Pharmaceuticals Influence on *Phragmites australis* Phytoremediation Potential in Cu Contaminated Estuarine Media

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Abstract: The aim of the present work was to evaluate the influence of two different pharmaceutical compounds (bezafibrate and paroxetine) on the phytoremediation potential of *Phragmites australis* in an estuarine medium contaminated by copper. Plants were exposed for seven days to a simplified estuarine medium, elutriate solution, with or without sediment. The medium was doped with copper and bezafibrate or paroxetine. *P. australis* plants were able to accumulate a significant amount of Cu, particularly in their roots (600 and 250 times increase in copper levels in the absence and presence of sediment, respectively), corroborating the phytostabilization potential of salt marsh plants. Metal uptake and translocation was significantly lower in the presence of sediment (Cu in leaves increased 20 times in the absence and only 4 times in the presence of sediment). An effect of either pharmaceutical compound on metal accumulation was not observed (levels of Cu in plants tissues were in general identical) but, in the presence of sediment, both bezafibrate and paroxetine changed Cu solubility in elutriate solution, either decreasing or increasing it. The current study highlights the possible influence the presence of contaminants of different characteristics (inorganic and organic contaminants) can have on salt marsh phytoremediation potential in the long run, and the effects pollutants might have in the environment.

Keywords: estuaries; phytoremediation; salt marsh plants; metals; contaminants of emergent concern; bezafibrate; paroxetine

1. Introduction

Estuarine areas are highly productive ecosystems and one of the most sensitive and fragile ecosystems on Earth [1]. However, they suffer from high anthropogenic pressures, and several estuaries are often considered sinks for pollutants (metals, petroleum hydrocarbons, pesticides, surfactants, etc.) receiving all type of contaminants from industrial and metropolitan areas [2]. These inputs can also contain high concentrations of contaminants of emerging concern (CECs), such as human and veterinary pharmaceuticals, among others [3]. These contaminants can cause severe effects at the ecological or human level by bioaccumulating in organisms, accumulating in estuarine sediments and/or dissolving in water [4]. Moreover, most CECs are not regulated, and the continuous release of these into the environment can lead to resistance to antibiotics, endocrine disruption, and carcinogenicity [3,5,6]. Different types of pharmaceuticals, including antidepressants and lipid regulator, have been detected in Portuguese rivers and estuaries (e.g., [7–9]), as in other countries (e.g., [10,11]), mainly due to their reduced removal in wastewater treatment plants (WWTPs). In fact, WWTPs receive wastewaters from municipalities, hospitals, and...
livestock and pharmaceutical industries that can contain CECs, and these compounds and their metabolites can end up in environments receiving WWTPs effluents. Other CECs sources into the environment are illegal untreated effluent discharges, improper disposal of unused or expired pharmaceuticals, manufacture spill accidents, manure and sludge used as organic fertilizer, treatment of crop diseases, and sometimes through leachates from solid waste landfills. Paroxetine (an antidepressant) and bezafibrate (a lipid regulator) are two examples of pharmaceuticals regularly found in effluents from WWTPs in Portugal [12–14]. Their use by the general population leads to their presence in the wastewater entering WWTPs, for instance.

