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RESEARCH ARTICLE

Removal of contaminants from freshwaters and estuarine waters by constructed and natural wetlands towards river catchment protection through nature-based solutions

[version 1; peer review: awaiting peer review]

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Abstract

Background

Constructed wetlands (CWs) and saltmarshes are promising nature-based solutions for mitigating the release of metals and pharmaceuticals into aquatic ecosystems. Nevertheless, their performance under multi-contaminant conditions requires further in-depth investigation to better understand the underlying processes and interactions. This study evaluated the removal, retention and accumulation of Cu, Cd, ketoprofen and venlafaxine in freshwater CW mesocosms using *Canna indica* and in estuarine wetland microcosms with *Sporobolus maritimus* and *Juncus maritimus.*

Methods

CWs were operated under batch conditions for 4-weeks. Estuarine microcosms included combinations of plants, sediment and elutriate exposed to contaminants over 7-days. Metals and pharmaceuticals were determined in CW effluents using AAS and SPE-HPLC, respectively. Metals in plant tissues and substrate/sediment were determined by AAS after high pressure acid microwave digestion.

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Results

CWs achieved very high removal efficiencies, >99% for metals and > 99.9% for pharmaceuticals. In estuarine microcosms, sediments acted as the primary sink for Cu, Cd and venlafaxine, with metals removal from elutriate solution frequently >90%. Both halophytes predominantly accumulated metals in their belowground tissues, indicating limited translocation to their aerial organs and a phytostabilisation strategy. In contrast, the contribution of plants to the removal of pharmaceuticals was limited. Venlafaxine showed high attenuation in sediment-containing systems (elutriate removal >90%), whereas ketoprofen exhibited low and variable removal from elutriate, particularly in the absence of sediment (<10%).

Conclusions

Overall, contaminants attenuation was primarily determined by system characteristics rather than interactions between co-occurring contaminants. In CWs, *C. indica* presence provides an efficient barrier limiting the transfer of metals and pharmaceuticals from freshwater to downstream ecosystems. In estuarine environments, remediation was largely driven by sediment retention, complemented by metal accumulation in saltmarsh plants. These findings highlight the importance of selecting nature-based remediation strategies according to the specific environmental context and to the behaviour of contaminants under realistic multi-contaminant conditions.

Keywords
Metals, Pharmaceuticals, Copper, Cadmium, Ketoprofen, Venlafaxine, saltmarshes; estuarine environment



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Introduction

Freshwater and estuarine ecosystems are under increasing threat from anthropogenic pollution, including through the discharge of metals and contaminants of emerging concern (CECs) such as pharmaceuticals (Mateus et al., 2016; Hejna et al., 2022; Ortuzar et al., 2022). Estuaries are particularly vulnerable because they act as a transition zone between riverine and marine environments. Due to urban runoff, untreated wastewaters, industrial discharges, and agricultural inputs, these waters frequently contain complex mixtures of pollutants. For instance, several contaminants have been detected in Lima and Douro estuaries waters in northern Portugal (Gorito et al., 2024; Freitas et al., 2025).

Nature-based solutions (NbS) have gained increasing recognition as sustainable and environmentally sound approaches for mitigating aquatic pollution (Debele et al., 2023; Ferreira et al., 2023; Pastor-Lopez et al., 2024). In this context, constructed wetlands (CWs) and natural wetlands, including estuarine saltmarshes, have emerged as effective systems for reducing contaminant loads and reducing environmental impacts (Verlicchi & Zambello, 2014; Vymazal, 2014; Pastor-Lopez et al., 2024). Despite differences in their degree of design, hydrological control and management, both systems rely on similar natural processes to attenuate contaminants.

The removal of contaminants in wetland-based systems is driven by a combination of physical, chemical and biological mechanisms. These include sedimentation, particle retention, adsorption and precipitation in sediments and substrates, sorption to organic matter, microbial transformation within the rhizosphere and uptake and accumulation by plants (Vymazal, 2014; Malyan et al., 2021; Wang et al., 2024). These mechanisms operate in both, constructed and natural wetlands, where vegetation and associated microbial communities play a fundamental role in contaminants removal efficiency (Fernandes et al., 2017; Gorito et al., 2017). The importance of each process depends on specific factors, such as hydrological conditions, sediment and substrate properties, physicochemical characteristics and plant species composition (Verlicchi & Zambello, 2014; Vymazal, 2014; Zhang et al., 2014; Gorito et al., 2017).

CWs are engineered treatment systems that take advantage of natural wetlands processes, being specifically designed for the treatment of contaminated waters. Their performance depends on the interaction between the substrate, microbial consortia and wetland macrophytes, allowing for the effective, cost-efficient and sustainable removal of conventional and emerging contaminants. Growing body of evidence highlighting the capacity of CWs to attenuate complex pharmaceutical compounds alongside traditional contaminants (Vymazal, 2011; Zhang et al., 2014; Gorito et al., 2017; Ilyas et al., 2020; Ravikumar et al., 2022). Among the macrophytes used in CWs, *Canna indica* has demonstrated a high potential for the removal of both inorganic (metals) and organic contaminants, the performance being attributed to the rapid growth of the species, the significant biomass production, and the extensive root system (Karungamye, 2022; Angmo et al., 2024). However, removal assessment for contaminants mixtures is still scarce.

Alongside engineered systems, natural wetlands such as estuarine saltmarshes play a crucial role in controlling contamination within the aquatic environments. In these systems, contaminants are often initially retained in sediments through adsorption and precipitation processes (Weis & Weis, 2004; Cunha et al., 2024). While this retention contributes to water quality improvement, the accumulation of contaminants in sediments may represent a long-term environmental risk due to potential remobilisation and increased toxicity if not removed/degraded. In this context, phytoremediation in estuarine saltmarshes, integrating the action of marsh vegetation and their rhizosphere-associated microbial communities, represents a promising strategy for the stabilisation, uptake and transformation of both organic and inorganic contaminants (Fernandes et al., 2017; Cunha et al., 2024). Furthermore, the protection and restoration of saltmarshes through actions such as habitat creation, restoration and adaptive management can themselves function as NbS, contributing to the prevention, mitigation and recovery of impacted estuarine areas (Ganju, 2019; Debele et al., 2023; Ferreira et al., 2023).

The effectiveness of phytoremediation in estuarine environments depends on the careful selection of saltmarsh plant species and on a comprehensive understanding of local physicochemical conditions (Weis & Weis, 2004). Key controlling factors include sediment properties (such as organic matter content and grain size), contaminant characteristics and the simultaneous presence of inorganic contaminants, such as metals, and organic contaminants, including pharmaceutical compounds (Dias et al., 2021; Fraga-Santiago et al., 2022). Together, these factors influence contaminant bioavailability, plant uptake and microbial activity, ultimately determining remediation efficiency, which requires further research.

In recent years, emerging contaminants, particularly pharmaceutical residues, have received increasing attention due to their widespread consumption, continuous discharge into aquatic environments, and potential ecological and health risks (Ma et al., 2018; Wang et al., 2018; Chen et al., 2022; Ahmed et al., 2025). Pharmaceuticals such as ketoprofen (KPF) and venlafaxine (VFX) are now commonly detected in municipal wastewater and receiving surface waters in various regions, often at concentrations ranging from nanograms to micrograms per litre (Ma et al., 2018; Chen et al., 2022; Tyumina et al.,

2023; Gorito et al., 2024). At the same time, metals remain persistent contaminants in estuarine ecosystems, where they can accumulate in sediments and organisms (Weis & Weis, 2004; Cave et al., 2005; Martin et al., 2012). The co-occurrence of organic and inorganic contaminants may modify individual removal pathways and give rise to complex interactions that influence both phytoremediation performance and environmental toxicity (Almeida et al., 2009; Dias et al., 2021; Fraga-Santiago et al., 2022).

Previous laboratory-scale and mesocosm studies have demonstrated the capacity of saltmarsh species such as *Juncus maritimus* and *Phragmites australis* to remove Cd and Cu from aquatic systems, either through accumulation in plant tissues or through retention within the rhizosphere-associated sediments (Oliveira et al., 2014; Silva et al., 2014; Fraga-Santiago et al., 2022). Experiments conducted with elutriate solutions have further shown that *P. australis* can contribute to the removal of pharmaceutical compounds, including paroxetine and bezafibrate, from the aqueous phase (Dias et al., 2021). In addition, laboratory studies have indicated that Cu phytoremediation by saltmarsh plants such as *Halimione portulacoides* and *P. australis* may be influenced by the presence of organic contaminants, including pesticides, hydrocarbons and pharmaceuticals (Almeida et al., 2009; Fraga-Santiago et al., 2022), and that pharmaceutical removal may itself be affected by Cu (Fraga-Santiago et al., 2022). Crucially, these interactions appear to depend on the specific properties of the organic contaminants involved, highlighting the need for further investigation under combined exposure scenarios.

Within this context, this study aimed to evaluate the phytoremediation potential of three macrophyte species to remove a contaminants mixture (one metal and one pharmaceutical) under laboratory-scale conditions. The selected species, *C. indica*, *J. maritimus* and *Sporobolus maritimus*, represent a spatial gradient from freshwater to brackish environments within the estuarine system. While *J. maritimus* and *S. maritimus* are native species commonly found in saltmarshes and brackish zones of the estuaries in North of Portugal, *C. indica* is a non-native species often used in CWs for wastewater treatment. It was included here as a CWs model species for potential application of CWs in freshwater streams draining into the estuarine zone, preventing therefore the input of contaminants into these areas, in a source-to-sea approach.

The target contaminants, two metals (Cu and Cd) and two pharmaceutical compounds (VFX and KPF), were selected based on recent monitoring data from the Lima and Douro River estuaries (Cunha et al., 2024; Gorito et al., 2024; Freitas et al., 2025). Copper is an essential micronutrient, whereas cadmium is a toxic metal with no known biological function. Both have recently been detected in estuarine waters (Freitas et al., 2025), as well as in sediments and native saltmarsh vegetation (Cunha et al., 2024). Similarly, VFX and KPF, two widely used pharmaceutical compounds belonging to distinct therapeutic classes, have also been detected in estuarine waters and/or sediments (Gorito et al., 2024) and are commonly reported in aquatic environments worldwide.

Material and methods

a) **Experimental setup**

1. **Freshwater treatment by constructed wetlands mesocosms**

Experiments were conducted under controlled laboratory conditions using mesocosms simulating subsurface vertical-flow CWs in batch mode to evaluate the removal efficiency of metals and pharmaceuticals, alone or mixture, from freshwater using *C. indica* (Botanist/Author: Carl Linnaeus; Specimen Reference Number: Herb. A. van Royen No. 912.356–390) as the phytoremediation species.

Each mesocosm consisted of a plastic container measuring $40 \times 30 \times 30\ cm^3$ with a drainage tap connected to a perforated silicone tubing in the bottom (Figure 1) The system consisted of three layers of substrate: 1 cm of coarse gravel at the bottom, 4 cm of expanded clay in the middle and 8 cm of sand on top.

Between 10 and 30 *C. indica* plants were transplanted into the sand layer in each container. *C. indica* were acquired from a horticulture facility (Flor do Norte - Comércio de Plantas e Flores, Lda.) in Arcozelo, Portugal. In the lab the plants' roots were separated from any soil attached, washed first with tap water and then with deionised water until all soil was removed. Plants were kept in a nutrient solution for one day before being transplanted into the mesocosms.

To prevent the photodegradation of the target compounds, aluminium foil was wrapped around the external surfaces of the containers (Figure 1, A). All systems were placed in a greenhouse and exposed to natural light and environmental temperature conditions (9.3–32.3 °C). CW mesocosms systems were irrigated daily with water from a nearby freshwater lake (Porto Botanical Garden, Porto, Portugal), simulating a freshwater stream

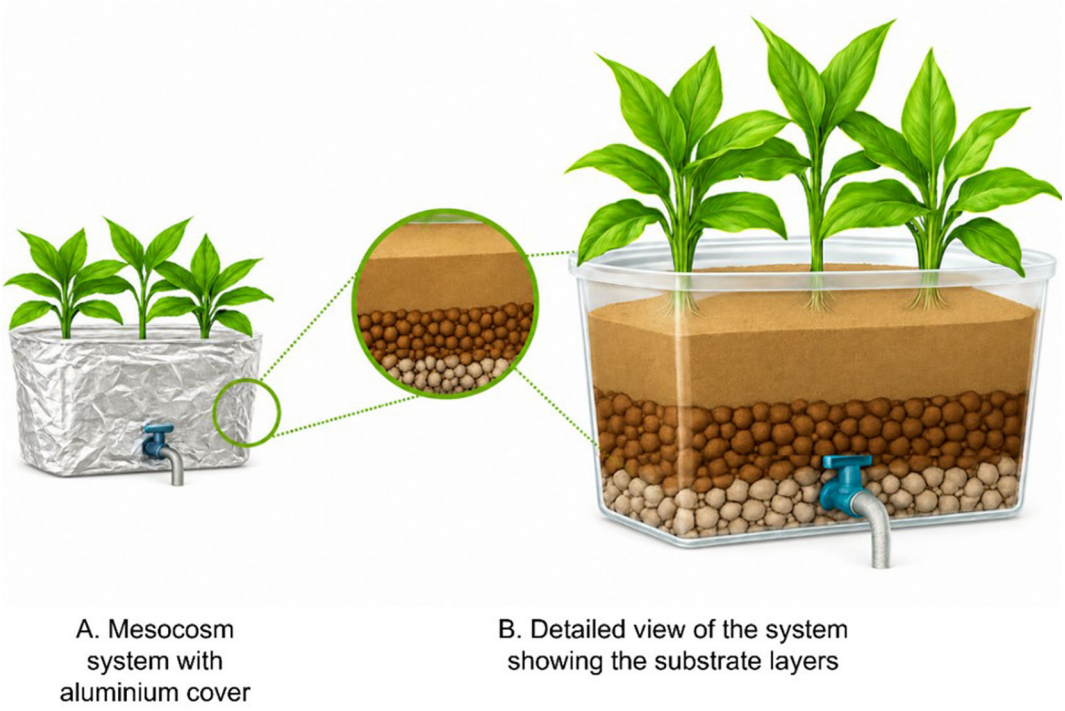

Figure 1. Experimental constructed wetland microcosm system. Schematic representation of CW's systems with the aluminium-covered experimental system (A) and enlarged cross-sectional view showing the internal substrate layers (B).

that could drained into an estuarine area. The systems have been assembled previously being involved in different experiments, after which, a long period of freshwater irrigation was carried out to allow the full recovery of the systems (Figure 1).

