Influence of exposure time, physicochemical properties, and plant transpiration on the uptake dynamics and translocation of pharmaceutical and personal care products in the aquatic macrophyte Typha latifolia

Débora Jesabel Pérez, Lucas Rodrigo Lombardero, William Joseph Doucette

PII: S0048-9697(23)03730-0

DOI: https://doi.org/10.1016/j.scitotenv.2023.165107

Reference: STOTEN 165107

To appear in: *Science of the Total Environment*

Received date: 17 April 2023

Revised date: 22 June 2023

Accepted date: 22 June 2023

Please cite this article as: D.J. Pérez, L.R. Lombardero and W.J. Doucette, Influence of exposure time, physicochemical properties, and plant transpiration on the uptake dynamics and translocation of pharmaceutical and personal care products in the aquatic macrophyte Typha latifolia, *Science of the Total Environment* (2023), https://doi.org/10.1016/j.scitotenv.2023.165107

Influence of exposure time, physicochemical properties, and plant transpiration on the uptake dynamics and translocation of pharmaceutical and personal care products in the aquatic macrophyte *Typha latifolia*

Débora Jesabel Pérez [a,b,c*], Lucas Rodrigo Lombardero [d], William Joseph Doucette [c]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Godoy Cruz 2290 (C1425FQB), Ciudad Autónoma de Buenos Aires, Buenos Aires, Argentina.

[b] Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible (INTA Balcarce – CONICET), Ruta Nacional 226 Km 73,5 (Zip Code 7620), Balcarce, Buenos Aires, Argentina.

[c] Utah Water Research Laboratory, Utah State University, Logan, Utah (Zip Code 834341), USA.

[d] Instituto de Investigaciones Marinas y Costeras (IIMYC), CONICET, Universidad Nacional de Mar del Plata, Dean Funes 3350, Mar del Plata (Zip Code 7600), Buenos Aires, Argentina.

Corresponding author: Débora Jesabel Pérez

E-mail address of the corresponding author: perez.debora@inta.gob.ar; deborajperez@yahoo.com.ar

Abstract

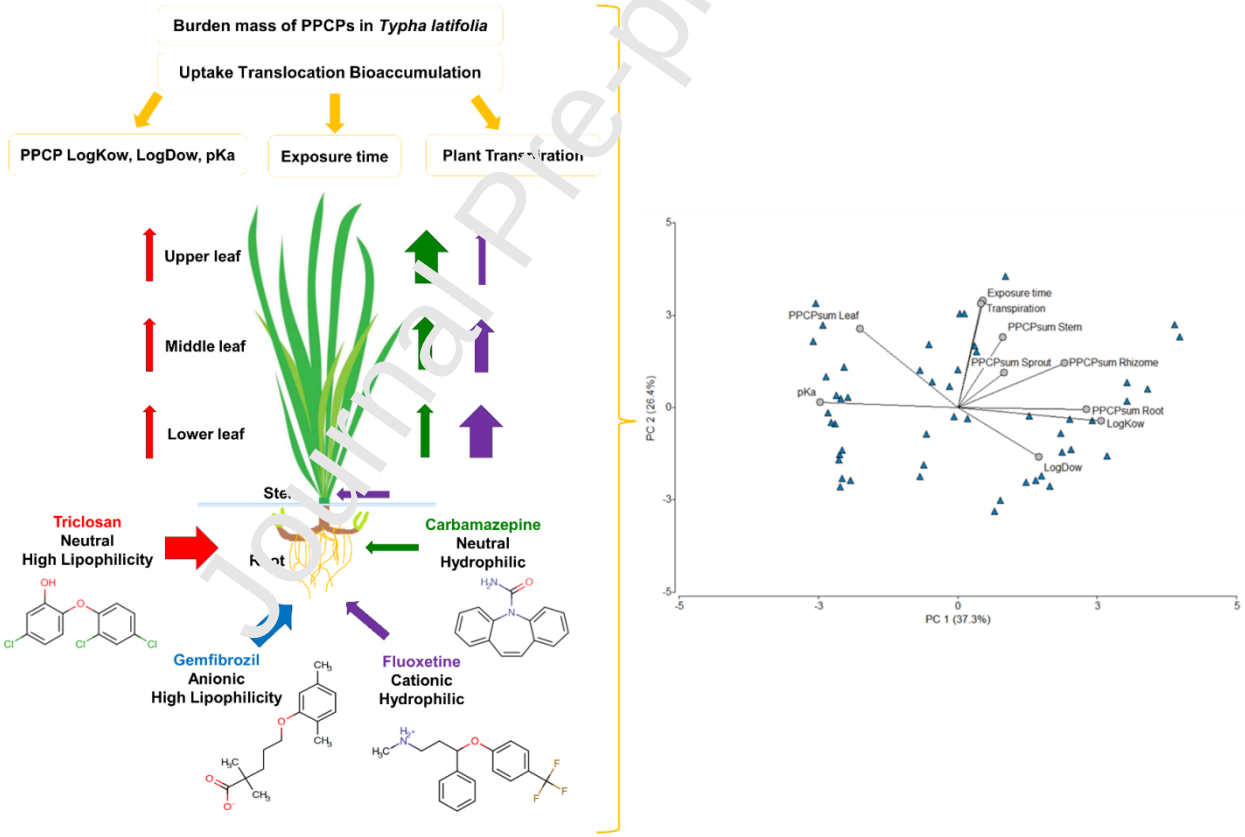
Typha latifolia is widely used as a phytoremediation model plant for organic compounds. However, the dynamic uptake and translocation of pharmaceutical and personal care products (PPCPs) and their relationship with physicochemical properties, such as lipophilicity (LogKow), ionization behavior (pKa), pH-dependent lipophilicity (LogDow), exposure time and transpiration, are scarcely studied. In the current study, hydroponically grown *T. latifolia* was exposed to carbamazepine, fluoxetine, gemfibrozil, and triclosan at environmentally relevant concentrations (20 μg/L each). Eighteen out of thirty-six plants were exposed to the PPCPs and the other eighteen were untreated. Plants were harvested at 7, 14, 21, 28, 35, and 42 days and separated into root, rhizome, sprouts, stem, and lower, middle, and upper leaf sections. Dry tissue biomass was determined. PPCP tissue concentrations were analyzed by LC-MS/MS. PPCP mass per tissue type was calculated for each individual compound and for the sum of all compounds during each exposure time. Carbamazepine, fluoxetine, and triclosan were detected in all tissues, while gemfibrozil was detected only in roots and rhizomes. In roots, triclosan and gemfibrozil mass surpassed 80% of the PPCP mass, while in leaf carbamazepine and fluoxetine mass represented 90%. Fluoxetine accumulated mainly in the stem and the lower and middle leaf, while carbamazepine accumulated in the upper leaf. The PPCP mass in roots and rhizome was strongly positively correlated with LogDow, while in leaf it was correlated with water transpired and pKa. PPCP uptake and translocation in *T. latifolia* is a dynamic process determined by the properties of contaminants and plants.

Keywords: Macrophyte, pH-dependent lipophilicity, ionization behavior, plant development, tissue function, contaminant of emerging concern

Highlights

- PPCP burden mass is a good bioaccumulation metric in *T. latifolia* tissues.
- Triclosan and gemfibrozil bioaccumulated more highly in roots.
- Carbamazepine and fluoxetine bioaccumulated highly in leaf, and fluoxetine in stem.
- In root and rhizome, PPCP mass was correlated with LogKow and LogDow.
- In the leaf, PPCP mass was correlated with plant transpiration and pKa.

Graphical abstract



1. INTRODUCTION

Aquatic plants are key components of freshwater ecosystems, providing multiple benefits that are recognized within the paradigm of ecosystem services. Nowadays, water purification is among the most important ecosystem services provided by aquatic plants (Thomaz, 2021). These services have a great impact due to water shortages in arid and semiarid regions and the loss of water quality worldwide (Ahmadi and Merkley, 2017). Water purification by aquatic plants occurs through the uptake and tissue bioaccumulation of nutrients and inorganic and organic contaminants present in the water body (Di Luca et al., 2019; Bertrand et al., 2019; Pérez et al., 2017).

The Millennium Ecosystem Assessment (MEA, 2005) concluded that contaminants of emerging concern, such as pharmaceutical and personal care products (PPCPs) and pesticides, are the most important organic pollutants affecting water resources. The ubiquitous occurrence of unregulated and bioactive PPCPs in aquatic environments (USEPA 2022) jeopardizes the vitality of these valuable ecosystems (Elliot et al., 2017). In this environmental scenario, and based on their intrinsic capability of removing contaminants, aquatic plants are an excellent mitigation option to prevent the entry and adverse impacts of PPCPs on freshwater ecosystems (Dhir, 2009).

The uptake dynamic and movement of organic contaminants are incessantly studied in the soil-plant continuum. Besides, most existing models have been adjusted in terrestrial plants to predict the uptake and movement of neutral organic compounds (Brigg et al., 1982; Travis and Arms, 1988; Dettenmaier et al., 2009). These prediction models are based on the criterion that the lipophilicity in the neutral state of the compounds (measured by LogKow) is the main physicochemical property that governs the root uptake and shoot translocation. However, PPCPs are highly ionizable compounds (i.e., anionic, cationic, amphoteric) under environmentally relevant pH conditions. A recent study showed that the movement of PPCPs

can be predicted through existing models for neutral states and bases. However, for weak to moderate acids (pKa ≤ 6), the uptake showed a strong dependency on soil pH (Trapp et al., 2023). In this sense, more experimental studies could be conducted to verify these predictions (Carter et al., 2022; Trapp et al., 2023).

In general terms, the uptake, movement, and distribution of PPCPs in plants depend on the dissociation constant (pKa), the charge of the chemical, the pH of the various plant compartments, and the exposure media (Miller et al., 2016). Given that the pH changes the lipophilicity of ionizable compounds, pH-dependent lipophilicity was defined as the octanol-water distribution coefficient (LogDow) (Lee et al., 2011). Moreover, the exposure time, environmental conditions, and plant properties (e.g., transpiration rate, lipid contents, and biodegradation capacity) influenced PPCP behavior in terrestrial plants (Miller et al., 2016; Pérez et al., 2022a).

To predict the uptake and translocation of organic contaminants in emergent rooted aquatic plants through existing models, hydroponic conditions that simulate the water body environment are often used (Wilson et al., 2001; Turgut, 2005; Pérez et al., 2013). Using emergent aquatic plants to quantify and predict PPCP transfer from water body to plants is important for assessing ecological risks, evaluating their use as biomonitors, and predicting the effectiveness of phytoremediation in waste-water treatment facilities (Pi et al., 2017). The uptake of PPCPs in plants is often expressed in terms of tissue concentrations or bioaccumulation factors, i.e., the ratio between PPCP concentrations in tissues and the exposure medium, such as soil water, soil, or nutrient solution (Miller et al., 2016; Pérez et al., 2022b). Moreover, the PPCP bioaccumulated mass or PPCP body burden mass is often used as a biometric descriptor, which relates the PPCP concentrations to the total tissue weight (D. Zhang et al., 2013a; Pérez et al., 2022b). The translocation factor has also been

used to assess root-to-shoot translocation. This factor is defined as the ratio of PPCP concentrations in the shoot to root (Wang et al., 2021; Pérez et al., 2022a, b)

Typha latifolia, or common broadleaf cattail, is an emergent aquatic plant and an excellent bioindicator organism of ecotoxicology of organic contaminants in aquatic systems (Sesin et al., 2021). *T. latifolia* is also commonly used in constructed wetlands to increase the phytoremediation efficiency of PPCPs. However, its use to assess the PPCP root uptake, shoot translocation, and bioaccumulation behavior in the whole-body plant, under experimental conditions, is scarce and limited to no more than twenty compounds (Supplementary Material, Table S1).