Most estuaries present large salt marsh areas colonized by different plants. Salt marsh vegetation influences the dynamics of the estuarine ecosystem and often efficiently retains anthropogenic pollutants discharged to the system [15], namely metals. Plants are known to be able to oxidize the sediment through the movement of oxygen towards the roots [16] or acidify its rhizosphere through the release of root exudates [17], which can contribute to the retention/elimination of pollutants. If salt marsh plants can immobilize metals in their belowground structures, they may act as phytostabilizers, reducing metal bioavailability. On the other hand, plants that accumulate metals in their aboveground tissues can act as phytoreductors reducing metal content in the estuarine medium [18]. Nevertheless, the role of a plant depends on multiple factors, including environmental characteristics, plant species, and pollutant type and amount. Phytoremediation is a possible method to treat contaminated environments. This methodology is based on naturally occurring processes, using plants and associated microorganisms to degrade, metabolize, accumulate, and/or absorb organic and inorganic contaminants from polluted media (water, air, and soil) [19]. It is a cost-effective, promising, and trustworthy technology and represents a sustainable solution to recover damaged ecosystems, such as estuarine areas [19,20]. Salt marsh plants have been studied to control pollution by phytoremediation [19,21,22], concluding that plants such as Juncus maritimus, Phragmites australis, Spartina patens, Triglochin striata, and Spartina alterniflora have the potential to treat estuaries contaminated with metals and hydrocarbons. More recently, the potential of a plant that can also be found in salt marsh, e.g., P. australis, to remove CECs from water or sediment contaminated with pharmaceuticals, has been evaluated in a few studies, showing promising results [13,23–25]. Frequently, sites polluted with metals are also polluted with other chemicals of different natures, for instance, petroleum hydrocarbons, pesticides and surfactants [26,27], and CECs can also be expected at these environments. The simultaneous presence of inorganic and organic contaminants has been shown to affect the response of the salt marsh communities and, consequently, the phytoremediation process [13,26,28–32]. Indeed, the response of plants to the surrounding chemical environment, in terms of metal accumulation and contaminants bioavailability, may be influenced by the concomitant presence of different chemicals [28]. Therefore, more research is need on this topic to fully assess the phytoremediation potential of salt marsh plants to remove different metals in the presence of other pollutants, namely CECs, a subject still not explored for these emergent pollutants.

The aim of the present work was to evaluate the influence of two different pharmaceutical compounds on the phytoremediation potential of P. australis in an estuarine medium contaminated by copper. For that, controlled laboratory experiments (with elutriates) that simulated estuarine environmental interactions among plant roots, water, and sediments were carried out. The pharmaceuticals chosen to be tested are representatives of extensively used ones: bezafibrate and paroxetine. P. australis was chosen due to its potential of phytoremediation of different types of compounds, including metals (e.g., [21,22,33]) and pharmaceuticals (e.g., [13,24,25]). In parallel, this study also aimed to confirm in situ the role of different salt marsh plants on metal retention previously observed by collecting sediment samples in vegetated and non-vegetated locations.
2. Materials and Methods

2.1. Materials and Reagents

Sampling and labware materials were submerged for 24 h in a 20% (v/v) HNO₃ solution, washed with bi-deionized water (conductivity < 0.1 mS cm⁻¹) several times, and dried at 30 °C in an oven. This procedure was conducted to prevent contamination.

For copper analytical determination, a stock Cu standard solution (as Cu(NO₃)₂) was obtained from Merck (Darmstadt, Germany). For experiments, CuCl₂ (CAS No.: 7447-39-4, from Sigma-Aldrich (Darmstadt, Germany) was dissolved in deionized water to attain 20 mL of a 1g/L concentration solution.

Paroxetine, acquired from Enzo Life Sciences (Nova York, USA) (CAS No.:110429-35-1), was used, whereas bezafibrate used was acquired from Sigma-Aldrich. Individual standard stock solutions were prepared for each pharmaceutical by dissolving known amounts of bezafibrate or paroxetine in methanol. Standard working solutions, also in methanol, were prepared from these individual standard stock solutions. All solutions were maintained in amber vials at −20 °C.

All remaining reagents used were analytical grade or equivalent.

2.2. Evaluation of the Role of Different Salt Marsh Plants on Metal Retention

Sediment in contact with plant roots (rhizosediment) was collected in March 2018, at low tide, at three different sites each vegetated by one plant species: Halimione portulacoides at Câvado River Estuary (41°31′22.2″ N, 8°47′05.9″ W in Ofir, NW Portugal), Phragmites australis at Lima River Estuary (41°41′23.4″ N, 8°48′58.6″ W in Viana do Castelo, NW Portugal), and Juncus maritimus at Lima River Estuary (41°41′19.8″ N, 8°47′37.8″ W in Darque, NW Portugal). At each site, a sample of non-vegetated sediment, at a distance of about 50 m from the vegetated location was also collected. Sediment was retrieved by means of a plastic shovel, placed in individual sterile bags, and immediately transported to the laboratory in a refrigerated ice box.

