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Performance of *Iris pseudacorus* and *Typha domingensis* for furosemide removal in a hydroponic system

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ABSTRACT

The potential of *Iris pseudacorus* and *Typha domingensis* to remove the pharmaceutical active compound (PhAC) Furosemide from a nutrient solution was assessed. Both plants were exposed to 2 mg L^{-1} of furosemide during 21 days and the removal of furosemide was monitored. Vessels without furosemide were also implemented as control systems for plants development. Likewise, unplanted vessels with furosemide were employed to assess abiotic removal mechanisms. All vessels were covered with aluminum foil to avoid photodegradation of the compound. Both plants showed potential to remove Furosemide, attaining, at the end of the experiment, a removal of 42.0–66.9% and 40.5–57.8%, for *Typha* and *Iris,* respectively. The plants do not presented a visible negative stress response to the exposure to furosemide, having a positive growth rate at the end of the experiment. Biodegradation seems to play an important role in furosemide removal, being enhanced by the presence of the plants. The two macrophytes presented different removal behaviors, particularly in the first 48 h of contact time. FUR removal by *Iris* follows a pseudo-first order while by *Typha* is divide in different phases. These results indicate that different plants species seem to have different mechanisms to remove pollutants from water.

HIGHLIGHTS

- PhACs removal potential of *Iris pseudacorus* and *Typha domingensis* was assessed.
- Plants were exposed to 2 mg L^{-1} of furosemide during 21 days.
- Both macrophytes showed good removal efficiencies.
- Biodegradation of furosemide seems to be the main removal mechanism.
- Plants demonstrated different removal behavior along the experiment.
- Removal mechanisms of plants seem to differ between species.

KEYWORDS

Biodegradation; furosemide; hydroponic system; pharmaceutical; phytoremediation

Introduction

Aquatic ecosystems pollution by pharmaceutical active compounds (PhACs) is a current environmental issue. PhACs appear in surface water, groundwater, drinking water and sediments (Barbosa *et al.* 2016; Paíga and Delerue-Matos 2016; González-Alonso *et al.* 2017; Thiebault *et al.* 2017; Yang *et al.* 2017). They are considered "pseudo" persistent pollutants because of their continuous introduction into the environment. PhACs presence in these ecosystems can lead to adverse effect on the biota such as antibiotic resistance, endocrine disruption and behavioral changes (Schwartz *et al.* 2003; Aubertheau *et al.* 2017; Azzam *et al.* 2017).

The main source of PhACs in the water bodies is mainly via wastewater treatment plants (WWTPs) effluents and septic systems (Alonso *et al.* 2010; Aubertheau *et al.* 2017). WWTPs were designed to remove bulk pollutants, hence not being able to effectively remove emergent pollutants which are present at much lower concentrations, such as PhACs, along the implemented conventional treatments. As a result, a sizable part of these substances reach the aquatic ecosystems. Implementation of treatments such as ozonation, advanced oxidation processes or nanofiltration has proven to enhance the removal efficiency for these compounds (Rosman *et al.* 2018; Azuma *et al.* 2019). However, this would imply an additional cost to an already expensive process. Additionally, some of these advanced treatments do not lead to a complete degradation of the pollutants and may in some cases yield transformation products that are even more toxic than the original compounds.

Green technologies, such as constructed wetlands (CW), have been gaining more attention in the last decades given that they (i) have the capability to remove a wide range of pollutants, since they encompass physical, chemical and biological processes, (ii) require low implementation and maintenance costs because they do not need highly specialized equipment and personnel and have low energy requirements (iii) and can also represent a positive impact on the environment and landscape. CW can be applied as an integrated system in WWTPs, acting as secondary or

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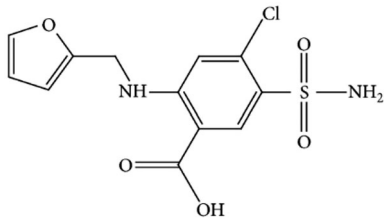
Table 1. Structure, physical and chemical properties of furosemide.

