6) were supplied by TCI as standards with purity >99 %, >98 %, and >98 %, respectively. Ibuprofen (2-(4-isobutylphenyl)propanoic acid; CAS: 15687-27-1) and PCT (N-(4-hydroxyphenyl)acetamide; CAS: 103-90-2) were supplied by Sigma Aldrich as standards, both with purity >98 %. The chemical structure and main physicochemical characteristics are presented in Table S1.

2.3. Biofiltration experiments

Each pharmaceutical was tested individually, at a concentration of 0.5 mg.L^{-1} and 1.0 mg.L^{-1}. Dechlorinated tap water (pH ≈ 7.7, conductivity ≈ 410 μS cm^{-1}, hardness ≈ 59 mg.L^{-1} as $CaCO_3$) were used both for the preparation of stock solutions (2 mg.L^{-1}) and as dilution medium. Tests were performed in glass beakers containing 10 clams in 500 mL of test medium, in the dark, at 20 ± 1 °C under constant aeration.

The following treatments were considered for testing with each chemical: clams in dechlorinated tap water containing the chemical at 0.5 or 1.0 mg.L^{-1} (4 replicates *per* treatment); clams in dechlorinated tap water (blank; 4 replicates); and dechlorinated tap water containing the chemical at 0.5 or 1.0 mg.L^{-1} (control; 3 replicates *per* treatment). The initial test concentrations were selected i) to allow for a precise quantification of the contaminants in the aqueous phase, in line with the limits of detection and quantification of the applied analytical methods and ii) to allow obtaining measurable results at the tested scale. Given the known effect of clams size on their biofiltration rate (Castro et al., 2018), clams used in the experiments were selected based on their length, which ranged between 19 and 23 mm and varied by no more than 3 mm within each experiment (mean size ± standard deviation of all clams used in the experiments was 21 ± 1 mm). At the start of the experiments, a suspension of the microalgae *R. subcapitata* was added to all beakers, to reach a density of 8 x 10^4 cells.mL^{-1}. Test vials were covered with a cling film to prevent water loss. The exposure period was 48 h, with aliquot water samples for chemical analyses being taken at the start of the experiment, as well as after 6 h, 24 h and 48 h of exposure, and frozen at −20 °C until quantification. Samples for ecotoxicological assessment were also taken, both at the start of the experiment and after 48 h of exposure and were vacuum-filtered with a glass fiber membrane (1.2 μm) before being stored at −20 °C until testing.

2.4. Biosorption experiments

The biosorption experiments with the milled shells were performed in 15 mL polypropylene Falcon tubes, containing 15 mL of medium and the milled shells. The medium consisted of dechlorinated tap water spiked with the stock solutions previously mentioned and diluted with dechlorinated tap water to achieve concentrations of 0.5 mg.L^{-1} and 1.0 mg.L^{-1}. Shells were tested at a dose of 50 g.L^{-1} (0.75 g in 15.0 mL of medium). This dose was selected as it corresponds to the weight of the dry shells of the clams as tested in the biofiltration experiments, thus offering additional information for discussing the efficacy of clams' filtration (part of the removed compounds can actually be sorbed to the shells).

The tubes were shaken in an overhead shaker (Heidolph, Reax 2; 80 rpm) at 20 °C for 24 h. This contact time was selected to be reasonable in the context of a WWTP management. Experiments were performed in triplicate. Control treatments, consisting of each chemical at both concentrations without shells, were carried out simultaneously and used as reference for the calculation of adsorption percentages. After the 24 h period, the tubes were centrifuged for 5 min at 4000 rpm and the supernatant was collected for further ecotoxicological assessment and chemical quantification.

The milled shells were characterized regarding the point of zero charge and the specific surface area, as described in Supplementary Section S1.

2.5. Quantification of the compounds in water

Previously to the chemical analyses, samples were filtered using Whatman Puradisc (hydrophilic, PVDF, 13 mm diameter, 0.22 μm pore) syringe filters; for fluoxetine, the filtration was performed using PTFE Hydrophilic syringe filters (Labfil, 13 mm diameter, 0.22 μm pore). The concentration of compounds in aqueous samples was determined by High Performance Liquid Chromatography using UV–Vis detection (HPLC-UV-Vis), except for MET, which was quantified by Capillary Zone Electrophoresis (CZE), as detailed in Supplementary Section S2.

The removal percentage (removal %), for each initial concentration, promoted either by biofiltration or by biosorption, was determined through Eq. (1), where C_0 is the average concentration of the chemical in the control (no shells; no clams) and C_f is the concentration of the chemical in each corresponding replicate after biofiltration or biosorption. Regarding the biosorption experiment, the adsorption capacity (Q_e) for each replicate was determined according to Eq. (2), where Q_e is expressed as μg.g^{-1}, and m (expressed in g) is the mass of milled shell *per* volume (V; expressed in L).

$$\text{Removal}\ (\%) = \frac{C_0 - C_f}{c_0} \times 100, \qquad \text{Equation 1}$$

$$Q_e = \frac{(C_0 - C_f)}{m} \times V \times 1000 \qquad \text{Equation 2}$$

2.6. Ecotoxicological assessment

The ecotoxicological assessment was performed using the microalgae *R. subcapitata* and the bacteria *A. fischeri*, both considered sensitive species, representing groups of organisms potentially affected by the discharge of treated wastewater in aquatic systems, and commonly employed in the environmental assessment of processes for wastewater treatment (e.g., Gomes et al., 2021; Jesus et al., 2022). A composite sample of replicates within each treatment in the biofiltration and biosorption experiments was prepared and used for the ecotoxicological tests.

The growth inhibition test with *R. subcapitata* followed the OECD guideline 201 (OECD, 2006) with the modifications for the use of 24-well microplates as detailed by Gomes et al. (2019). Tests started with 10^4 cells.mL^{-1} and each sample was tested in triplicate. Water samples were enriched in nutrients required for growth of the test species, complying with the standard MBL medium recipe (Stein et al., 1973), hence assuring that any observed ecotoxicological effect was not due to nutrient scarcity. The nutrient spiking caused a slight dilution of the samples, which were tested at 98.2 % strength. The control consisted of dechlorinated tap water, nutrient spiking and the *R. subcapitata* inoculum. Microplates were incubated under artificial continuous light for 96 h at 23 ± 1 °C. After this period, the microalgae growth was assessed based on the absorbance of each sample at 440 nm (spectrophotometer Shimadzu UV-1800, Japan), for samples testing CAF, DIC and IBU. The absorbance was converted to cell density using a specific calibration equation previously developed in our laboratory (Castro et al., 2018). Regarding the remaining CECs, the microalgae density was determined by counting under a microscope in a Neubauer hemocytometer, as these chemicals interfere with absorbance measurements at 440 nm. Cell densities were used for yield inhibition and growth rate inhibition calculations (OECD, 2006).