In this context, the aims of this study were as follows: (1) to evaluate the root uptake, translocation to shoot, and bioaccumulation mass distribution patterns in the whole vegetative tissues (root, rhizome, stem, sprout, and leaf) of four PPCPs (carbamazepine, fluoxetine, gemfibrozil, and triclosan) over 42 days under hydroponic conditions; (2) to analyze the relationships between PPCP bioaccumulation mass in tissues, exposure time, transpiration rate, and physicochemical properties of the target compounds, in order to understand how these parameters influence uptake and bioaccumulation dynamics. The hypothesis tested was that under controlled greenhouse conditions, there would be differential uptake and bioaccumulation of carbamazepine, fluoxetine, gemfibrozil, and triclosan in all vegetative tissues of *T. latifolia*, influenced by the physicochemical properties of each compound, the exposure time, and the plant transpiration.

2. MATERIALS AND METHODS

2.1. PPCPs selected, chemicals, reagents, and stock solution

Four PPCPs, the anticonvulsive carbamazepine, the antidepressant fluoxetine, the antihyperlipidemic gemfibrozil, and the antibacterial triclosan were selected as test compounds in this study. The selection of these PPCPs was based on three criteria. Firstly, previous literature indicated that the compounds are extensively used worldwide and frequently detected in aquatic ecosystems (Valdés et al., 2016; Ma et al., 2018; Tete et al., 2020; Pintado-Herrera et al., 2014). Secondly, few previous studies evaluated the uptake and translocation of these PPCPs in *T. latifolia*. A review of the available literature (Supplementary Material, Table S1) showed that no published uptake studies are available for fluoxetine and gemfibrozil. Besides, a few studies were found for carbamazepine and triclosan, but they did not evaluate the uptake dynamics over long-term exposure or the influence of PPCP physicochemical properties and transpiration. Thirdly, the target compounds differ significantly in the following physicochemical properties: lipophilicity (measured by LogKow), ionization behavior (measured by pKa), and pH-dependent lipophilicity (measured by LogDow), as described in Table 1.

The PPCPs (purity > 95%) were purchased from Sigma-Aldrich (St Louis MO, US). A stock solution of 2000 mg/L of each PPCP standard was prepared in HPLC grade methanol and stored at 4°C in darkness. Isotopically labeled versions of each compound (carbamazepine-d10, fluoxetine-d5, gemfibrozil-d6, and triclosan-d3) were purchased from CDN Isotopes (Quebec, Canada). Methanol, formic acid, and acetonitrile (HPLC grade) were purchased from Thermo Fisher Scientific (Logan, Utah, USA).

Table 1: Physicochemical properties of the target pharmaceutical and personal care products

Target compounds (CAS Number)[a]	**Molecular mass (g/mol)**	**Molecular formula**	**Aqueous solubility (mg/mL) at pH 5.5[b]**	**pKa [a]**	**Charge and ionizable species distribution at pH 5.5 (%)[b]**	**LogKow[a]**	**LogDow at pH 5.5[b]**
Carbamazepine (298-46-4)	236.274	$C_{15}H_{12}N_2O$	0.0382	15.96	Neutral (100%)	2.77	2.77
Fluoxetine (56296-78-7)	309.332	$C_{17}H_{18}F_3NO$	208.9773	9.4	Cationic (99.99%), Neutral (0.01%)	4.17	1.01
Gemfibrozil (25812-30-0)	250.338	$C_{15}H_{22}O_3$	1.6077	4.42	Anionic (92.37%), Neutral (7.63%)	4.39	3.27
Triclosan (3380-34-5)	289.54	$C_{12}H_7Cl_3O_2$	0.0015	7.68	Neutral (99.34%), Anionic (0.66%)	4.98	4.98

used in the present study.

[a] Data obtained and calculated from Chemicalize (https://chemicalize.com/) developed by ChemAxon (http://www.chemaxon.com).

[b]The pH value of 5.5 corresponds to the condition of the hydroponic solution during exposure.

2.2. Plant material and hydroponic bioassay

2.2.1. *Typha latifolia* propagation and growth

Bare-root plants were obtained from Aquatic and Wetland Company (Fort Lupton, CO, USA). Plants were grown hydroponically in a greenhouse until roots developed and then transplanted into individual 3.78-L glass jars containing a nutrient solution (see monocotyledon starting solution composition in Supplementary Material, Table S2). The nutrient solution was modified when young plants developed the second pair of leaves (see vegetative growth solution composition in Supplementary Material, Table S2). Atmospheric air was supplied to each container at a flow rate of 50 mL/min to maintain adequate mixing and oxygen levels in the root zone. A 15-cm-thick closed-cell foam seal was used to support plants, and aluminum foil was used to shield the root zone from light. Plants were grown under greenhouse conditions with a natural summer/fall photoperiod (14-h day and 10-h night), a mean temperature of 27.24 ± 7.25 °C, and mean relative humidity of 42.30 ± 9.63%. Daytime photosynthetic photon flux averaged ~795 µmol m^{-2} s^{-1} for 1 month before the start of the exposure study.

2.2.2. Experimental design and hydroponic bioassay

Thirty-six plants of uniform size were selected for the PPCP hydroponic exposure study (N = 36), for a period of 42 days. Eighteen plants were used as non-exposed controls (Control treatment, n = 18 plants) and eighteen plants were dosed with a mixture of the four PPCPs (Exposure treatment, n = 18 plants). Three experimental units were assigned to each treatment, for each exposure time (7, 14, 21, 28, 35, and 42 days). At the beginning of the bioassay (day 0), a single dose of the PPCP stock solution (37.8 µL of 2000 mg/L) was added to each exposed plant container to yield an initial nominal exposure concentration of 20 µg/L. A single dose of PPCP-free methanol (37.8 µL) was added to the untreated control plants.

The dosage procedure was repeated on days 7, 14, 21, 28, and 35, when the solution in each container was completely replenished with a freshly prepared nutrient solution containing the PPCP mixture (Exposure treatment) or PPCP-free methanol (Control treatment) to maintain semi-static hydroponic exposure. Then, after each exposure time (7, 14, 21, 28, 35, and 42 days), three control and three exposed plants were removed from the hydroponic medium to determine dry biomass weights and PPCP tissue concentrations. In addition, two unplanted glass containers were dosed with the same PPCP mixture to evaluate their stability in the hydroponic environment. Details of the experimental design are shown in Fig. S1, Supplementary Material.

Samples of the hydroponic nutrient solution were collected immediately after each dosing and at regular intervals (1, 2, 3, 4, 5, and 6 days) between exposure times throughout the experiment to monitor PPCP concentrations in the exposure medium over time. In the unplanted glass containers, the differences between the initial and final concentrations of PPCPs were lower than 5%, indicating that the PPCPs were stable in the nutrient solution without plants. The nutrient solution lost to evapotranspiration was replenished daily, and the difference in the volumes added to the unplanted and planted systems was used to determine the amount of water transpired by each plant per day and week (transpiration rate). The transpiration rates increased as the plants grew, ranging from 2.95 ± 0.91 L at 7 days to 20.44 ± 1.74 L at 42 days. The initial pH of the hydroponic nutrient solution was 5.5, and NaOH or HCl was added to maintain this pH over time during the experiment.

2.2.3. Tissue plant collection

At the end of each exposure time (7, 14, 21, 28, 35, and 42 days), three control and three exposed plants were removed from the hydroponic medium and separated into submerged tissues (root, rhizomes, and sprout) and aerial tissues (stem and leaf). Leaves were separated into three sections (lower, middle, and upper) to study foliar PPCP differential patterns. Sprouts were considered submerged tissues because they developed inside the glass containers in direct contact with the hydroponic nutrient solution.

The collected plant tissues were oven-dried at 40 °C until constant weight, and the dry weights were obtained to determine dry tissue and whole-plant biomass. After drying, tissue samples were stored in desiccators containing silica gel until analysis.

2.3. PPCP extraction and analytical determination

2.3.1. Hydroponic nutrient solution

Samples (0.9 mL) were filtered through a 0.22-µm nylon filter and dispensed into a 1.5-mL vial along with 0.1 mL of methanol before direct injection analysis by liquid chromatography coupled to a triple quadrupole mass spectrometer (LC-MS/MS). Calibration standard curves (0.1 to 100 µg/L) for each target compound were made in the hydroponic nutrient solution with methanol (90/10 % v/v). Calibration standards (0.1 to 100 µg/L) for each deuterated target compound were made in methanol. Details of the LC-MS/MS procedure are described in the following subsection.

2.3.2. Plant material

PPCP extraction and analytical methodology were carried out following Pérez et al. (2022a). Briefly, 0.5 g of dried plant tissue was covered with liquid nitrogen and ground to a fine powder. Ground tissues were transferred to 50-mL polypropylene centrifuge tubes and spiked with a 20-µL aliquot of a methanol solution containing 10 mg/L of the deuterated-

labeled PPCP mixture. Later, 5 mL of methanol was added to the centrifuge tubes and vortexed for 1 min, shaken for 60 min at 250 rpm, and sonicated twice for 15 min. The methanol extract was removed after centrifugation at 3000 rpm for 30 min. The methanol extraction was repeated three times, and the extracts were combined before reducing the final volume to approximately 0.5 mL by evaporation under a gentle nitrogen stream using a TurboVap®II. Finally, the extract was reconstituted into 1 mL of methanol:acetonitrile (50:50, v/v) and transferred into a 1.5-mL vial. The final sample clean-up step was performed by adding 0.6 mL of the concentrated sample into a 2-mL vial containing a mixture of sorbents (150 mg MgSO4, 25 mg primary and secondary amine exchange material, and 7.5 mg graphitized carbon black, Phenomenex roQ QuEChERS). Later, 0.3 mL acetonitrile and 0.3 mL basic methanol were added and the pH was brought up to over 11 by adding KOH. The vials were vortexed for 30 s and centrifuged for 10 min at 4000 rpm. An aliquot of 0.6 mL was placed into a 1.5-mL vial for LC-MS/MS determination.

An Agilent 1290 Infinity LC system with an Agilent 6490 Triple Quadrupole MS system, operated in positive and negative electrospray ionization mode, was used for PPCP analysis. PPCPs were separated using an Agilent Eclipse Plus C18 column (2.1 x 50 mm, 1.8 µm I.D., 0.45 mL/min flow rate, 13 min run time, 3 µL injections) maintained at 30 °C. The mobile phase consisted of organic-free ultra-pure water containing 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The percentage of B was changed linearly as follows: 5% at 0 min; 95% at 10 min; 95% at 13 min; and 5% at 13.01 min. PPCPs were identified by retention time and using one or two ion products from the corresponding precursor ion. The most intense ion product (Q) was selected for quantification, and a secondary transition (q) was used for confirmation purposes. PPCP identification was considered confirmed when the sample Q/q ratio matched that of the reference standard by 20%. The MassHunter Workstation Software Version B.07.00 /Build 7.0.457.0 (Agilent Technologies) was used to

process quantitative data obtained from calibration standards and samples. Calibration standard curves (0.1 to 100 µg/L) for each PPCP and its deuterated-labeled version were made in a hydroponic nutrient solution with methanol. Linear relationships were observed for all compounds (R^2 > 0.99). Details of the LC-MS/MS conditions for the target PPCPs are shown in Table 2.