Common name	Furosemide
Chemical structure	
CAS-Number	54-31-9
Molecular formula	$C_{12}H_{11}ClN_2O_5S$
Molecular weight (g mol^{-1})	330.75[a]
Melting Point (°C)	206[a]
Ionisation constant, pKa	pKa = 3.8[b]
Octanol/Water Partition Coefficient, log Kow	2.03[c]
Water solubility, at 30 °C ($mg.L^{-1}$)	73.1[d]

[a]O'Neil (2001); [b]Berthod *et al.* (1999); [c]Sangster (1994); [d]Yalkowsky and Dannenfelser (1992).

tertiary treatment. They are also a particularly interesting solution for small communities and isolated populations where the access to the sewage network is scarce and expensive (Verhoeven and Meuleman 1999; Machado *et al.* 2016). Their good removal efficiency is commonly recognized in terms of organic matter, nutrients and metals (Haberl *et al.* 2003; Vymazal 2007; Vymazal and Kröpfelová 2015). In the last decades, the potential of CWs to remove other emergent pollutants like PhACs has also demonstrated their good potential (Verlicchi and Zambello 2014; Carvalho *et al.* 2014; Lv *et al.* 2016; Dordio and Carvalho 2018).

CWs are complex systems that incorporate different elements (filling materials, vegetation and microbial community) which influence the system's global removal efficiency. Therefore, to enhance their performance, the study of their different components is needed. This is especially true for vegetation where its contribution on the removal efficiency is not yet fully understood. The presence of vegetation generally provides a positive effect on the PhACs removal capacity of CWs (Brisson and Chazarenc 2009; Vymazal 2011). Moreover, different plants seem to promote different PhACs removal values (Mackul'Ak *et al.* 2015; Zhang *et al.* 2016). Thus, plant selection criteria should be considered in CWs studies.

Several different macrophytes are used in CWs, the most common ones being the *Phragmites australis,* followed by *Typha* spp. (Kadlec and Wallace 2009; Vymazal 2013) since these are resistant and proliferative species. However, the generic employment of plants can also lead to an environmental issue if they are not native species, due to displacement of native vegetation in CWs surrounding areas. Moreover, the use of native plants on these systems can also promote CW efficiency since they are already well adapted to the region climate conditions and pest infestations.

In Portugal, the wetland plants *Iris pseudacorus* and *Typha domingensis* are considered native species, being well represented along the country. They are known for being tolerant to flooded soils and polluted waters, being therefore suitable for phytoremediation (Wu *et al.* 2013; Wang *et al.* 2014). They already demonstrated their capacity for removing pollutants such as nutrients, organic matter and metals (Gomes *et al.* 2014; Di Luca *et al.* 2015; Ediviani *et al.* 2018; Huang *et al.* 2018), and, more recently, also their potential to remove some PhACs (Mackul'Ak *et al.* 2015; Dordio and Carvalho 2018) although, in the latter case, the mechanisms involved in the removal of such pollutants are not so well characterized.

The chemical parameter log K_{ow} has been suggested as an indicator for plant uptake propensity (Dietz and Schnoor 2001; Pilon-Smits 2005). Compounds with moderate hydrophobicity ($0.5 < \log K_{ow} < 3.5$) can be uptaken and translocated within the plant tissues. Therefore, for the present study the diuretic drug Furosemide (FUR) with a log K_{ow} of 2.03 was selected (Table 1). Furosemide, included in the cardiovascular system therapeutic group, is one of the compounds with the highest sales in Portugal (INFARMED 2014), having this pharmacotherapeutic group the highest consumption rate in Europe (OECD 2017). After ingestion, up to 30% of FUR is excreted, whereof 90% appear as parent compound (Zuccato *et al.* 2005). In many countries, FUR has been detected in many environmental samples (*i.e.* surface water, groundwater and wastewater) at concentration levels of ng L^{-1}–µg L^{-1} (Cabeza *et al.* 2012; Verlicchi *et al.* 2013; Vymazal *et al.* 2017; Cantwell *et al.* 2018). In Portugal, FUR presence can be found in the WWTPs water bodies, especially if they receive effluent from hospital facilities (Salgado *et al.* 2010; Santos *et al.* 2013).

Therefore, the present work compares the capacity of two different Portuguese macrophytes *I. pseudacorus* and *T. domingensis*, to remove the PhAC furosemide from water. The study aims to provide further knowledge on the capacity of different riparian wetland plants in removing PhACs from water and on the potential use of native plants for constructed wetland systems.