The bioluminescence inhibition test with *A. fischeri* was performed following the Whole Effluent Toxicity (WET) testing, as outlined in the manufacturer protocol, using a Microtox Model 500 Analyzer (Modern Water Inc, USA). Each sample, including controls, was tested at 15 °C in duplicate. Sodium chloride was added to each sample to adjust the osmotic pressure to 2 % NaCl, ensuring that the test is run at optimal osmotic conditions for the bacteria. The addition of the reconstituted bacteria caused a slight dilution of the samples, which were tested at 99 % strength. The control consisted of dechlorinated tap water, NaCl and the bacteria. Measurements of the luminescent output of the bacteria were recorded after 15 min of exposure and compared to the light output of the control sample to determine bioluminescence inhibition (%).

2.7. Statistical analyses

Regarding the biofiltration experiments, and to assess whether the initial concentration of contaminant and the time of exposure affected the removal percentage, a two-way ANOVA was performed, using the initial concentration of contaminant and the time of exposure as factors, and the removal percentage as dependent variable, followed by the Holm-Sidak method for multiple comparisons. If the normality (Shapiro-Wilk) or equal variance (Brown-Forsythe) failed, an ANOVA on ranks was performed instead, followed by the Dunn's method for multiple comparisons. Considering the removal by the milled shells, and to assess whether the initial concentration of each contaminant affected the removal percentage and adsorption capacity by the shells, a *t*-test was used to compare these endpoints for each initial concentration (0.5 mg.L^{-1} and 1.0 mg.L^{-1}). If the normality or equal variance failed, the Wilcoxon Rank Sum test was performed instead. Aiming to test whether the removal of the compounds by *C. fluminea* or the milled shell were related to physicochemical properties of the compounds, Pearson correlation analyses were applied between the removal endpoints (removal % and removal rate *per* clam; removal % and adsorption capacity of the shell) and the physicochemical descriptors (molar mass; log Dow (octanol-water distribution coefficient, which corresponds to the log Kow at the system pH)). The comparison of the mass of each compound removed by clams and the milled shells was performed using a *t*-test (normality assumption was met). The statistical analyses were performed using an α value of 0.05.

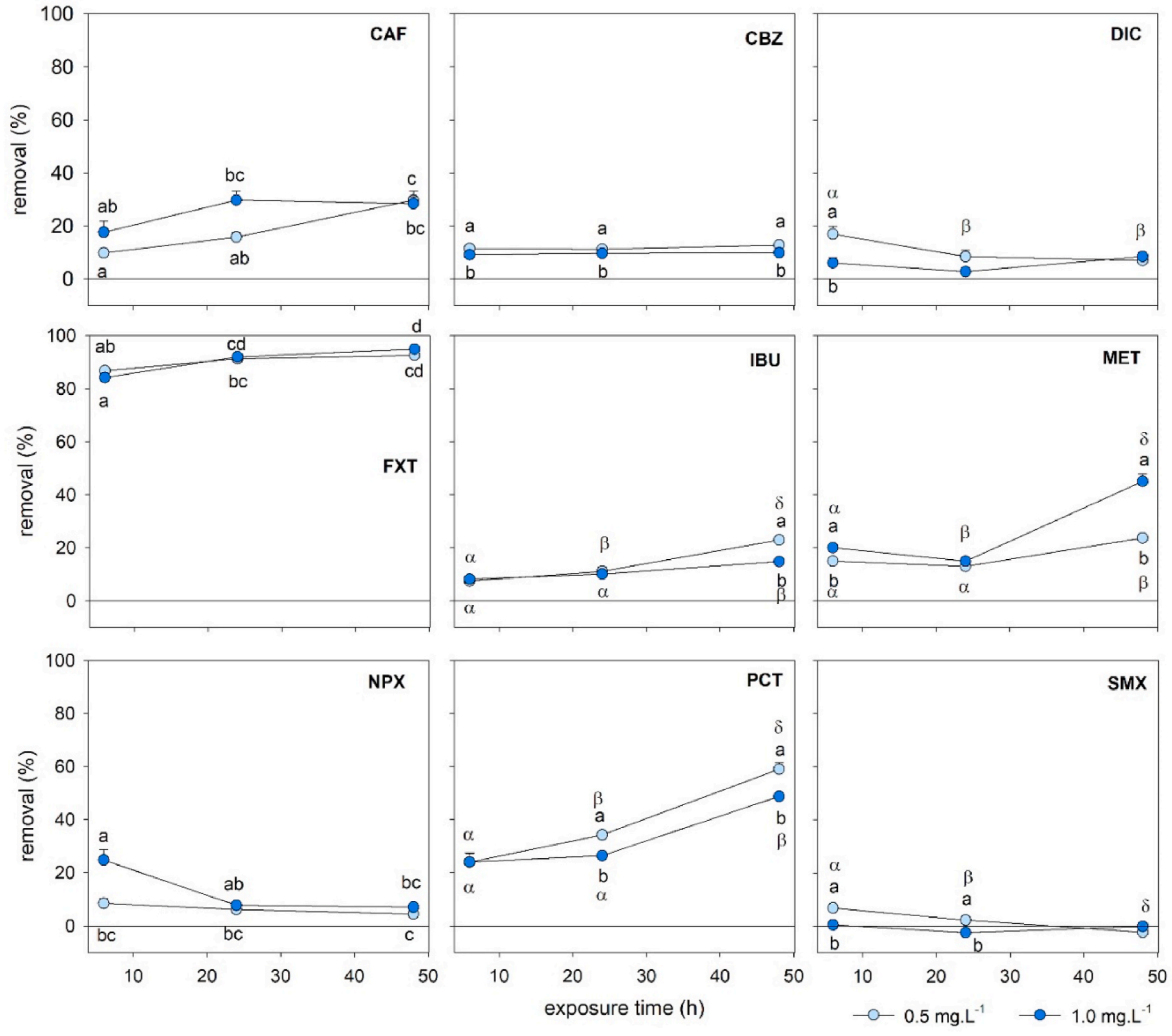

Fig. 1. Removal percentage of the tested compounds by *C. fluminea* after 6 h, 24 h and 48 h of exposure to a solution containing the compounds at an initial concentration of 0.5 mg.L^{-1} and 1.0 mg.L^{-1}. Symbols represent the mean and error bars represent the standard error. Lines connecting the experimental points are only shown for easier interpretation of the data. For CAF, FXT and NPX, treatments denoted by different letters are statistically significantly different among each other. For the remaining compounds, different Latin letters denote significant differences between both concentrations at each exposure time, whereas different Greek letters denote significant differences among the exposure times within each concentration.

