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Accumulation and metabolization of the antidepressant venlafaxine and its main metabolite *o*-desmethylvenlafaxine in non-target marine organisms *Holothuria tubulosa*, *Anemonia sulcata* and *Actinia equina*

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ABSTRACT

The assessment of exposure to the antidepressant venlafaxine and its major metabolite *o*-desmethylvenlafaxine in *Holothuria tubulosa*, *Anemonia sulcata* and *Actinia equina* is proposed. A 28-day exposure experiment (10 μg/L day) followed by a 52-day depuration period was conducted. The accumulation shows a first-order kinetic process reaching an average concentration of 49,125/54342 ng/g dw in *H. tubulosa* and 64,810/93007 ng/g dw in *A. sulcata*. Venlafaxine is considered cumulative (BCF > 2000 L/kg dw) in *H. tubulosa*, *A. sulcata* and *A. equina* respectively; and o–desmethylvenlafaxine in *A. sulcata*. Organism-specific BCF generally followed the order *A. sulcata* > *A. equina* > *H. tubulosa*. The study revealed differences between tissues in metabolizing abilities in *H. tubulosa* this effect increases significantly with time in the digestive tract while it was negligible in the body wall. The results provide a description of venlafaxine and *o*-desmethylvenlafaxine accumulation in common and non-target organisms in the marine environment.

1. Introduction

Pharmaceuticals active compounds (PhACs) include a diverse and wide group of chemical substances used for the treatment and prevention of diseases in human and animals (Badawy et al., 2023). There are >4000 substances with different chemical and therapeutic properties that have greatly improved global public health in recent decades (Duarte et al., 2023; Fonseca et al., 2021). However, their high consumption means that the excretion of these drugs from the human body contributes to their release into wastewater, allowing them to enter the environment and more specifically the marine ecosystem, which has led to them being considered a group of emerging pollutants (EPs) of high concern (Archer et al., 2023; Antonopoulou et al., 2022).

Among the PhACs, antidepressants are a family of drugs widely prescribed to treat depression, anxiety and even chronic pain (Zheng et al., 2023). The antidepressants consumption was doubled between 2000 and 2017 in OECD countries and this trend will probably continue to increase (OECD Health Statistics, 2019; Fabbri et al., 2023). Thus, gaining further insight into their bioaccumulation capacity is critical to prioritize chemicals and evaluate their chronic risks to non-target organisms. Specifically, venlafaxine (VFX) and *O*-desmethylvenlafaxine (O-VFX) belong to the family serotonin-norepinephrine reuptake inhibitors (SNRIs) and they are two of the most widely used treatment for early-onset depression (Ahmadimanesh et al., 2020). According to the Decision 2022/1307/EC, the European Commission has included both compounds in a watch list of substances as emerging pollutants (European Union, 2022). These drugs are long-term treatments and are classified as genotoxic and carcinogenic, and it is therefore appropriate to assess their environmental toxicity (Castillo-Zacarías et al., 2021). VFX has become an environmental pollutant because of its wide consumption and incomplete degradation in wastewater treatments (Golovko et al., 2020; Gomes-Moreira et al., 2022; Boulard et al., 2020; Solaun et al.,

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2022).

In recent years, biomonitoring strategies have focused on the use of bioindicators to assess the impact of these compounds in marine ecosystem (Chahrour et al., 2021). Bioconcentration is the accumulation of chemicals in an organism that is constantly exposed to the contaminated environment. The bioconcentration factor (BCF) is a term used to assess the amount of accumulated contaminants in an organism in relation to the environment (Nikokherad et al., 2022). Absorption of chemical compounds by marine organisms can take place through various pathways, such as the dermal, gut, gill or pulmonary surfaces (Chidya et al., 2022). There are several studies of bioconcentration of neuroactive pharmaceuticals in aquatic organisms, e.g. Qu et al. (2019) evaluated the BCF of VFX and its metabolite in loach with values between 0.04 and 0.14 L/kg, or Maulvault et al. (2018), Grabicova et al. (2014) and Lajeunesse et al. (2011) studied the bioconcentration of different antidepressants in fish species such as rainbow trout, loach or brook trout. Other studied have focused in the bioconcentration in bivalves (Serra-Compte et al., 2018; Gomez et al., 2021) obtaining BCFs between 213.3 and 528.1 and 265 L/kg, respectively. The importance of this type of study lies in understanding the behavior of animals in controlled laboratory experiments in order to be able to know the potential effects of the presence of these pollutants in nature and the harm or risk they may cause on a large scale (Alvarez-Mora et al., 2022). However, to the best of our knowledge, an important gap within marine environment is that the accumulation pattern of PhACs has been studied only in a few of the many species that exist.

Cotton spinner sea cucumber *(Holothuria tubulosa)* belongs to the class Holothuroidea, echinoderms found in almost all marine environments (Künili and Çolakoglu, 2019). They probe to be ideal bioindicators for biomonitoring EPs since its feeding mode consists of ingesting sediments from which it extracts organic matter (Bulleri et al., 2021). Some studies have found the presence of pollutants in different body compartments such as the digestive tract or gonads (Martín et al., 2020), suggesting that higher trophic levels can be reached. On the other hand, snakelocks anemone *(Anemonia sulcata)* and beadlet anemone (*Actinia equina*) are sessile organisms that can be affected by the presence of pollutants in the environment they inhabit, which also makes them interesting for the study of bioaccumulation of PhACs and as informants of the state of the water (Morais et al., 2020).

In this context, the present study was conducted to investigate the bioconcentration of the antidepressant VFX and its metabolite in non-target but common organisms found in the marine environment, exposed under controlled laboratory conditions. The study aimed to determine the bioaccumulation kinetics and their BCF in sea cucumber, snakelocks anemone and beadlet anemone. Selected species are excellent sentinels for monitoring bioavailable contaminants as can reflect changes in the concentration of the contaminant from the surrounding environment, accumulate contaminants without being seriously affected by the concentrations as well as are considered representative and abundant in the marine environment (OSPARCOM, 2012).