Material and method

Chemicals and materials

Analytical-grade furosemide (99.8% purity) was obtained from Sigma-Aldrich (Lisbon, Portugal). Phosphoric acid (>85% purity), HPLC-grade solvent acetonitrile and

methanol were acquired from Enzymatic, S.A. (Loures, Portugal). Ultra-pure water was prepared from a Millipore Milli Q system. All filters used in the experiments as well as all chemicals used for the nutrient Hoagland solution were purchased from Enzymatic, S.A. (Loures, Portugal).

Modified Hoagland nutrient solution

For the present hydroponic experiment was used a modified Hoagland nutrient solution with the following composition: 2.5 mmol L^{-1} K^{+}; 2 mmol L^{-1} Mg^{2+}; 2 mmol L^{-1} Ca^{2+}; 2 mmol L^{-1} SO_4^{2-}; 6 mmol L^{-1} NO_3^{-}; 0.5 mmol L^{-1} $H_2PO_4^{-}$; 10 µmol L^{-1} Fe^{3+}, 10 µmol L^{-1} H_3BO_3; 1 µmol L^{-1} Mn^{2+}; 0.5 µmol L^{-1} Cu^{2+}; 0.1 µmol L^{-} MoO_4^{2-}.

Furosemide solutions

For HPLC-UV FUR quantification, a FUR stock standard solution containing 4 mg L^{-1} was prepared. Furosemide was dissolved in 1 mL of methanol and made up to the mark of 1 L with ultra-pure water. Ultrasonic bath was used to help the dissolution of furosemide. Standards solutions were prepared within the range of 0.1–4 mg L^{-1}. All solutions were stored at room temperature and covered with aluminum foil to avoid photodegradation. Modified nutrient Hoagland solution spiked with 2 mg L^{-1} of FUR was prepared adding to each 3 L of Hoagland solution a pre-dissolved 6 mg of FUR in 1 mL of methanol.

Plant material and experiment setup

Iris pseudacorus and *T. domingensis* were obtained from the banks of a pond in Tapada da Ajuda Botanical Park, within Lisbon, Portugal (coordinates: 38°42′58″N; 9°11′20″W). The plants collection area is under the classification of Csa according to Köppen-Geiger, being characterized as a hot Mediterranean dry summer. Three adult plants from each specie were collected in October 2015. Plant's roots were rinsed with tap water to eliminate residual soil and debris from the root system. Plants were separated in 30 L recipients filled with Hoagland nutrient solution and kept in a greenhouse to obtain new sprouts to perform the experiment. Along one and a half year new grown sprouts were separated and also maintained in the nutrient solution.

The experiment was carried out in a greenhouse at May 2017. Greenhouse temperature and humidity data wwew monitored during the experiment using a Comark RF313-TH sensor. From the new sprouts, for each specie, 10 juvenile plants with similar physical characteristics were selected. Plant's roots were rinsed with de-ionized water and their physical characteristics were measured: weight, height, number of leaves, number of sprouts, root length and width. Plants were inserted in 5-L containers that were covered with aluminum foil to avoid light exposure. To access plant removal capacity, five plants of each specie (*I. pseudacorus*: IF1-IF5; *T. domingensis*: TF1-TF5) had their root system immersed in 3 L of aerated nutrient Hoagland solution spiked with 2 mg L^{-1} of FUR and other five plants (*I. pseudacorus*: I1-I5; *T. domingensis*: T1-T5) with their roots immersed in 3 L of nutrient Hoagland solution without FUR. Vessels were disposed in a randomized order. The tested concentration, although higher than the expected environmental concentrations, was selected to allow the detection of the compound along the study. Additionally, to investigate possible external FUR removal mechanisms, a set of five covered containers without plants were filled with 3 L of nutrient Hoagland solution spiked with 2 mg L^{-1} of FUR (B1-B5). In the beginning and end of the experiment pH, electrical conductivity (EC), temperature and dissolved oxygen (DO), were measured in the water. Along the 21 days, greenhouse temperature was measured. After the 21 days, plants were collected, frozen in liquid nitrogen and stored at −80 °C.

Macrophytes characterization

Iris pseudacorus and *T. domingensis* plants used in the present study were distributed randomly by the two different treatments. Initial growth parameters of all the plants were measured to evaluate plants tolerance to Furosemide exposure.