3. Results and discussion

3.1. Characterization of the milled shells

The milled shells showed a PZC of 9.1, meaning that below this value the shells' surface is overall positively charged, thus promoting electrostatic attraction with negatively charged chemical species. The specific surface area (S_{BET}), the total pore volume (V_p), and the average diameter of the pores were 4.1 $m^2\ g^{-1}$, 0.03 $cm^3\ g^{-1}$, and 127.6 Å, respectively, whereas the micropore volume was 0.002 $cm^3\ g^{-1}$ and the average micropore width was 2.15 nm (according to the Dubinin-Astakhov equation), as determined by N_2 adsorption/desorption isotherms. The S_{BET} and V_p are similar or higher to most values reported in literature (Table S2), namely to those obtained for cockle shells (3.4 $m^2\ g^{-1}$ and 0.0017 $cm^3\ g^{-1}$, respectively; Kim et al. (2018)). The only exception is the marine bivalve *Mytella falcata* shells (Silva et al., 2017), which showed a S_{BET} 16-fold higher and a V_p twice higher than those observed herein for *C. fluminea* shells.

3.2. Removal of contaminants by biofiltration

The removal of the tested compounds by the clams showed a large variation among the tested CECs (Fig. 1), with the highest value observed for FXT (≥84 ± 3 % for both concentrations at any exposure period). The removal is much higher than any of the other compounds which might be related to its mode of action. Being an antidepressant, it relaxes muscles (Fong et al., 2023), thus promoting the opening of the valves and allowing the clams to filter for longer periods, which may potentiate its removal from water. This is in agreement with a previous study that also reported a full removal of FXT from water by *C. fluminea* after 72 h of exposure to a solution containing several psychoactive drugs (Bourioug et al., 2018). Burket et al. (2019) also reported that FXT was the second most accumulated analyte in *C. fluminea* exposed to a wastewater effluent dependent stream (6.7 μg.kg^{-1}), and this compound was amongst the most frequently detected analytes (maximum 5.4 μg.kg^{-1}) in freshwater bivalves collected from the Great Lakes, USA (Kimbrough et al., 2018).

The second highest removal percentage was observed for PCT, with a maximum removal of 59 ± 9 % after 48 h in a 0.5 mg.L^{-1} solution, followed by CAF and MET. It is interesting to note that the 4 compounds removed to the highest extent are neutral (CAF and PCT) or cationic (FXT and MET) at the test pH: 7.7 (see additionally Table S1). Cationic and neutral forms are more permeable through the membranes as a result of their attractive, or non-repulsive, charge-based interactions (Fu et al., 2009; Cravo et al., 2022), hence showing higher likelihood for accumulation. Oppositely, anionic species (DIC, IBU, NPX and SMX; Table S1) have a more difficult permeation through membranes owing to their conflicting charge with phospholipids (Cravo et al., 2022). Among the tested compounds, there is only one showing a neutral form at the test pH, and low removal: CBZ. Indeed, the removal of this compound in WWTPs is very low either through biodegradation or adsorption, which has been related to its chemical structure and moderate hydrophobicity (Min et al., 2018; Lee et al., 2022). Interestingly, the compounds FXT, CAF and PCT were also those that accumulated to a higher extent in the marine clam *Ruditapes decussatus* exposed to water from a lagoon receiving urban WWT effluents (Cravo et al., 2022), suggesting that the removal and accumulation processes might be similar between these species.

The different removal of PCT and CAF is supported by previous studies reporting a noticeable accumulation of PCT, but a much lower one for CAF in *C. fluminea* exposed to a wastewater effluent dependent stream (Burket et al. 2019, 2020), namely 30.8 μg kg^{-1} and 2.8 μg kg^{-1}, respectively (Burket et al., 2019). Moreover, PCT was also the compound with the highest concentration decrease (>4000 μg) among 13 pharmaceuticals in a wastewater sample following a 24 h biofiltration treatment by the bivalve *Dreissena polymorpha* (Binelli et al., 2014). Regarding MET, it was reported to be hardly accumulated in the mussel species *Lasmigona costata* downstream of a WWTP (de Solla et al., 2016), which suggests that the removal percentage observed in the present study might be related to other mechanism, such as metabolism or adsorption to shells by the clams. On the other hand, this interpretation should be held carefully considering that we tested a single component MET solution while the quoted authors tested a complex mixture of pollutants where chemical interactions may occur, as well as competition among chemical species for internalization and similar biological targets.

The removal of the remaining compounds was lower than 25 %, which agrees with the lack of removal reported in the literature for *C. fluminea* exposed to IBU (Ismail et al., 2014), and the non-accumulation of DIC (Burket et al., 2019), as well as the low accumulation of CBZ (2.5 μg kg^{-1}; Burket et al., 2019). Studies with other bivalves also report low/no removal of CBZ, NPX and DIC from wastewater (Binelli et al., 2014), and of SMX from a synthetic effluent (Gomes et al., 2020). Regarding CBZ, a removal of 30 % from a synthetic effluent was previously reported, but using 20 clams in 0.5 L (Gomes et al., 2020), which seems consistent to the 10–13 % removal observed in the present study, where halved clam density was used.

It is noticeable that CAF, FXT, IBU, MET and PCT were steadily removed from the water, unlike the remaining compounds which seem to have saturated the clams or triggered their valve closure protection mechanism (Castro et al., 2018), early in the test timeline. Despite this apparent differential trend, the removal of the tested compounds (Table S3) was always more pronounced during the period 0–6 h, decreasing during the following periods. This might have been motivated by the existence of food (microalgae) in suspension, which may stimulate valve opening and filtration for feeding. Indeed, the effect of the exposure period on the removal rate was statistically significant for all compounds except CBZ (Fig. 1; Table S4). The initial concentration also showed a statistically significant effect on the removal percentage, except for CAF and FXT (Fig. 1; Table S4). For instance, the removal percentage at 6 h increased with concentration for MET and NPX. The opposite was observed for SMX, DIC and CBZ. In particular, for DIC and SMX the removal rate at 1.0 mg.L^{-1} was lower than at 0.5 mg.L^{-1} (Fig. 1; Table S3), suggesting that increased concentration of these compounds might have triggered the defense mechanisms of valve closure, which prevents filtration. Moreover, the fact that a significant interaction between both factors (time and initial concentration) was observed for most compounds suggests that their removal by *C. fluminea* in real wastewater will be difficult to predict.