2. Materials and methods

2.1. Chemical and reagents

Ultrapure water (18.2 MΩ-cm) was obtained by using a Milli-Q Plus® system (Millipore, Madrid, Spain). Analytical-grade formic acid (≥98 %) used as mobile phase additive (Honeywell, Charlotte, USA). LC-MS grade methanol (MeOH) was purchased from VWR Prolabo CHEMICALS (Barcelona, Spain). VFX (≥98.0 %) and O-VFX (≥98.5 %) were purchased by Sigma Aldrich (Madrid, Spain). Cinchophen (CIN, surrogate standard) was provided from Alfa Aesar (Massachusetts, MA, USA). Chemical structures and the physicochemical properties of the analytes are shown in Table S1. Nitrate, nitrite and ammonium tests were conducted with Hanna instruments® test kits (HI 3874, 3873 and 3826, respectively). Stock solutions were prepared in MeOH and stored at −20 °C until use.

2.2. Laboratory test

2.2.1. Specimens' collection and acclimation period

The specimens under study were captured in February 2022 by divers at a depth of 10–15 m at random locations along the coast of Granada (Spain). Then, they were transported in refrigerated containers, at 4 °C, to the laboratory facilities where the experiment was carried out. They were then placed in the experimental tanks and left to acclimatize and depurate for a period of 15 days.

2.2.2. Up-take and depuration test

The study was developed at the Zoology Department of the University of Granada (Spain), where a 300 L glass tank containing recirculating natural seawater was installed with artificial illumination by a 6000 K metal halide light bulb. The system was filtered with a biological filter with foam rubber and bioballs and aeration was performed with compressed air. Photoperiods of 10 h of light and 14 h of darkness were carried out. During the experiment, the physicochemical characteristics of the water were checked two times a week, with mean values of temperature 19.7 ± 0.2 °C, pH 8.20 ± 0.01, salinity 37.5 ± 0.2 PSU, dissolved O_2 6.3 ± 0.1 mg/L, ammonium 0.20 ± 0.03 mg/L, nitrites 0.23 ± 0.01 mg/L, nitrates 51 ± 3 mg/L.

Feeding. A spoonful containing a mixture of fishmeal and dried spirulina (1:1) was added to the aquarium bi-weekly to feed the animals.

Experimental design. The bioconcentration assay (exposure concentration, exposure time and depuration time) was designed following the OECD 305 technical guidance on aqueous exposure for testing chemicals (Gomez et al., 2021; Maulvault et al., 2018; Molina-Fernandez et al., 2017; Molina-Fernández et al., 2021; OECD, 2012). A low exposure concentration was used, 1 % of LC_{50}, according with the OECD 305 method (LC_{50} 10–16 mg/L in algae, fish and *Daphnia*) (Golovko et al., 2020). During the first 28 days of the experiment (uptake), after acclimation of the specimens over two weeks, 3 mg of each compound was added daily. As the volume of the tank was 300 L, the final concentration added was 10 μg/L per day. Every four days, sample specimens were collected from the tank. In the case of sea cucumber, body compartments (body wall and digestive tract) were separated. After day 28, all remaining specimens were moved to a new tank with the same characteristics as the previous one, starting the depuration period, which took place for 52 days, with a total duration of 80 days for the experiment. In the same way, samples were collected every 4 days and the same pre-treatment of the specimens was carried out, which consisted of freeze-drying and pulverization. All samples were stored at −20 °C until analysis.

Sample collection. In each sampling, 3 sea cucumbers, 3 snakelocks anemones and 2 beadlet anemones were collected as well as 100 mL vessel containing sediments and water. Digestive tract and body wall were separated from the sea cucumbers, and all samples were stored in plastic measuring specimen cup sterile containers (Molina-Fernández et al., 2021; Nunes et al., 2020; Martín et al., 2017a,b; Chen et al., 2015; Du et al., 2015). Once in the laboratory, samples were freeze-dried except for the water, which was frozen at −20 °C. Freeze-drying took place for 48 h at −109 °C. After drying, the samples were first pulverized with a mortar and pestle to reduce the particle size as much as possible, and then with a ball mill for 15 min with a frequency of 25.0 s^{-1}. Then, they were stored at −20 °C until analysis.

2.3. Analytical methods

Animal and sediment samples were aliquoted (0.2 g dw) and weighed into 10 mL glass tubes together with a methanolic solution of surrogate (CIN), obtaining a final concentration of 200 ng/g in the sample. The samples were left in the dark for 24 h to ensure contact between the matrix and the internal standard. Subsequently, 2.2 mL of

MeOH were added, vortexed for 30 s and an ultrasound-assisted extraction (UAE) was performed for 12 min at 40 % amplitude. The samples were then centrifuged for 5 min at 4000 rpm and the supernatant was removed to another tube. This procedure was performed in duplicate, mixing the collected supernatants. Afterwards, the extracts were dried under a N_2 stream. The dried residue was reconstituted in an 80/20 (v/v) H_2O/MeOH solution to a final volume of 0.2 mL and 2 μL were injected into the LC apparatus.

Water samples were collected in 100 mL sterilized water bottles. Aliquots of 10 mL of sample were separated and 50 μL of a methanolic solution of CIN was added to obtain a final concentration of 200 ng/mL of the internal standard. The sample was then frozen and freeze-dried for 48 h at −109 °C. After drying, 1 mL of MeOH was added and vortexed for 1 min. Subsequently, it was centrifuged for 5 min at 4000 rpm and the supernatant was collected. The procedure was repeated and the supernatants mixed and then evaporated under a N_2 stream. After drying, the same reconstitution procedure was performed as for the solid matrices.