Iris pseudacorus, commonly called yellow iris and yellow flag, is native to Europe, western Asia and northwest Africa. In Portugal, it is well represented along the mainland, being found in the banks along slow flowing water courses and in the margins of lakes, ponds and wetlands. *Iris pseudacorus* is considered an exotic plant in Madeira Island and do not exist in Azores Archipelagos. Prefers sandy and loamy soils, and is located in warmer regions and low altitudes 0–1250 m (Castroviejo 1986/2013).

Plants from the genus *Iris* are characterized for having a stock as a rhizome or bulb. Usually are caulecent plants and have equitant leaves and actinomorphic flowers. *Iris pseudacorus* are differentiated by their yellow flowers (4–12), the lower ones with long and subrerect peduncles. Have pedicels with 20-50 mm and spathes ranging from 40–100 mm. Adult plants can present steams from 40–150 cm, slightly compressed, with several basal leaves with 50–90 cm × 10–30 cm (Franco and Rocha Afonso 1994). This plant is also designated as *Limniris pseudacorus* (L.) Fuss, Fl. Transsilv. 636 (1866) on the Flora Iberica (Castroviejo 1986/2013).

The *Iris* plants used in the experiment had on average an initial biomass of 149.16 ± 27.3 g and a height of 140.41 ± 7.17 cm. The plants number of leafs ranged from six to seven, while the roots had average area of 623.73 ± 126.48 cm^2.

Typha domingensis, has a cosmopolitan distribution, and is commonly known as southern cattail, appears in tropical, temperate and Mediterranean regions of Europe, Asia, America, Africa and Australia. This perennial plant is native in Portugal mainland, exotic in Azores Archipelagos and do not exist in Madeira Island. *Typha domingensis* prefers soils that are humid or flooded and is adapted to both fresh and saline water. Tolerate contaminated waters well and soil rich in nutrients. Appear in locations below 1100 m of altitude (Castroviejo 1986/2013).

Plants from the genus *Typha* have fibrous roots and creeping rhizomes. The stems are erect with a corn-like form at the base. The leaves are basal, linear, distichous and erect. *Typha domingensis* plants can reach up to 300 cm and are characterized by their yellowish-green leaves with 5-12 mm wide. Flowering stems are similar or shorter than leaves. Female and male inflorescence parts have a cinnamon-brown color and are separated by 0.5–6 cm.

For the present experiment, the *Typha* plants had an average initial biomass of 131.77 ± 21.71g, and height of 163.94 ± 21.89 cm, a root area of 189.78 ± 102.95cm^2 and the number of leaves varied from five to eight.

Plant growth and PhAC tolerance

In the beginning and in the end of the experiment, plant growth parameters (plant weight and height, root length and width, number of leafs) were measured. Plant visual effects (yellow leafs, plant mortality) and the plants relative growth rate (RGR) parameter, calculated according to Equation (1), were used to evaluate plants tolerance to FUR presence along the experiment.

$$\mathrm{RGR} = (\ln Wf - \ln Wi)/t \quad (1)$$

where *Wf* and *Wi* are the weights at the end and beginning of the experiment, respectively, and *t* is the experimental period (Dordio *et al.* 2009).

HPLC-UV apparatus

Furosemide quantification was accessed through a HPLC - Beckman Coulter System Gold, coupled with a Solvent Module 126 and a Diode Array Detector 168, using the 32 Karat Software version 8.0, with a variable wavelength detector and 20 µm volume injector loop. Samples flow rate was 1 mL min^{-1}. A reversed phase analytical column Zorbax Eclipse XDB-C8 (4.6 × 150 mm; 5 µm) was used. Furosemide analyses were previously tested in a gradient mobile phase of acetonitrile and ultra-pure water acidified with 0.1% (v/v) phosphoric acid, being subsequently selected the ideal mixture of 60% of acetonitrile and 40% of ultra-pure water acidified with 0.1% (v/v) phosphoric acid to be used in isocratic elution mode. Three replicates of each sample were injected with an automatic injector Spark Holland BV – MIDAS, at room temperature (16–20 °C).

Statistical analysis

Data were checked for normality through the Kolmogorov–Smirnov test. Removal data along the experiment did not show a normal distribution, hence differences between the two tested plant species along the experiment were analyzed through the Kruskal–Wallis test. Average removals after 21 days of FUR exposed had normal distribution and were related to the initial plant growth parameters for the studied two plant species through Pearson test. Results were found statistically significant different for $p < 0.001$.