Interestingly, the removal of NPX at 1.0 mg.L^{-1} decreased significantly after 48 h compared to 6 h, and the same trend was observed for SMX and DIC at the concentration 0.5 mg.L^{-1}, although variations were less marked. This could be explained by excretion of the compounds via (pseudo)feces, as previously postulated for other contaminants and other bivalve species (e.g., Ismail et al., 2014). The Asian clam, as a bivalve suspension feeder, can sort edible seston from inorganic, nutritionally poor or even toxic particles (Beninger et al., 1999; Kooijman, 2006). The rejection of filtered non-edible materials results in rejection before ingestion followed by a counter-current mucociliary-assisted transport through the mantle and excretion (Beninger et al., 1999). This is a bypass to the digestive tract resulting in the outer sinking of mucilaginous masses, in the present case, in the bottom of test flasks. While these masses trap contaminants and particles filtered, when in outer medium, it is likely that aeration-induced turbulence might have caused the excreted (pseudo)feces to resuspended in the medium (Ismail et al., 2014), and release contaminants back to the water column; this would lead to an increase of its concentration in the aqueous media compared to the previous sampling times, and a consequently lower removal percentage. Desorption from the shells of living bivalves is unlikely to have contributed to the decreased removal after longer periods. The removal of the adsorbate from water will increase up to the point when the maximum adsorption capacity is achieved, remaining stable as long

as the test conditions are kept unmodified (Murphy et al., 2023), which was the scenario in the experiments.

The accumulation of ionizable compounds in aquatic species is commonly related to the physicochemical properties of the compounds, namely log Dow (e.g., Ismail et al., 2014; Meador et al., 2017). Ismail et al. (2014) reported increased accumulation by bivalves for compounds with log Dow higher than 1. In the present study, there was no significant correlation between log Dow and removal percentage (Table S5), but only two (CBZ and FXT) out of nine compounds tested have a log Dow higher than 1 (Table S2). Considering all tested compounds, no significant correlation was found between the molar mass and the removal percentage (Table S5). However, excluding DIC and FXT, which have molar mass above 300 g mol^{-1} (Table S1), a significant correlation was found, stronger for the removal % after 48 h of exposure to the compounds at the initial concentration of 1.0 $mg.L^{-1}$ ($\rho = -0.982$, $p = 8.06 \times 10^{-5}$, $n = 7$; Table S5). For these 7 compounds, which have a molar mass in the range 150–253 g mol^{-1}, removal can be feasibly predicted based on a linear inverse relationship with molar mass (Fig. 2). A negative effect of increasing molar mass on the bioaccumulation of pharmaceuticals and personal care products in the freshwater mussel *L. costata* was previously reported by de Solla et al. (2016). The results here reported are in good agreement with that study and are a consequence of the difficulty of large molecules, with higher molar mass, to cross biological membranes.

3.3. Removal of contaminants by biosorption

The removal percentage of the compounds by the *C. fluminea* milled shells was low, with only CAF, FXT and NPX showing removal percentages above 25 % for both tested concentrations (Table 1). The highest removal was observed for CAF, which achieved values of 62 % (±3 %) and 49 % (±4 %), respectively at 0.5 and 1.0 $mg.L^{-1}$. The compounds NPX and FXT showed moderate removal (26–43 %), whereas the remaining compounds were removed by no more than 6 %. Increasing the concentration led to a statistically significant decrease on the removal percentage of CAF, CBZ and MET (Table 1), which suggests that the adsorbent might be closed to saturation at these concentrations. An increased adsorption capacity with increased concentration is observed for most compounds, but it is statistically significant only for CAF (Table 1; Table S7). This denotes that, apart from CAF, the adsorption capacity is not significantly affected by the compound concentration within the tested range due to the adsorbent saturation. For CAF, it suggests that the adsorption capacity will increase with further increasing the initial CAF concentration. It is worth mentioning that the observed removal % values are relative to the tested conditions, not reflecting the maximum adsorption capacity of the bivalve shells. Given that adsorption capacity increases with surface area, and considering that different milling processes result in differential surface areas and porosity (Thind et al., 2022), increasing the superficial area of the milled shells through an improved milling process is achievable and might worth being tried in future experiments to improve the adsorption capacity.

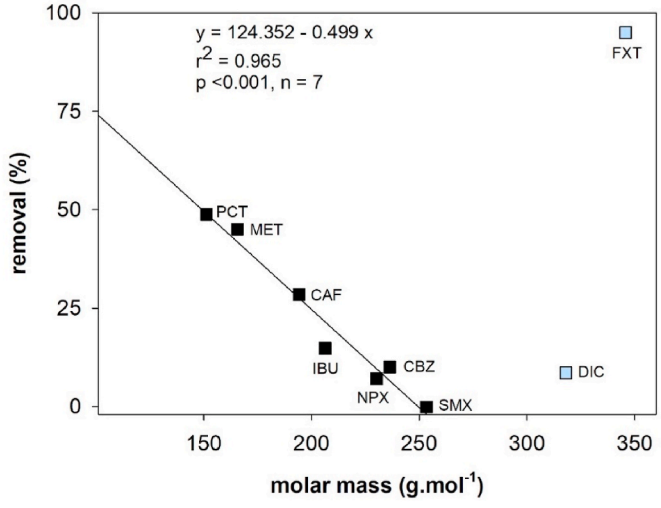

Fig. 2. Relationship between the molar mass of the tested compounds and the removal percentage by *C. fluminea* after a 48 h exposure period to an initial concentration of 1.0 $mg.L^{-1}$. The regression line and the corresponding equation refers to compounds with molar mass values in the range 150–253 g mol^{-1}. The compounds with molar mass above 300 g mol^{-1} are represented in blue (DIC and FXT).

Table 1
Removal percentage (mean ± standard deviation) and adsorption capacity (mean ± standard deviation) of the tested compounds by the *C. fluminea* milled shells after a 24 h contact time with solutions at an initial concentration of 0.5 and 1.0 $mg.L^{-1}$. Compounds with a removal percentage above 25 % are highlighted in bold. Asterisks represent statistically significant differences between both initial concentrations (details on Tables S6 and S7).

Compound	Initial concentration (mg. L^{-1})	Removal (%)	Adsorption capacity (μg.g^{-1})
CAF	0.5	**62±4** *	5.2 ± 0.3 *
	1.0	**49±4**	8.3 ± 0.4
CBZ	0.5	3 ± 1 *	0.3 ± 0.1
	1.0	1 ± 1	0.26 ± 0.2
DIC	0.5	– [a]	– [a]
	1.0	– [a]	– [a]
FXT	0.5	**42±2**	3.8 ± 0.2
	1.0	**44±13**	8 ± 3
IBU	0.5	– [a]	– [a]
	1.0	6 ± 6	1.3 ± 0.7
MET	0.5	– [a]	0.7 ± 0.3
	1.0	– [a]	– [a]
NPX	0.5	**35±14**	4 ± 2
	1.0	**26±12**	5 ± 3
PCT	0.5	– [a]	– [a]
	1.0	– [a]	– [a]
SMX	0.5	– [a]	– [a]
	1.0	2 ± 1	0.4 ± 0.2

[a] The contaminant concentration in the treated water did not differ from the concentration in the control, and thus no removal was observed.