Liquid chromatography-tandem mass spectrometry analysis was performed using an ACQUITY HSS T3 column (100 mm × 2.1 mm internal diameter, 1.8 μm particle size). The compounds were separated using a mobile phase gradient consisting of an aqueous buffer with 0.05 % formic acid (v/v) as phase A and MeOH as phase B. The method was carried out in 8 min with the following gradient program: 0–1.5 min, isocratic gradient 80 % of A. 1.5–3 min, linear gradient from 80 % to 60 % A, 3–4 min, linear gradient from 60 to 15 % A, and 4–6 min, isocratic 15 % A. 6–6.1 min, return to initial conditions for 2 min in order to conditioning for the next injection. Two multiple reaction monitoring (MRM) transitions were selected for each analyte, the first one for quantification, and the second one for confirmation. All these data are shown as supplementary material together with the spectrometer parameters (Table S2). The mass spectrometer was operated in positive ESI mode. The method was successfully validated according to the ICH guideline (ICH Quality Guidelines, 2005) in terms of linearity, range, sensitivity and accuracy (trueness and precision). The validation process is explained in Supplementary Information (Validity requirements). Table S3 summarizes the limits of detection and quantification, as well as linearity and linear dynamic range. Accuracy assays showed recoveries close to 100 % for all compounds and matrices, with standard deviations below 15 % in all cases, which proves that the method is accurate.

2.4. Bioconcentration factor calculation

The general aquatic bioaccumulation model described in OECD (2012, Annex 5) was followed. Bioaccumulation can be described in terms of uptake and loss processes, ignoring uptake with food. Two different forms were used. The first involves the relationship between measured concentration levels in water and in biota at steady state (SS) using Eq. (1):

$$BCF_{SS} = \frac{C_b}{C_w} \quad (1)$$

C_b is the concentration in the biota at ng/g dry weight (dw) and C_w is the concentration in the water at ng/mL, both measured at the same time. The steady stated was estimated at 28 days in all cases. Moreover, the C_w was kept within 20 % of the measured mean (n = 3). Two different parts of the organisms (body wall and digestive tract) were considered in sea cucumber, while for anemones the whole body of the organism was considered.

The second way to calculate BCF is by the kinetic parameters, the uptake rate constant (k_1) and the clearance rate constant (k_2). The differential equation describing the rate of change in animal concentration (ng/g·d) is described mathematically as a first order process (Mackay and Fraser, 2000) with Eq. (2):

$$\frac{dC_b}{dt} = k_1 C_w - k_2 C_b \quad (2)$$

With the integration

$$C_b = \frac{k_1}{k_2} C_w \left(1 - e^{-k_2 t}\right) + C_{b,0}\left(e^{-k_2 t}\right) \quad (3)$$

$C_{b,0}$ is the concentration when the depuration phase starts (ng/g dw), and t the exposure time (days).

For the depuration phase, C_w is assumed to be zero and Eq. (3) can then be reduced to:

$$C_b(\text{depuration}) = C_{b,0} e^{-k_2 t} \quad (4)$$

By performing a linear regression of Ln (concentration) versus time, it can be obtained k_2 from the slope of the regression line. And, 50 % purification will then be reached at time ($t_{1/2}$):

$$t_{1/2} = \frac{ln2}{k_2} \quad (5)$$

Then, k_1 was calculated as follows:

$$k_1 = \frac{C_b k_2}{C_w(1 - e^{-k_2})} \quad (6)$$

The BCF through the kinetic parameters was calculated as:

$$BCF_k = \frac{k_1}{k_2} \quad (7)$$

Note that BCF_k should be equal to BCF_{ss}, although deviations may occur if the steady state was uncertain or if growth corrections have been applied to the kinetic BCF. Since k_1 and k_2 are constants, it is not necessary to reach steady state to obtain a BCFk.

2.5. Quality and exposure control

Before the experiment VFX and its metabolite were measured in the water and sediment of the aquaria as well as in control organisms (N = 5). Organisms' mortality in the experiment was lower than 1 % for all species. The condition index (control and contaminated) remained stable, with low variability, being thus optimal for studied animals during the experiment.

To guarantee the quality assurance of the results, a protocol involving the use of control samples including fortified organisms samples (at 100 ng/g dw), a mixture of the standards of the target compounds in pure solvent (at 100 ng/mL), solvent (methanol:water 50:50 v/v) and procedural blanks injections were included in each analytical batch. For quantification purposes, eight-point matrix-matched calibration curves were prepared containing VFX and O-VFX in the range from method quantitation limit (LOQ) to 500 ng/g dw.

3. Results

3.1. Bioconcentration in cotton spinner sea cucumber

The mean concentrations measured, with their relative standard deviation, in water and sediment samples during the experiment are shown in Table 1.

The concentration levels measured for VFX and O-VFX before the first addition of compounds to the experimental system (t = 0) were 2.40 and 4.59 ng/mL in the water phase, and 0.38 and 2.97 ng/g dw in the sediments, respectively. These concentrations are negligible compared to those added during the exposure period. The concentration levels measured during the exposure period were maintained relatively stable and meet the 20 % maximum variation commitment of OECD 305. Similar variations have been observed in other comparable studies in which the concentration was monitored during the experiment (Molina-Fernández et al., 2021; Molina-Fernandez et al., 2017).

Table 1
Mean concentration and relative standard deviation of VFX and O-VFX in the aqueous phase and in the sediments throughout the experiment.

Sampling (days)	Water phase				Sediments			
	VFX		O- VFX		VFX		O- VFX	
	Mean (ng/mL)	RSD (%)	Mean (ng/mL)	RSD (%)	Mean (ng/g)	RSD (%)	Mean (ng/g)	RSD (%)
0	2.40	11	4.59	5	0.38	16	2.97	12
4	11.0	10	2.42	1	62.1	3	63.4	11
8	19.4	13	29.5	8	86.0	2	65.2	9
12	14.3	5	4.20	3	88.2	12	83.2	10
16	11.7	4	35.9	5	189	3	158	2
20	26.8	6	39.9	6	171	6	188	2
24	17.7	3	30.8	6	236	2	212	9
28	15.7	8	29.4	4	227	10	214	9
32	8.32	8	7.45	13	56.5	4	29.4	5
36	4.25	1	<LOD	–	41.0	12	9.15	2
40	3.94	16	6.34	9	36.4	9	13.2	8
44	2.46	11	<LOD	–	35.3	5	14.9	13
48	3.37	14	7.57	8	29.1	1	15.7	5
52	2.24	2	6.95	1	23.9	4	14.6	13
56	3.50	1	7.32	13	64.7	5	17.0	6
60	1.10	12	5.07	3	19.1	9	9.26	7
70	0.55	6	<LOD	–	26.7	14	12.6	8
80	<LOD	–	<LOD	–	8.90	8	5.67	4

<LOD: below the limit of detection.