2.3. Elutriate Experiments Assembly and Samples Preparation

Elutriate experiments were assembled as described in [13]. P. australis plants and the respective rhizosediment were collected from Lima River Estuary, in the north of Portugal, in March 2018. At the sampling site, plant roots were separated from the rhizosediment and washed with estuarine local water. Estuarine water was also collected in decontaminated plastic water bottles.

Elutriates were prepared according to the protocol of the Environmental Protection Agency (EPA) of the United States of America, as described in [13]. For that, 200 mL of estuarine water was mixed with 50 g of rhizosediment per flask. Flasks were shaken and left to settle for 24 h. A total of 50 flasks were prepared. Flasks were divided into 2 groups: a group was left with the sediment for experiments "sediment plus elutriate", the other group was used for experiments with elutriate solution only. For the latter, elutriate solutions were filtrated (0.45 µm pore size cellulose nitrate membrane, Millipore). After 24 h of settling, P. australis roots were washed with deionized water, and three individual plants were placed in each flask. Flasks without plants (control) were also prepared.

For the experiment, 3 different treatments (each in triplicate) were prepared: copper, copper + bezafibrate, and copper + paroxetine. For that, the flasks were doped with the respective contaminant: 100 mg/L of copper without or with 100 µg/L of paroxetine or 100 µg/L of bezafibrate.

Flasks were wrapped in aluminum foil and maintained in the lab for 7 days (one week) under a natural day: night regime with natural sunlight.

During the week, a 2nd (at day 3) and a 3rd (at day 5) doping of 100 µg/L of bezafibrate or paroxetine was conducted aiming to simulate a continuous discharge of pollutants. So, the total doping for each pharmaceutical compound was 300 µg/L. This concentration was much higher than that commonly found in rivers or WWTP effluents [12,14]. However, this
was conducted to simulate a worst-case scenario, exposing \textit{P. australis} to an extreme situation. Copper was added only at the beginning of the experiment, as inorganic compounds do not degrade over time as can occur for organic compounds. The copper concentration was three times higher than the ERM, the concentration above which effects are frequently or always observed among most species of biota for Sediment Effect Concentrations [34]. Although not being frequently found in estuaries [21], this copper concentration was selected taking into consideration the capacity of \textit{P. australis} to uptake metals, namely Cu, and to observe a clear response by the selected plant. This Cu concentration also simulates a worst-case scenario, where a significantly higher proportion of metal in relation to pharmaceutical compounds is normally observed. In a different type of experiment, with sediment, this copper concentration was already tested with no significant effect on plant vitality [22]. Experiments with this plant and with lower copper concentrations close to ERL (Effects Range Low [34]) were previously carried out, and no interference of organic compounds (petroleum hydrocarbons) on copper phytoremediation potential was observed [27,28].

Removal efficiency of Cu after the experiments was evaluated by measuring the metal concentration in elutriate solutions, in sediments (when present), and in plant tissues (when present).

At the end of the experiment, after 7 days, pH was determined in all elutriate solutions. Elutriate solutions were then collected and acidified with HNO$_3$ (1% v/v). Sediment samples, when present, were collected from each flask and stored at $-20 \degree C$, being lyophilized prior to Cu analysis. The lyophilization was carried out in a VirTis BenchTop Pro freeze dryer. Plants, when present, were removed and dried at room temperature until constant weight. Afterwards, they were divided into different tissues: leaves, stems, rhizomes, and roots. Plants collected in the estuary and not exposed were also dried and divided in the different tissues to determine plants’ Cu initial levels.

2.4. Metals Analysis

Cu, Pb, Fe, Mn, Zn, and Cr concentrations were measured in vegetated (by different salt marsh plants) and non-vegetated sediments collected at different estuarine sites, and Cu concentrations in elutriate solutions, sediments, and plant tissues (leaves, stems, rhizomes, and roots) were measured before and at the end of the experiment. Solid samples (sediments and plant tissues) were firstly digested with concentrated HNO$_3$ (plus H$_2$O$_2$ 30% solution for plant tissues) by a high-pressure microwave system (Ethos 1, Milestone, Bomby, Denmark). Metals were analyzed by atomic absorption spectrometry either with flame atomization (AAnalyst 200, PerkinElmer, Waltham, MA, USA) or with electrothermal atomization (PinAAcle 900Z, PerkinElmer (Waltham, MA, USA) coupled to an AS 900 furnace autosampler), depending on metal levels. More details are described in Almeida et al. [26]. Recoveries between 80 and 105% were obtained for samples spiked with known amounts of copper and for the certified reference material “Estuarine Sediment BCR 277”.