Table 2: LC-MS/MS operating parameters for the pharmaceutical and personal care products (PPCPs) analyzed in *Typha latifolia* tissues.

PPCPs	RT (time)	Q transition	Ion polarity	Cone (V)	C. E. (eV)	q transition	Ion polarity	Cone (V)	C. E. (eV)	LOD (µg/kg)	LOQ (µg/kg)
Carbamazepine	4.02	237.0 > 193.9	Positive	4	15	237.0 > 179.0	Positive	4	35	0.5	35
Carbamazepine-d10	3.95	247.0 > 204.0	Positive	4	31						
Fluoxetine	4.26	310.14 > 148.1	Positive	3	5	310.14 > 44.1	Positive	3	10	0.5	2
Fluoxetine-d5	4.21	315.0 > 153.0	Positive	3	5	315.0 > 44.0	Positive	3	5		
Gemfibrozil	6.79	249.2 > 121.0	Negative	4	25	249.2 > 83	Negative	4	25	40	40
Gemfibrozil-d6	6.75	255.0 > 121.0	Negative	4	25						
Triclosan	7.26	286.9 >	Negativ	4	28	288.9 >	Negativ	4	28	4	4

		35.0	e			37.1	e		
Triclosan-d3	7.23	289.9 > 35.0	Negativ e	4	28	292.0 > 35.0	Negativ e	4	28

LC-MS/MS: Liquid chromatography-tandem mass spectrometry; PPCPs: Pharmaceutical and personal care products; LOD: Limit of detection; LOQ: Limit of quantification; RT (min): Retention time; Q transition: Transition of the major ion product; q transition: Transition of the secondary ion product; Cone (V): Cone voltage; C.E. (eV): Collision energy.

2.3.3. Quality controls

Triplicate solvent blanks, spiked solvent control samples, and spiked tissue samples were analyzed along with each batch of plant tissue samples. Blanks were monitored to identify contamination or carryover. Spiked solvent controls and spiked tissue samples were fortified with deuterated and non-deuterated PPCPs (10 ng) and extracted as described above. Continuous calibration verification samples were analyzed every five samples. The limit of detection (LOD) was established as the lowest concentration that could be reliably differentiated from background levels with a signal-to-noise ratio of three or greater. The limit of quantification (LOQ) was determined as the lowest fortification level in the matrix sample that had a mean recovery between 70 and 120% of the spiking concentration, a relative standard deviation of 15% or less, and a signal-to-noise ratio of 10 or greater.

2.4. Data obtained and analysis

2.4.1. PPCP bioaccumulation mass in tissues

The distribution of the target compounds in the *T. latifolia* tissues was expressed as µg of each compound per plant tissue dry weight. Firstly, the mass of each individual PPCP per tissue per exposure time ($PPCP_{ind}$) was calculated as shown in Equation 1.

$$PPCP_{ind}\ \text{mass} = \text{PPCP tissue concentration (µg/kg)} * \text{Total tissue weight (kg)} \quad (1)$$

where PPCP represents carbamazepine, fluoxetine, gemfibrozil, or triclosan, and tissue represents root, rhizome, sprout, stem, lower leaf, middle leaf, or upper leaf. The leaf tissue was considered the sum of the three leaf sections (lower, middle, and upper). Secondly, the PPCP total mass ($PPCP_{sum}$) was calculated as the sum of all $PPCP_{ind}$ at each exposure time per tissue. The $PPCP_{sum}$ was determined to describe the distribution of the total PPCP mass at each exposure time in the analyzed tissues. Thirdly, the PPCP overall mass ($PPCP_{overall}$) was calculated as the mean of $PPCP_{sum}$ including all values obtained over 42 days per tissue. The $PPCP_{overall}$ was used to describe the overall steady-state bioaccumulation patterns in the analyzed tissues.

The statistical analysis was done as follows:

a) $PPCP_{sum}$ bioaccumulation patterns: To analyze the changes in the $PPCP_{sum}$ between tissues over time, the normality and homoscedasticity of the data set were determined, using the Shapiro-Wilk and Levene´s tests, respectively, with a significance level of $\alpha = 0.05$. Later, a two-way ANOVA was applied, where one factor was exposure time, the other factor was tissue, and the dependent variable was the $PPCP_{sum}$. A Tukey test was applied *a posteriori* with a significance level of $\alpha = 0.05$.

b) $PPCP_{ind}$ bioaccumulation patterns: To analyze the changes in $PPCP_{ind}$ in each tissue over time, the $PPCP_{ind}$ contribution was initially expressed as a percentage of the total including all PPCPs. The $PPCP_{ind}$ data were then transformed using the arcsine-square root transformation to meet the normality and homogeneity of variance assumptions, which were

verified through Shapiro-Wilk and Levene´s tests, respectively, with a significance level of α = 0.05. Later, two-way ANOVAs were applied, where one factor was exposure time, the other factor was compound, and the dependent variable was the percentage of $PPCP_{ind}$. A Tukey test was applied *a posteriori* with a significance level of α = 0.05.

Statistical analyses and graphical results were carried out using Infostat 2020e software developed by Córdoba National University, Argentina, which implements user-friendly interface on R to carry out multivariate analysis. A total of seven tissue sections and 252 tissue samples (126 for exposed plants and 126 for non-exposed control plants) were analyzed to obtain PPCP mass levels, indicating the robustness of this research.

2.4.2. PPCP translocation factors

First, PPCP mass in shoot, i.e., stem plus leaf, was calculated for each compound. Then, translocation factors (TFs) were calculated for each individual compound as the ratio between the PPCP in shoot and root, following Equation 2.

$$TF = \frac{\text{PPCP mass in shoot (stem + leaf) (μg)}}{\text{PPCP mass in roots (μg)}} \quad (2)$$

Moreover, the overall TF was calculated as the ratio between $PPCP_{overall}$ in shoot and $PPCP_{overall}$ in root.

2.4.3. Relationship between PPCP bioaccumulated mass and physicochemical properties

The PPCP bioaccumulated mass was evaluated over 42 days and dynamic interactions were found between exposure time, transpiration rate, and PPCP physicochemical properties,

such as LogKow, LogDow, and pKa (explanatory variables). Therefore, a Principal Component Analysis (PCA) was applied to study the relationships between PPCP mass in tissues (observations) and the five explanatory variables, as well as to determine to what extent the set of explanatory variables could account for the variability in the observations. A Matrix Correlation and Correlation Coefficients (PCA_{COEF}) were determined, and a Cophenetic Correlation Coefficient was calculated between the Euclidean distances to determine the PCA adjustment. If the cophenetic distances between variables are high, the PCA_{COEF} is close to 1, and the correlations are strong (Clarke et al., 2016).

The PCA was performed with the carbamazepine, fluoxetine, and triclosan bioaccumulation mass data because these compounds were determined in all tissues over time. Given that gemfibrozil was detected only in root and rhizome, this compound was excluded from the PCA to avoid an unbalanced data matrix. Thus, the $PPCP_{sum}$ for each tissue was determined using only carbamazepine, fluoxetine, and triclosan masses. The PCA and biplot graph were carried out using Infostat 2020e software.

3. RESULTS

3.1. PPCPs in the hydroponic nutrient solution

The initial and average concentrations of carbamazepine, fluoxetine, gemfibrozil, and triclosan in the hydroponic nutrient solution are shown in Fig. S2, Supplementary Material. The initial concentration of each compound was approximately 20 µg/L on days 0, 7, 14, 21, 28, and 35 (i.e., every time the hydroponic nutrient solution was renewed). After dosing, PPCP concentrations in the exposed plant containers decreased over time, indicating root uptake. The average nominal concentrations at the initial time were as follows: carbamazepine: 19.34 ± 0.49 µg/L; fluoxetine: 19.06 ± 0.25 µg/L; gemfibrozil: 19.86 ± 0.80

µg/L; and triclosan: 19.82 ± 2.47 µg/L. Average concentrations during the exposure time were as follows: carbamazepine: 10.45 ± 6.90 µg/L; fluoxetine: 4.00 ± 5.16 µg/L; gemfibrozil: 4.95 ± 5.79 µg/L; and triclosan: 3.55 ± 7.46 µg/L. In general, the concentrations of gemfibrozil and triclosan in the nutrient solution were below the detection limits on day 2 after each dosing while carbamazepine and fluoxetine mean concentrations were 2.24 ± 2.18 µg/L and 0.49 ± 0.59 µg/L, respectively, on day 6 after each dosing. These results indicate that 20 µg/L of gemfibrozil and triclosan was sorbed to the roots after 48 h, due to the high lipophilicity of both compounds. However, for the more hydrophilic compounds, i.e., carbamazepine and fluoxetine (LogDow = 1.01 for fluoxetine), measurable concentrations remained in the root zone solution for at least 3 days after dosing (Supplementary Material, Fig. S2). This suggests that sorption to the roots was faster for gemfibrozil and triclosan than translocation to shoots was for carbamazepine and fluoxetine. In contrast, in the unplanted glass containers, PPCP levels were stable for 7 days, indicating that the losses due to evaporation and degradation were negligible under greenhouse conditions.

3.2. PPCP extraction method in *T. latifolia* tissues

The extraction/clean-up method was found to be precise and accurate, with a matrix effect of < 20% and a relative standard deviation between 5 and 20%. Recovery percentages for spiked tissue samples with the deuterated-labeled PPCPs are shown in Table S3, Supplementary Material.

3.3. Overall PPCP bioaccumulated mass patterns in *T. latifolia*

In the tissues of control plants, no PPCPs were detected over time. In exposed plants, carbamazepine, fluoxetine, and triclosan were detected and quantified in all tissues, while gemfibrozil was detected only in roots and rhizomes (Table 3). Overall distribution of PPCP

patterns was as follows: (a) Roots: triclosan > gemfibrozil > fluoxetine > carbamazepine; (b) Rhizome: triclosan > fluoxetine > gemfibrozil > carbamazepine; (c) Sprouts: fluoxetine > triclosan > carbamazepine; (d) Stem: fluoxetine > triclosan > carbamazepine; (e) Leaf: carbamazepine ~ fluoxetine > triclosan. The highest PPCP mass was observed in roots and leaves over time, with values > 40 µg of PPCPs, while in rhizome, stem, and sprouts, the PPCP mass was less than 10 µg (Table 3). The TFs mean value was 1.24 ± 0.09, indicating that PPCP mass in aerial tissues (leaf + stem) and roots was in the same order of magnitude (Table 3).

Fig. 1A describes the dry biomass change over time up to 42 days for each tissue, while Fig. 1B describes the $PPCP_{sum}$ mass distribution per tissue at each exposure time. The two-way ANOVA showed statistical differences in the PPCP mass between tissues and exposure time, and a strong interaction between both variables ($p < 0.001$). At 7 days, there were no statistical differences in PPCP mass between tissues (Fig. 1B). At 14, 21, and 28 days, the highest PPCP mass was determined in roots and leaves in the same magnitudes ($p > 0.05$), while at 35 and 42 days, higher PPCP mass was observed in leaves than in roots ($p < 0.001$). At 28 days, a slight decrease, although not significant, in PPCP mass in root and leaf was observed, which could be attributed to intraspecific variability in the bioaccumulation potential between plants.