Overall, VFX and O-VFX were detected at higher concentrations in the sediment compartment than in water samples, which suggest that sediments may be an important source of selected compounds for benthonic biota like is the case of sea cucumber. The experimental partition factor between the marine sediment and the water, calculated as the ratio between the concentration measured in both compartments was 1.16 and 0.86 L/kg (log K_d) for VFX and O-VFX, respectively (in concordance with their octanol-water partition coefficient (log Kow 3.2 and 2.7 for VFX and O-VFX, respectively)). To our knowledge, this is the first K_d data reported in marine sediments. The calculated values are similar or slightly lower (1.21–2.19) to those reported by Osorio et al. (2016) and Boulard et al. (2020), who studied VFX sorption in Iberian and German Rivers, respectively. Some higher values up to 3.2 have been published in water and sediment from Lake Malaren, Sweden (Golovko et al., 2020). VFX was only found at one of three locations at concentration ranges between 2.8 and 7.8 ng/g dw while O-VFX was quantified at all sampling locations (up to 3.7 ng/g dw). Authors concluded that O-VFX has stronger persistency than its parent compound. The accumulation and depuration profile of VFX and O-VFX in sea cucumber are drawn in Fig. 1.

In the uptake phase, the concentrations kept increasing, and a rapid uptake was observed. After 28 days, the concentration of VFX and O-VFX reached as high as 40,823 and 54,342 ng/g dw, respectively, in the digestive tract and 11,577 and 12,870 ng/g dw, respectively, in the body wall. These concentrations were almost 100-fold higher than those found in the water and sediment phase. To understand the tissue distribution in sea cucumbers, the percentages of antibiotic concentration in the gastrointestinal tract and body wall were calculated after 28 days of exposure, as shown in Fig. 3.

VFX and O-VFX were predominantly concentrated (>75 %) in the digestive tract of *H. tubulosa* in comparison with the body wall. Both compounds showed similar accumulation patterns, however, at the initial stage of the experiment the concentration measured for VFX was higher than O-VFX, while along days the order was reverted in the digestive tract which can be explained by a possible metabolization of VFX. Meanwhile, during the depuration phase, the concentrations of VFX and O-VFX showed a slow drop. Up to 17 days were needed to eliminate half of the amount ($t_{1/2}$) in the digestive tract and even a longer time (30 and 20 days for VFX and O-VFX, respectively) was estimated in the body wall, probably explained by the low metabolization and degradation in this tissue. At the end of the experiment, after 52 days of depuration, concentrations in *H. tubulosa* were 8982 and 12,257 ng/g dw for VFX and O-VFX, respectively, in the digestive tract and 1231 and 1258 ng/g dw for VFX and O-VFX, respectively, in the body wall. Table 2 summarizes the kinetic parameters after adjusting to the first-order kinetic model.

The accumulation rate constants (k_1) were relatively high, especially in the digestive tract 122 and 103 L/kg·d for VFX and O-VFX, respectively, while the contrary effect was observed for depuration constants (k_2) with a rate of were 0.04 and 0.06 1/d for VFX and O-VFX, respectively. The estimated BCF_k varied slightly between studied tissues, been notably higher in the digestive tract in comparison with the body wall pointing out the absorption (by diet) as the main up-take route of studied compounds. The BCF was also calculated at the steady stated,

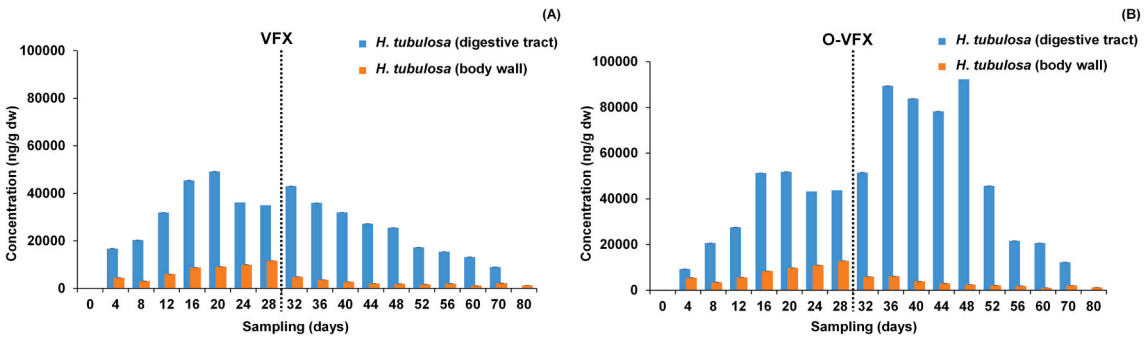

Fig. 1. Kinetics of accumulation (days 0–28) and depuration (days 32–80) of VFX (A) and O-VFX (B) in sea cucumber (digestive tract and body wall).

Table 2
Kinetic data of VFX and O-VFX in studied animals.