2.5. Data Analysis

Elutriate and sediment samples of the different treatments were analyzed for the respective compounds. Each sample was treated independently, calculating the mean and standard deviation of the three experimental replicates.

For metal concentration in sediments collected at different estuarine sites, mean and standard deviation of the triplicates were also calculated.

For metal concentrations, statistically significant ($p < 0.05$) differences among samples were evaluated through a parametric one-way analysis of variance (ANOVA) followed by Tukey pair wise comparisons test using SPSS.

3. Results and Discussion

3.1. Role of Different Salt Marsh Plants on Metal Retention

Regarding metal levels in sediments collected at the three different estuarine sites (Table 1), metal concentrations of Cu, Pb, Fe, Mn, and Cr were, in general, higher in
the vegetated sediments than in non-vegetated sediments, although differences were not always significant. Zn, on the other hand, presented higher concentrations in the non-vegetated sediments collected in two of the sampling places (Ofir and Viana do Castelo). However, at the Darque point, Zn concentrations were identical between vegetated and non-vegetated sediments.

Table 1. Metal levels in vegetated and non-vegetated sediments in three different estuarine sites (mean and standard deviation, n = 3).

<table>
<thead>
<tr>
<th>Site</th>
<th>Plant Type</th>
<th>Sediment Type</th>
<th>Cu (µg/g)</th>
<th>Pb (µg/g)</th>
<th>Fe (mg/g)</th>
<th>Mn (mg/g)</th>
<th>Zn (mg/g)</th>
<th>Cr (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFIR, Cavado river</td>
<td>Halimione portulacoides</td>
<td>Vegetated</td>
<td>61 (3)</td>
<td>44 (8)</td>
<td>23.1 (0.6)</td>
<td>0.70 (0.04)</td>
<td>0.27 (0.02)</td>
<td>41 (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-vegetated</td>
<td>15 (1)</td>
<td>19 (1)</td>
<td>17.2 (0.4)</td>
<td>0.56 (0.02)</td>
<td>0.36 (0.02)</td>
<td>15 (7)</td>
</tr>
<tr>
<td>Viana C, Lima river</td>
<td>Phragmites australis</td>
<td>Vegetated</td>
<td>14 (1)</td>
<td>20 (10)</td>
<td>18.9 (0.2)</td>
<td>0.35 (0.07)</td>
<td>0.40 (0.05)</td>
<td>21 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-vegetated</td>
<td>6 (2)</td>
<td>10 (10)</td>
<td>14 (2)</td>
<td>0.30 (0.05)</td>
<td>0.48 (0.05)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Darque, Lima river</td>
<td>Juncus maritimus</td>
<td>Vegetated</td>
<td>22 (1)</td>
<td>29 (8)</td>
<td>21 (1)</td>
<td>0.38 (0.04)</td>
<td>0.30 (0.02)</td>
<td>27 (2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-vegetated</td>
<td>14 (1)</td>
<td>20 (10)</td>
<td>15.2 (0.4)</td>
<td>0.34 (0.05)</td>
<td>0.29 (0.03)</td>
<td>14.0 (0.7)</td>
</tr>
</tbody>
</table>