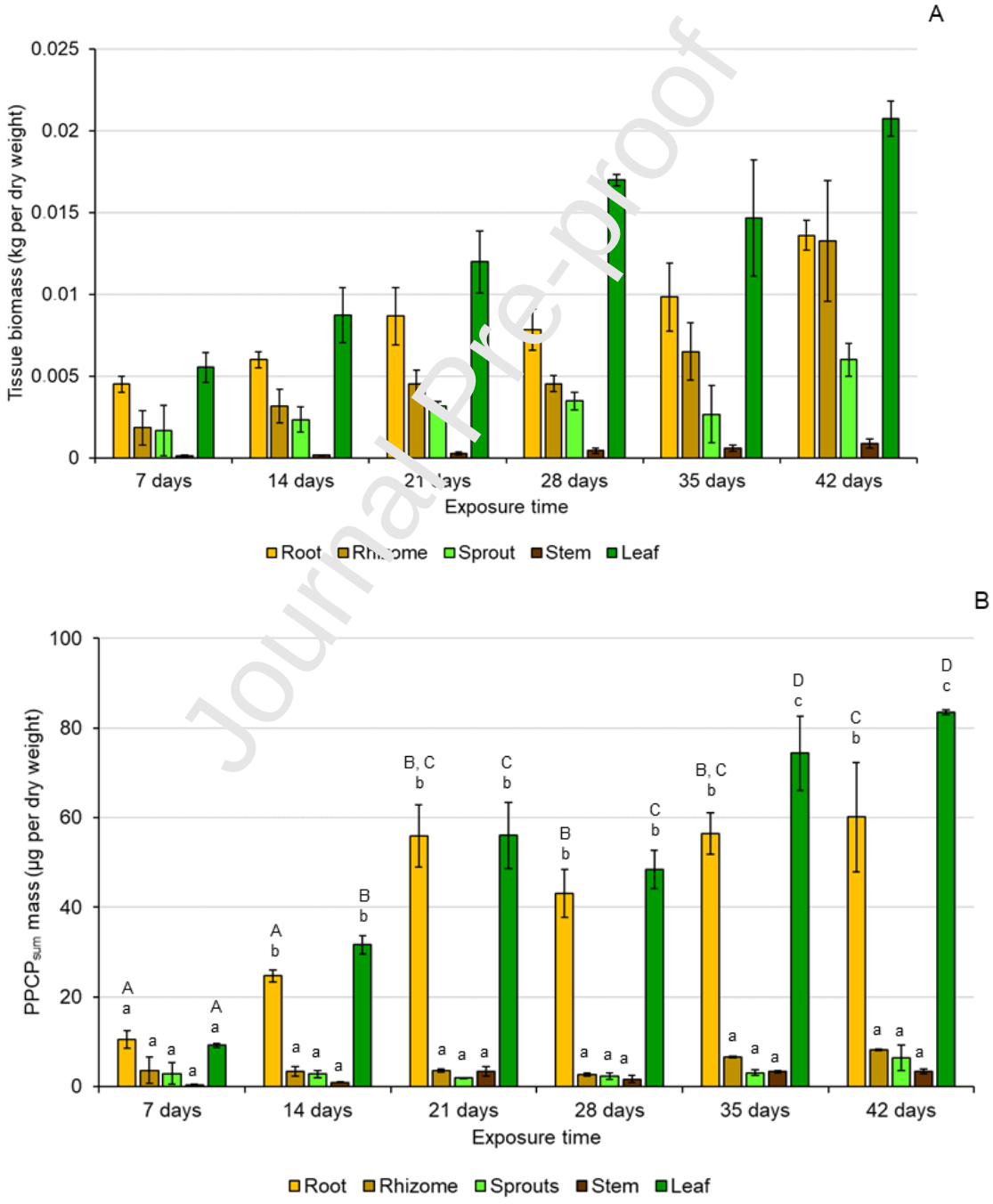
A relationship between dry biomass and PPCP mass was observed only in root and leaf tissues. For rhizome, sprout, and stem, the increases in the dry biomass did not reflect the PPCP mass increases over time. PPCP mass in the rhizome, sprouts, and stem did not vary with exposure times (Fig. 1B). The obtained results shown in Fig. 1B are in agreement with the enhanced bioaccumulation potential of root and leaf.

Table 3: Overall bioaccumulation mass patterns over 42 days in the analyzed tissues, and translocation factors (TFs) of pharmaceutical and personal care products (PPCPs) in tissues (µg per dry weight) of exposed *Typha latifolia* plants over 42 days (mean ± SE).

Target compound	Submerged tissues			Aerial tissues		TFs
	Root	Rhizome	Sprout	Stem	Leaf	
Carbamazepine	1.13 ± 0.15	0.54 ± 0.06	0.27 ± 0.04	0.05 ± 0.01	23.88 ± 4.33	22.02 ± 2.62
Fluoxetine	4.34 ± 0.42	1.49 ± 0.22	2.24 ± 0.57	1.68 ± 0.29	21.53 ± 2.67	5.92 ± 0.95
Gemfibrozil	9.78 ± 1.30	0.69 ± 0.09	n.d.	n.d.	n.d	n.a.
Triclosan	26.54 ± 3.63	1.99 ± 0.36	0.75 ± 0.14	0.65 ± 0.09	1.87 ± 0.28	0.10 ± 0.01
$PPCP_{overall}$	41.80 ± 4.98	4.71 ± 0.65	3.25 ± 0.66	2.21 ± 0.37	48.61 ± 6.18	1.24 ± 0.09

n.d.: not detected; n.a.: not analyzed

Figure 1: A) Distribution pattern of dry tissue biomass (expressed in kg per dry weight). B) Distribution pattern of $PPCP_{sum}$ mass (expressed in µg per dry weight) over 42 days of exposure time in *Typha latifolia* tissues. For (B), different lowercase letters indicate statistically significant differences between tissues at the same exposure time. Different capital letters indicate statistically significant differences for roots or leaf between exposure times with ANOVAs and Tukey test with $\alpha = 0.05$.



3.4. Accumulated PPCP mass in *T. latifolia* tissues over time

Fig. 2 shows the bioaccumulated mass of each $PPCP_{ind}$ in percentages in both submerged and aerial tissues over the exposure time. For all tissues, significant differences were observed between $PPCP_{ind}$ mass contributions and exposure time, as well as an interaction between both variables ($p < 0.05$) (details of statistical two-way ANOVA are shown in the Supplementary Material).

In roots, triclosan showed the highest contributions to the $PPCP_{ind}$ mass ($p < 0.001$). Moreover, triclosan showed a gradual statistically significant increase with exposure time, reaching more than 70% of mass contribution at 42 days ($p < 0.001$). In rhizome, fluoxetine and triclosan mass showed the highest contributions to the total PPCP mass. At 42 days, triclosan reached more than 50% of the total PPCP mass ($p < 0.001$). Carbamazepine and gemfibrozil mass contributions were the lowest observed and similar at each exposure time ($p > 0.05$). In sprouts, fluoxetine contributions represented more than 60% of PPCP mass, except at 35 days when triclosan was higher ($p < 0.001$). Carbamazepine mass was lower than 20% of the total PPCP mass (Fig. 2).

In stem, fluoxetine contributions represented nearly 70% of PPCP mass at each exposure time ($p < 0.001$). Triclosan contributions were higher than those of carbamazepine throughout the exposure time ($p < 0.010$). Carbamazepine contributions were lower than 10% of the PPCP mass (Fig. 2), while in leaf, carbamazepine and fluoxetine masses represented more than 90% of the total PPCP mass ($p < 0.001$). At 7, 14, and 28 days, fluoxetine contributions were higher than those of carbamazepine ($p < 0.001$). However, gradual increases were observed in carbamazepine, which represented more than 55% of the total PPCP mass ($p < 0.001$) at 35 and 42 days (Fig. 2).

The two-way ANOVAs for PPCP mass contributions to leaf sections (lower, middle, and upper leaf) showed a differential distribution pattern, with a strong interaction between PPCP mass contributions and exposure time ($p < 0.001$), except in the upper leaf section (Fig. 3). In the lower leaf, fluoxetine contributions represented more than 85% of the total PPCP mass over time ($p < 0.001$). In the middle leaf, at 7 days, carbamazepine and fluoxetine contributions were similar ($p > 0.05$). However, for the rest of the exposure times, fluoxetine contributions surpassed 62% of the total PPCP mass ($p < 0.001$) (Fig. 3). In the upper leaf, no interaction was observed between exposure time and PPCP mass ($p > 0.05$). Carbamazepine mass represented more than 90% of the total PPCP mass over time (Fig. 3).

The highest TF values were determined for carbamazepine due to the increased mass in the leaf (Table 3). The reductions in TF in fluoxetine compared to carbamazepine were due to the higher values of fluoxetine in root than those of carbamazepine. The TF values for triclosan were the lowest observed due to high root bioaccumulation.

Figure 2: Bioaccumulated mass of individual PPCP ($PPCP_{ind}$, expressed in % of µg per dry weight) in *Typha latifolia* tissues over exposure time (mean ± SE). Two-way ANOVAs and Tukey test with $\alpha = 0.05$ for each tissue are further described in the Supplementary Material. CBZ: carbamazepine; FLX: fluoxetine; GBZ: gemfibrozil; TRI: triclosan.