Compound	k_2 (1/d)	R^2 (k_2)	$t_{1/2}$ (d)	k_1 (L/kg d)	R^2 (k_2)	$BCF_{kinetic}$ (L/kg)	BCF_{ss} (L/kg)	BCF_{ss} sed
H. tubulosa (digestive tract)								
VFX	0.041	0.941	17	121.7	0.686	2947	1831	287
O-VFX	0.053	0.905	13	103.8	0.823	1515	1298	275
H. tubulosa (body wall)								
VFX	0.023	0.608	30	21.74	0.688	949	738	51
O-VFX	0.033	0.716	20	14.32	0.735	431	437	60
A. sulcata								
VFX	0.027	0.808	26	125.4	0.784	4728	4133	286
O-VFX	0.042	0.727	16	100.1	0.688	2392	3160	434
A. equina								
VFX	0.051	0.799	13	106.9	0.834	2080	2307	160
O-VFX	0.055	0.558	13	83.92	0.816	1522	1837	252

k_1: first-order uptake constant; k_2: first-order elimination rate constant; R^2: Determination coefficient of linear representations; BCF_k: bioconcentration factor based in kinetic parameter; BCF_{ss}: bioconcentration factor based in concentration measured at the steady state; BCF_{ss} sed: bioconcentration factor in the sediment compartment.

estimated after 28 days, getting BCF_{SS} and BCF_k for comparison purposes. Some slightly differences could be observed between the two ways of calculation, probably explained because the steady state was not ready reached. The 305 guideline in 2002 suggests to use toxicokinetic models as the first-order two-compartment model to estimate the BCF if the steady state is not clearly reached. However, kinetic model is not commonly applied in the previous reported studies (Molina-Fernández et al., 2021).

3.2. Bioconcentration in snakelocks anemone and beadlet anemone

The accumulation and depuration profile of VFX and O-VFX in snakelocks anemone and beadlet anemone are drawn in Fig. 2.

The bioaccumulation pattern in anemones showed a similar trend that in the digestive tract of sea cucumber. VFX and O-VFX were detected in control anemones samples (not exposed) at concentration levels between 17 and 24 and 116–661 ng/g dw, respectively. Both compounds bioaccumulated, increasing their concentration with the increasing of the exposure time, resulting in 64,810 and 93,007 ng/g dw uptake for VFX and O-VFX, respectively, in snakelocks anemone and in 36,176 and 54,054 ng/g dw uptake for VFX and O-VFX, respectively, in beadlet anemone after 28 days of exposure. This phenomenon suggests the latent high accumulation of these compounds in anemones without being seriously affected.

On the other hand, depuration was very slow for both compounds. Within 26 and 16 days, the concentration in whole organisms decreased 50 % of the initial at the depuration phase in snakelocks anemone for VFX and O-VFX, respectively, and a few days less (13) in beadlet anemone. As is shown in Table 2, the calculated BCF of VFX and O-VFX varied slightly between organisms. This might be due to the different capacities to accumulate and/or metabolize compounds of the different species. In fact, at difference of *A. equina*, *A. sulcata* is an endosymbiotic species that interchanges nutriments and metabolites with its endosymbiotic microalgae (Stambler and Dubinsky, 1987).

4. Discussion

Considering REACH classification that estimates BCFs >2000 L/kg for cumulative compounds, VFX would be considered as such (2947, 4728 and 2080 L/kg dw in *H. tubulosa*, *A. sulcata* and *A. equina*, respectively) or O-VFX (2392 L/kg dw) in *A. sulcata* and consequently they may represent a potential risk in the marine environment (Molina-Fernández et al., 2021). Then, sea cucumber and anemones represent interesting models for studying bioconcentration.

On the one hand, the anemones studied are sessile organisms (like mussels) and can be associated with a specific place, unlike fish. Indeed, as they are secondary or tertiary consumers (they feed on carrion or animals that may be herbivores -primary consumers- or carnivores -secondary consumers-), they may have a greater capacity for bioconcentration of each trophic level. Sea cucumber although is not sessile, it is sedentary with low mobility and it can also be associated with a specific place because its locomotion capacity is much reduced. In this case, it is a detritivorous deposit-feeding animal that obtains food particles by ingesting the surface layer of the marine sediment on which it crawls and lives and where it accumulates (Palmer et al., 2022; Jiang et al., 2015; Martín et al., 2017a,b; Sugni et al., 2007). Although

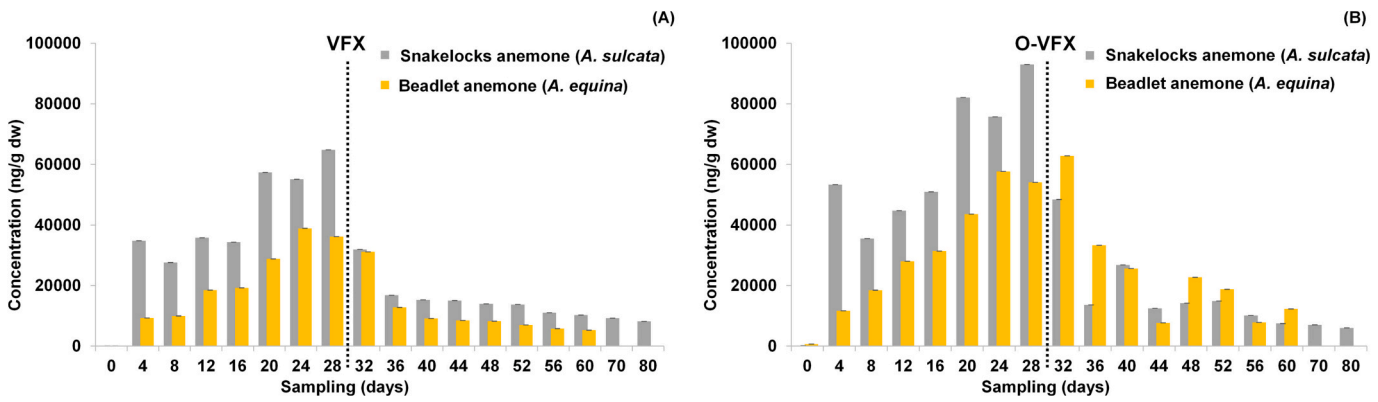

Fig. 2. Kinetics of accumulation (days 0–28) and depuration (days 32–80) of VFX (A) and O-VFX (B) in snakelocks anemone and beadlet anemone.