Two experimental series were conducted, the first with KPF contaminated water (doping the freshwater with 0.1 mg L^{-1} of KPF (adding 100 µL of a stock solution of 1 g L^{-1}, prepared by dissolving 10 mg of KPF (KPF 5 g FujiFilm Wako, catalogue n° 118–01091) in 10 mL of methanol, into 1 L of lake water)) and the second with VFX contaminated water (doping the freshwater with 0.1 mg L^{-1} of VFX (adding 100 µL of a stock solution of 1 g L^{-1}, prepared by dissolving 10 mg of VFX (Venlafaxine Hydrochloride 500 mg, FujiFilm Wako, catalogue n°227–01951) in 10 mL of methanol, into 1 L of lake water)). Each series comprised two independent four-week trials, one with Cd (1 mg L^{-1}, adding of 1 mL of a stock solution of 10 g L^{-1}, prepared by dissolving 100 mg of cadmium sulphate (8/3-hydrate, 500 mg, ACS REAGEN, Acros Organics, catalogue n° ACRO403765000) in 10 mL of deionised water, into 1 L of lake water)) and one with Cu (10 mg L^{-1}, adding of 1 mL of a stock solution of 100 g L^{-1}, prepared by dissolving 1 g of copper chloride ($CuCl_2$ dihydrate, ACS REAG, Sigma-Aldrich, catalogue n° 307483-100G) in 10 mL of deionised water, into 1 L of lake water)), separated by a cleaning period using uncontaminated freshwater to minimise carry-over effects. The first series (Cd + KPF and Cu + KPF) was followed by the second series (Cd + VFX and Cu + VFX) in a subsequent experiment. These pollutants concentration represented a worst-case contamination scenario.

All systems were maintained under the same operational conditions, with contaminated water dosed weekly and recirculated throughout each one-week cycle. The treatments, each of which was tested in duplicate, included control conditions (non-contaminated freshwater), single-contaminant conditions (Cd, Cu, KPF or VFX) and mixed-contaminant conditions (metal + pharmaceutical). The experimental series with KPF were conducted between June and July 2024, whereas the experimental series with VFX were performed between October and November 2025.

Samples of water were collected at the beginning (CW inlet) and end (CW outlet) of each one-week cycle to determine contaminants removal percentages. Sediment (collected near the roots) as well as plant leaves were

collected at the start (T_0) and end (T_4) of each experimental series to investigate metal distribution within the system. In addition, plant leaves were collected for the analysis of photosynthetic pigments.

To evaluate the operational performance and stability of the CW systems over the experimental period, conventional water quality parameters were also monitored weekly in the influent (CW inlet) and effluent (CW outlet) waters. These included nitrates (NO_3^-) and ammonia (NH_4^+) ions, total phosphorus (TP) and organic matter content (assessed through chemical oxygen demand (COD), all measured through Hanna kits (NO_3^- ions kit: HI93766–50; NH_4^+ ions kit: HI93764A-25; TP kit: HI93758C-50; COD kit HI93754A-25 (Hanna instruments, Smithfield, EUA)). Detailed results are provided in the Dataset Interdisciplinary Centre of Marine and Environmental Research (2026).

2. **Estuarine natural treatments in wetland microcosms**

Estuarine plant species, *S. maritimus* ((Curtis) P.M.Peterson & Saarela botanists) and *J. maritimus* (botanist Jean-Baptiste Lamarck; public herbarium of the Muséum National d'Histoire Naturelle in Paris P-LA), were collected from the Lima and Douro estuaries in northern Portugal. Plants species were identified by the authors based on team experience from previous works on Lima and Douro estuaries and subsequently confirmed by genetic analysis. Voucher specimens are retained by the authors in their laboratory. Experiments were conducted using an elutriate medium, prepared by mixing estuarine water with vegetated sediment, both collected from the estuary area with the respective plant just prior to the experiment. This followed the procedure described by Dias et al. (2021), ensuring a simplified realistic simulation of interactions between estuarine water, sediment, and saltmarsh vegetation. All treatments were performed in triplicate.

Each replicate consisted of three to five individual plants exposed to 200 ml of elutriate solution, with or without 50 g of estuarine sediment (see Figure 2). To simulate highly polluted discharge events, contaminants were added directly to the elutriate at concentrations of 50 mg L^{-1} Cu or 5 mg L^{-1} Cd, combined with 0.1 mg L^{-1} VFX or KPF (reagents used were the same as described for the freshwater experiments). Pharmaceutical compounds were added three times during the one-week experimental period to reach a total of 0.3 mg L^{-1}. Metal and pharmaceutical concentrations were selected to represent a worst-case contamination scenario; however, Cd levels were lower than Cu levels due to Cd′s higher toxicity to plants. Pharmaceuticals were added periodically to simulate continuous discharge conditions, since these compounds can suffer degradation, whereas metal remain in the elutriate-plant-sediment system.

Plants (*S. maritimus* and *J. maritimus*) were exposed to elutriate (with or without sediment) for 7 days. Samples of elutriate, sediment (when present), and plant tissues were collected at the beginning and end of the

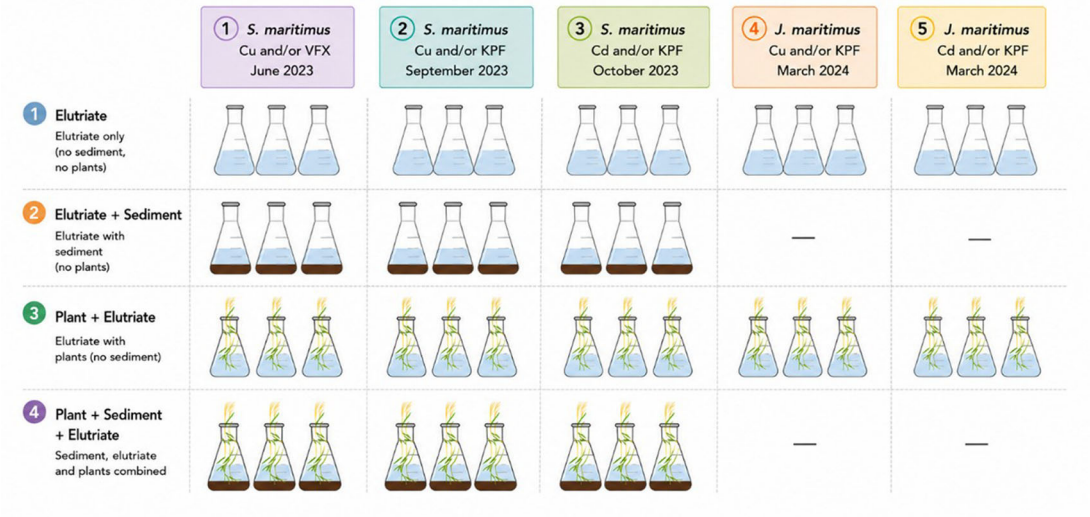

Figure 2. Experimental design of the elutriate exposure assays. Experimental series in wetland microcosms with elutriate (with/without sediment) using Sporobolus maritimus or Juncus maritimus. Cd, Cu, KPF and VFX were added (in combination or alone) in the different conditions.

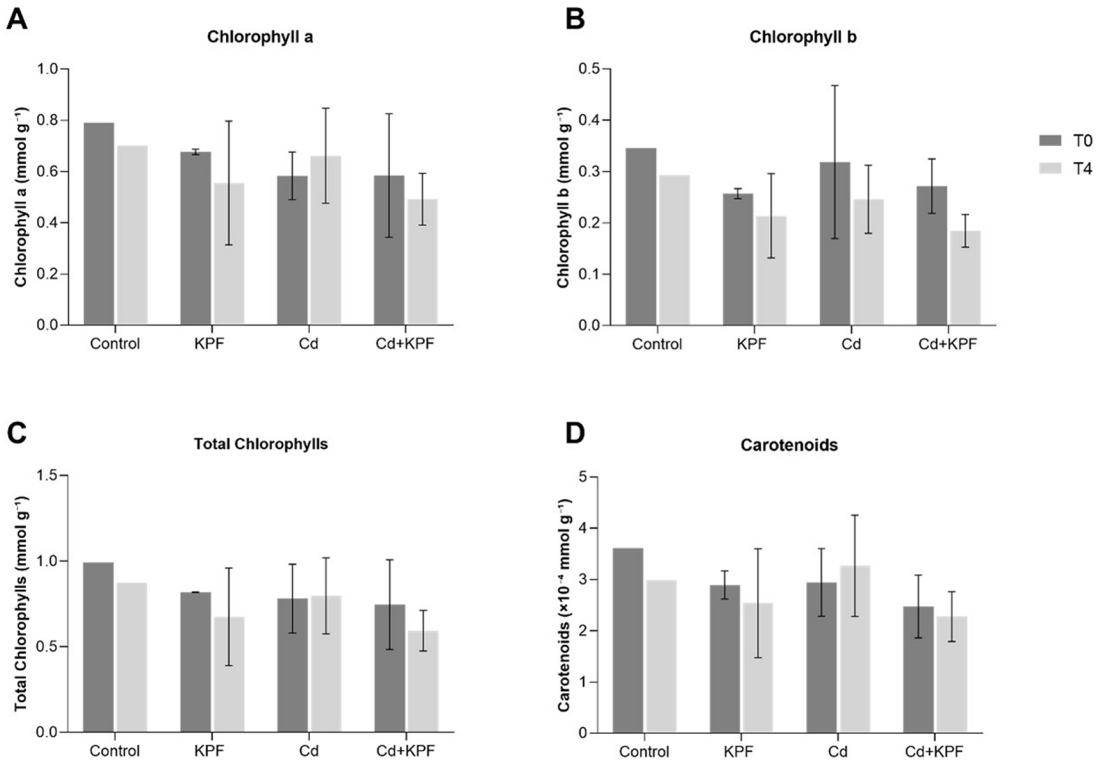

Figure 3. Pigment content in Canna indica during the Cd + KPF assay. Values are means ± SD, n = 2, except for Control with n = 1 (shown for descriptive purposes and not included in the statistical analyses). No significant differences were detected among treatments or sampling times (GLM, $p > 0.05$).

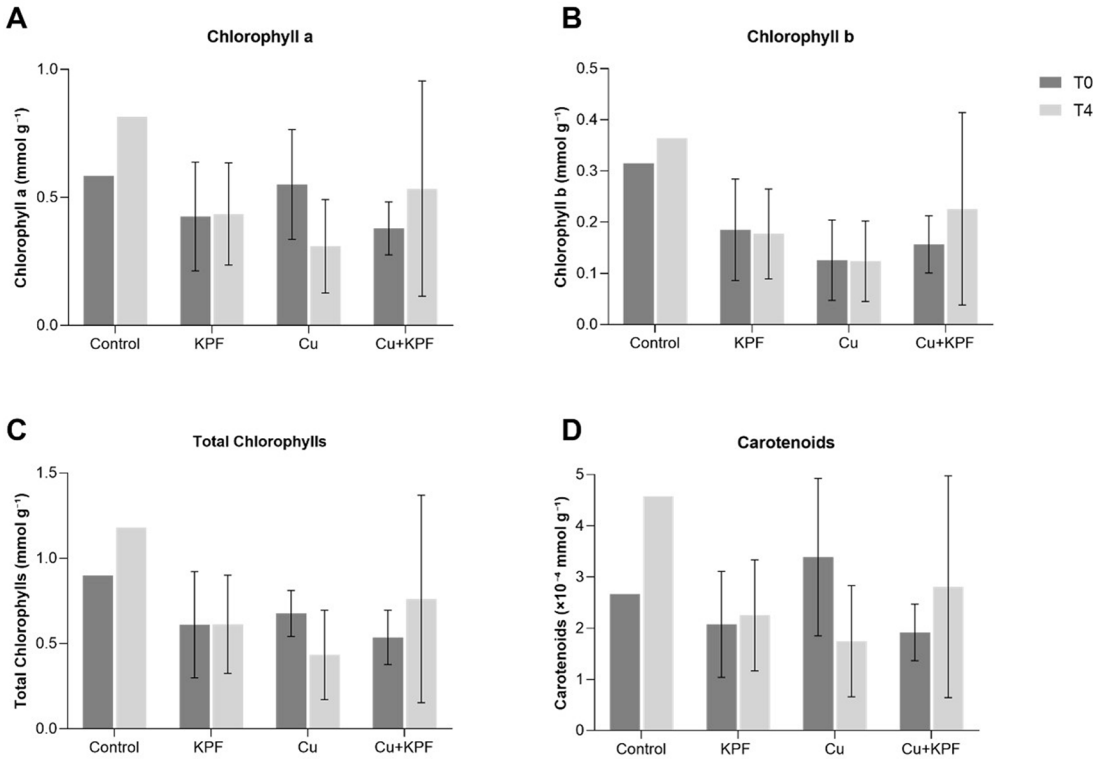

Figure 4. Pigment content in Canna indica during the Cu + KPF assay. Values are means ± SD, n = 2, except for Control with n = 1 (shown for descriptive purposes and not included in the statistical analyses). No significant differences were detected among treatments or sampling times (GLM, $p > 0.05$).