Table 2. Macrophytes growth parameters after 21 days.

	IF	I	TF	T
Leaf number increment (%)	22.0 ± 7.6	33.3 ± 0	2.6 ± 23.3	−1.0 ± 30.7
Leaf height increment (%)	10.3 ± 3.7	17.3 ± 1.7	40.9 ± 36.2	6.7 ± 9.4
Root area increment (%)	22.4 ± 9.3	42.4 ± 42.8	62.7 ± 30.3	179.6 ± 69.0

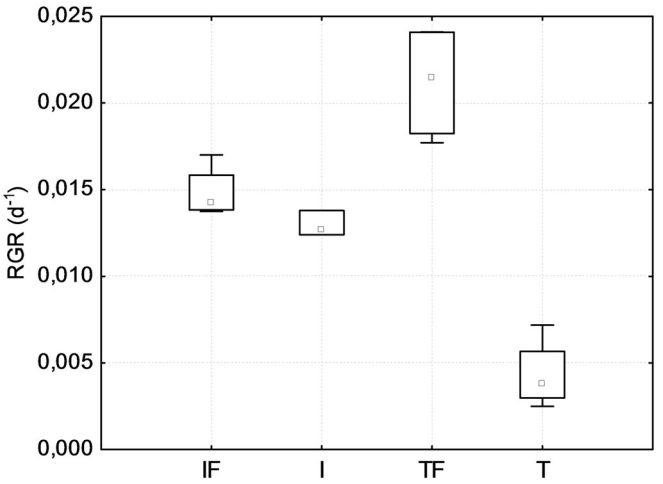


Figure 1. Plants relative growth rate (d^{-1}) after 21 days with and without FUR exposure.

Results and discussion

Macrophyte's growth parameters

Along the experiment, neither *Iris* nor *Typha* plants presented visible phytotoxic effects induced by the presence of FUR. Plant mortality was null and all plants had a positive biomass increment with and without furosemide exposure (Table 2). Nevertheless, no tests were undertaken on the cellular level or of enzyme activity. Plant growth patterns were different for both species. *Iris* plants with FUR (IF) showed slightly lower growth values than control *Iris* (I) for both leafs and roots, indicating that FUR exposure could inhibit plants development.

Nevertheless, when looking to the relative growth rate values (Figure 1) this difference is not evident. On the other hand, *Typha* plants with FUR exposure (TF) showed higher growth values than the control set for the parameters measured on aerial parts, while the root area increment presented significantly lower values.

Moreover, the RGR values found for *T. domingensis* when exposed to FUR are on the same order as the ones for *I. pseudacorus* plants with or without FUR exposure. These values are also of the same magnitude of other similar studies. Dordio *et al.* 2009 found comparable values for *Typha* when exposure to clofibric acid (0.032–0.035 d^{-1}). Additionally, the same pattern was also found where the *Typha* control set had a lower value of RGR. Nevertheless, the differences found were not so pronounced such as the present study where the control *Typha* plants have values of one magnitude below. No visual stress indicators (roots system necrosis and discoloration) were found to explain these results.

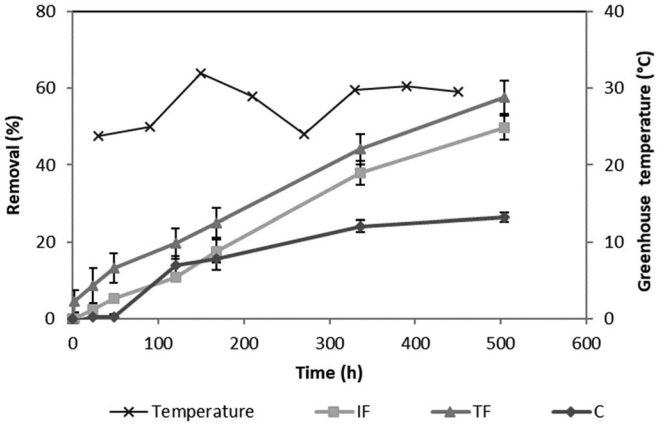


Figure 2. Furosemide removal in the vessels with *Iris* (IF), with *Typha* (TF) and in the control vessels as well as the greenhouse average temperature along the 21 days.