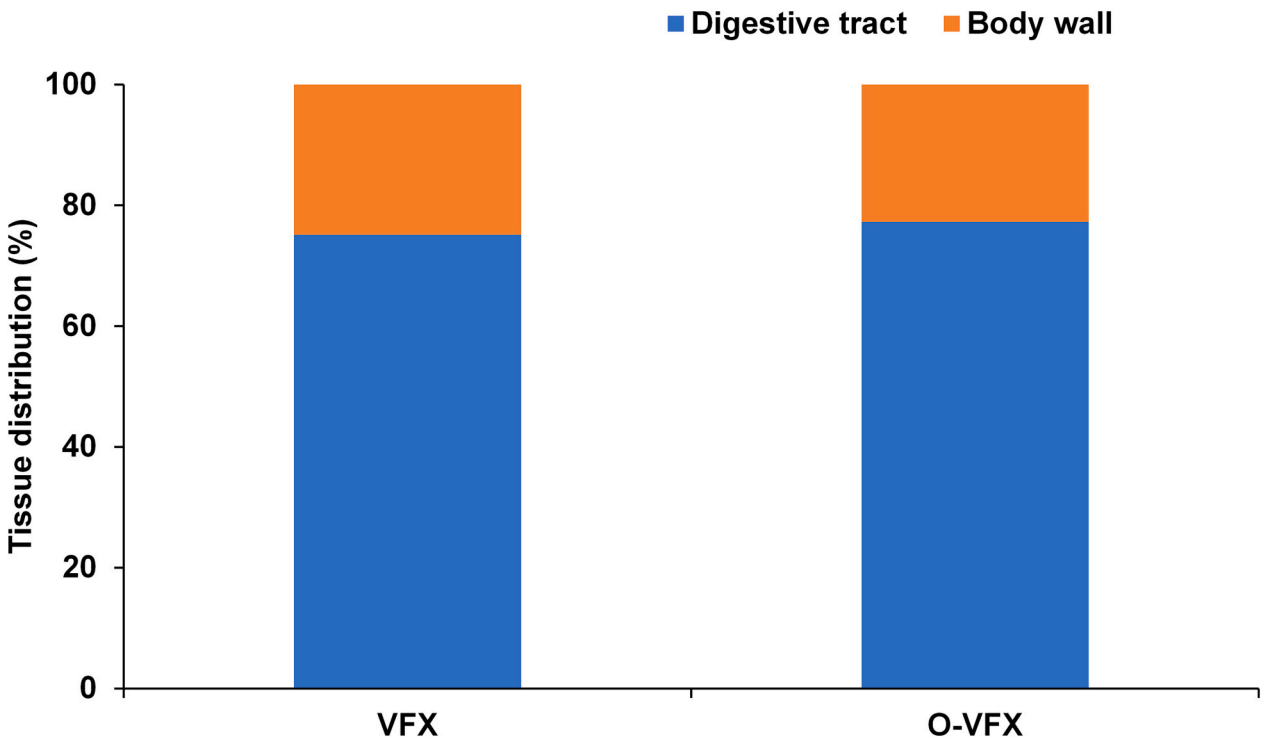

Fig. 3. Tissue distribution of VFX and O-VFX in *H. tubulosa* after 28 days exposure in term of concentration.

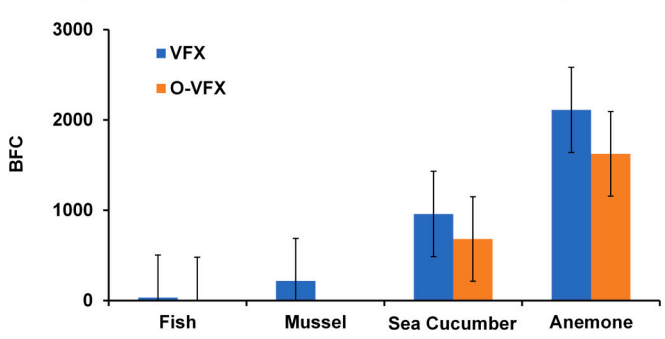

Fig. 4. Mean-whisker plots of BCF observations of VFX and O-VFX in different aquatic organisms.

extensive bioconcentration studies have been undertaken in organisms like fish or mussels, studies occurring in other nontarget organisms have been limited in number of species and/or type of contaminant, also including metabolites. The log K_{ow} has been the most widely used descriptor to predict the bioaccumulation potential, especially for lipophilic compounds (higher log K_{ow} corresponds to a higher BCF) (Arnot and Gobas, 2006). Meylan et al. (1999) developed an improved screening-level BCF estimation method and proposed that BCF values were 3.16 for the ionizable compounds with log $K_{ow} < 5$. However, in the present study, VFX (log K_{ow}: 2.47) presented higher bioaccumulation (up to 1831 and 4133 L/kg dw in sea cucumber and snakelocks anemone) than predicted with Meylan et al. (1999) or the QSAR models of Mackay and Veith (i.e., 26.3–42.6), as reported in Serra-Compte et al. (2018). Relying only on the K_{ow} may lead to a degree of underestimation (Gómez-Regalado et al., 2022; Kowalska et al., 2021). For example, in ionized chemicals such as pharmaceuticals, the BCF is pH dependent (Molina-Fernández et al., 2021). Nakamura et al. (2008) reported that BCF values of fluoxetine (secondary amine) measured in Japanese medaka *(Oryzias latipes)* increases as the pH increases from 9 to 260 L/kg at pH 7 and 9, respectively.

Fig. 4 shows a comparison of BFC observations, obtained in this study and from the literature, of VFX and O-VFX in different types of aquatic organisms. Studies with fish yield BCF values lower than the ones obtained in this study, but quite different between them can be found in the literature. The availability of quality information is essential to improve accuracy and reduce uncertainty in hazard and risk assessments. Table 3 summarizes the research found in the scientific literature dedicated to the evaluation of the bioaccumulation potential of VFX and O-VFX in different aquatic organisms.