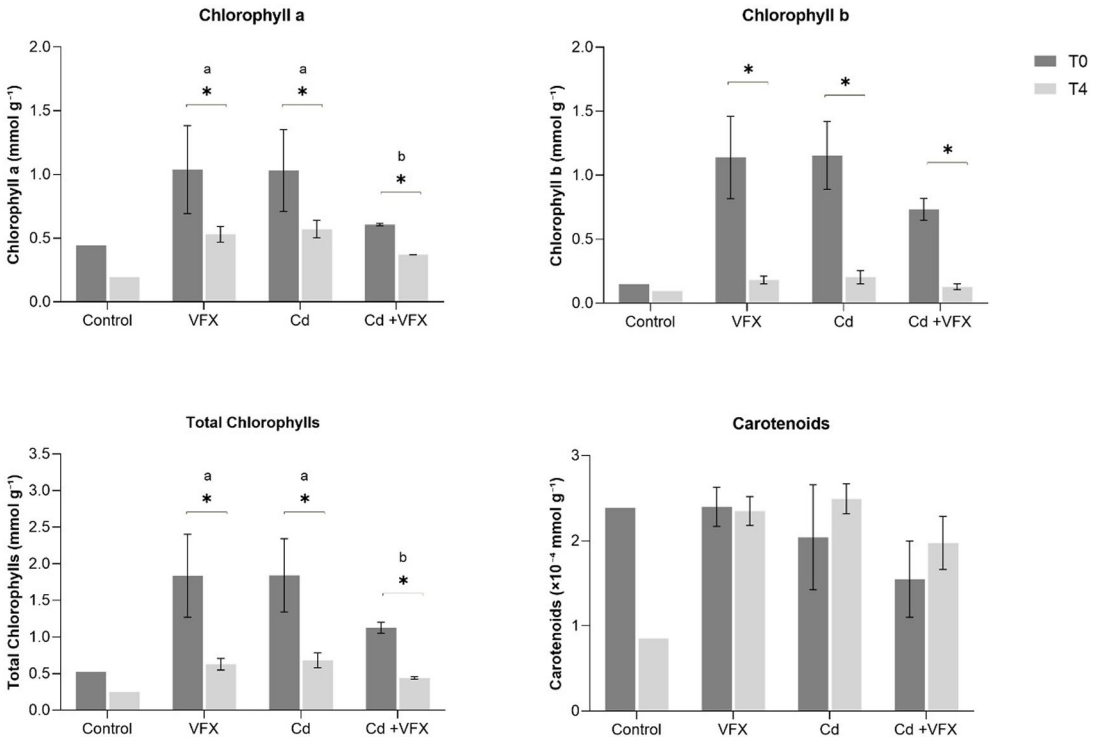

Figure 5. Pigment content in Canna indica during the Cd + VFX assay. Values are means ± SD, n = 2, except for Control with n = 1 (shown for descriptive purposes and not included in the statistical analyses). Different lowercase letters indicate significant differences among treatments according to Tukey's post hoc test ($p < 0.05$). Asterisk indicate the overall effect of sampling time determined by GLM analysis ($p < 0.05$). Bars without letters or asterisks indicate no significant differences.

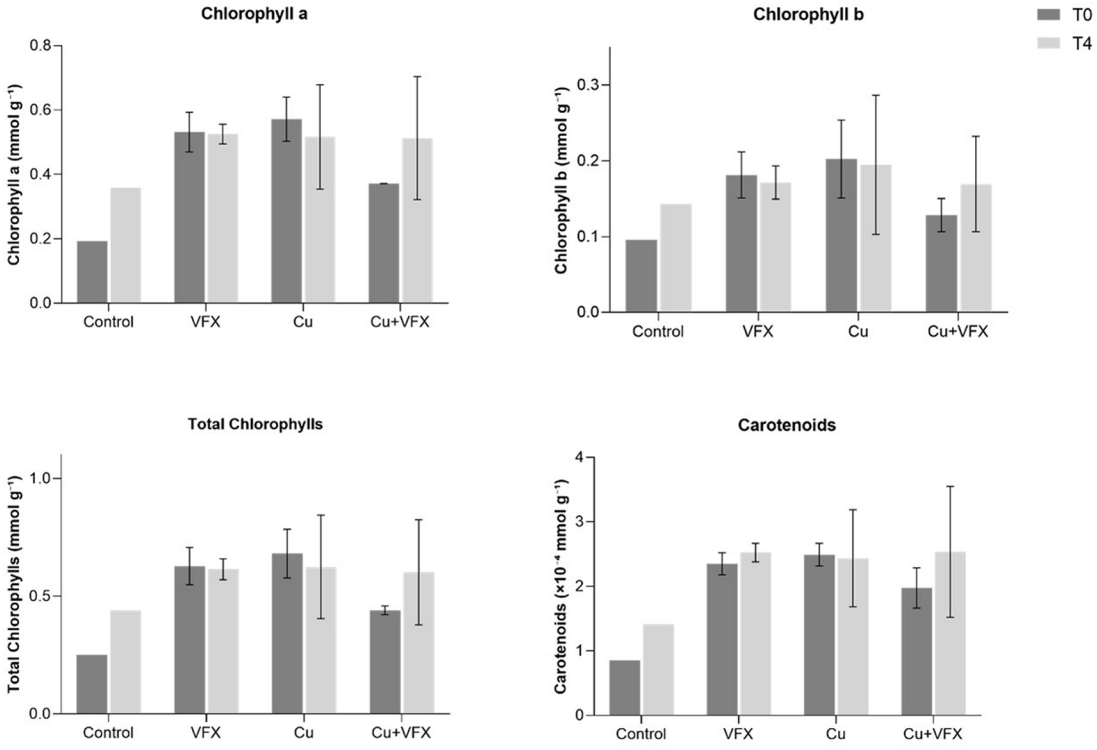

Figure 6. Pigment content in Canna indica during the Cu + VFX assay. Values are means ± SD, n = 2, except for Control with n = 1 (shown for descriptive purposes and not included in the statistical analyses). No significant differences were detected among treatments or sampling times (GLM, $p > 0.05$).

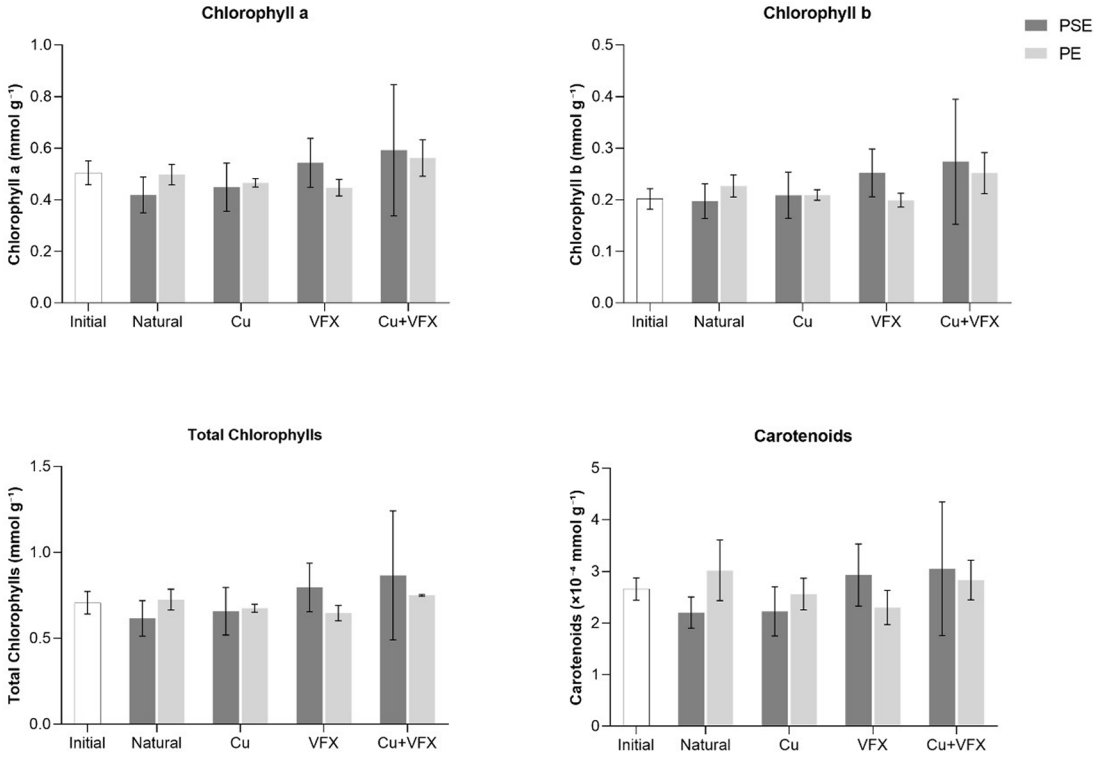

Figure 7. Pigment content in Sporobolus maritimus exposed to Cu and VFX. Values are means ± SD (n = 3). No significant differences were detected among treatments or experimental conditions. PSE and PE indicate the presence and absence of sediment, respectively. "Initial" represents the beginning of the experiment; "Natural" indicates the control, with no contaminant addition, at the end of the experiment; Cu, VFX and Cu + VFX indicates the different treatments at the end of the experiment.

experiment to quantify contaminant concentrations and determine removal percentages and contaminant distribution within the system. In addition, plant shoots were collected for the analysis of photosynthetic pigments.

Several experimental series were conducted:

- *S. maritimus* was exposed to

(1) Cu and/or VFX (June 2023); (2) Cu and/or KPF (September 2023); (3) Cd and/or KPF (October 2023) (Figure 2).

Both elutriate alone and sediment + elutriate with or without plants conditions were used.

- *J.* m*aritimus* was exposed to:

(4) Cu and/or KPF; (5) Cd and/or KPF, in elutriate without sediment in March 2024 (Figure 2).

For *J. maritimus* experiments only KPF in elutriate alone was tested, considering the results obtained for *S. maritimus.* VFX tends to persist in the sediment phase, which would limit the evaluation of plant phytoremediation potential and its interaction with metals in systems containing sediment. In contrast, KPF is frequently detected in the water column (Gorito et al., 2024) and is therefore more suitable for assessing interactions with plants and co-occurring contaminants in aqueous systems, as it is also susceptible to aquatic dispersion and degradation processes (Chen et al., 2022). Therefore, testing in elutriate alone allows higher interaction with plants as well as with other contaminants, such as metals, present in the medium. Moreover, metals are easily retained on sediments, which reduces their bioavailability.