Comparatively, the differences in the RGR values between *Typhas* with and without FUR exposure could imply a new nutrition source for the plants with the presence of FUR. Having this PhAC in its structure nitrogen (N) (Table 1), the degradation of the FUR along the assay could provide an increase in the availability of this nutrient for the plant and promote plant growth. Nevertheless, the same grade of response is not seen for *Iris* plants. This could indicate that the two species differ on the composition of Plant Growth-Promoting Bacteria (PGPB) population present in the roots system or differ in the exudates released that would promote the PGPB population in different levels (Rovira 1969). Zhang *et al.* (2016) compared the removal of ibuprofen and iohexol by four wetland plant species (*Typha latifolia*, *P. australis, I. pseudacorus* and *Juncus effuses*) and also found that *I. pseudacorus* growth did not seem to be significantly affected by the exposure to the tested PhACs. For the tested plant of the genus *Typha*, both PhACs exposure affected negatively the plant growth in terms of biomass, what did not occur in our study.

Furosemide removal

In all control plants (I and T), no traces of furosemide were found along the experiment. Figure 2 shows good FUR removal performances for the planted vessels. After 21 days of exposure, vessels with *Typha* reached 42.0–66.9% of FUR removal while vessels with *Iris* achieved 40.5–57.8%. As previously referred, the two studied plant species show different growth behavior with the presence of FUR. While *Iris* growth is only slightly affected by the exposure to the PhAC, *Typha* growth was highly stimulated. Therefore, the slightly higher removal values found for the vessels with *Typha* in comparison with the vessels of Iris could be related with the growth spurt that *Typha* showed in the presence of FUR.

Plants are able to release root exudates or rhizodeposits that will stimulate the microbial population activity since they will deliver a more nutrient rich environment for this bacteria. The composition and quantity of these exudates can differ with plant species (Rovira 1969). Therefore, our two species could have achieved different results due to the different released root exudates that would have promoted different levels of FUR biodegradation. Furthermore, contaminants with amines ($-NH_2$) and hydroxy (–OH) functional groups can be enzymatically transformed by the root surface extracellular enzymes or by the membrane-bound enzymes (Dietz and Schnoor 2001). FUR has the amines group and therefore can be potential degraded by these enzymes.

The microbial biodegradation of FUR is corroborated with the removal values also found for the control vessels. Initially, in the first 48 h of the assay, control vessels did not present FUR removal what indicates that processes such as adsorption to the vessels walls or hydrolysis does not seem to affect FUR removal. Moreover, the vessels were covered with aluminum foil, hence FUR photodegradation can also be considered negligible in our assay.

After the first 2 days, in the control vessels, FUR removal starts with a steep rate where at 120 h of contact time, the control had a removal average of 14%. Hereafter, the removal rate slows down and is gradual until the end of the experiment. After the 21 days, the control vessels achieved 23–28.5% of FUR removal. Since previously to the experiment the vessels were sterilized and the nutrient Hoagland solution was fresh made, the microbial population can be considered absent at the initial time of the experiment. Meanwhile the experiment was performed in an open greenhouse and not in a sterile environment, therefore, it is possible that microbial population grew in the vessels along the assay. Additionally, the FUR removal behavior found in the control vessels could be associated with the typical pattern of microbial population growth that would be promoted by the aerated and nutrient rich conditions.

On the other hand, for the vessels with plants, although the roots of the selected plants had been thoroughly washed some residual microbial population can remain attached to the roots surface. Therefore, additionally to the potential plant uptake fraction we could have faster biodegradation from the residual microbial population present in the roots.

Another trigger for FUR removal in the control vessels after 48 h could be related to the temperature and humidity values reached during the experiment. Along the 21 days, average temperatures in the greenhouse ranged from 23.7 to 32 °C (Figure 2), while relative humidity varied from 46.5% to 66%. These values promoted high evapotranspiration values, which entailed the refill of this solution to the initial volume (3 L) in all vessels, with or without plants along the experiment. Silvestrini *et al.* 2019 evaluate the tolerance of *T. domingensis*, *Scirpus californicus* and *I. pseudacorus* to landfill leachate in lab scale microcosms. *Typha domingensis* was the plant that present highest tolerance, not being affected when exposed to the diluted leachate. Likewise, according with the same study *T. domingensis* presented a higher evapotranspiration rate compared with *I. pseudacorus*. This characteristic could explain the higher FUR removal values of this plant compared with *I. pseudacorus* in our study. Organic pollutants such as PhACs can be uptake by the plant root system by diffusion, being the uptake

potential and subsequently pollutant translocation within the plant tissues linked to the evapotranspiration rate (Madikizela *et al.* 2018). Between the sampling data of 48 h and 120 h average temperatures in the greenhouse changed from 25 to 32 °C. This drastic change could have promoted the conditions for microbial population proliferation leading to the increase of FUR biodegradation. Nevertheless, FUR removal rate on planted vessels do not seem to be affected by this temperatures rise.