One of the factors influencing the adsorption of ionizable compounds relates to their protonation state as well as to the surface charge of the adsorbent. The surface of the milled shells is mainly positively charged under the tested conditions (test pH was 8.17 ± 0.05), as shown by the PZC results, hence promoting electrostatic attraction with negatively charged chemical species (DIC, IBU, NPX, SMX; Table S2). Among these compounds, only NPX was moderately removed, which suggests that electrostatic interactions are not ruling the adsorption mechanism and other interactions might play a significant role in their adsorption. For instance, the ability of the compounds to establish hydrogen bonds with the adsorbent (which is mostly constituted by calcium carbonate; Domingues et al., 2022) could play a relevant role in the adsorption mechanism in this type of systems. However, the compounds with higher number of hydrogen bond donors to establish H-bond with oxygens from calcium carbonate, such as MET (with 4) and SMX (with 3) are among the pharmaceuticals with a lower adsorption percentage/adsorption capacity. In fact, a negative correlation was observed between number of H-bond donors and adsorption, with the pharmaceuticals with the lower number of H-bond donors being the ones that were most adsorbed. Yet, the correlation coefficient is not high enough to draw solid conclusions on the implications of this fact ($\rho = -0.711$, $\rho = 0.0317$, Table S8; $r^2 = 0.506$). Other factors were also analysed such as H-bond acceptors, molecular weight, molar volume, polar and non-polar surface area of the tested pharmaceuticals and no significant correlations were found. Other approaches, such as thermodynamic studies to further explore if the adsorption is being ruled by enthalpic or entropic

processes were not considered, as a deep discussion on the adsorption mechanisms falls out of the scope of the present study, which aims to compare the removal efficiency of biofiltration and biosorption using clams and clam shells, respectively.

Bivalve shells have been successfully used to remove metals and dyes from water (e.g., Summa et al., 2022), but their efficiency for removing pharmaceuticals remains poorly studied, with the exception of two recent works that reported adsorption of the antibiotic rifampicin and an endocrine disruptor from water using shells of the bivalve *M. falcata* (Henrique et al. 2020, 2021).

The observed adsorption patterns and the relative differences among compounds are generally consistent with previous evidence. Similarly to CAF adsorption by the milled shells herein, CAF adsorption to river sediments was about 40 % after 24 h (Lin et al., 2010). The authors also reported that CAF adsorption was much higher than that of PCT. It is important to highlight that further comparisons with other organic adsorbents are impaired by the dominating role that the type of adsorbent/substrate has on the removal/adsorption of the compounds, which is even more important than the chemical properties of the compounds.

The removal efficiencies of the clams and the milled shells were compared considering the 24 h period, and the concentration 0.5 mg.L^{-1}, which is contextually more relevant. It was observed that shells were able to remove more CAF and NPX than clams (3.3- and 5.7-fold, respectively), whereas the opposite was observed for FXT and PCT, with clams removing 2.1- and 376-fold more mass than the shells (Fig. 3). For the remaining compounds, the removal is very low but, in general, clams performed better than the shells for CBZ, DIC and MET. The trend observed for the initial concentration 1.0 mg.L^{-1} is similar (Fig. S1). The higher removal of CAF and NPX by the shells compared to the clams agrees with the known low bioaccumulation of CAF and low removal of NPX by bivalves (Binelli et al., 2014; Burket et al., 2019). This suggests that adsorption to the clam shells might be playing an important role on the removal of these compounds by the bivalves, which is further promoted by the increased superficial area of milled shells compared to integer shells present in the living clams system. The higher removal of FXT and PCT by the clams rather than by the milled shells was expected based on pronounced removal and accumulation as reported in the literature (Binelli et al., 2014; Burket et al., 2019). Shell adsorption will play different roles in the removal observed in biofiltration experiments, depending on the chemical tested and its properties.

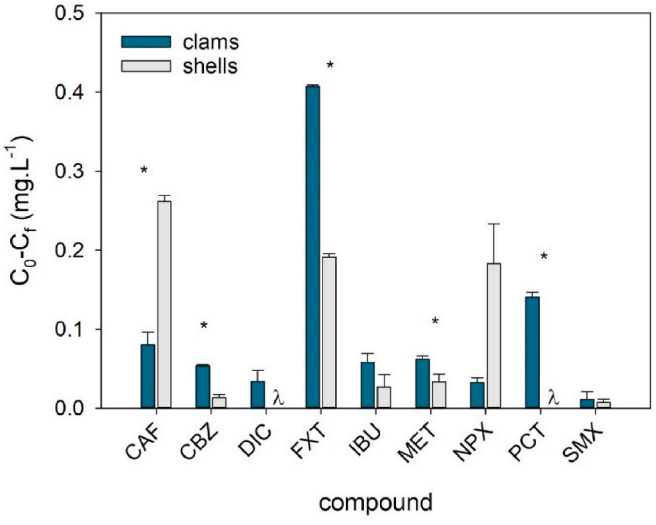

Fig. 3. Decrease on the compound concentration, expressed as the difference between the contaminant concentration in the control (C_0) and the concentration in the treated sample (C_f), after treatment by *C. fluminea* (biofiltration) and by *C. fluminea* milled shells (biosorption), during 24 h, considering a solution with an initial concentration of 0.5 mg.L^{-1}. Bars represent the mean and error bars represent the standard error. The asterisks represent statistical differences between both approaches for each compound (*t*-test; $p < 0.05$). λ: The contaminant concentration in the treated water did not differ from the concentration in the control, and thus no removal was observed.

3.4. Ecotoxicological assessment

The effects of the biofiltration treatment on the water toxicity to *R. subcapitata* and *A. fischeri* are shown in Figs. S2 and S3. Regarding toxicity to the microalgae, among the initial samples, FXT and SMX showed the highest toxicity (growth rate inhibition above 30 %; Fig. S2), which agrees with the toxicity reported to *R. subcapitata* (lowest EC_{50} values among all compounds; Table S9). Regarding toxicity to the bacteria *A. fischeri*, samples before biofiltration were low to moderately toxic, with bioluminescence inhibition mean values above 25 % only for MET (both concentrations), and for DIC, SMX and NPX at 1.0 mg.L^{-1} (Fig. S3). This is consistent with the low toxicity of the compounds to the bacteria (15 min-EC_{50} values $\geq$ 15 mg.L^{-1}; Table S10).

In the biosorption experiment, the toxicity of the untreated samples to the microalgae was also pronounced for FXT and SMX (Fig. S4), similarly to the results found for the biofiltration experiment. Regarding toxicity to the bacteria, untreated samples were barely toxic for the bacteria, with bioluminescence inhibition above 25 % only for IBU at 1.0 mg.L^{-1} (28 % inhibition; Fig. S5).