Metals’ mean concentrations ranged from: 14–23 mg/g Fe; 0.30–0.70 mg/g Mn; 0.27–0.48 mg/g Zn; 661 µg/g Cu; 13–44 µg/g Pb; and 11–40 µg/g Cr (Table 1). Cu, Pb, Fe, Mn, and Cr presented the lowest concentrations in sediments collected at Viana do Castelo (without plant) and the highest in Ofir (with plant). In contrast, Zn reversed this relationship, presenting the lowest level in the sediments collected in Ofir (with plant) and the highest in Viana do Castelo (without plant). Metal sources in estuarine areas are diverse. Lima River Estuary, an urban-industrialized estuary, is impacted by navigation activities, agricultural runoff, and urban and industrial treated and untreated wastewaters, as well as dredging activities, which can introduce pollutants into the water column. Different sources can affect the selected sites differently [21]. However, this is still considered an estuary with low levels of metal contamination. Cávado River has been affected by untreated domestic wastewater as well as mining activities, and pollutants can end up in the estuary [26]. In the past, this estuary was considered contaminated [26]. Metal levels at the three sites were within those previously observed in these areas, with the exception of Zn and Mn that showed higher values [21]. Cu and Pb levels were similar to those considered as background or good, whereas Zn sediment was considered good to moderate quality [35].

The results showed that selected salt marsh plants contributed to the retention of metals in sediments around their roots, i.e., their rhizosphere, as previously observed [21]. However, Zn presented a different dynamic than the other metals, showing higher concentrations in non-vegetated sediments. Thus, the results seem to show that the selected plants did not facilitate Zn retention, or that the plant promoted its uptake. In fact, these plants are known to accumulate this metal (e.g., [21]).

3.2. Elutriate Experiments—pH Variation

The pH of elutriates without sediment or plants was ca. 5.5 (Figure 1), probably due to the Cu standard solution used to dope the solutions. When sediment was present, either with or without plants, the mean pH values in elutriates increased to 7.30, particularly in the presence of the pharmaceutical compounds. This indicates that sediments promoted a neutral pH. When only plants were present, a pH of ca. 6.00 was observed, indicating that plants contributed to a decrease in elutriate pH relatively to the presence of sediments. Plants are known to release exudate compounds in the presence of metals, including low molecular organic acids, which can contribute to pH decrease (e.g., [36]). This was only observed in the absence of sediment, probably because sediment has a buffering capacity.
Figure 1. pH (mean and standard deviation, n = 3) in elutriates without sediment or plants, elutriates with plants, and elutriates with sediment and plants at the end of the experiments. All elutriates were amended with 100 mg/L of Cu, without or with addition of one of the pharmaceuticals tested (bezafibrate or paroxetine, at 300 µg/L).

3.3. *P. australis* Phytoremediation Potential for Cu Contaminated Medium

In all cases, initial and final Cu levels were measured in elutriates, sediment (when present), and plant tissues (when present).

As can be seen in Figure 2a, accumulation of Cu in leaves and stems was observed, particularly when sediment was not present, indicating that metal was translocated within the plant in the short period of exposure (seven days). The Cu concentration was higher in leaves than in stems (ratio [Cu]leaves/[Cu]stems ca. 2). So, translocation was more significant to leaves than to stems, although the difference was not significant (p > 0.05). In the presence of sediment, Cu accumulation was only observed in leaves not in stems, and in a significantly (p < 0.05) lower amount than when sediment was absent, probably because metal had a lower bioavailability.

![Figure 2](image-url)

**Figure 2.** Levels of Cu (mean and standard deviation, n = 3) in leaves and stems (a) and rhizomes and roots (b) of *Phragmites australis* observed at the end of the different experiments for plants exposed to elutriate, with or without sediment, amended with 100 mg/L Cu, without or with addition of paroxetine or bezafibrate. Control shows the levels of Cu initially presented in the plants: 2.0 µg/g, <LOD (2.0 µg/g), 1.5 µg/g, and 5.3 µg/g for leaves, stems, rhizomes, and roots, respectively. Cu concentrations in stems in the presence of sediments were <LOD (2 µg/g).

*Phragmites australis* clearly accumulated Cu mainly in belowground tissues as expected and in significantly (p < 0.05) higher amounts in its roots than in its rhizomes (Figure 2b). In fact, Cu concentrations were much lower in the aerial tissues compared to the belowground ones, a result previously observed for this and other salt marsh plants, which generally
behave as phytostabilizers [21]. Accumulation in belowground tissues was significantly ($p < 0.05$) lower when the plant was in the presence of sediment than when it was exposed to elutriate solution without sediment as expected, probably because the bioavailable metal fraction was higher in the second case.