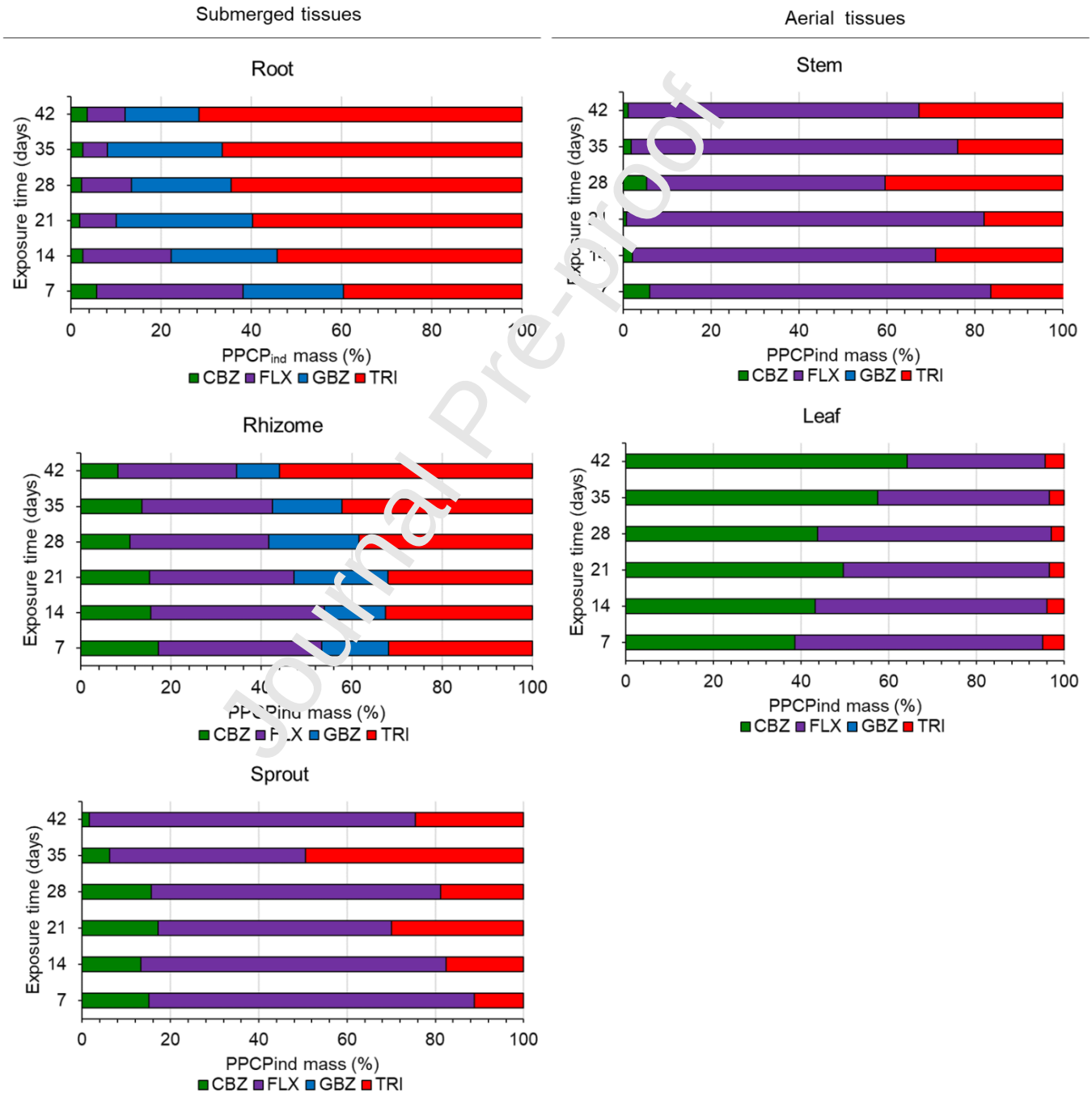
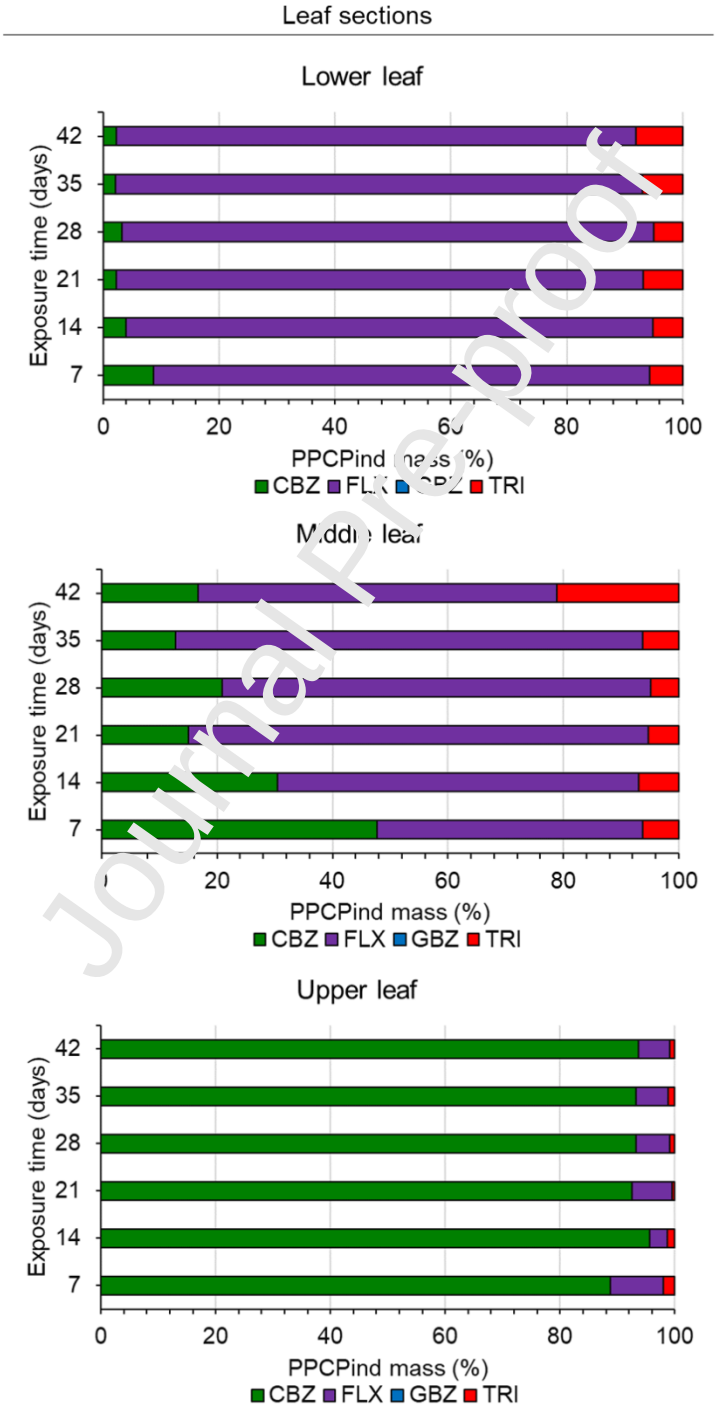


Figure 3: Bioaccumulated mass of individual PPCP ($PPCP_{ind}$, expressed in % of µg per dry weight) in *Typha latifolia* leaf sections over exposure time (mean ± SE). Two-way ANOVAs and Tukey test with $\alpha = 0.05$ for each tissue are further described in the Supplementary Material. CBZ: carbamazepine; FLX: fluoxetine; GBZ: gemfibrozil; TRI: triclosan.



3.5. Relationship between the bioaccumulated mass of PPCPs, physicochemical properties, and biological indicators

The biplot graph describes the relationships between the PPCP mass in tissues and exposure time, transpiration rate, pKa, LogKow, and LogDow (Fig. 4). Both principal components explained 63.7% of the total variability in the observations, and the Cophenetic Correlation Coefficient = 0.828 indicated a strong adjustment in the PCA analysis (details are shown in the Supplementary Material).

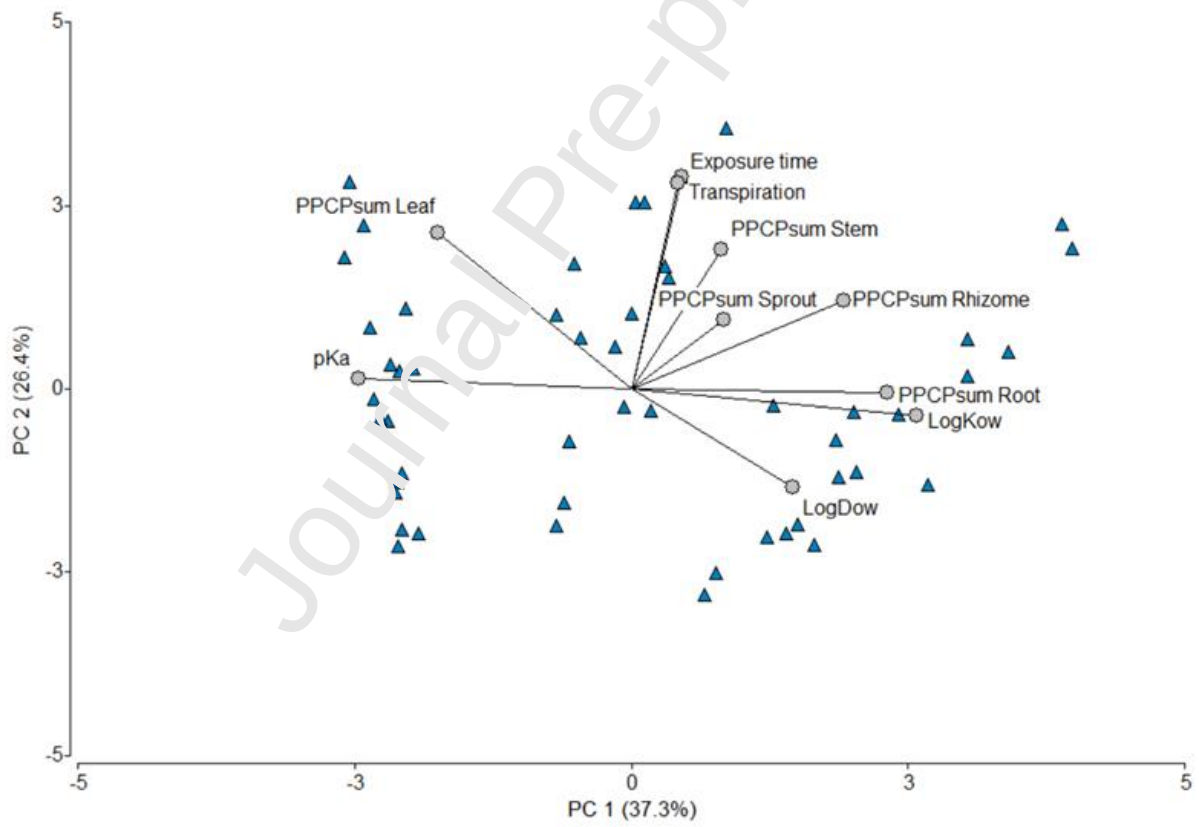
The following correlations were significant ($p < 0.05$) and their PCA_{COEF} is detailed below. The root $PPCP_{sum}$ was weakly positively correlated with exposure time ($PCA_{COEF} = 0.28$) and transpiration ($PCA_{COEF} = 0.24$), and it was strongly positively correlated with LogKow ($PCA_{COEF} = 0.68$) and LogDow ($PCA_{COEF} = 0.67$), and less positively correlated with exposure time ($PCA_{COEF} = 0.28$) and transpiration ($PCA_{COEF} = 0.24$). It was also negatively correlated with pKa ($PCA_{COEF} = -0.59$). Rhizome $PPCP_{sum}$ was weakly positively correlated with exposure time, transpiration, and LogDow ($PCA_{COEF} = 0.37$, $PCA_{COEF} = 0.32$, $PCA_{COEF} = 0.19$), and moderately positively correlated with LogKow ($PCA_{COEF} = 0.49$). Moreover, it was moderately negatively correlated with pKa ($PCA_{COEF} = -0.49$). Sprout $PPCP_{sum}$ was weakly negatively correlated with pKa and LogKow ($PCA_{COEF} = -0.32$, $PCA_{COEF} = -0.37$) (p values < 0.05).

Stem $PPCP_{sum}$ was weakly positively correlated with exposure time, transpiration, and LogKow ($PCA_{COEF} = 0.35$, $PCA_{COEF} = 0.32$, $PCA_{COEF} = 0.30$), and negatively correlated with pKa and LogDow ($PCA_{COEF} = -0.39$, $PCA_{COEF} = -0.38$). Leaf $PPCP_{sum}$ was moderately positively correlated with exposure time and transpiration ($PCA_{COEF} = 0.48$, $PCA_{COEF} = 0.43$), and strongly positively correlated with pKa ($PCA_{COEF} = 0.52$). Moreover, it was strongly negatively correlated with LogKow ($PCA_{COEF} = -0.58$) and LogDow ($PCA_{COEF} = -0.53$) (p values < 0.05).

In brief, in all tissues, except for sprout, $PPCP_{sum}$ was positively correlated with exposure time. Moreover, the most significant and strongest relations involved the bioaccumulation of PPCPs in root and leaf. In roots, PPCP bioaccumulation was favored by the lipophilicity of the target compound and diminished by the pKa, which was represented by the close distances between PPCPsum root, LogKow and LogDow vectors, and the opposite vector direction with pKa (Fig. 4). In leaf, there were inverse relations, and the PPCP bioaccumulation was moderately favored by plant transpiration and more strongly facilitated by the pKa of the target compound, represented by the close distance between PPCPsum leaf and pKa vectors (Fig. 4). However, PPCP bioaccumulation was attenuated by the lipophilicity of the target compounds, indicated as opposite vector directions with the LogKow and LogDow (Fig. 4).