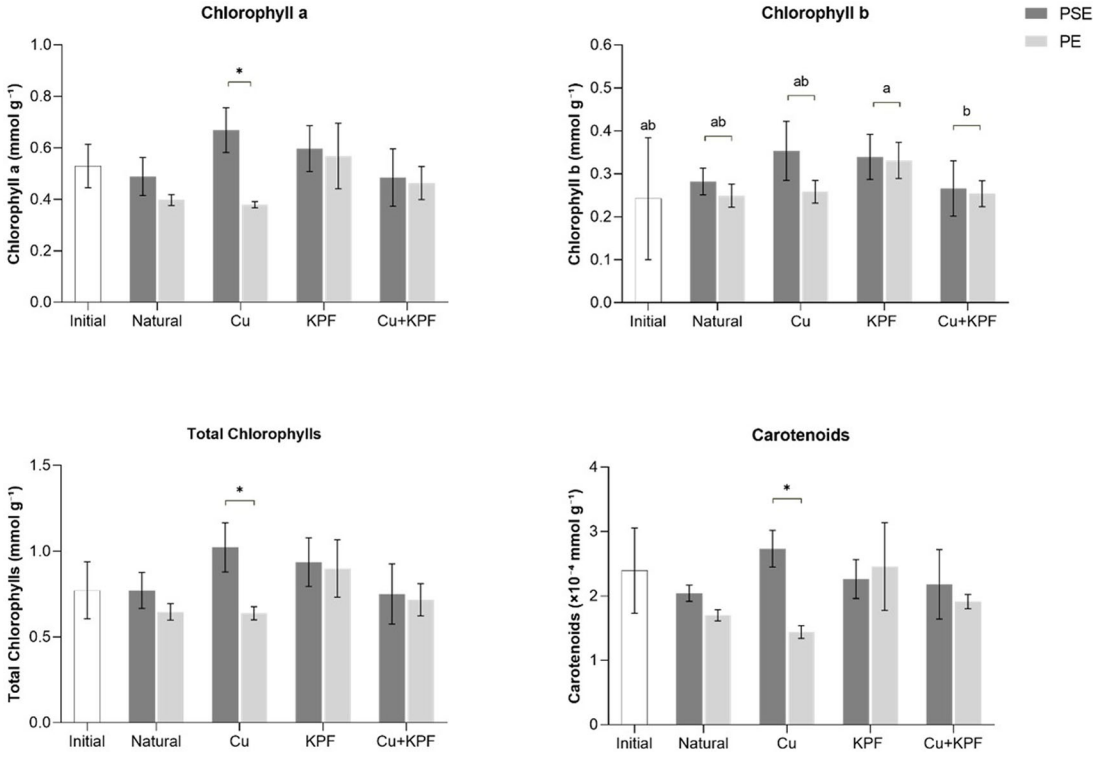

Figure 8. Pigment content in Sporobolus maritimus exposed to Cu and KPF. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments according to Tukey's post hoc test ($p < 0.05$). Asterisks indicate significant effects detected by GLM analysis ($p < 0.05$). Bars without letters or asterisks indicate no significant differences. "Initial" represents the beginning of the experiment; "Natural" indicates the control, with no contaminant addition, at the end of the experiment; Cu, KPF and Cu + KPF indicates the different treatments at the end of the experiment.

b) **Analytical procedures**

Water/elutriate samples collected for metal analysis were immediately acidified with 1% nitric acid concentrated solution (Nitric acid, puriss. p.a., 65.0% - 67.0%, Sigma-Aldrich, 84378–2.5 L) and kept at room temperature until analysis. Sediment and plant tissues collected for metal analysis were put to dry at room temperature until constant weight and subject to a high-pressure microwave digestion with nitric acid (for sediments) or nitric acid and hydrogen peroxide solution (Hydrogen peroxide, for analysis, 35 wt.% solution in water, stabilized, 1 L, Acros, 202460010) (for plant tissues) in accordance with the protocol described by Fraga-Santiago et al. (2022).

The concentrations of metals (Cd and Cu) were quantified using atomic absorption spectrometry with flame atomisation (AAS; Analyst 200, PerkinElmer). Measurements were performed directly in water/elutriate samples and in digestion extracts of solid matrices (sediments and plant tissues). Metal quantification was carried out by aqueous standard solution calibration (Cu concentrations between 0.1 and 1 mg L^{-1} and Cd concentration between 0.025 and 1 mg L^{-1}) (Fraga-Santiago et al., 2022).

The limit of detection (LOD) was estimated from the linear calibration curve using the 3-sigma criterion. The standard deviation of the response was calculated from the residual standard deviation of the regression, $s_{y/x}$, and the LOD was calculated as:

$$LOD = \frac{3 s_{y/x}}{a} \quad (1)$$

where a corresponds to the slope of the calibration line. The residual standard deviation was calculated as:

$$s_{y_{/x}} = \sqrt{\frac{\sum \left(y_i - y_{i,calc}\right)^2}{n-2}} \quad (2)$$

where y_i is the experimental response, $y_{i,calc}$ is the response predicted by the calibration model, and n is the number of calibration points. This approach applies the classical 3σ criterion for detection limits described by IUPAC (1987) and discussed by Long and Winefordner (2008) to a linear calibration model.

Water/elutriate samples collected for pharmaceutical analysis were immediately subjected to solid-phase extraction (SPE) using Oasis HLB cartridges (60 mg, 3 cc; Waters, USA). The extracts were evaporated to near dryness using a vacuum concentrator (Christ, RVC 2–18 CDplus) at 45 °C and subsequently reconstituted in the appropriate mobile phase according to the target compound (for KPF: 99.9% H_2O with 0.1% formic acid (98% PA ACS 1 L, Labkem, FORA-A0A-1 K0); for VFX: 20% H_2O with 0.1% formic acid and 80% acetonitrile (ACN, HPLC (gradient grade) 2,5 Lt, Chemlab, 15003CL00017524). The procedure followed an internally optimized laboratory methodology (unpublished), with SPE recoveries ranging between 90–100% for KPF and 99–100% for VFX.

Concentrations of pharmaceuticals (KPF and VFX) were determined using high-performance liquid chromatography (HPLC). KPF was analysed by HPLC with diode-array detection (HPLC-DAD; Hitachi LaChrom Elite system) and a 250 × 4.6 mm C18 (5 µm) Kinetex™ column (Phenomenex, USA), equipped with a C18 SecurityGuard™ precolumn (4.6 mm ID, Phenomenex, USA). The mobile phase consisted of water with 0.1% formic acid and ACN under isocratic conditions (70% ACN and 30% aqueous phase), at a flow rate of 0.5 mL min^{-1}, with detection at 254 nm and a retention time of approximately 6.3 min. VFX was analysed by HPLC with fluorescence detection (HPLC-FLD; UltiMate 3000 HPLC system, Thermo Scientific, USA) using a 100 × 4.6 mm C18 (5 µm) Acclaim column (Thermo Fisher, USA), equipped with an OPTI-GUARD® precolumn (1 mm, Merck, Germany). The mobile phase consisted of water with 0.1% formic acid and ACN under isocratic conditions (80% ACN and 20% aqueous phase), at a flow rate of 0.4 mL min^{-1}. Fluorescence

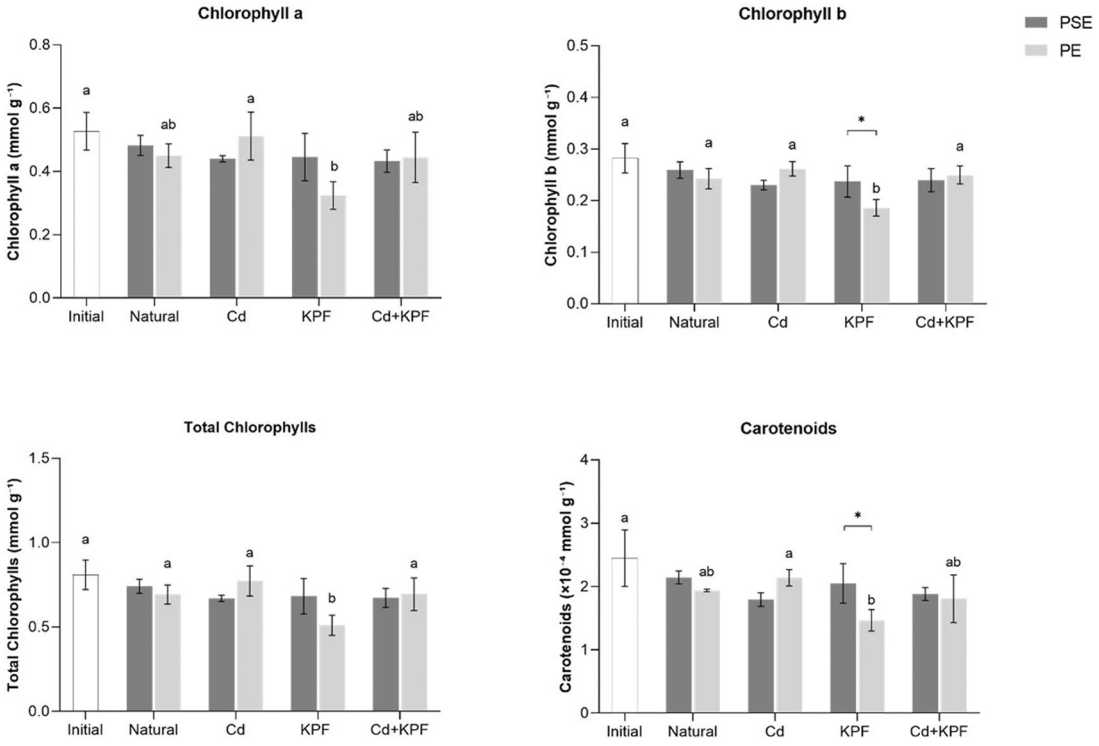

Figure 9. Pigment content in Sporobolus maritimus exposed to Cd and KPF. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among PE condition according to Tukey's post hoc test ($p < 0.05$). Asterisks indicate significant effects detected by GLM analysis ($p < 0.05$). Bars without letters or asterisks indicate no significant differences. "Initial" represents the beginning of the experiment; "Natural" indicates the control, with no contaminant addition, at the end of the experiment; Cu, KPF and Cu + KPF indicates the different treatments at the end of the experiment.

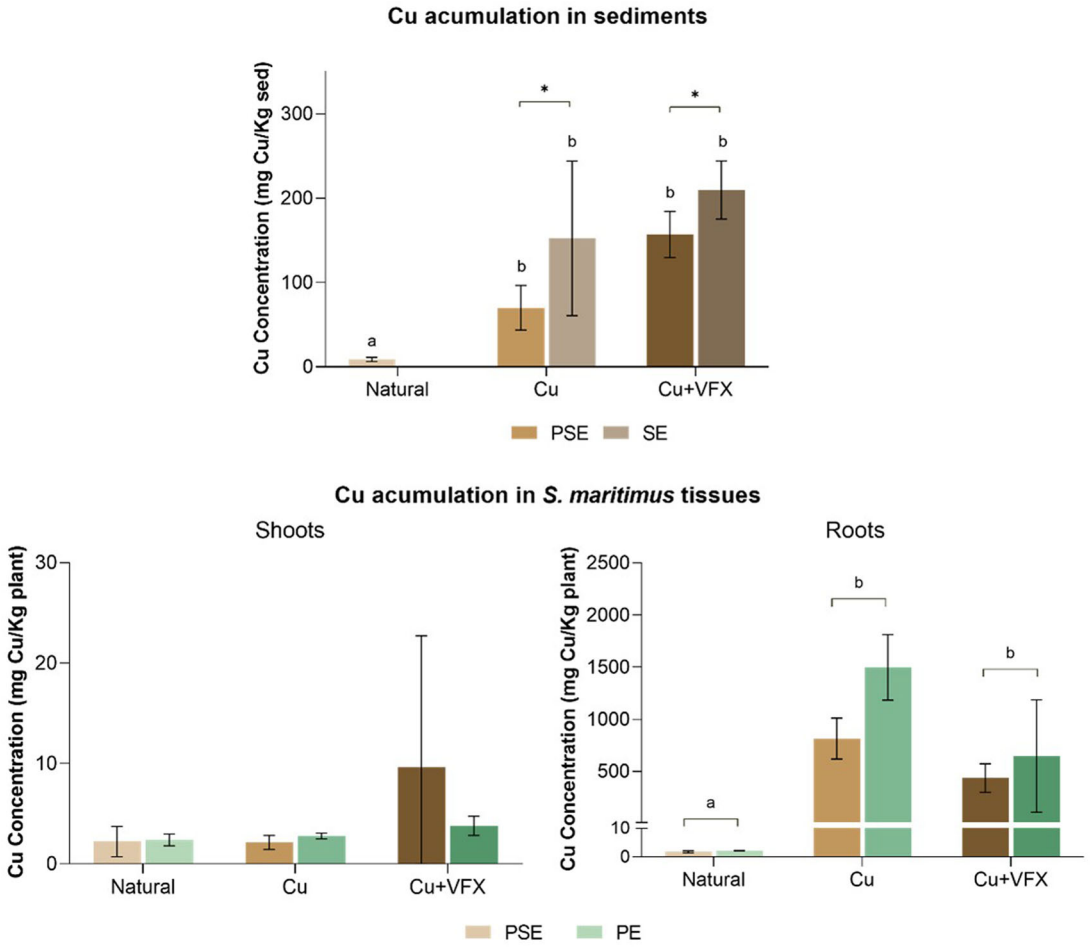

Figure 10. Cu concentration in Sporobolus maritimus during the Cu + VFX experiment. Cu concentrations measured in sediment and plant tissues (shoots and roots) under the different experimental conditions: PSE, plant + sediment + elutriate; SE, sediment + elutriate; and PE, plant + elutriate. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within each experimental condition according to Tukey's post hoc test ($p < 0.05$). Asterisks indicate significant effects detected by GLM analysis ($p < 0.05$). Bars without letters or asterisks indicate no significant differences.

detection was performed at an excitation wavelength of 278.5 nm and an emission wavelength of 304.9 nm, with a flow cell temperature of 45 °C, column temperature of 30 °C, autosampler temperature of 22 °C, and an injection volume of 10 µL. The retention time for VFX was approximately 5.0 min. All analyses were performed following an internally optimized laboratory methodology (unpublished). Pharmaceuticals were not analysed in solid samples (plant tissues and sediments) due to the lack of validated protocols.