Looking at the Cu levels in the elutriate solution (Figure 3), the metal retention potential of sediments is clear. In fact, when sediments were present, Cu concentration in elutriates was significantly ($p < 0.05$) lower, which was observed either in the presence or absence of plants. Cu can be removed from solution by precipitation and adsorbed at Fe and Mn oxides and organic matter known to be present in sediments [21]. Fe and Mn oxides are present in oxidized sediments, such as vegetated sediments, and these oxides can sorb metals [37]. In addition, vegetated sediments have high organic matter levels and organic ligands (namely exuded by salt marsh plants [36]) that can complex metals [38]. In the absence of sediment, Cu solubility is affected by pH and by adsorption/absorption to colloid particles (which are removed by elutriate filtration through 0.45 µm filters), leading to slightly lower Cu concentration than the added one of 100 mg/L (Figure 3). Plants may also uptake Cu, decreasing its concentration in solution, and Cu concentration elutriate was significantly lower in the presence of plants.

![Figure 3](image1.png)

**Figure 3.** Levels of Cu (mean and standard deviation, $n = 3$) in elutriate solutions, with or without sediment, at the end of the experiment with elutriate amended with 100 mg/L Cu, without or with addition of paroxetine or bezafibrate.

Cu concentration in sediments was also slightly lower in the presence of plants probably due to metal accumulation in plant tissues (Figure 4).

![Figure 4](image2.png)

**Figure 4.** Levels of Cu (mean and standard deviation, $n = 3$) in sediments observed at the end of the different experiments carried out in elutriate amended with 100 mg/L Cu, without or with addition of paroxetine or bezafibrate.
*P. australis* uptake of Cu, mainly in plant roots, has been previously observed (e.g., [21,22]), corroborating halophyte plants’ potential as phytostabilizers, with smaller amounts being translocated to stems and leaves [18,39–41]. The results also showed the high capability that *P. australis* has to accumulate Cu. Despite the high levels to which the plant was exposed, the plants maintained their visual vitality and were able to accumulate significant amount of the metal, including in their aboveground tissues, confirming *P. australis* phytoremediation potential.

### 3.4. Influence of Pharmaceuticals on *P. australis* Phytoremediation Potential for Cu Contaminated Medium

#### 3.4.1. Bezafibrate

The accumulation of Cu by plant tissues was not significantly influenced by the presence of bezafibrate, either in the absence or in the presence of sediments (Figure 2).

This is compatible with Cu concentration in elutriate solution in the absence of sediment, where the addition of bezafibrate did not cause a significant change in the Cu levels in solution (Figure 3). In the elutriate experiments without plants, Cu concentrations in solution were also similar between the experiments with or without bezafibrate. Thus, bezafibrate did not influence the amount of Cu in the elutriate solutions in the absence of sediment.

However, in the presence of sediment, Cu levels in solution were either lower or higher in the medium amended with bezafibrate (the difference was only statistically significant in the absence of plants, as shown in Figure 3), indicating that bezafibrate influenced the solubility of Cu and its retention in sediment. However, these small solubility variations, in general, were not reflected in sediment levels or in Cu accumulation by the plant with similar levels being found (Figure 2a,b).

#### 3.4.2. Paroxetine

When paroxetine was added to the medium, Cu accumulation in aboveground plant tissues and rhizomes slightly increased, slightly decreasing in plant roots (Figure 2, differences only significant in rhizomes, as a high variability was observed). So, paroxetine probably promoted Cu translocation, which agrees with the fact that metal levels in elutriate solution were identical in the presence and in the absence of the pharmaceutical compound. This feature was only observed in the absence of sediment when pollutants were more available. Sediment can retain not only metals but also organic pollutants, namely pharmaceutical compounds [13,24]. In the presence of sediment, metal accumulation in plant tissues was identical in the absence or in the presence of paroxetine. However, as observed for bezafibrate, paroxetine influenced Cu levels in elutriate solution when sediment was present, particularly when plants were present (Figure 3). Cu levels in sediment were not influenced by paroxetine presence (Figure 4).

#### 3.4.3. Pharmaceutical Influence

The influence of organic pollutants on plant metal uptake, including Cu uptake, has been observed in other studies, including for *P. australis*.