While the above-mentioned results contribute to the body of knowledge on the ecotoxicity of the tested compounds, the focus in the present study is placed on understanding whether the clams or their shells have a beneficial effect on the toxicity abatement. For this purpose, the toxicity of treated (with clams) and untreated (no clams) samples, both at the end of the 48 h experimental period, were compared and integrated in a toxicity removal endpoint (Figs. 4 and 5, respectively for the biofiltration and biosorption approaches). Biofiltration caused a very pronounced decrease in the toxicity of the FXT sample to microalgae (Fig. 4), from about 73 % to −1 % (mean values from both concentrations), reflecting a beneficial effect of this approach after 48 h. Such an effect was expected given the high toxicity of the untreated sample (Fig. S2) and the high removal of this compound by the clams (about 90 %). In opposite, biofiltration increased toxicity to microalgae concerning NPX samples. This was unexpected given the low toxicity of the untreated sample and the very low removal of NPX. We hypothesized that this toxicity increment could be due to the excretion of some compounds by the clams while exposed to NPX (no differences in microalgae growth were found between test controls and blank samples, i.e., with clams and no chemical, from the biofiltration experiment; Fig. S6) that inhibited the microalgae growth, driving the enhanced toxicity records. Moreover, besides the concentration of the target compound, abiotic factors during the microalgae growth inhibition experiments (such as light, presence of nutrients), as well as the bioremediation effect of the microalgae (Zhou et al., 2023), may interact and influence the ecotoxicological results. For the remaining compounds, the variation in the growth rate inhibition caused by the biofiltration treatment was below 20 %, thus negligible (Fig. 4). Despite the moderate removal of PCT and CAF by the clams, no evident decrease of the toxicity was observed for the treated samples, which relates to the poor resolution provided by the low toxicity of these compounds to the microalgae (Table S9). The biofiltration treatment had a mild effect on the toxicity to *A. fischeri*, causing a variation above 20 % only for MET at 1.0 mg.L^{-1} that denotes a toxicity decrease (Fig. 4). The beneficial effect for MET can be explained by its removal from the water. Despite the pronounced removal of FXT by the clams, followed by PCT, no pronounced decrease on the toxicity to the bacteria was visible, which relates to the low toxicity of these compounds towards this model species (Table S10).

The effects of the biosorption treatment on the water toxicity to *R. subcapitata* and *A. fischeri* are shown in Fig. 5 (details are provided in Figs. S4 and S5). The high toxicity of the FXT and SMX untreated

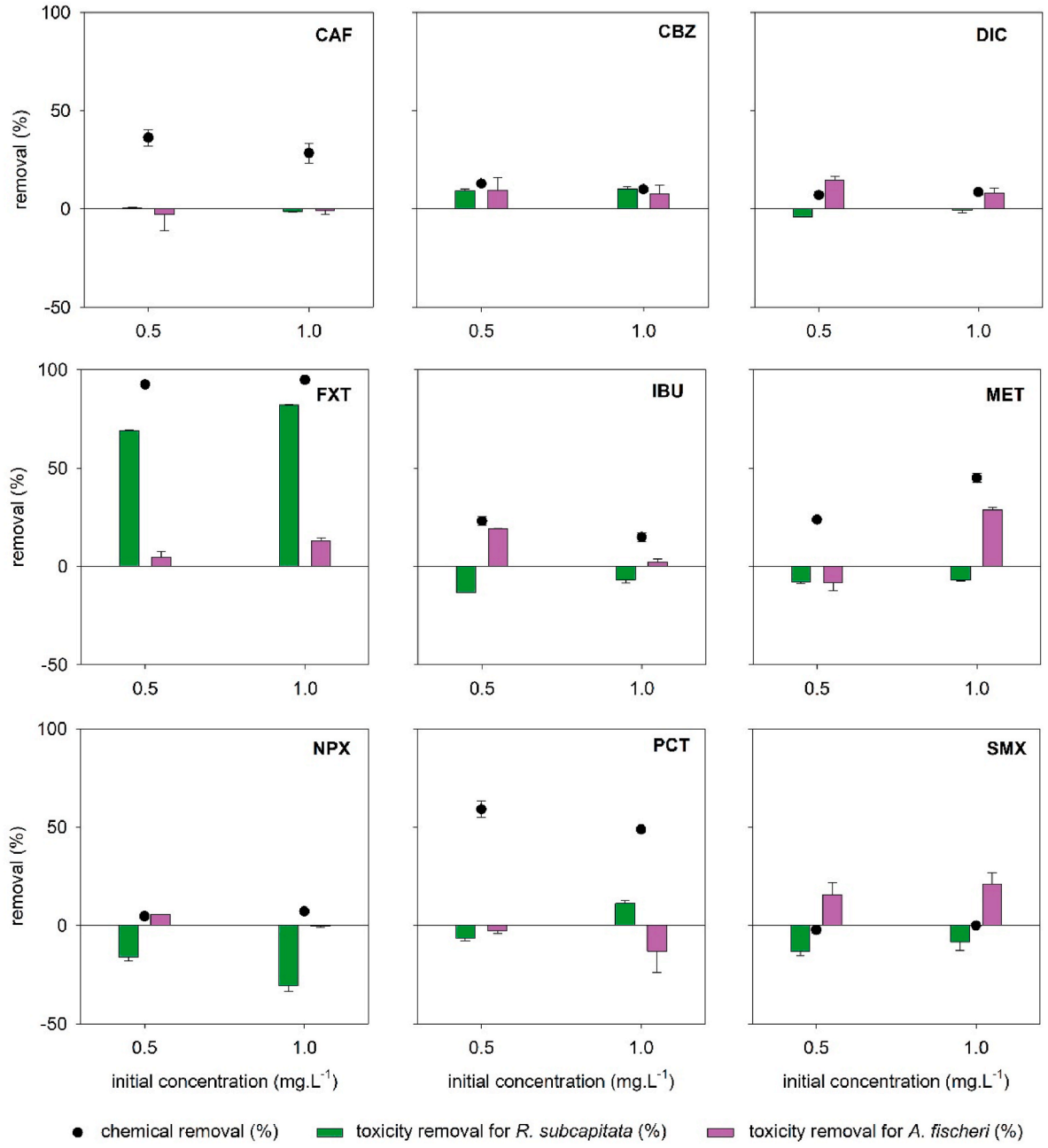

Fig. 4. Removal of the toxicity to *R. subcapitata* (measured as the variation in the growth rate inhibition, %) and to *A. fischeri* (measured as the variation in the bioluminescence inhibition, %) caused by the biofiltration treatment. Bars represent the mean and error bars represent the standard error. Removal values were obtained by subtracting inhibitions obtained with the treated samples from the mean inhibition of the untreated sample, both regarding samples collected after 48 h of biofiltration. Hence, positive variation values refer to a toxicity decrease, i.e., a beneficial effect of the biofiltration treatment, whereas negative variation values refer to a toxicity increase, i.e., an adverse effect of the biofiltration treatment. To facilitate an integrated analysis of the results, the CECs removal (%) after the same period is also presented (black circles).

samples to the microalgae (see above), did not vary pronouncedly after being in contact with the milled shells, which agrees with the negligible removal of SMX and moderate removal of FXT (Fig. 5). Regarding the latter, despite the removal about 43 %, FXT concentration in the treated samples was still high enough (compared to the low EC_{50} values - Table S9) to trigger toxicity. For the remaining compounds, the toxicity variation caused by the biosorption treatment was $\leq$ 25 %, except for NPX at 0.5 mg.L^{-1}, for which the variation was slightly higher (29 %). Regarding toxicity to the bacteria, given the low toxicity of untreated samples (see above) there is a low resolution in general to appraise the effects of the biosorption treatment on the toxicity to the bacteria, and variations below 25 % were recorded for all samples (Fig. 5).