The relations between PPCP bioaccumulation in rhizome and transpiration, lipophilia, and pKa of the PPCPs were less relevant. However, these relations followed a similar pattern in rhizome and root, while transpiration and lipophilia were similar in stem and leaf, except for the pKa, which was negatively correlated.

Figure 4: Principal component analysis (PCA) biplot graph of PPCP bioaccumulated mass in vegetative tissues (root, rhizome, sprouts, stem, leaf) and explanatory variables (Exposure time, Transpiration, pKa, LogKow, LogDow). The biplot shows the PCA scores of the explanatory variables as vectors (black lines) and the bioaccumulated mass of each PPCP in tissues (gray triangles) of the first principal component (PC 1, x-axis) and second principal component (PC 2, y-axis). The magnitude of the vectors represents the strength of their contribution to each PC. Vectors pointing in similar directions indicate positively correlated variables, vectors pointing in opposite directions indicate negatively correlated variables, and vectors at approximately right angles indicate low or no correlations.



4. DISCUSSION

4.1. PPCP bioaccumulation mass in *T. latifolia*

Most previous studies using *T. latifolia* have focused on phytoextraction and phytoremediation processes, using removal rate, tissue concentrations, or bioaccumulation factors as metrics (Supplementary Material, Table S1). In this way, the range of exposure concentrations of PPCPs was higher (in the order of mg/L) than those that could be expected to occur more frequently in aquatic ecosystems, in contrast to the findings of L. Zhang et al. (2017), Y. Zhang et al. (2017), and Adesanya et al. (2021), who used exposure concentrations in the µg/L order, between 5 and 100 µg/L. The present study evaluated the uptake and translocation of a mixture composed of carbamazepine, fluoxetine, gemfibrozil, and triclosan, at a detectable range level in aquatic ecosystems of 20 µg/L each. Similar levels of PPCP mixtures have been applied to analyze the root uptake and shoot translocation using the aquatic plants *Eichornia caprisses* and *Echinodorus horemanii* (Pi et al., 2017).

In the current study, PPCP mass in the whole-plant body, commonly known as burden mass, was used as a bioaccumulation metric in *T. latifolia*. This measurement accurately describes the plant bioaccumulation capacity because it considers the total body biomass. Sometimes, the standard metrics (i.e., tissue concentrations and bioaccumulation factors) can overestimate or underestimate the real tissue levels. For example, this occurs when the contaminants have a non-homogeneous distribution in the tissue/organs or when the growth dilution effect overlaps with the tissue concentration (Mitton et al., 2014; Pérez et al., 2022a).

In the calculation of the bioaccumulation factor in static and semi-static bioassays, the most common experimental difficulty lies in controlling exposure medium concentrations over time (Ribbenstedt et al., 2017). In many reports, it is assumed that the exposure concentration is constant after the initial pulse, and the bioaccumulation factor is calculated using the initial dosing (D. Zhang et al., 2013a; 2013b). Ideally, bioaccumulation factors

should be determined under steady-state conditions (i.e., concentration ratios between plant compartments and exposure media are constant over time) or by kinetically using uptake and elimination rates for consistency and comparability (Doucette et al., 2018). In general, the studies do not consider the changes in the exposure medium concentrations that occur over time, such as root uptake and adsorption, losses by degradation or biodegradation, plant elimination to the surrounding medium, or irrigation dilution (Y. Zhang et al., 2016; Pérez et al., 2022b). There are some alternatives to reduce this experimental error. One of them is to monitor the medium concentration throughout the exposure time and add or dilute the exposure medium concentration (Pérez et al., 2013). Another alternative is to replenish the hydroponic solution every one or two days (Pi et al., 2017) or use a passive dosing method (Ribbenstedt et al., 2017).

In the present study, to reduce errors in tissue concentration, the whole-body plant was harvested, dissected into seven sections (i.e. root, rhizome, sprout, stem and lower, middle, and upper leaf sections), dried, and homogenized, and each section was subsampled to analyze the tissue concentrations (Tables S4-S7, Supplementary Material). Moreover, obtaining the dry biomass reduces the potential water content differences between tissues or phenological stages, which can be another source of error in the calculation metrics. Pérez et al. (2022b) used the accumulated dry mass of atrazine and its metabolites in *T. latifolia* tissues, while D. Zhang et al. (2013a) used the caffeine assimilated mass in roots and shoots per fresh weight in *Scirpus validus* as bioaccumulated metrics.

Body burden mass of the target PPCPs displayed differential bioaccumulation patterns between tissues (Table 3, Figs. 1 and 2). Roots and leaves were the preferential bioaccumulation tissues where the PPCP mass surpassed 70 µg at 42 days (Fig. 1). In roots, triclosan mass was higher than that of the other PPCPs, namely more than 20 times higher than that of carbamazepine, seven times higher than that of fluoxetine, and only three times

higher than that of gemfibrozil. In contrast, carbamazepine and fluoxetine mass values in leaf were more than ten times higher than that of triclosan (Table 3). In rhizome, sprout, and stem, the total PPCP mass was reduced between 10 and 20 times in comparison with roots and leaf (Table 3, Fig. 2). In a previous study, the bioaccumulation of triclosan in *T. latifolia* was analyzed in constructed wetlands. The bioaccumulation factors were 0.03 ± 0.01 µg/g for roots and 0.02 µg/g for leaf, without significant differences between tissues (Zarate et al., 2012).

Previous studies using *Typha spp.* exposed to carbamazepine showed that this pharmaceutical accumulated in the leaf, but the roots were not analyzed (Dordio et al., 2011). Moreover, the uptake of carbamazepine in *T. latifolia* was analyzed by the Pitman chamber method, but tissue bioaccumulation was not measured (Cui et al., 2015). In a study on *Eichornia crassipes* (free-floating root), the PPCP distribution patterns observed for carbamazepine and triclosan (Pi et al., 2017) were similar to those described in our study. *Eichornia crassipes* exposed to 20 µg/L (semi-static bioassay) of a PPCP mixture reached, at 20 days, nearly 10,500 µg/kg and 7000 µg/kg of carbamazepine in leaf and roots, respectively, while 20,000 µg/kg and 40000 µg/kg of triclosan were found in leaf and roots, respectively. Carbamazepine concentrations quantified in *T. latifolia* were lower in roots over time (< 160 µg/kg) than those reported for *E. crassipes*, while in leaf, carbamazepine concentrations were in the same order of magnitude in the upper leaf (Supplementary Material, Table S4). Moreover, in the roots and leaf of *T. latifolia*, triclosan concentrations (Supplementary Material, Fig. S1) were much lower than those in *E. crassipes*. In addition, gemfibrozil was only detected in the roots and rhizome of *T. latifolia* (Table 3, Fig. 2) and in the root and leaf of *E. crassipes*, with root levels 10 times higher than in leaf, based on steady-state bioconcentration factors (Pi et al., 2017). Gemfibrozil concentrations in *T. latifolia* roots at 21 days were ~2000 µg/kg (Supplementary Material, Table S5), similar to

those reported in *E. crassipes* (Pi et al. 2017). However, Wang et al. (2019) did not detect gemfibrozil in submerged or aerial tissues of *T. angustifolia* in a field experiment.

Concerning fluoxetine, there are no previous reports on the bioaccumulation process in *Typha spp*. In the present study, fluoxetine concentrations in *T. latifolia* roots reached the maximum values of ~800 µg/kg at 14 days, in stem ~10000 µg/kg at 21 days, and in leaf ~ 4400 µg/kg (Supplementary Material, Table S6). In another study, using cauliflower exposed in the soil-plant system, fluoxetine accumulated mainly in the stem, rather than the leaf, and it was not detected in the roots (Redshaw et al., 2008), in agreement with our results with respect to the high bioaccumulation in the stem. Another recent study using a hydroponic system with *Zea mays* at 20 µg/L of fluoxetine showed greater bioaccumulation in roots than in leaf and low bioaccumulation in stem (Pérez et al. 2022a). The data obtained in *T. latifolia* are relevant since the stem has not been extensively studied to determine its bioaccumulation of contaminants.

The lower levels of PPCPs in the rhizome, sprout, and stem of *T. latifolia* can be explained by the biological function of each tissue. For rhizome, a submerged stem from which young shoots (sprouts) grow has its main activity as a nutrient sink during the senescence stage (Garver et al., 1988). Given the relationship between the biological function and the bioaccumulation process of PPCPs, it makes sense that the PPCP accumulated mass in the rhizome could be increased at the end of the vegetative growth. In the sprouts, PPCP accumulation was similar to that of the rhizome (Table 3, Fig. 2). This fact is probably related to the close relationship between both tissues. For the stem, it is clear that the PPCP mass was lower because this tissue acted as a dynamic transporter of water and nutrients from roots to shoot. In the genus *Typha,* the stem is small and hard and the vascular system is composed of vessel elements and narrow tracheids that retain a primary wall meshwork in the end wall pits. The narrow tracheids and vessel elements are thick-walled cells that, when dying at

maturity, exhibit lignified walls (Carlquist, 2012). This suggests that the stem generally has a low capacity to accumulate PPCPs, except for fluoxetine, which was highly bioaccumulated in this organ. It could be hypothesized that fluoxetine was compartmentalized in the lignified cell wall of the stem and the lower and middle leaf sections. Lignin is a natural amorphous polymer that is greatly negatively charged by its end-groups, such as alcohols and aldehydes (Katahira et al., 2018). In this sense, fluoxetine, which is positively charged, may be absorbed by the negatively charged end-groups of lignin. There is limited information about the compartmentalization of organic contaminants in the plant wall cells of monocotyledons. Q. Zhang et al. (2021) demonstrated in the monocotyledon *Oryza sativa* the sequestration and compartmentalization of biotransformation products of 2,4,6-tribromophenol in the wall cells and vacuoles of root cells. In this sense, further studies on the possible compartmentalization of fluoxetine in lignified stem wall cells could be carried out.

Moreover, a differential PPCP distribution pattern was observed in the leaf sections (Fig. 3). In lower and middle leaf sections, fluoxetine contributed more than 80% and 50% of the total PPCP mass, respectively, while in the upper section, carbamazepine mass represented more than 90% of the total PPCPs. A similar distribution pattern was observed in *T. latifolia* leaves after exposure to sulfolane (Doucette et al., 2005). This differential PPCP bioaccumulation pattern is likely the result of the linear development and growth of the *Typha spp.* leaf (Grace and Harrison, 1986). Moreover, the greater amounts of water being transpired by the upper leaf due to their older age and the additional sunlight received relative to the lower leaf sections could explain this distribution (Doucette et al., 2005). Moreover, concerning tissue concentrations, if the whole leaf were used to quantify the PPCP levels, the results would change carbamazepine levels, while fluoxetine levels would be similar in the whole leaf, and triclosan would probably not be detected due to dilution. Thus, before the experimental design, it is important to take into account the plant status (i.e., submerged,

free-floating, rooted emergent), plant growth, and tissue development, to understand the dynamics of organic contaminants and their relationship with root uptake, translocation, bioaccumulation, and biodegradation in the target species.