Additionally, the chlorophyll content of plants leaves/shoots was assessed as an indicator of potential plant stress. For that, plant leaves/shoots collected at the beginning and end of each experiment were immediately extracted in methanol (HPLC 99,9 + % (Gradient Grade) Chem-Lab 2,5 Lt (EC 200–659-6/UN: 1230), 15000CL00136424) for 48 h, being the pigments determined spectrophotometrically as described in Silva et al. (2014).

c) **Statistical analysis**

Mean and standard deviation (SD) were calculated for each parameter measured in each treatment of each experiment.

Statistical analyses were performed using R version 2026.04.0 + 526 (R Foundation for Statistical Computing, Vienna, Austria). Differences among treatments and experimental conditions were assessed using generalised

linear models (GLMs), with the model structure adapted to each experimental design. In CW experiments, treatment and time (T0 and T4) were included as fixed factors. In microcosm experiments, treatment, experimental condition and tissue (when applicable) were considered as fixed factors, with interaction terms included where appropriate. Error distributions and link functions were selected according to the characteristics of each response variable and the performance of the model, and were then validated using residual diagnostics.

For pigments, due to the experimental design, only one biological replicate was available for the 'Control' treatment, whereas two replicates were analysed for each contaminant treatment. Consequently, the control treatment was included for descriptive purposes only and was not considered in the statistical analysis of the pigment data.

Model assumptions and goodness of fit were evaluated using simulation-based residual diagnostics implemented in the DHARMa package, including tests for residual uniformity, dispersion and outliers. Depending on the experimental design, Type II analysis of deviance was used to assess the significance of model terms. When significant interaction effects were detected, full models were retained; otherwise, reduced models including only main effects were considered.

Post-hoc pairwise comparisons were performed using estimated marginal means (EMMs) with a Tukey adjustment for multiple comparisons (emmeans package). Statistical significance was accepted at $p < 0.05$.

Results and discussion

1. **Freshwater treatment by constructed wetlands mesocosms**

In the Cu + VFX experiment, pigment levels generally increased over time, again with the exception of the Cu alone treatment as in the Cu + KPF assay (Figure 6). However, once again no significant differences were found between treatments containing Cu alone and those combined with VFX. This indicates that pharmaceutical co-contamination does not seem to exacerbate the physiological stress induced by metals in *C. indica.*

Water samples (CW effluents) collected at the end of each seven-days exposure cycles had metal (Cd and Cu) concentrations below the detection limit (<0.01 mg L^{-1} for Cd and < 0.05 mg L^{-1} for Cu), indicating removal >99%. Similarly, KPF and VFX were generally below detection limit (<0.05 mg L^{-1}) and occasionally below quantification limit (<0.1 mg L^{-1}), indicating removals >99.9%.

These results suggest that the contaminants were effectively retained within the system, being adsorbed and/or degraded (in the case of the organic contaminants) by microorganisms and/or accumulated in plant tissues. The high contaminants removal is consistent with previous reports demonstrating the effectiveness of CW in removing metals via adsorption, precipitation, and plant uptake and organic contaminants mainly by adsorption and degradation (Marchand et al., 2010; Vymazal, 2011, 2014; Gorito et al., 2017; Ilyas et al., 2020). The substrates used in CWs, such as sand and expanded clay, are known to facilitate the removal of contaminants from the water by increasing the surface area for adsorption and supporting microbial activity (Wang et al., 2024). The rhizosphere also contributes to contaminant attenuation through complex physicochemical and biological interactions occurring around plant roots.

Moreover, contaminants mixture did not affect CWs removal performance with no significant different among systems treating water contaminated with one (metal or pharmaceutical) or two contaminants (metal + pharmaceutical).

Monitoring of conventional water quality parameters confirmed that the CW systems remained operational throughout the experimental period. The concentrations of nitrate and ammonia ions, total phosphorus and COD exhibited the expected behaviour for functioning CW systems, indicating that treatment performance remained consistent throughout all experimental assays, without significant impact of the contaminants presence (see Dataser in Interdisciplinary Centre of Marine and Environmental Research, 2026).

Plants and substrate samples

In the experiments with KPF and Cd, Cd was initially detected in plant leaves and substrate of the systems treating Cd contaminated water (Table 1), likely due to residual contamination from previous experiments involving the same CW systems. Cd does not have a known biological function. In fact, Cd was not detected in leaves or substrate in the control system (always irrigated with water without contaminants). At the end of the experiment, Cd concentrations in plant leaves decreased or remained similar to initial values, with no clear temporal trends observed. Cd was not detected in the substrate at the end of the experiment. No differences were observed, in general, between the metal-only and combined metal and pharmaceutical treatments at the end of the experiment.

In the experiments with KPF and Cu, Cu was also initially detected in plant leaves, result expected as Cu is a micronutrient commonly present in biological matrixes. Cu concentration showed a tendency to increase from T0 to T4 in Cu exposed treatment (Table 1), which may indicate Cu accumulation and translocation in *C. indica* over the four-week experimental period. However, no statistically significant effects of treatment, time or contaminants interaction were observed ($p > 0.05$), although a marginal treatment effect was detected (GLM, $p = 0.055$). In the substrate, at T0, Cu concentrations were below the detection limit (LOD = 0.8 mg kg^{-1}). At T4, Cu was only quantifiable in the Cu + KPF treatment, while the remaining treatments remained below the detection limit. Although statistical comparisons were not performed due to the high proportion of non-detects, these results may suggest partial Cu retention within the CW substrate over time.

In the Cd + VFX experiment, Cd concentrations in the plant's leaves were below the limit of detection (LOD = 0.3 mg kg^{-1}) in control treatment. In contrast, a slight upward trend in Cd concentration was observed in the leaves of the Cd-only and Cd + VFX treatments from the start to the end of the experiment (Table 1). Cd was only detected in substrate in the Cd treatment at the end of the experiment (T4). In all other cases, levels were below the limit of detection (0.8 mg kg^{-1}).

Similarly, in the experiment involving Cu and VFX, no significant differences in Cu concentration were observed in the leaves of the plants between the Cu and Cu + VFX treatments at either T0 or T4, with no significant Cu increase over time. In the substrate, Cu concentrations were below the detection limit (LOD = 4 mg kg^{-1}) at T0 for all treatments and at T4 for Cu treatment. However, at T4, in the combined metal and pharmaceutical treatment, Cu increased (Table 1), indicating Cu retention within the CW system, as observed before for the Cu + KPF treatment.

It should be noted that metals were analysed only in the leaves of the plants tissues, as the systems were not disassembled after each experiment and as so no plant roots were collected. So, metal concentration in plant leaves may be low, as in general only a low amount of metals absorbed by the roots is translocated to the leaves (Marchand et al., 2010). Moreover, the only substrate collected at the beginning and end of the experiment was sand in contact with plant roots. Expanded clay has also potential to adsorbed metals and could contribute for the retention of the metal within the CW system. However, as the system was not dismantled it was not possible to collect substrate from this layer. Pharmaceutical concentrations were not determined in plant tissues or substrate samples. Therefore, although the high removal rates observed in the effluents suggest efficient retention and/or transformation of contaminants within the CW systems, it was not possible to determine the relative contribution of plant uptake, substrate adsorption and microbial degradation. Consequently, the mechanisms responsible for contaminants removal can only be deduced based on previous studies reporting the importance of these processes in constructed wetlands (Fernandes et al., 2017; Malyan et al., 2021).

Overall, systems efficiently retained contaminants from the freshwater. In general, no significant differences were observed between treatments involving only metals and those combining metals with pharmaceuticals (KPF or VFX). This suggests that no clear effect was observed on the retention, accumulation or translocation of metals within the CW systems, although a potential impact of organic contaminants in Cu retention in the substrate might have occurred, with higher Cu retention. In all cases, metals were efficiently retained within the CWs and distributed among the different compartments of the systems over time. This indicates the effectiveness of *C. indica*-based constructed wetlands for removing metals from contaminated water, contributing to prevent metal inputs into the environment.

Photosynthetic pigment content

The levels of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were measured in plant leaves at the start (T0) and finish (T4) of each experimental assay.

In the Cd + KPF experiment, a general decrease trend in pigment content was observed across all treatments over the experimental period, particularly for chlorophyll b; although no statistically significant differences were detected (GLM,

Table 1. Metal concentrations in Canna indica leaves and substrate during the experimental assays. Metal concentrations in leaves and substrate measured at the beginning, T0, and end, T4, of each experimental assay. Concentrations are expressed as mg kg^{-1} dry weight. Values are means ± SD, n = 2, except control with n = 1. ND indicates values below the detection limit (LOD for Cd in leaves: 0.3–0.4 kg^{-1}; LOD for Cd in substrate: 0.8 kg^{-1}; LOD for Cu in leaves: 1.8–5.5 mg kg^{-1}; LOD for Cu in substrate: 3–8 mg kg^{-1}). NA indicates values that were not analysed due to technical problems.

Assay	Matrix	Treatment	T0	T4
Cd + KPF	***Leaves***	Control	ND	ND
		Cd	1.29	ND
		Cd + KPF	0.96	1.00
	Substrate	Control	ND	ND
		Cd	1.1 ± 0.4	ND
		Cd + KPF	ND	ND
Cu + KPF	***Leaves***	Control	3.5	4.5
		Cu	6 ± 3	10 ± 5
		Cu + KPF	8.9 ± 0.4	10 ± 3
	Substrate	Control	ND	ND
		Cu	ND	ND
		Cu + KPF	ND	7.29
Cd + VFX	***Leaves***	Control	ND	ND
		Cd	0.58	0.85 ± 0.03
		Cd + VFX	0.7 ± 0.3	1.0 ± 0.2
	Substrate	Control	ND	ND
		Cd	ND	1.12
		Cd + VFX	ND	ND
Cu + VFX	***Leaves***	Control	NA	3.66
		Cu	7.74 ± 0.09	7 ± 2
		Cu + VFX	7.8 ± 0.8	6 ± 1
	Substrate	Control	ND	ND
		Cu	ND	ND
		Cu + VFX	ND	8.2 ± 0.5

$p > 0.05$, Figure 3). A similar trend was observed in the control treatment, suggesting a general decline in the physiological condition of the plants over time due to experimental conditions rather than a specific effect of contaminant exposure. Nevertheless, the combined Cd and KPF treatment consistently showed the lowest pigment values, suggesting a potential higher level of physiological stress in this treatment, although these differences were not statistically significant.

In contrast, in the Cu + KPF experiment, pigment concentrations showed a tendency to increase at the end of the experiment (T4) in all treatments, except those with Cu alone, although no statistically significant differences were again observed (Figure 4). This pattern is consistent with the role of Cu as an essential micronutrient, that can be toxic at high concentrations. The highest pigment values were observed in the control, which may indicate a subtle negative effect of contaminant exposure on plant physiological status. This further supports the idea that CW systems receiving moderate Cu loads may retain the metal efficiently while sustaining plant physiological performance.

As in the Cd experiments with KPF, the Cd + VFX assay revealed a general reduction in the levels of several photosynthetic pigments at the end of the experiment (T4) (Figure 5). However, similar temporal changes were also observed in the control treatment, suggesting that these responses cannot be attributed exclusively to Cd exposure. Although treatment and time effects were detected for some pigments, no consistent pattern of pigment reduction was observed in Cd exposed plants compared to the other treatments. Overall, the results suggest that *C. indica* was able to

Table 2. Pharmaceutical removal from elutriate solutions under different experimental conditions. Pharmaceutical removal percentages (%) from elutriate solution obtained under the different assays and experimental conditions. Values are means ± SD, n = 3. NR indicates no removal.

Plant	Pharmaceutical	Treatment	Plant + Sed + Elu	Sed + Elu	Plant + Elu	Elu
Sporobolus maritimus	VFX	VFX	92 ± 12	93 ± 3	47 ± 19	30
		Cu + VFX	94 ± 2	94 ± 3	79 ± 18	73
Sporobolos maritimus	KPF	KPF (Cu assay)	60 ± 9	51 ± 18	1 ± 11	8 ± 2
		Cu + KPF	49 ± 3	35 ± 2	6 ± 7	NR
Sporobolos maritimus	KPF	KPF (Cd assay)	13 ± 14	NR	NR	NR
		Cd + KPF	8 ± 10	8 ± 9	10 ± 20	NR
Juncus maritimus	KPF	KPF	-	-	NR	8 ± 43
		Cu + KPF	-	-	NR	9 ± 37
		Cd + KPF	-	-	NR	NR

Table 3. Metal removal from elutriate solutions in Sporobolus maritimus experiments. Metal removal percentages (%) from elutriate solution obtained under the different assays and experimental conditions. Values are means ± SD, n = 3. NR indicates no removal.

Metal	Treatment	Plant + Sed + Elu	Sed + Elu	Plant + Elu	Elu
Cu	Cu (VFX assay)	92 ± 1	96 ± 1	73 ± 3	73 ± 16
	Cu + VFX	97 ± 1	96 ± 1	77 ± 0.5	80 ± 4
Cu	Cu (KPF assay)	97 ± 0.1	98 ± 1	87 ± 1	48 ± 1
	Cu + KPF	98 ± 0.3	99 ± 1	90 ± 5	17 ± 17
Cd	*Cd*	82 ± 11	91 ± 2	95 ± 1	NR
	Cd + KPF	81 ± 8	93 ± 1	97 ± 2	2 ± 4

maintain its photosynthetic performance despite showing some variation in pigment content over time when exposed to Cd.