In overall, along the experiment, a gradual removal rate of FUR is visible for both plant species. However, *Typha* always has a higher removal compared to *Iris*. Along the experiment the two plant species had statistically different FUR removal ($p < 0.001$). This difference is mostly due to the initial exposure time. After 2 h of contact time, while *Iris* almost did not present FUR removal (average of 0.05%), *Typha* already achieved 5%. After 48 h, *Typha* still have a higher removal rate than *Iris*, the first reached 13.2% while the later only attained 5.1%. This initial fast removal capacity of *Typha* is also verified in the work of Amaya-Chávez et al. (2006) and Dordio *et al.* (2009). FUR adsorption to *Typha* roots surface could be the first mechanism of FUR removal from the nutrient solution. However, the same would be expected to occur in the *Iris* vessels. The root systems of this two plant species are distinctly different. *Typha* roots are more divided and filamentous, whereas *Iris* roots are more bulky but less extensive. As previously discussed, *Iris* plants used in the present study had a root area three times bigger than *Typha* plant root system. Therefore, one could hypothesize that FUR adsorption to the root surface area is not the key mechanism for the FUR removal initial differences between the plants.

Initial growth plant parameters were correlated with the achieved removal values for each plant (Table 3). *Typha* removal potential does not seem related with the plant characteristics. Whereas for *Iris*, initial plants size seems to affect FUR removal. Root length was the parameter that attained a better correlation with $r = 0.917$. Plant weigh and length also presented good correlation with FUR removal, $r = 0.603$ and $r = 0.687$ respectively.

Therefore, our results suggest that for *Iris*, FUR removal could be ruled by mechanisms such as adsorption to root surface and plant uptake, since plants can uptake organic xenobiotics by diffusion through the root membrane in the xylem apoplast (Dhir 2013). While the initial faster FUR removal efficiency by *Typha* could be related to the plant specific released exudates. In overall, the results show that plants PhACs removal potential is plant species dependent.

Taking into account the control set, we can infer that the presence of *T. domingensis* and *I. pseudacorus* on average promoted effectively 31.3% and 23.3% of Furosemide removal, respectively, being this removal a contribution of both biodegradation by released enzymes, plant uptake capacity and adsorption to the roots surface. However, this hypothesis should be further validated by the determination of FUR in the different plant tissues (roots and leaves).

Since a high concentration (2 mg L^{-1}) of FUR was used in our work, an overall lower removal capacity was found as compared to the use of a lower typically environmental concentration. This fact can be supported by the work of Lin and Li (2016) that tested the removal of six different PhACs by two different aquatic plants, *Pistia stratiotes* and *Eichhornia crassipe* for a high concentration of 10 mg L^{-1} and a low concentration of 0.8 mg L^{-1}. The authors found that for exposure to high concentration of all PhACs except Triclosan, extremely low removal percentages (1.4–3.8%) were attained. However, for the lower concentration, Sulfadiazine, Sulfamethazine, Sulfamethoxazole, Ibuprofen, Triclosan had high removals between 42.0% and 99.8%. For Carbamazepine a moderate removal was found with 36.2% and 34.3% for *Eichhornia crassipe* and *Pistia stratiotes*, respectively. Moreover, Dordio *et al.* (2009) with a similar experimental design to our present study, tested the potential of plant also of the genus *Typha* for the removal of clofibric acid from water. Using a low concentration of 20 µg L^{-1}, after 21 days of exposure the plants vessels achieved a high removal of 80%, which results in a removal of 16 µg L^{-1} of clofibric acid.

Hence, in terms of quantity of FUR removed, our planted vessels achieved good results, with *Iris* and *Typha* vessels

Table 3. Correlations values between removal efficiency and macrophytes initial growth parameters.