Qu et al. (2019), for example, estimated a BCFss between 0.04 and 0.14 for VFX and between 0.15 and 0.97 for O-VFX in liver of loach (*Misgurnus anguillicaudatus*) exposed to a nominal VFX concentration of 16 μg/L day, similar to those used in the present study. BCF variability between tissues was also noted between liver, plasma and brain of trout in two different exposure studies with animals exposed to treated effluent wastewater (Grabicova et al., 2014; Lajeunesse et al., 2011). In both cases, authors found higher bioaccumulation patterns in liver (BCF 53 and 17 L/kg) followed by brain (9 and 11 L/kg) compared with muscle tissue (2 L/kg). Maulvault et al. (2018) uses both water and dietary exposure sources to study VFX accumulation in juvenile meagre (*Argyrosomus regius*) after 20 days besides the influence of environmental climate conditions. Authors concluded that VFX bioaccumulation (BCF = 64.6, exposure via water) in fish plasma was enhanced under the combination of warming and acidification. Their results also showed that VFX bioaccumulation in fish plasma was positively correlated with the fish growth, regardless of the exposure pathway. BCF levels similar to those estimated in the present study in sea cucumber have been reported for VFX in laboratory studies conducted on mussels (*Mytilus galloprovincialis*) (178–189 L/kg (Serra-Compte et al., 2018) and 178–189 L/kg (Gomez et al., 2021), although the exposure and depuration times were seven and twenty days, respectively).

In comparison and according with reported data summarized in Table 3, our results demonstrate that anemones and sea cucumber tend to have the highest BCF. Similar behavior has been reported for other emerging pollutants in the literature (Xie et al., 2017; Martín et al.,

Table 3
BCF observations for VLF and O-VLF in aquatic organisms belonging to different trophic levels.

Compound	Organism	Type of study	Nominal exposure concentration (ng/mL)	Exposure/depuration period	Tissue; dry weight (dw) or wet weight (ww)	BCF	Reference
VFX	Loach (*Misgurnus anguillicaudatus*)	Steady state	500	16	Liver (ww)	0.04–0.14	Qu et al., 2019
			500 (co-exposure with microplastics at 50 mg/L)	16		0.06–0.92	
	Juvenile meagre (*Argyrosomus regius*)	Steady state	20 (for exposure via water) and 160 μg/kg dw for feed exposure	28	–	65	Maulvault et al., 2018
	Juvenile rainbow trout (*Oncorhynchus mykiss*)	Steady state	Trout were exposed to treated effluent wastewater	13	Plasma	8	Grabicova et al., 2014
			Trout were exposed to treated effluent wastewater		Liver (ww)	63	
			Trout were exposed to treated effluent wastewater		Brain (ww)	9	
	Brook trout (*Salvelinus fontinalis*)	Steady state	Trout were exposed to 10 % and 20 % v/v of effluent treated with 15 mg/L of ozone.	3 months	Liver (ww)	18	Lajeunesse et al., 2011
			Trout were exposed to 10 % and 20 % v/v of effluent treated with 15 mg/L of ozone.		Brain (ww)	11	
			Trout were exposed to 10 % and 20 % v/v of effluent treated with 15 mg/L of ozone.		Muscle (ww)	2	
	Mussel (*Mytilus galloprovincialis*)	Steady state	Up to 15.7	20/20	Whole organism (dw)	213–258	Serra-Compte et al., 2018
	Mussel (*Mytilus galloprovincialis*)	Kinetic	10	7/7	Whole organism (dw)	275	Gomez et al., 2021
	Mussel (*Mytilus galloprovincialis*)	Steady state	1, 10, 100 (unique initial exposure concentration)	7	Whole organism (dw)	178–189	Gomez et al., 2021
	Sea cucumber (*Holothuria tubulosa*)	Kinetic	10	28/52	Digestive tract (dw)	87	The present study
	Sea cucumber (*Holothuria tubulosa*)	Steady state		28	Digestive tract (dw)	1831	
	Sea cucumber (*Holothuria tubulosa*)	Kinetic		28/52	Body wall (dw)	91	
	Sea cucumber (*Holothuria tubulosa*)	Steady state		28	Body all (dw)	738	
	Sea anemone (*Anemonia sulcata*)	Kinetic		28/52	Whole organism (dw)	91	
	Beadlet anemone (*Actinia equina*)	Steady state		28	Whole organism (dw)	2307–4133	
O-VFX	Loach (*Misgurnus anguillicaudatus*)	Steady state	500	16	Liver (ww)	0.15–0.97	Qu et al., 2019
			500 (co-exposure with microplastics at 50 mg/L)			4.31–22.81	
	Sea cucumber (*Holothuria tubulosa*)	Kinetic	10	28/52	Digestive tract (dw)	65	The present study
	Sea cucumber (*Holothuria tubulosa*)	Steady state		28	Digestive tract (dw)	1298	
	Sea cucumber (*Holothuria tubulosa*)	Kinetic		28/52	Body wall (dw)	91	
	Sea cucumber (*Holothuria tubulosa*)	Steady state		28	Body all (dw)	437	
	Sea anemone (*Anemonia sulcata*)	Kinetic		28/52	Whole organism (dw)	91	
	Beadlet anemone (*Actinia equina*)	Steady state		28	Whole organism (dw)	1837–3160	

2019). However, it should be noted that there are inherent difficulties in interpreting and comparing BCF. This variability, in addition to natural dispersion due to different species, may be due to the lack of uniformity in reported studies such as exposure concentrations. In the case of ionizable compounds it is found that bioaccumulation depends on concentration (Burden et al., 2014), exposure and depuration times (Gómez-Regalado et al., 2022), temperature (Maulvault et al., 2018) or pH (Maulvault et al., 2018).

Another important limitation found is that most of the accumulation studies did not examine suspended solids or sediments on which contaminants are often adsorbed. Many authors (Oetken et al., 2005; Lagesson et al., 2016; Xie et al., 2017; Wilkinson et al., 2018) have emphasized that deposit-feeding organisms concentrate higher amounts of PhACs, due to their ingestion of organic matter from sediments. Recently, Fonseca et al. (2021) observed higher detection frequencies in benthic and demersal species living directly on or just above the substrate, supporting the combined roles of sediment and dietary routes of pharmaceutical uptake. In the present work, the sediments were also collected in the same sampling point as the water samples. A BCF from sediment up to 287 in the digestive tract of the sea cucumber for VFX and up to 434 in snakelocks anemone for O-VFX were noted.