Concerning biodegradation and metabolization, it is important to analyze their dynamics within the plants. In a previous study, phase I atrazine metabolites were analyzed in *T. latifolia* tissues (Pérez et al., 2022b), but in the present study it was not possible to evaluate PPCP metabolites and their implications in the uptake and translocation. The biodegradation process of PPCPs in *T. latifolia* is scarcely explored (Supplementary Material, Table S1). Transformation products of diclofenac, such as OH-diclofenac, 4-O-glucopyranosyloxy-diclofenac, and 4-OH–glutathionyl-diclofenac, were analyzed in roots and shoot (Bartha et al., 2014). Methylbiguanide, a metformin metabolite, was analyzed in roots, rhizome, and leaf (Cui and Schroder, 2016). Twenty-three transformation products of iopromide were identified, including hydroxy-metabolites and carboxylic-metabolites (Cui et al., 2017). Moreover, Zarate et al. (2012) did not detect the main metabolite of triclosan, methyl-triclosan, in *T. latifolia*, in constructed wetlands. The formation of metabolites as a consequence of plant activity biodegradation needs further study to know their fate in the plant, identify those PPCPs that remain recalcitrant in biomass, and examine further decomposition steps of the plant.

4.2. PPCP mass and its relation to exposure time, transpiration, and physicochemical properties

In the present work, four PPCPs with contrasting physicochemical properties were selected (Table 1). Triclosan, gemfibrozil, and fluoxetine are highly lipophilic compounds at neutral pH (LogKow = 4.98, 4.39, 4.17, respectively). However, gemfibrozil and fluoxetine lipophilicity was negatively and positively charged, respectively, under the experimental

hydroponic conditions, pH = 5.5. In this sense, lipophilicity for gemfibrozil and fluoxetine was LogDow = 3.17 and LogDow = 1.01, respectively. These changes occurred because both compounds were ionized in the experimental conditions. Gemfibrozil has a pKa = 4.42, and 92.37% of its molecules may be expected to be in anionic ionization and only 7.63% may be neutral molecules. In contrast, fluoxetine has a pKa = 9.4, and its ionized molecule distribution could be expected to be 100% cationic. On the other hand, triclosan and carbamazepine are neutral compounds (100% neutral molecules), and their lipophilicity and ionization behavior did not change under the experimental conditions. Moreover, carbamazepine has a LogKow = 2.77, which was the least lipophilic compound used in the present study (Table 1).

As detailed above, the general distribution pattern was as follows: triclosan and gemfibrozil bioaccumulated highly in roots, fluoxetine accumulated in stem and lower and middle leaf, and carbamazepine accumulated highly in leaf, mainly in the upper section (Table 3, Figs. 2 and 3). To understand the relationship between exposure time, water transpired, and physicochemical properties of target PPCPs, a PCA was carried out (Fig. 4). To avoid the unbalanced matrix, gemfibrozil was excluded from the PCA, because it only accumulated in root and rhizome, as mentioned above.

Three important relationships were observed in the PCA analysis. First, PPCP accumulation in tissues increased with exposure time, following plant growth, except in the sprout, which was explained by the lower growth during the experiment (Fig. 1A-B). Second, lipophilia favored the bioaccumulation of PPCPs in roots and attenuated their translocation to shoot and bioaccumulation. Third, plant transpiration and PPCP pKa favored the translocation to shoot and bioaccumulation, but the pKa diminished bioaccumulation in root (Fig. 4). In this sense, it is reasonable to expect the highly lipophilic and neutral triclosan to be mainly bioaccumulated in root and less translocated to shoot (Fig. 3). For triclosan, it was

possible to apply the translocation models, as proposed by Briggs et al. (1982) and Dettermaier et al. (2009), to predict the uptake and translocation dynamics.

Based on the predictive models cited above, fluoxetine was expected to accumulate mainly in the roots. However, under the experimental conditions used in this study, it was hydrophilic and cationic. This favored root-to-shoot translocation, and therefore fluoxetine bioaccumulated in aboveground tissues. Three processes could impact the uptake and translocation of cationic PPCPs, the electrical attraction between cationic fluoxetine with a negative charge on the biomembranes, the movement with the transpiration stream, and the bioaccumulation in the vacuole by the ion trap mechanism (Al-Farsi et al., 2017). The third process is in agreement with the possible compartmentalization of fluoxetine in the stem lignified wall cells.

Carbamazepine had the greatest root-to-leaf transfer due to its neutral state and optimal LogKow for passive uptake and translocation. Trapp et al. (2023) considered carbamazepine an ideal PPCP to study plant uptake and translocation dynamics because of its properties. Carbamazepine had the highest bioaccumulation in the upper leaf (Fig 3) probably because it received the greatest amount of water via the transpiration stream.

Gemfibrozil was excluded from the analysis, but its distribution pattern was in agreement with the PCA relationships. Its high lipophilicity and anionic condition favored root accumulation and limited shoot translocation, possibly because it was repelled by the negative charges of the biomembranes (Al-Farsi et al., 2017).

In general, the obtained results are in agreement with the passive uptake and shoot translocation of neutral organic compounds, such as triclosan and carbamazepine. However, translocation is limited by lipophilicity, as in the case of triclosan, and it is favored by transpiration stream, as in the case of carbamazepine (Dettenmaier et al., 2009). However, in PPCPs, the ionization behavior plays a key role in the translocation from root to shoot. For a

wide range of PPCPs with different ionization behaviors, Dodgen et al. (2015) showed a strong positive relationship between transpiration rate and neutral charge and a weaker relation to cationic compounds. Nevertheless, these authors did not evaluate fluoxetine, and based in the present results, the cationic state favors the shoot translocation through the transpiration stream.

The results of this study indicate that the uptake and root-to-shoot transfer of PPCPs is influenced by the physicochemical properties of the target compound, the biological characteristics of the studied plant, and the exposure conditions. It is important to take into account the environmental conditions since, under different scenarios (e.g., natural wetlands, constructed wetlands, waste-water treatment plant), the PPCPs physicochemical properties can be modified, thus impacting uptake and translocation dynamics.

5. CONCLUSION

The current study proposes the use of PPCP burden mass as an appropriate bioaccumulated metric in *Typha latifolia*. Moreover, the results obtained showed that the PPCP uptake, translocation, and distribution in vegetative *T. latifolia* tissues must be studied and analyzed as a dynamic process strongly related to plant growth and tissue development, as well as to the exposure conditions and physicochemical properties of the target compounds. In this sense, it is essential to know the life cycle, growth status, plant growth, tissue development, transpiration rate, as well as the physicochemical properties of target compounds, to understand the PPCP movement inside the plant.

Credit author statement

Débora Jesabel Pérez contributed to the original idea, designed the study, organized the greenhouse experiment, conducted the writing and reviewed the original draft, conducted the PPCP residues analysis, the statistical analysis, and the visualization of the draft, and

contributed to funding acquisition. **Lucas Rodrigo Lombardero** contributed to the writing and revision of the draft and to the statistical analysis of PPCP residues in plant tissues. **William Joseph Doucette** contributed to the original idea, designed the study, organized the greenhouse experiment, conducted the writing, reviewed the original draft, and contributed to funding acquisition.

Declaration of competing interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments: This research was supported by project WR-2201 granted to W. Doucette from Utah State University (USA), project PICT 2014–1460 of the Fondo para la Investigación Científica y Tecnológica (Argentina) and PIP 0967 of the CONICET granted to D. Pérez, the Fellowship Program for Young CONICET Researches (Argentina). We thank Jeffrey Flashinski, Autumn Slade, Joe Stewart, Leila Ahmadi, and Ivonne Cardo Harris for their assistance with various aspects of the project.

REFERENCES

Adesanya, T., Zvomuya, F., Farenhorst, A., 2021. Phytoextraction of ciprofloxacin and sulfamethoxazole by cattail and switchgrass. Chemosphere, 279, 130534. https://doi.org/10.1016/j.chemosphere.2021.130534

Ahmadi, L., Merkley, G.P., 2017. Wastewater reuse potential for irrigated agriculture. Irrigat. Sci., 35, 275–285. https://doi.org/10.1007/s00271-017-0539-7

Al-Farsi, R.S., Ahmed, M., Al-Busaidi, A., Choudri, B.S., 2017. Translocation of pharmaceuticals and personal care products (PPCPs) into plant tissues: a review. Emerg. Contam., 3, 132–137. https://doi.org/10.1016/j.emcon.2018.02.001

Bartha, B., Huber, C., Schröder, P., 2014. Uptake and metabolism of diclofenac in *Typha latifolia*–how plants cope with human pharmaceutical pollution. Plant Sci., 227, 12-20. https://doi.org/10.1016/j.plantsci.2014.06.001

Bertrand., L, Monferrán., M.V., Valdés, M.E., Amé, M.V., 2019. Usefulness of a freshwater macrophyte (*Potamogeton pusillus*) for an environmental risk assessment in a multi-source contaminated basin. Chemosphere, 222, 1003-1016. https://doi.org/10.1016/j.chemosphere.2019.02.018

Briggs, G.G., Bromilow, R.H., Evans, A.A., 1982. Relationships between lipophilicity and root uptake and translocation of non-ionized chemicals by barley. Pestic. Sci., 13, 495:504. https://doi.org/10.1002/ps.2780130506

Carlquist, S., 2012. Monocot xylem revisited: new information, new paradigms. Bot. Rev., 78, 87-153. https://doi.org/10.1007/s12229-012-9096-1

Carter, L.J., Armitage, J.M., Brooks, B.W., Nichols, J.W., Trapp, S., 2022. Predicting the accumulation of ionizable pharmaceuticals and personal care products in aquatic and terrestrial organisms. Environ. Toxicol. Chem., 00, 1–11. https://doi.org/10.1002/etc.5451

Clarke, K.R., Somerfield, P.J., Gorley, R.N., 2016. Clustering in non-parametric multivariate analyses. J. Exp. Mar. Biol. Ecol., 483, 147-155. https://10.1016/j.jembe.2016.07.010

Cui, H., de Angelis, M.H., Schröder, P., 2017. Iopromide exposure in *Typha latifolia* L.: Evaluation of uptake, translocation and different transformation mechanisms in planta. Water Res., 122, 290-298. https://doi.org/10.1016/j.watres.2017.06.004

Cui, H., Hense, B.A., Müller, J., Schröder, P., 2015. Short term uptake and transport process for metformin in roots of *Phragmites australis* and *Typha latifolia*. Chemosphere, 134, 307-312. https://doi.org/10.1016/j.chemosphere.2015.04.072

Cui, H., Schröder, P., 2016. Uptake, translocation and possible biodegradation of the antidiabetic agent metformin by hydroponically grown *Typha latifolia*. J. Hazar. Mater., 308, 355-361. https://doi.org/10.1016/j.jhazmat.2016.01.054

Dettenmaier, E.M., Doucette, W.J., Bugbee, B., 2008. Chemical hydrophobicity and uptake by plant roots. Environ. Sci. Technol., 43, 324–329. https://doi.org/10.1021/es801751x

Dhir, B., Sharmila, P., Saradhi, P.P., 2009. Potential of aquatic macrophytes for removing contaminants from the environment. Crit. Rev. Environ. Sci. Technol., 39, 754-781. http://dx.doi.org/10.1080/10643380801977776

Di Luca, G.A., Mufarrege, M.M., Hadad, H.R., Maine, M.A., 2019. Nitrogen and phosphorus removal and *Typha domingensis* tolerance in a floating treatment wetland. Sci. Total Environ., 650, 233-240. https://doi.org/10.1016/j.scitotenv.2018.09.042

Dodgen, L.K., Ueda, A., Wu, X., Parker, D.R., Gan, J., 2015. Effect of transpiration on plant accumulation and translocation of PPCP/EDCs. Environ. Pollut., 198, 144–153. https://doi.org/10.1016/j.envpol.2015.01.002