The ecotoxicological assessment is commonly overlooked in biofiltration/biosorption studies for water treatment. In the present study we showed that, despite some unexpected minor changes in the toxicity of treated samples, there was an agreement between the removal of contaminants from water and the corresponding toxicological effect. The biofiltration treatment proved to efficiently reduce the toxicity of the samples contaminated with FXT to the microalgae *R. subcapitata* and of the samples contaminated with MET for the bacteria *A. fischeri*, whereas the biosorption treatment did not cause remarkable decreases in the toxicity of the samples to the tested microalgae or to the bacteria.

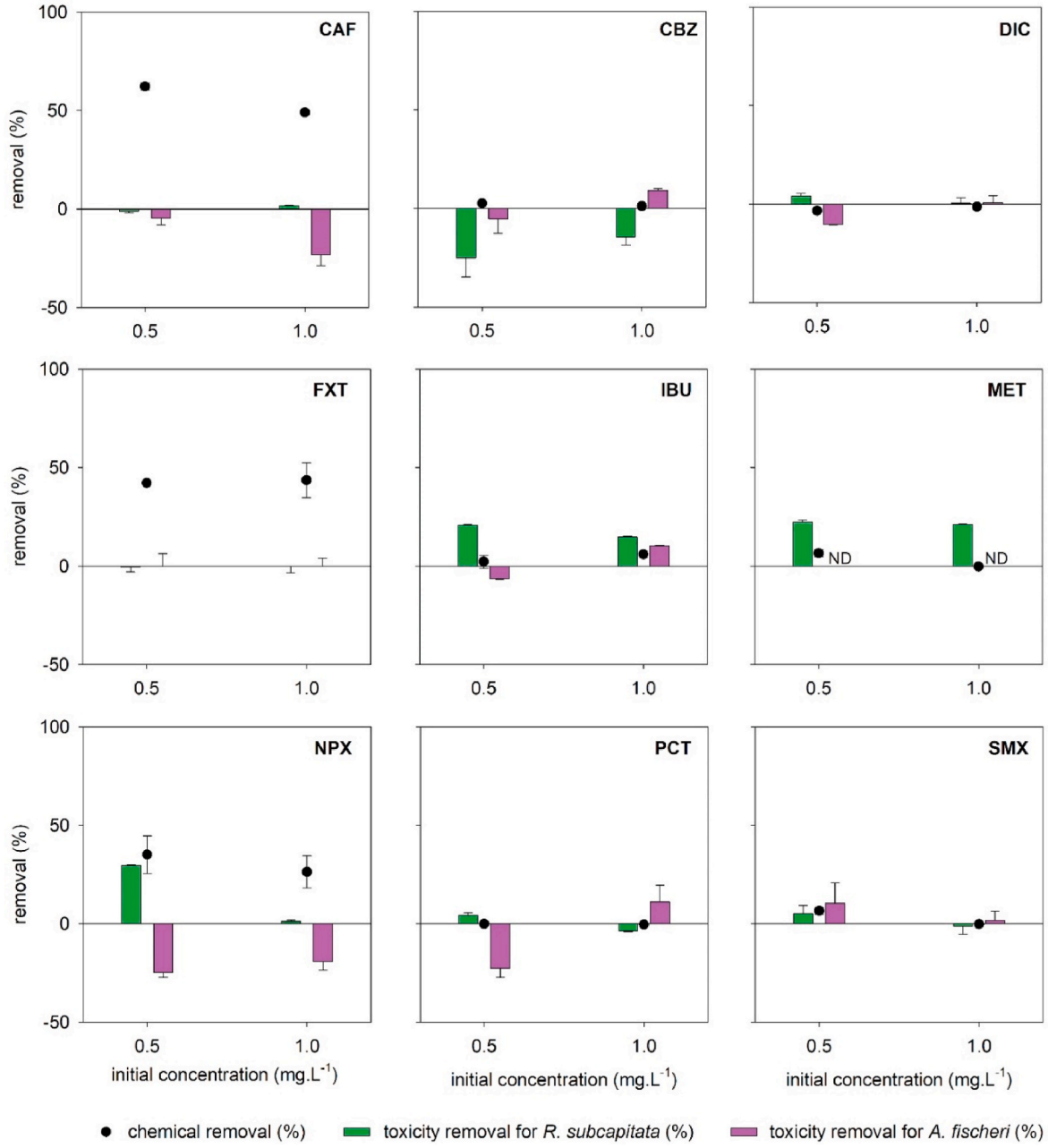

Fig. 5. Removal of the toxicity to *R. subcapitata* (measured as the variation in the growth rate inhibition, %) and to *A. fischeri* (measured as the variation in the bioluminescence inhibition, %) caused by the biosorption treatment (milled shells of *C. fluminea*). Bars represent the mean and error bars represent the standard error. Removal values were obtained by subtracting inhibitions obtained with the treated samples from the mean inhibition of the untreated sample, both regarding samples collected after 48 h of biofiltration. Hence, positive variation values refer to a toxicity decrease, i.e., a beneficial effect of the biofiltration treatment, whereas negative variation values refer to a toxicity increase, i.e., an adverse effect of the biofiltration treatment. To facilitate an integrated analysis of the results, the CECs removal (%) after the same period is also presented (black circles). ND: not determined.

In a previous study, Gomes et al. (2021) assessed the toxicity of a swine wastewater untreated and treated (4 h) by *C. fluminea* and found a decrease on the bioluminescence inhibition of *A. fischeri* (91.9 %–65.4 %) after treatment, also representing a consistency between chemical removal and toxicity reduction. However, the tested treatments treatment do not necessarily reflect in an improved water quality for all tested endpoints. Indeed, the negative removal observed for some compounds (Figs. 4 and 5), despite not very pronounced (commonly below 25 %), means that the treated samples were more toxic than the corresponding untreated samples. Such effect might be due to the production of metabolites by clams under chemical stress (e.g., Xiao et al., 2014), or by perturbation of the cells due to the presence of small particles from the milled shells (Hund-Rinke et al., 2022; Kováts et al., 2021). For instance, a previous study reported that despite filtration of wastewater by *D. polymorpha* decreased the acute toxicity of the wastewater for the mussels, chronic toxicity biomarkers showed contradictory results (Binelli et al., 2015). Such results support the unexpected toxicity variations observed in the present study, concomitantly highlithing the extreme importance of performing ecotoxicological tests to assess if the removal translates into a significant toxicity abatement.