	Pearson Correlations	
Macrophytes initial growth parameters	*Iris*	*Typha*
Plant weight	0.603	0.422
Plant length	0.687	0.376
Root length	0.917	0.0998
Root width	−0.183	0.268
Root area	0.379	0.286

Table 4. Kinetic fitting of FUR removal by the two plant species.

Treatment	Time range (h)	Kinetic order	Kinetic equation	r	k
IF	0–48	Pseudo-first order	$\ln(q_0 - q_t) = -kt + \ln(q_0)$	0.9972	0.024 h^{-1}
	48–504			0.9606	0.0056 h^{-1}
	120–504			0.9961	0.0056 h^{-1}
TF	0–48	Pseudo-second order	$\frac{t}{q_e} = \frac{1}{k_2 q^2_e} + \frac{1}{q_e} t$	0.8307	0.12 g mg^{-1} h^{-1}
	48–504	First order	$\ln(q_t) = -kt + \ln(q_e)$	0.9928	0.0015 h^{-1}
	120–504	Second order	$\frac{1}{q_t} = \frac{1}{q_e} + kt$	0.9998	0.0023 g mg^{-1} h^{-1}
Control	0–48	–	–	–	–
	48–504	First order	$\ln(q_t) = -kt + \ln(q_e)$	0.9961	0.0017 h^{-1}
	120–504			0.9997	0.0017 h^{-1}

having removals between 0.7–1.2 mg L^{-1} and 0.8–1.4 mg L^{-1}, respectively.

Furosemide removal kinetics

From Figure 2, the removal of FUR along the experiment seems to be divided in different phases for all the different tested treatments (*Iris*, *Typha* and control). Therefore, the removal kinetics of FUR was studied for all the tested treatments (Table 4). *Iris pseudacorus* seems to follow a pseudo-first order kinetic along the experiment, although it is visible that it is in the first 48 h interval that the removal has the better fitting ($r = 0.9972$) and the highest removal rate ($k = 0.024\ h^{-1}$) compared to the subsequent time ($k = 0.0056\ h^{-1}$). *Typha domingensis* removal is clearly divided in 3 phases. FUR removal in the first 48 h follows a pseudo-second-order kinetics with a pronounced high rate ($k = 0.12\ g\ mg^{-1}\ h^{-1}$). Moreover, the remaining time seems also to be divided in different phases. When fitting the time range between 48–504 h the removal follows a first-order kinetics. However, when we look only for the interval from 120 to 504 h the kinetics change and the removal follows a second order kinetics. This can be a result of the removal rate decrease between 48 and 120 h.

Meanwhile, the control vessels having removal values only after the first 48 h, starts with a steep removal from 48–120 h followed a more mild removal until the end of the experiment. However, this is not reflected in the kinetic behavior where the removal rates are similar in both studied interval (48–504 h and 120–504 h) and follow a first order kinetics. The graphically difference that can be seen in Figure 2 is only reflected in the minor difference on the kinetic fitting quality.

Dordio *et al.* (2009) also found distinct phases on the removal behavior of a *Typha* plant for clofibric acid, but the first 96 h followed a first order kinetics showing that the *Typha* plants of both studies could have different removal mechanisms acting in the removal of diverse PhACs.

Overall, it seems that the two studied plants have different removal mechanisms acting in the removal of Fur. The first 48 h appears to be the main responsible for the achieved removal differences. From the kinetic behavior, FUR removal in *Typha* and control vessels after the 48 h of contact time seems to be ruled by similar removal mechanisms, previously suggested to be microbial degradation. FUR removal by *Iris* although enhanced in the first 48 h seems to be ruled by the same mechanisms along the experiment. *Typha* high removal values in the first 48 h could be a result of a more heterogeneous mechanisms set.

Conclusion

Iris pseudacorus and *T. domingensis* were studied for the removal of FUR in a hydroponic system. Both plants showed tolerance to the exposure to the tested FUR concentration (2 mg L^{-1}) during the 21 days of the experiment. Results indicated that both macrophytes have the potential to remove FUR from water. Furthermore, the results showed that the studied plants displayed different removal behaviors, especially in the first 48 h of contact time.

The present study indicates that different plants species have different mechanisms to remove pollutants from water and therefore plant selection criteria should be taken in account for phytoremediation treatments, and native plants are a viable solution. For further conclusions, plant tissues should be analyzed to determine the effective uptake potential of this two plant species.

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