Overall, the pigment responses observed in *C. indica* suggest a balance between physiological stress under metal exposure and functional resilience within the CW system. These results emphasise the dual function of *C. indica*-based CWs, which combine effective metal retention with potential active plant uptake while maintaining sufficient physiological performance under contaminant pressure. Despite the observed physiological responses, the plants remained functional throughout the experimental period, which supports the long-term suitability of *C. indica* for use in CWs for treating - freshwater contaminated either with metals or pharmaceutical or a mixture of the two.

2. **Estuarine natural treatments in wetland microcosms**

Sporobolus maritimus.

Photosynthetic pigment content

In the Cu + VFX experiment, no significant differences in photosynthetic pigment content were observed between the contaminant treatments and the natural control, regardless of the exposure medium used (elutriate alone or elutriates combined with sediment) (Figure 7). Experimental conditions (comparison between initial and natural control after 7 days of experiment) also did not impact pigments contents. However, an overall increase in pigment levels was detected in the combined Cu and VFX treatment, suggesting a potential stimulatory effect when both contaminants were present. Nevertheless, this pattern was not statistically significant.

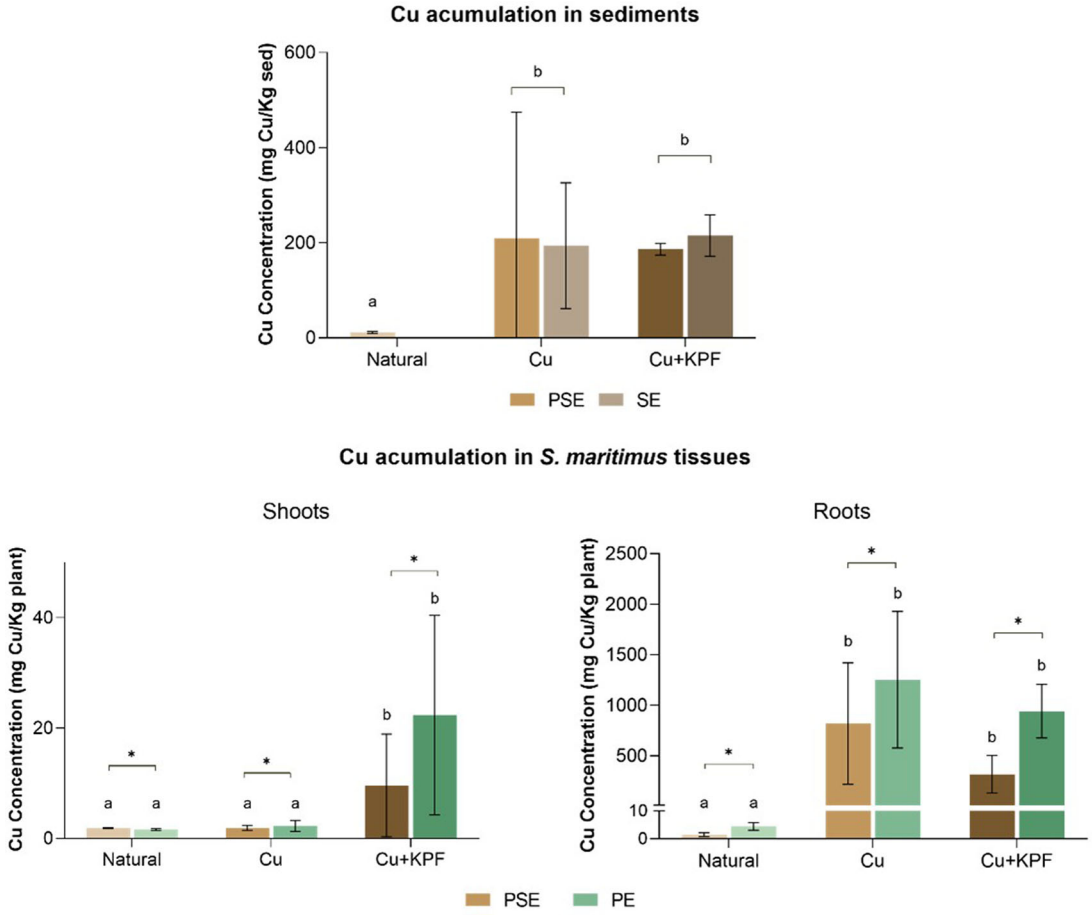

Figure 11. Cu concentration in Sporobolus maritimus during the Cu + KPF experiment. Cu concentrations measured in sediment and plant tissues (shoots and roots) under the different experimental conditions: PSE, plant + sediment + elutriate; SE, sediment + elutriate; and PE, plant + elutriate. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within each experimental condition according to Tukey's post hoc test ($p < 0.05$). Asterisks indicate significant effects detected by GLM analysis ($p < 0.05$). Bars without letters or asterisks indicate no significant differences.

By contrast, the content of photosynthetic pigments in the Cu + KPF experiment was significantly influenced by the treatment and exposure conditions (Figure 8). Significant interactions were observed between the treatment and conditions for chlorophyll a, total chlorophyll, and carotenoids levels. However, despite these statistical effects, pairwise comparisons revealed only limited differences between the control and contaminant treatments. No consistent pattern of pigment reduction was observed under exposure to Cu or Cu + KPF. Instead, several differences were associated with the exposure medium, suggesting that the presence of sediment contributed more to the variability in pigment levels than exposure to the pollutants themselves. Overall, pigment concentrations remained within the range of natural variability, indicating that photosynthetic performance was not substantially affected by the experimental conditions, with no differences between initial and final conditions. The differences observed with the Cu + VFX experiment might just reflect the fact that the new pool of plants was collected in a different month of the year (one in spring and another in late summer) which can impact plant physiology.

Similarly, in the Cd + KPF experiment, plants exposed to KPF under elutriate-only conditions generally exhibited lower concentrations of photosynthetic pigments than those observed in the other treatments (Figure 9). This trend was particularly clear for chlorophyll b, total chlorophyll and carotenoids, whereas the response of chlorophyll a was less pronounced. In contrast, pigment concentrations were comparable among treatments when sediment was present, suggesting that sediment may mitigate the physiological effects associated with pharmaceutical exposure. Overall, these

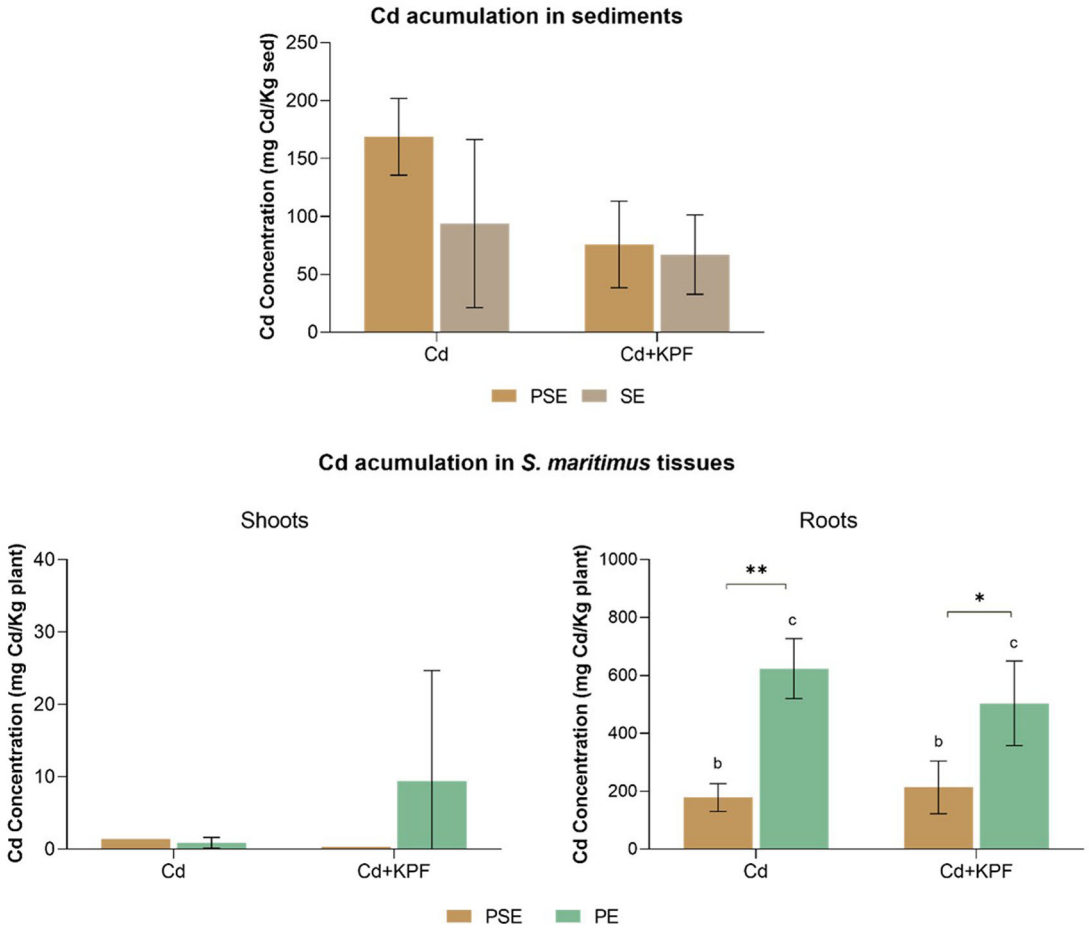

Figure 12. Cd concentration in Sporobolus maritimus during the Cd + KPF experiment. Cd concentrations measured in sediment and plant tissues (shoots and roots) under the different experimental conditions: PSE, plant + sediment + elutriate; SE, sediment + elutriate; and PE, plant + elutriate. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within each experimental condition according to Tukey's post hoc test ($p < 0.05$). Asterisks indicate significant effects detected by the statistical analysis ($p < 0.05$). Bars without letters or asterisks indicate no significant differences. Cd concentrations in the natural treatment were below the limit of detection (LOD; sediment = 0.92 mg kg^{-1} and plant tissue = 0.232 mg kg^{-1}).

results suggest that KPF might have a moderate impact on the photosynthetic status of *S. maritimus*, particularly when contaminant bioavailability is high.

Pharmaceutical removal from elutriates solutions

In the VFX experiment, in the treatment without Cu, significant differences in pharmaceutical removal from elutriate solution were observed among the exposure media. Higher removal was observed in systems containing sediment, particularly in the plant + sediment + elutriate and sediment + elutriate treatments (92% and 93%, respectively), compared to the plant + elutriate and elutriate-only treatments (47% and 30%, respectively, Table 2). These results emphasise the important role of sediment in enhancing VFX removal from water in estuarine environments, likely through adsorption processes and increased microbial activity.

Conversely, when Cu was present, the removal efficiency of VFX remained generally high across all exposure media. The overall pattern of higher removal in sediment-containing treatments remained evident (plant + sediment + elutriate: 94%; sediment + elutriate: 94%; plant + elutriate: 79%; elutriate-only: 73%, Table 2). These results suggest that Cu may modulate VFX removal to some level, but sediment presence remains the primary determining factor in removal efficiency.

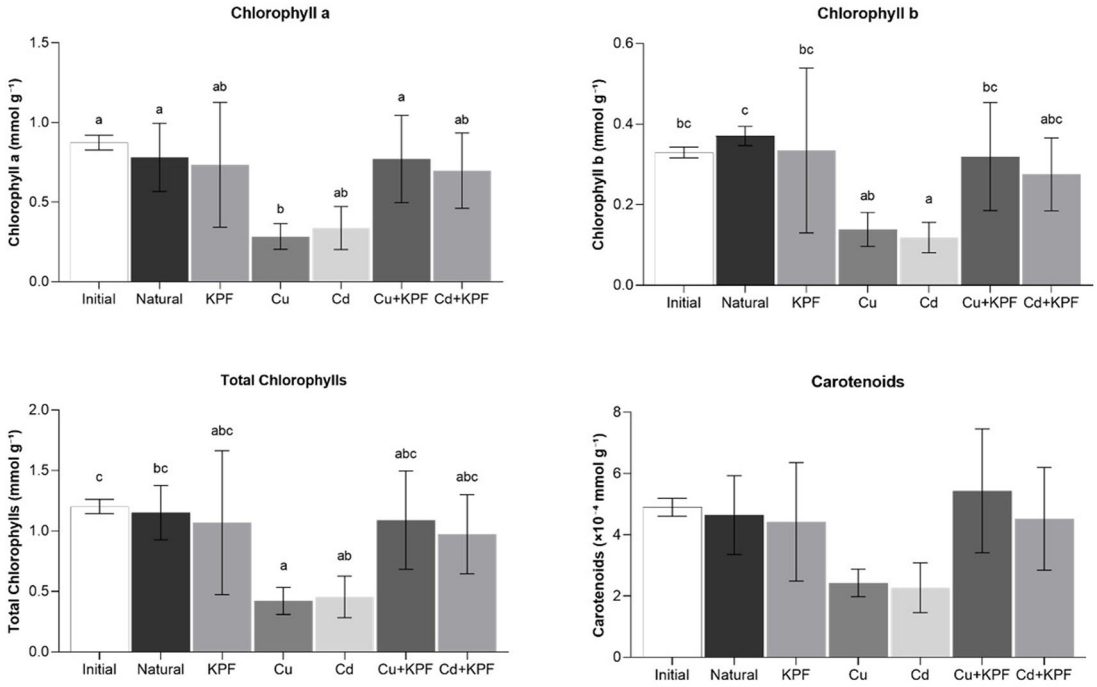

Figure 13. Pigment content in Juncus maritimus exposed to Cu, Cd and KPF. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments according to Tukey's post hoc test ($p < 0.05$). Bars sharing the same letter are not significantly different. "Initial" represents pigment concentrations at the beginning of the experiment, whereas "Natural" corresponds to the uncontaminated control at the end of the exposure period.