Finally, besides the accumulation of the VFX parent compound, the presence of its related metabolites in biota is a focus of interest, as some metabolites can be biologically active. The O-VFX metabolite shares the primary biochemical actions of its parent compound and appears to contribute to the therapeutic efficacy of those medications in humans (Rudorfer and Porter, 1997). Thus, the presence of this metabolite in marine organisms could have adverse effects. We have determined the ratio between the metabolite concentration and the concentration of the parent compound in the studied animals at the different exposure times.

This ratio increases with time, especially in the bioaccumulation experiment carried out in the digestive tract of *H. tubulosa* while a constant value was observed in the body wall, reflecting a low metabolic activity in this tissue and highlighting between-tissues differences in metabolization capacities. Therefore, the BCF must be supplemented with knowledge of the metabolism to have a clear view of their environmental risk.

The mechanisms inherent to VFX and the role of metabolites in absorption and elimination still require further understanding. The study by Kingbäck et al. (2012) concluded that CYP2D6 enzymes catalyze the metabolism of VFX to its major metabolite O-VFX. In a co-exposure study in the loach *Misgurnus anguillicaudatus*, Qu et al. (2019) observed that malondialdehyde (MDA) and superoxide dismutase contents in loach liver were elevated when the animal was exposed 40 days to VFX and O-VFX. Both compounds were detected in loch tissues and liver subcells. However, these two compounds had a different effect on the loch liver. O-VFX could produce more oxygen radicals than its parent compound and this fact may induce lipid peroxidation reaction, which increases liver tissue damage.

The MDA content in the O-VFX assay was higher than in the parent compound assay Furthermore, in the same study, the authors suggested that co-exposure with microplastics may have more adverse effects against loach. The authors stated that, in the subcellular structure of the liver, microplastics may help to transport more compounds to subtle areas and delay the metabolism of contaminants in organisms.

On the other hand, results presented by Maulvault et al. (2018) confirmed that VFX can accumulate in juvenile fish, not only from water, but also from diet. However, the lower levels of VFX in fish exposed through diet suggest that diet may, in fact, play a minor role in uptake compared to other routes of exposure (such as inhalation). In addition, Maulvault et al. observed that when animals were exposed through feeding, an increase in temperature and pCO2 levels enhanced VFX bioaccumulation in the animal. This fact was related to metabolic changes induced by the altered abiotic variables, as well as possible tissue damage, which could facilitate the entry into cells of the contaminant (Sampaio et al., 2016, 2018). In another attempt, Serra Compte et al. (2018) stated that acidification decreased the ability of mussels to metabolize VFX, while the effects of warming were less clear. These studies suggest that changes in environmental conditions may not only affect the physicochemical properties of contaminants, but also alter the physiological state of the animal (Nardi et al., 2017), which may affect their bioconcentration potential. A moderate increase in water temperature increases the metabolic activity of aquatic organisms, i.e. food adsorption in mussels (Navarro et al., 2016; Serra Compte et al., 2018).

Some gaps were found regarding toxicological and behavioral similarities (or distinctions) between organisms, especially in sea cucumber and anemone species. It is important to assess potential ecological implications and cascading effects to marine biota due to environmental contamination related to human pharmaceuticals (Maulvault et al., 2018).

5. Conclusions

The bioconcentration of the antidepressant VFX and its main metabolite O-VFX was studied in lower non-target marine organisms under controlled laboratory conditions. Both compounds accumulated rapidly from the first days to reach 40,823 and 54,342 ng/g dw of absorption in the digestive tract of sea cucumber in 28 days and 64,810 and 93,007 ng/g dw, respectively, in the snakelocks anemone showing a first-order kinetic process in all cases.

The kinetic BCF reached 2947 (VFX) and 1515 L/kg dw (O-VFX) in the digestive tract of the sea cucumber, and between 2080 and 4728 (VFX) and 1522–2392 L/kg dw (O-VFX) in anemones which classified the VFX as cumulative. The animal-specific BCF generally followed the order of snakelocks anemone > beadlet anemone > sea cucumber. The results also highlighted between-tissues differences in metabolization capacities, being noteworthy this effect in the digestive tract while irrelevant in the body wall of sea cucumber.

The potential adverse effects of VFX and O-VFX on non-target organisms due to exposure in the marine environment should therefore not be overlooked. Our results demonstrate that anemones and sea cucumber tend to have the highest BCF in comparison with other target organisms like fish or mussels, although the reported data are quite different between them. Up to now, no criteria have been proposed to evaluate the quality of accumulation assays and, as such, there is a lack of uniformity in the reported studies.

The need for greater standardization of experimental settings is key to improving the accuracy of environmental risk assessments. Given the growing need to propose animal models, the use of echinoderms and cnidarians represents a promising alternative for future studies of bioaccumulation and ecotoxicology of emerging contaminants. To our knowledge, this is the first study to determine the BCF in sea cucumber and anemone species. This study proves to be a good basis for research on the quality of the marine environment, as well as for the use of benthic bioindicators as good candidates for inclusion in both laboratory and field environmental studies to contribute to a more biodiverse assessment of the effects of pharmaceuticals on ecosystems. It is also a promising approach for regulatory agencies to conduct risk assessments and understand the long-term consequences of the continued entry of these contaminants into the environment. However, future research is still needed to assess trophic transfer in aquatic ecosystems, as well as the metabolic or biotransformation behavior of pharmaceuticals in the marine environment, in order to conduct a proper environmental risk assessment.

CRediT authorship contribution statement

MCGR: Methodology, resources, conceptualization, writing, review & editing; JM: Methodology, resources, conceptualization, writing, review & editing; FH: Methodology, resources, conceptualization, review & editing; JLS: Conceptualization, supervision, review & editing; IA: Conceptualization, supervision, writing, review & editing; EA: Conceptualization, supervision, funding acquisition and project administration; AZG: Conceptualization, writing, review & editing, supervision, funding acquisition and project administration.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.115055.

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