Dordio, A.V., Belo, M., Teixeira, D.M., Carvalho, A.P., Dias, C.M.B., Picó, Y., Pinto, A.P., 2011. Evaluation of carbamazepine uptake and metabolization by *Typha spp*., a plant

with potential use in phytotreatment. Bioresour. Technol., 102, 7827-7834. https://doi.org/10.1016/j.biortech.2011.06.050

Doucette, W.J., Chard, J.K., Moore, B.J., Staudt, W.J., Headley, J.V., 2005. Uptake of sulfolane and diisopropanolamine (DIPA) by cattails (*Typha latifolia*). Microchem. J., 81, 41-49. https://doi.org/10.1016/j.microc.2005.01.015

Doucette, W.J., Shunthirasingham, C., Dettenmaier, E.M., Zaleski, R.T., Fantke, P., Arnot, J.A., 2018. A review of measured bioaccumulation data in terrestrial plants for organic chemicals: metrics, variability, and the need for standardized measurement protocols. Environ. Toxicol. Chem., 37, 21–33. https://doi.org/10.1002/etc.3992

Elliott, S.M., Brigham, M.E., Lee, K.E., Banda, J.A., Choy, S.J., Gefell, D.J., Minarik, T.A., Moore, J.M., Jorgenson, Z.G., 2017. Contaminants of emerging concern in tributaries to the Laurentian Great Lakes: I. Patterns of occurrence. PloS One 12, e0182868. https://doi.org/10.1371/journal.pone.0182868

Garver, E.G., Dubbe, D.R., Pratt, D.C., 1988. Seasonal patterns in accumulation and partitioning of biomass and macronutrients in *Typha spp*. Aquat. Bot., 32, 115-127. https://doi.org/10.1016/0304-3770(88)90092-7

Grace, J.B., Harrison, J.S., 1986. The biology of Canadian weeds.: 73. *Typha latifolia* L., *Typha angustifolia* L. and *Typha xglauc*a Godr. Can. J. Plant Sci., 6, 361-379. https://doi.org/10.4141/cjps86-051

Katahira, R., Elder, T.J., Beckham, G.T., 2018. A brief introduction to lignin structure, in: Beckham, G,T. (Ed.), Lignin valorization: Emerging approaches. Royal Society of Chemistry, pp. 1 – 21. https://doi.org/10.1039/9781788010351

Lee, S., Kang, S.I., Lim, J.L., Huh, Y.J., Kim, K.S., Cho, J., 2011. Evaluating controllability of pharmaceuticals and metabolites in biologically engineered processes, using corresponding octanol-water distribution coefficient. Ecol. Eng., 37, 1595–1600. https://doi.org/10.1016/j.ecoleng.2011.04.007

Ma, L.D., Li, J., Li, J.J., Liu, M., Yan, D.Z., Shi, W.Y., Xu, G., 2018. Occurrence and source analysis of selected antidepressants and their metabolites in municipal wastewater and receiving surface water. Environ. Sci. Process. Impacts, 20, 1020-1029. https://doi.org/10.1039/C8EM00077H

Millennium Ecosystem Assessment. Ecosystems and human well-being: synthesis (2005). Island Press, Washington, DC

Miller, E.L., Nason, S.L., Karthikeyan, K.G., Pedersen, J.A., 2016. Root uptake of pharmaceuticals and personal care product ingredients. Environ. Sci. Technol., 50, 525–541. https://doi.org/10.1021/acs.est.5b01546

Mitton, F.M., Miglioranza, K.S.B., Gonzalez, M., Shimabukuro, V.M., Monserrat, J.M., 2014. Assessment of tolerance and efficiency of crop species in the phytoremediation of DDT polluted soils. Ecol. Eng., 71, 501–508. https://doi.org/10.1016/j.ecoleng.2014.07.069

Pérez, D.J., Doucette, W.J., Moore, M.T., 2022a. Contaminants of emerging concern (CECs) in *Zea mays*: Uptake, translocation and distribution tissue patterns over the time and its relation with physicochemical properties and plant transpiration rate. Chemosphere, 288,132480. https://doi.org/10.1016/j.chemosphere.2021.132480

Pérez, D.J., Doucette, W.J., Moore, M.T., 2022b. Atrazine uptake, translocation, bioaccumulation and biodegradation in cattail (*Typha latifolia*) as a function of exposure time. Chemosphere, 287, 132104. https://doi.org/10.1016/j.chemosphere.2021.132104

Pérez, D.J., Menone, M.L., Doucette, W.J., 2013. Root-to-Shoot transfer and distribution of endosulfan in the wetland macrophyte *Bidens laevis* L. Environ. Toxicol. Chem., 32, 2478-2481. https://doi.org/10.1002/etc.2343

Pérez, D.J., Okada, E., Menone, M.L., Costa, J.L., 2017. Can an aquatic macrophyte bioaccumulate glyphosate? Development of a new method of glyphosate extraction in *Ludwigia peploides* and watershed scale validation. Chemosphere, 185, 975-982. https://doi.org/10.1016/j.chemosphere.2017.07.093

Pi, N., Ng, J.Z., Kelly, B.C., 2017. Bioaccumulation of pharmaceutically active compounds and endocrine disrupting chemicals in aquatic macrophytes: Results of hydroponic experiments with *Echinodorus horemanii* and *Eichhornia crassipes*. Sci. Total Environ., 601, 812-820. https://doi.org/10.1016/j.scitotenv.2017.05.137

Pintado-Herrera, M.G., González-Mazo, E., Lara-Martín, P.A., 2014. Determining the distribution of triclosan and methyl triclosan in estuarine settings. Chemosphere, 95, 478-485. https://doi.org/10.1016/j.chemosphere.2013.09.101

Redshaw, C.H., Wootton, V.G., Rowland, S.J., 2008. Uptake of the pharmaceutical fluoxetine hydrochloride from growth medium by Brassicaceae. Phytochemistry, 69, 2510-2516. https://doi.org/10.1016/j.phytochem.2008.06.018

Ribbenstedt, A., Mustajärvi, L., Breitholtz, M., Gorokhova, E., Mayer, P., Sobek, A., 2017. Passive dosing of triclosan in multigeneration tests with copepods–stable exposure concentrations and effects at the low μg/L range. Environ. Toxicol. Chem., 36, 1254-1260. https://doi.org/10.1002/etc.3649

Sesin, V., Davy, C.M., Freeland, J.R., 2021. Review of *Typha spp*. (cattails) as toxicity test species for the risk assessment of environmental contaminants on emergent macrophytes. Environ. Pollut., 284, 117105. https://doi.org/10.1016/j.envpol.2021.117105

Tete, V.S., Nyoni, H., Mamba, B.B., Msagati, T.A., 2020. Occurrence and spatial distribution of statins, fibrates and their metabolites in aquatic environments. Arab. J. Chem., 13, 4358-4373. https://doi.org/10.1016/j.arabjc.2019.08.003

Thomaz, S.M., 2021. Ecosystem services provided by freshwater macrophytes. Hydrobiologia, 1, 21. https://doi.org/10.1007/s10750-021-04739-y

Trapp, S., Shi, J., Zeng, L., 2023. Generic model for plant uptake of ionizable pharmaceuticals and personal care products. Environ. Toxicol. Chem., 00, 1–12 https://doi.org/10.1002/etc.5582

Travis, C.C., Arms, A.D., 1988. Bioconcentration of organics in beef, milk, and vegetation. Environ. Sci. Technol., 22, 271-274.

Turgut, C., 2005. Uptake and modeling of pesticides by roots and shoots of parrotfeather (*Myriophyllum aquaticum*). Environ. Sci. Pollut. Res., 12, 342-346. https://doi.org/10.1065/espr2005.05.256

United State Environmental Protection Agency, 2022. Contaminants of emerging concern including pharmaceuticals and personal care products. [cited 2022, April 10]. Available in: https://www.epa.gov/wqc/contaminants-emerging-concern-including-pharmaceuticals-and-personal-care-products

Valdés, M.E., Huerta, B., Wunderlin, D.A., Bistoni, M.A., Barceló, D., Rodriguez-Mozaz, S., 2016. Bioaccumulation and bioconcentration of carbamazepine and other pharmaceuticals in fish under field and controlled laboratory experiments. Evidences of carbamazepine metabolization by fish. Sci. Total. Environ., 557, 58-67. https://doi.org/10.1016/j.scitotenv.2016.03.045

Wang, F., Li, X., Yu, S., He, S., Cao, D., Yao, S., Fang, Y., Yu, Y., 2021. Chemical factors affecting uptake and translocation of six pesticides in soil by maize (*Zea mays* L.). J. Hazard. Mater., 405, 124269. https://doi.org/10.1016/j.jhazmat.2020.124269

Wilson, P.C., Whitwell, T., Klaine, S.J., 2001. Metalaxyl toxicity, uptake, and distribution in several ornamental plant species. J. Environ. Qual., 30, 411-417. https://doi.org/10.2134/jeq2001.302411x

Zarate Jr, F.M., Schulwitz, S.E., Stevens, K.J., Venables, B.J., 2012. Bioconcentration of triclosan, methyl-triclosan, and triclocarban in the plants and sediments of a constructed wetland. Chemosphere, 88, 323-329. https://doi.org/10.1016/j.chemosphere.2012.03.005

Zhang, D.Q., Gersberg, R.M., Hua, T., Zhu, J., Goyal, M.K., Ng, W.J., Tan, S.K., 2013a. Fate of pharmaceutical compounds in hydroponic mesocosms planted with *Scirpus validus*. Environ. Pollut., 181, 98-106. https://doi.org/10.1016/j.envpol.2013.06.016

Zhang, D.Q., Gersberg, R.M., Hua, T., Zhu, J., Goyal, M.K., Ng, W.J., Tan, S.K., 2013b. Fate of caffeine in mesocosms wetland planted with *Scirpus validus*. Chemosphere, 90, 1568–1572. http://dx.doi.org/10.1016/j.chemosphere.2012.09.059

Zhang, L., Lv, T., Zhang, Y., Stein, O.R., Arias, C.A., Brix, H., Carvalho, P.N., 2017. Effects of constructed wetland design on ibuprofen removal–A mesocosm scale study. Sci. Total Environ., 609, 38-45. https://doi.org/10.1016/j.scitotenv.2017.07.130

Zhang, Q., Kong, W., Wei, L., Hou, X., Ma, Q., Liu, Y., Luo, Y., Liao, C., Liu, J., Schnoor, J.L., Jiang, G., 2021. Compartmentalization and excretion of 2, 4, 6-tribromophenol sulfation and glycosylation conjugates in rice plants. Environ. Sci. Technol., 55, 2980-2990. https://dx.doi.org/10.1021/acs.est.0c07184

Zhang, Y., Lv, T., Carvalho, P.N., Arias, C.A., Chen, Z., Brix, H., 2016. Removal of the pharmaceuticals ibuprofen and iohexol by four wetland plant species in hydroponic culture: plant uptake and microbial degradation. Environ. Sci. Pollut. Res., 23, 2890-2898. https://doi.org/10.1007/s11356-015-5552-x

Zhang, Y., Lv, T., Carvalho, P.N., Zhang, L., Arias, C.A., Chen, Z., Brix, H., 2017. Ibuprofen and iohexol removal in saturated constructed wetland mesocosms. Ecol. Eng., 98, 394-402. https://doi.org/10.1016/j.ecoleng.2016.05.0775