Overall, the results suggest that the presence of plants did not significantly impact VFX removal, as high attenuation occurred also in treatments lacking vegetation. The enhanced removal observed in sediment-containing systems suggests that sorption to the particulate and colloidal matter present in the elutriate substantially contributed to the attenuation of pharmaceuticals. Colloids are known to exhibit a strong affinity for organic contaminants, thereby facilitating their removal from aqueous phases (Xing et al., 2015), which can justify VFX removal observed in elutriate alone systems. This behaviour is further supported by the hydrophobic nature of VFX (log KOW = 3.2), which favours strong interactions with organic-rich sediments. Furthermore, the increased VFX removal observed in the presence of Cu, when sediment was absent, suggests a potential role of metal–pharmaceutical interactions, potentially through complexation processes that may enhance sorption or transformation within the system. Nevertheless, under these conditions, no additional significant contribution from plant uptake was evident, which reinforces the predominant role of sediment-associated processes in VFX removal under estuarine wetland conditions.

In KPF experiments, no significant differences were observed between treatments containing KPF alone and those supplemented with Cu, suggesting that there is no clear effect of the metal on the attenuation of the pharmaceutical. However, significant differences were observed among systems, with the plant + sediment + elutriate system exhibiting the highest removal efficiencies (60% for KPF alone and 49% for Cu + KPF, Table 2). This was followed by the sediment + elutriate system (51% and 35%, respectively). These results suggest that also sediment played a role in KPF attenuation, while plant uptake contributed less.

In the Cd + KPF experiments, low removal percentages were observed. In the absence of metals, KPF removal reached only 13% in the plant + sediment + elutriate system. When Cd was present, attenuation remained low across treatments, with removal of 8% in the plant + sediment + elutriate system, 10% in the plant + elutriate system, and 8% in the sediment + elutriate system (Table 2). Statistical analysis confirmed that KPF removal was significantly affected by experimental condition (GLM, $p < 0.001$), whereas no significant effect of Treatment was detected (GLM, $p = 0.146$), and the Treatment × Condition interaction was not statistically significant (GLM, $p = 0.084$). Overall, KPF attenuation remained low under all tested experimental conditions, with the lowest removal occurring in elutriate-only systems. This indicates that system composition had a stronger influence on pharmaceutical attenuation than the presence of Cd.

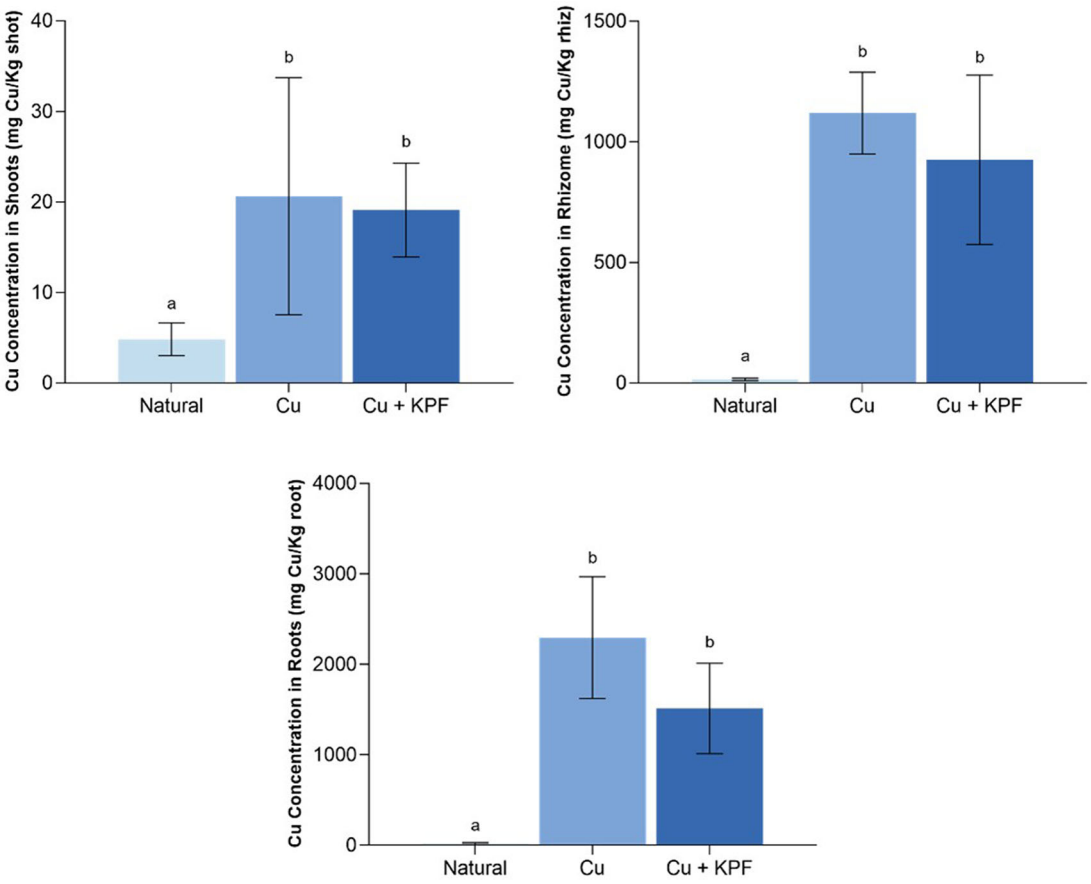

Figure 14. Cu concentration in Juncus maritimus tissues. Cu concentrations measured in shoots, rhizomes and roots of Juncus maritimus. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within each experimental condition according to Tukey's post hoc test ($p < 0.05$).

Despite KPF's hydrophobic nature (log KOW = 3.1), which would favour interactions with organic-rich substrates, removal was inconsistent and depended more on the presence of sediment than on plant contribution. Variations in the content of organic matter in sediments may partially explain the higher attenuation observed in Cu experiments relatively to those of Cd, as the capacity for sorption is influenced by the availability of organic matter and physicochemical conditions. However, given the limited magnitude of KPF removal and the lack of consistent metal effects, these mechanisms appear to play a secondary role compared to those governing VFX behaviour.

Overall, the results suggest that, as with VFX, the presence of plants did not significantly enhance KPF attenuation in elutriate systems. Removal occurring in the absence of vegetation suggests that sediment-associated processes predominate, including sorption to particulate and colloidal matter. However, unlike VFX, KPF exhibited lower retention efficiencies, suggesting weaker sediment affinity and highlighting its compound-specific behaviour within estuarine wetland systems.

Metal removal and accumulation

Elutriate analyses revealed very high Cu removal percentages from elutriate solutions in sediment-containing systems (>97%), followed by moderate removal in plant + elutriate systems, and substantially lower removal in elutriate-only treatments (Table 3). The presence of VFX or of KPF did not significantly affect Cu removal dynamics.

In systems exposed to Cu, significant Cu accumulation occurred in sediments (Figure 10). Concentrations increased significantly from natural conditions to Cu-only treatments, reaching the highest levels under combined Cu + VFX exposure. VFX seemed to promote Cu retention in sediment, although no significant differences were observed between the exposure media. Sediment Cu concentrations were higher in the sediment + elutriate systems than in the plant +

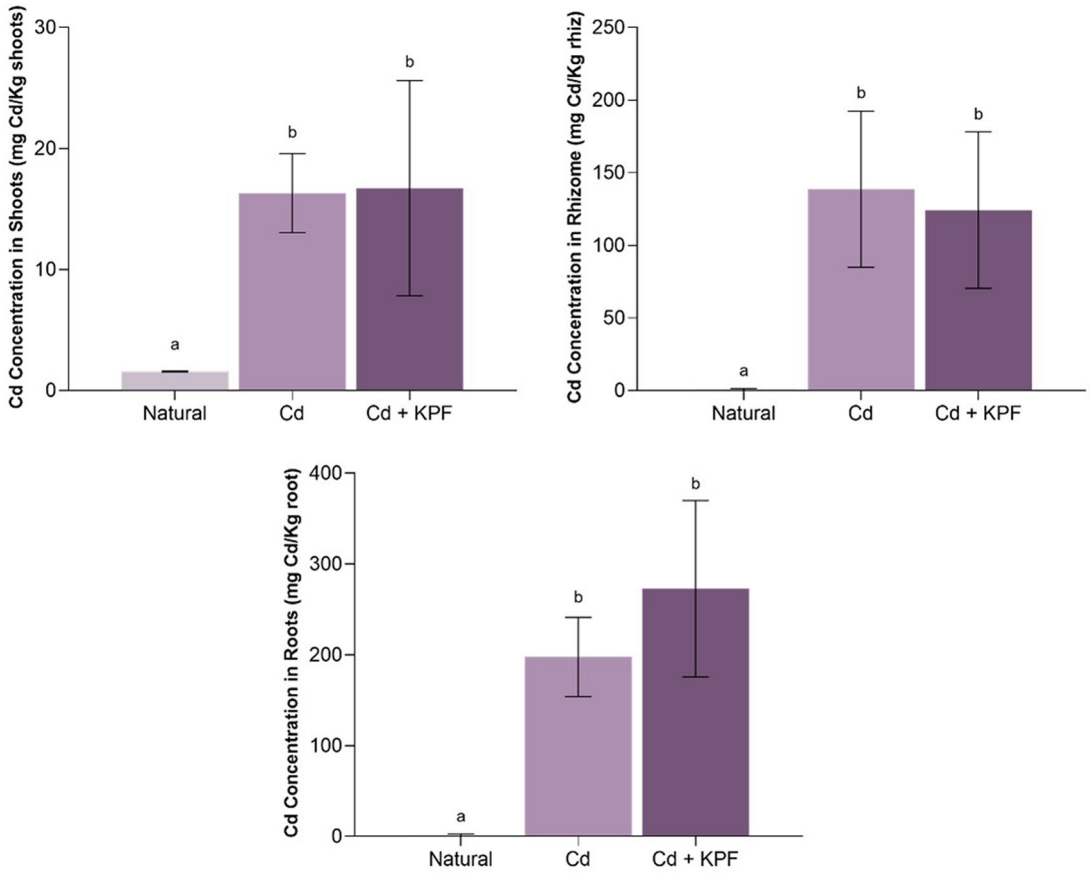

Figure 15. Cd concentration in Juncus maritimus tissues. Cd concentrations measured in shoots, rhizomes and roots of Juncus maritimus. Values are means ± SD (n = 3). Different lowercase letters indicate significant differences among treatments within each experimental condition according to Tukey's post hoc test ($p < 0.05$).

Table 4. Metal removal from elutriate solutions in Juncus maritimus experiments. Metal removal percentages (%) from elutriate solution. Values are means ± SD, n = 3. NR indicates no removal.

Metal	Treatment	Plant + Elu	Elu
Cu	Cu	81 ± 2	73 ± 2
	Cu + KPF	81 ± 2	68 ± 5
Cd	Cd	33 ± 6	NR
	Cd + KPF	38 ± 7	NR

sediment + elutriate systems. These results suggest that, while the presence of plants slightly reduced Cu accumulation in sediments, probably due to plant uptake, sediment remained the main sink for Cu, regardless of system configuration.

Cu accumulation in plant tissues was primarily confined to the roots (Figure 10). Significantly higher concentrations were observed in the Cu treatments than in natural conditions. In the Cu + VFX treatments, plant root accumulation was slightly lower, although not significantly, indicating a potential lower Cu availability due to VFX and accordingly to the slightly higher Cu retention in sediment. As mention before, metal-complexation with VFX might have occurred which can reduce Cu availability. Plant accumulation was slightly higher when sediment was absent, reflecting the higher Cu availability in this case. In contrast, Cu levels in shoots remained low and did not differ significantly between treatments, suggesting limited translocation to aerial tissues during the 7-day exposure period.

For Cu combined with KPF experiment, sediment again exhibited significantly higher Cu accumulation in both Cu and Cu + KPF treatments than in natural conditions, with no difference between the exposure media (Figure 11). Therefore, sediment is the main player in the removal of the metal from the aquatic media, but contrarily to VFX, KTF does not seem to have an impact on Cu retention in sediment. Cu accumulation in plant tissues was strongly dependent on specific tissue type (Figure 11). Root concentrations were again significantly higher than shoot concentrations in both Cu and Cu + KPF treatments, indicating that most of the accumulated metal remained belowground. As for VFX, plant accumulation was slightly higher when sediment was absent, reflecting the higher Cu availability in this case and slightly lower when KPF was present.