For instance, Sayen et al. [32] observed that the antibiotic enrofloxacin slightly reduced the uptake of Cu by *P. australis* due to the complexation of the metal by the antibiotic, changing Cu bioavailability. However, the formation of the Cu–enfloxacin complex led to an increase in Cu in solution. The authors concluded that the chemical forms of both pollutants changed (different speciation), which can have different impacts on metal uptake by plants in the long term. However, another study has shown that the presence of the antibiotic ceftiofur promoted Cu uptake by *P. australis*, probably due to the formation of a Cu–ceftiofur complex that potentiated this accumulation [42]. In the present study, one can suppose also the formation of Cu complexes with bezafibrate and/or paroxetine, although no information on the topic was found in the literature, and research on possible chemical interactions between Cu and bezafibrate or paroxetine is needed.
A higher accumulation of Cu by another salt marsh plant, *Halimione portulacoides*, when in the presence of hydrocarbons has been also observed, authors suggesting hydrocarbons can control to some extent Cu sorption by plants, namely the passive penetration of Cu into the root cells or how the plant controls the solubility of Cu [26]. Further, the non-ionic surfactant Triton X-100 markedly promoted Cu accumulation on plant roots, even in the presence of sediment [43]. However, the pesticide DDE and the organotin MBT did not significantly increase Cu uptake by *H. portulacoides*. However, the addition to the medium of these pollutants (DDE, MBT, and Triton X-100) favored Cu solubility from sediments [28].

In the present study, both pharmaceutical compounds could have interacted with Cu. In general, that was not reflected by a higher or lower Cu accumulation by *P. australis*, but both bezafibrate and paroxetine influenced the solubility of Cu and its retention in sediment. In a parallel study [13], Cu also prompted the retention of both pharmaceuticals in the sediment. One should also be aware that organic pollutants can be degraded by microorganisms present in sediments, which could lower pharmaceuticals’ impact. In the parallel study [13], at the end of the experiment, bezafibrate removal from elutriate solution was due to abiotic factors (ca. 20%), plants (ca. 40%), and adsorption to sediment (ca. 40% that increased to 75% in the presence of Cu), with probable degradation of the compound by the microbial communities present in the sediment, as bezafibrate was not detected in sediments. For paroxetine, 82% was removed from elutriate solution by both sediment adsorption and microbial degradation. This removal slightly decreased in the presence of Cu, as paroxetine levels in sediment were higher in the presence of Cu, probably due to some toxicity of Cu to the microbial communities.

The results of the current study, in line with the abovementioned studies, indicate that the presence of pharmaceuticals can affect metal behavior in estuarine environments, where the presence of mixed contamination can be expected. Thus, the interactions between pollutants of different characteristics (inorganic and organic) should be considered before the application of phytoremediation technologies.

4. Conclusions

The obtained results confirmed the role of different salt marsh plants on metal retention with Cu, Pb, Fe, Mn, and Cr levels being higher in the plant rhizosphere than in non-vegetated sediment. However, Zn presented a different dynamic, showing higher concentrations in non-vegetated sediments, which could be due to plant uptake. These plants are known to accumulate this metal.

The simultaneous presence of different types of pollutants can influence salt marsh plants’ phytoremediation potential. Pollutants of two different families were tested at high concentration levels to simulate a worst-case scenario, exposing the plant to an extreme situation. No significant effect on Cu accumulation by *Phragmites australis* was, in general, observed by either bezafibrate or paroxetine presence, although Cu translocation was improved by paroxetine but only in hydroponic conditions (in the absence of sediment). However, both compounds influenced Cu solubility in solution and Cu retention in sediment, which could promote changes in metal availability and its impacts in an estuarine medium. Although research was carried out with Cu as a model metal, one should also investigate possible effects on saltmarsh plants’ phytoremediation potential for the other metals found in these estuarine areas.

Therefore, this work illustrates that the simultaneous presence of pollutants from different families (inorganic and organic) in estuarine environments may change the composition in the water column or of the pore water, sediment, or biota, differentiating from what is expected considering the effect of each pollutant individually. This should be considered when planning remediation approaches and the effects pollutants might have in the environment.

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