3.5. Perspectives on the Asian clam and its shells as bioremediation tools

The ability of *C. fluminea* to remove some contaminants from water is

undeniable. This bivalve has been studied for the removal of metals from acid mine drainage (Rosa et al., 2014); phenols and amines from olive oil mill wastewater (Domingues et al., 2020); organic matter (as oxygen chemical demand) and toxicity reduction of swine wastewater (Domingues et al., 2021; Gomes et al., 2021) and winery effluents (Pipolo et al., 2017; Ferreira et al., 2018); CECs from wastewater (Ismail et al., 2014); *Escherichia coli* from wastewater (Gomes et al., 2018b) or from rivers when used simultaneously with mussels (Ismail et al., 2016); cyanobacterial blooms, used solely (Silva et al., 2020), or simultaneously with the fish species *Aristichthys nobilis* (Shen et al., 2020); as well as in the reduction of the eutrophication status of aquatic systems used simultaneously with other aquatic species (Li et al., 2010; Song et al., 2014). This species also removed organic compounds from laboratorial-prepared solutions (Ismail et al., 2014), including carbamazepine and lorazepam (Gomes et al., 2020). In the present study we reported, for the first time, the remarkable removal of FXT from water by *C. fluminea* (≥84 % after 6 h, and ≥91 % after 24 h). This suggests that biofiltration with *C. fluminea* might be considered for specific or tailored bioremediation strategies; although not common or widespread, wastewater mainly contaminated with FXT was already reported as a consequence of direct disposal from facilities handling large quantities of this pharmaceutical (Petrie et al., 2016), or possibly from pharmaceutical manufacturing facilities (e.g., Kleywegt et al., 2019).

It is hypothesized that the presence of this antidepressant pharmaceutical in the water might contribute to remove other contaminants as well, owing to the relaxation effect on the muscles and the valve-opening consequence. In addition, we showed that *C. fluminea* milled shells might also remove waterborne contaminants, even better than the clams for CAF and NPX (note that milling increased surface area for adsorption compared to the equivalent mass in living clams). Hence, the use of *C. fluminea* shells might be a potential solution for removal of these contaminants from effluents particularly rich in these compounds. However, for an urban WWTP, where a wide variety of contaminants can be found, it might be worth to test whether both approaches can be combined to retrieve the best removal efficiency. Obviously, the approach of the dry milled shells appears as more practical, economic and environmentally relevant, as the bivalves must be continuously monitored and batch-replaced to avoid reversing the benefits obtained driven by, for example, the death (natural or accelerated by toxicity of internalized contaminants) of organisms.

Using bivalve shells as adsorbents is an environmentally friendly solution which respects the basis of circular economy, reducing the challenges involved with solid waste management, improving the ecological status of invaded ecosystems and further reducing the cost of obtaining adsorbents for wastewater treatment. Still, the possibility of treating the bivalve shells, for instance by calcination or pyrolisis shoud be considered in future studies as these treatments might result in increased removal efficiency (Henrique et al., 2020). In the present study, the target contaminants were tested individually, since model systems with single compounds are more appropriate as a ground work enabling discussion of the processes involved. Moreover, tests were performed for concentrations above those found in WWTP effluents. With such baseline information acquired, future studies examining biofiltration *vs*. biosorption alone should be carried out using a real wastewater sample, representing environmentally relevant conditions. In a real wastewater sample, a wide variety of contaminants are expected to be present at $ng.L^{-1}$ to $\mu g.L^{-1}$ levels along with other constituints at much higher concentrations (e.g., dissolved organic matter), and different physicochemical conditions of the aqueous medium. All these factors affect both biofiltration and biosorption processess, a scenario that needs to be conspicuously studied before considering the application to upscaled settings.

4. Conclusions

This study aimed to compare the efficiency of *C. fluminea* and the corresponding milled shells for the removal of nine common wastewater contaminants. After 24 h, clams removed mainly FXT (≥91 %) and, to a moderate extent, PCT (≥26 %), with removal being inversely related to molar mass (for compounds with molar mass in the range 150–253 g mol^{-1}). Both the initial concentration of the compounds and contact period generally affected removal by biofiltration. Milled shells (at a dose of 50 $g.L^{-1}$) removed mainly CAF (≥49 %), FXT (≥42 %) and NPX, after 24 h of contact, with higher initial concentration promoting further adsorption for CAF. Comparing the living clams with their milled shells it was observed that clams were more efficient on removing FXT, PCT, CBZ, DIC and MET whereas the opposite was observed for CAF and NPX. Despite clams and milled shells being effective on removing some compounds, only few advantageous effects were observed on toxicity abatement of the treated water samples. The highest toxicity reduction was observed for the microalgae exposed to the biofiltered FXT sample, matching the highest removal % observed in the present study and thus confirming the beneficial effect of *C. fluminea* on the quality of water contaminated with this compound. The low toxicity of the remaining compounds and/or their low-moderate removal percentage by clams or shells constrained the resolution of the ecotoxicological assessment. This and the differential sensitivity observed herein between microalgae and bacteria highlight the need to consider different model species to improve the discriminatory power of the ecotoxicological tests applied to the assessment of bioremediation efficiency.

Overall, the biofiltration treatment performed better than biosorption, allowing a remarkable removal of FXT and a consequent pronounced toxicity reduction to the microalgae. However, due to the requirements for clams' maintenance and the management requirements to prevent the spreading this invasive species in non-native areas, this solution is hardly feasible, unless a very specific scenario of major contamination with FXT is on stage. Future experiments should address the improvement of the adsorption capacity of the milled shells, through e.g., calcination or pyrolysis, targeting the use of this inexpensive and widely available material, potentiated by their wide geographic distribution and the ecological benefits of removing them from impacted ecosystems.

CRediT authorship contribution statement

Fátima Jesus: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Érica Pascoal:** Investigation, Formal analysis. **Érika M.L. Sousa:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Diogo Mantas:** Investigation, Formal analysis. **Mariana Sousa:** Investigation, Formal analysis. **Bárbara M.C. Vaz:** Writing – review & editing, Methodology, Investigation. **Fernando J.M. Gonçalves:** Writing – review & editing, Resources. **João A.P. Coutinho:** Writing – review & editing, Resources. **Sónia P.M. Ventura:** Writing – review & editing, Resources. **Vânia Calisto:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Joana Luísa Pereira:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Data curation, 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 A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jenvman.2025.128039.

Data availability

The full dataset concerning the assessment of compounds removal by clams and milled shells, along with corresponding metadata are available in the institutional repository DUnAs (University of Aveiro), at https://doi.org/10.48527/DEP8ML.

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