Shoot concentrations generally remained low, again without metal translocation in the Cu treatment. However, plants exposed to Cu + KPF showed slightly higher Cu levels in shoots, indicating a potential metal translocation, accordingly to the a slightly lower metal content in plant roots compared with the Cu treatment.

In the Cd + KPF experiment, the removal of Cd from elutriates solutions followed a similar pattern to that observed for Cu, with the highest removal efficiencies recorded in systems containing sediment and/or plants, followed by systems containing plants + sediment + elutriate (Table 3). No removal was observed in systems containing elutriate alone, highlighting the importance of biological and sediment compartments in Cd attenuation. There were no significant differences observed between the Cd alone and Cd with KPF treatments, both performed similarly in terms of removal efficiency across all experimental conditions (Table 3).

The concentration of Cd in the sediments was also significantly higher in the treatments exposed to Cd than in the natural treatment, indicating again metal retention in the sediment (Figure 12). Although the Cd and Cd + KPF treatments showed similar sediment concentrations, there was a slight, non-significant trend towards lower Cd levels under combined exposure when plants were present.

Cadmium accumulation in plant tissues occurred mainly in the roots, while translocation to the stems remained low across all treatments, with no significant differences observed between treatments or between exposure conditions (Figure 12). This confirms that Cd mobility within the plant system was limited during the experimental period. However, a significant influence of both the treatment and the condition was observed in the roots. This indicates that Cd uptake was probably primarily driven by system-specific conditions and that the addition of KPF had no consistent effect. Specifically, higher accumulation was observed in the roots under Plant + Elutriate condition than in systems containing sediment, suggesting that Cd was more bioavailable in the absence of sediment, as already observed for Cu.

Overall, sediments played a dominant role in removing metals from elutriate solutions, acting as major sinks through adsorption processes. This is reflected by high sediment metal concentrations and removal efficiencies exceeding 90%, which is consistent with previous observations in estuarine environments (Fraga-Santiago et al., 2022). Consistently low levels of metals have been reported in Lima estuarine waters, which supports the idea of strong sediment retention (Freitas et al., 2025). Despite this dominance, *S. maritimus* demonstrated a clear capacity for metal accumulation, particularly in belowground tissues. Cu concentrations in roots increased by several hundredfold relative to natural conditions, while Cd levels increased by more than three orders of magnitude, confirming effective metal uptake even in sediment-rich environments. However, metal translocation to aerial tissues remained negligible, likely due to the short exposure period and the way in which metals are compartmentalised physiologically within root cells.

Metal accumulation patterns were strongly influenced by sediment, which supports the idea that sediment reduces metal bioavailability through adsorption and complexation mechanisms (Duarte et al., 2010). The presence of pharmaceuticals (KPF and VFX) had a slightly effect on metal accumulation. Similar compound-specific behaviour was observed for the pharmaceuticals: VFX showed slightly higher attenuation in sediment-containing systems, whereas KPF exhibited lower and more variable removal. These results are consistent with environmental observations indicating that VFX is predominantly retained in estuarine sediments, while KPF persists more in the water column (Gorito et al., 2024). This highlights the central role of sediment-associated processes in contaminant dynamics.

Taken together, these findings demonstrate the complementary roles of sediment retention and plant uptake in estuarine wetland systems. Sediment acts as the primary contaminant sink, while vegetation acts as an additional sink in belowground tissues, complementing the dominant role of sediments in the remediation process.

Juncus maritimus.

Based on the results obtained for *S. maritimus*, experiments were conducted with *J. maritimus* using only elutriate solutions without sediment, focusing on the combination of KPF with Cu and Cd, the conditions where more potential impact of contaminants interactions and bioavailability can be observed. This design aimed to maximise aqueous contaminant bioavailability and evaluate plant-mediated physiological responses under sediment-free conditions.

Photosynthetic pigment content

Pigment concentrations in *J. maritimus* were significantly affected by the treatment, with chlorophyll a, chlorophyll b, total chlorophyll and carotenoids levels showing significant differences among treatments ($p < 0.01$ in all cases). However, paired comparisons revealed a limited differences between specific treatments. Nevertheless, a consistent pattern emerged in which plants exposed to metals alone generally showed lower pigment concentrations than those exposed to KPF treatments or natural conditions. A general decrease in pigment content was mainly associated with exposure to Cu or Cd alone, particularly for chlorophyll b and total chlorophylls (Figure 13).

This reduction may be an early physiological response to metal exposure when metals are present in the aqueous phase, where bioavailability is higher in the absence of sediment (Almeida et al., 2004). Metal-induced impairment of photosynthetic processes has been widely associated with disruptions in chlorophyll synthesis and increased oxidative stress in wetland plants (Nikalje & Suprasanna, 2018). Pigment concentrations were maintained in treatments containing KPF, suggesting that the presence of the pharmaceutical did not exacerbate, or even reduced, the physiological stress caused by exposure to metals during the experimental period. These patterns were different from those observed in *S. maritimus*, suggesting that there are species-specific responses to exposure to contaminants. However, differences in the sampling period and the seasonal physiological status of the plants cannot be excluded, since the plants used in the different experiments were collected at different moments of the year, which may have affected their pigment content and stress responses.

Pharmaceutical removal from elutriate solutions

KPF concentrations in the elutriation solutions exhibited low and highly variable attenuation throughout the entire experimental period. Overall, no removal or very low removal was observed, and no consistent pattern was detected across the different treatments (Table 2). Statistical analysis revealed no significant differences between KPF alone and KPF combined with Cu or Cd. This indicates that the presence of metals did not significantly influence KPF attenuation under the analysed conditions.

Overall, removal was not significantly affected by the presence of plants, indicating that *J. maritimus* did not measurably contribute to pharmaceutical attenuation under these experimental conditions. The very low removal percentages observed suggest that natural dissipation processes in the aqueous phase are limited, regardless of metal presence (Table 2).

This behaviour is consistent with the reported resistance of KPF to phytoremediation, which has been attributed to its molecular structure limiting sorption and enzymatic transformation by plants (Tyumina et al., 2023). Efficient KPF degradation typically relies on specialized microbial or fungal communities capable of biodegradation, which were not incorporated in the present experimental design (Marco-Urrea et al., 2010; Almeida et al., 2013), which might explain the low and variable removal observed.

Furthermore, the low level of attenuation observed is consistent with field observations in estuarine systems, in which KPF is frequently found in the water column (Gorito et al., 2024).

Metal removal and accumulation

Both metals, Cu and Cd, accumulated mostly in belowground tissues of *J. maritimus*, following a consistent pattern of roots > rhizomes > shoots under metal exposure (either alone or combined with KPF) (Figure 14; Figure 15). Root concentrations increased markedly relative to the control group (increasing 130-fold for Cu and 160-fold for Cd), with lower, yet still substantial, accumulation in rhizomes and significantly lower levels in shoots (increasing 4-fold for Cu and 10-fold for Cd). Although aerial concentrations remained low, the presence of detectable metal levels in shoots indicates partial translocation from the belowground compartments. This distribution pattern is characteristic of short-term exposure (7 days).

Regarding metal attenuation in the elutriate solution, Cu removal was high when plants were present (81% for Cu and Cu–KPF treatments, Table 4). The substantial attenuation of Cu in the absence of vegetation suggests the involvement of abiotic processes, although the specific mechanisms could not be identified in this study. The higher removal rate observed in the planted medium exposure, together with the significant accumulation in plant tissues, indicates a biological contribution to Cu attenuation.

In contrast, Cd removal exclusively occurred in treatments containing plants, reaching 33% for Cd alone and 38% in Cd–KPF systems (Table 4). No removal was detected in elutriate-only controls. This suggests that Cd attenuation in the absence of sediment is primarily driven by plant uptake rather than by physicochemical processes.

The presence of KPF did not significantly affect metal concentrations in the elutriate solution, but there was a tendency towards reduced Cu uptake and slightly increased Cd accumulation in belowground tissues. While these effects were not statistically significant, this suggests that the presence of co-occurring organic contaminants may influence the bioavailability of metals and the dynamics of their uptake by plants, as has been previously observed in co-contaminated salt marsh systems (Almeida et al., 2009; Oliveira et al., 2014).

Taken together, these findings confirm the phytoremediation capacity of *J. maritimus* in estuarine environments (Almeida et al., 2004), highlighting the importance of belowground tissues (roots and rhizomes) in metal storage. They also indicate that, even when subtle, interactions between metals and pharmaceuticals should be considered when evaluating remediation strategies under multi-contaminant scenarios.

Conclusions

The present study shows that constructed wetlands with *C. indica* and natural estuarine systems with native halophytes, such as *S. maritimus* and *J. maritimus*, are effective in reducing the concentration of metals and pharmaceuticals under controlled experimental conditions, demonstrating significant contaminant attenuation potential. Nevertheless, contaminant removal occurred through different pathways depending on system structure and environmental context.

In freshwater mesocosms, *C. indica* constructed wetland promoted efficient attenuation of Cu, Cd, KPF and VFX over seven-day cycles. These contaminants were retained within the CW system through a combination of adsorption to the substrate, uptake by the plant and transformation processes. Metals were distributed among the different compartments of the system without significant interference from pharmaceuticals, while leaf pigment levels showed only moderate changes that did not compromise plant functionality. These results support the use of *C. indica*-based CWs as a potential upstream treatment strategy, which is capable of reducing contaminant loads before they are discharged into downstream aquatic ecosystems.

Estuarine microcosms revealed that sediment was the dominant sink for metals and VFX in *S. maritimus* systems, achieving removal efficiencies of over 90% independently of vegetation. KPF was associated with moderate changes in pigment content when exposed to specific conditions. In the absence of sediment, *J. maritimus* became indispensable for the uptake of Cd (33–38% removal), while Cu attenuation reflected both plant sequestration and abiotic losses. Across halophytes, belowground tissues acted as key storage sites, limiting the aerial translocation that is characteristic of phytostabilisation.

Pharmaceutical behaviour differed markedly between compounds. While VFX showed strong attenuation in sediment containing systems, KPF remained largely in the aqueous phase, with attenuation below 13%, highlighting its persistence under saltmarsh conditions. Interactions between metals and pharmaceuticals occasionally influenced uptake patterns, though without compromising overall retention trends.

Overall, exposure to a combination of metals and pharmaceuticals had a limited impact on the removal and accumulation of contaminants, as well as on the physiological responses of plants, in both freshwater and estuarine systems. The presence of pharmaceuticals did not affect metal retention, while metals had only minor, compound-specific effects on pharmaceutical attenuation. While some variations in uptake and pigment responses were observed under specific experimental conditions, the overall remediation performance of constructed and estuarine wetland systems was not significantly impacted by pollutant mixtures. These findings suggest that nature-based remediation strategies can remain effective in scenarios involving multiple contaminants.

These findings emphasise the importance of adapting nature-based solutions to specific environmental settings. Constructed wetlands with *C. indica* appear suitable for upstream freshwater treatment, whereas estuarine saltmarsh remediation benefits from the combined role of sediments and native halophytes. Local hydrology, sediment properties and contaminant mixtures should therefore guide system design to maximise remediation efficiency while maintaining ecosystem function.

Ethics and consent

Ethical approval and consent were not required.

Data availability statement

Zenodo. MAR2PROTECT DATASET Removal of contaminants from freshwaters and estuarine waters by constructed and natural wetlands towards river catchment protection through nature-based solutions [Dataset]. https://doi.org/10.5281/zenodo.21513608 (Interdisciplinary Centre of Marine and Environmental Research 2026).

This project contains the following underlying data:

- Data_Set_CW_Canna_indica_CdxKPF.xlsx. (data on the experiments in *Canna indica* constructed wetlands with Cd and ketoprofen)
- Data_Set_CW_Canna_indica_CdxVFX.xlsx (data on the experiments in *Canna indica* constructed wetlands with Cd and venlafaxine)
- Data_Set_CW_Canna_indica_CuxKPF.xlsx (data on the experiments in *Canna indica* constructed wetlands with Cu and ketoprofen)
- Data_Set_CW_Canna_indica_CuxVFX.xlsx (data on the experiments in *Canna indica* constructed wetlands with Cu and venlafaxine)
- Data_Set_Juncus_maritimus_CuxCdxKPF.xlsx (data on the experiments with *Juncus maritimus* elutriate with Cu, Cd and ketoprofen)
- Data_Set_Sporobolus_maritimus_CdxKPF.xlsx (data on the experiments with *Sporobolus maritimus* elutriate with Cd and ketoprofen)
- Data_Set_Sporobolus_maritimus_CuxKPF.xlsx (data on the experiments with *Sporobolus maritimus* elutriate with Cu and ketoprofen)
- Data_Set_Sporobolus_maritimus_CuxVFX.xlsx (data on the experiments with *Sporobolus maritimus* elutriate with Cd and venlafaxine)

Data is available under the terms of the Creative Commons Attribution 4.0 International license.

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