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RESEARCH ARTICLE

Removal of enrofloxacin using *Eichhornia crassipes* in microcosm wetlands

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Abstract

The global consumption of antibiotics leads to their possible occurrence in the environment. In this context, nature-based solutions (NBS) can be used to sustainably manage and restore natural and modified ecosystems. In this work, we studied the efficiency of the NBS free-water surface wetlands (FWSWs) using *Eichhornia crassipes* in microcosm for enrofloxacin removal. We also explored the behavior of enrofloxacin in the system, its accumulation and distribution in plant tissues, the detoxification mechanisms, and the possible effects on plant growth. Enrofloxacin was initially taken up by *E. crassipes* (first 100 h). Notably, it accumulated in the sediment at the end of the experimental time. Removal rates above 94% were obtained in systems with sediment and sediment + *E. crassipes*. In addition, enrofloxacin was found in leaves, petioles, and roots (8.8–23.6 µg, 11–78.3 µg, and 10.2–70.7 µg, respectively). Furthermore, enrofloxacin, the main degradation product (ciprofloxacin), and other degradation products were quantified in the tissues and chlorosis was observed on days 5 and 9. Finally, the degradation products of enrofloxacin were analyzed, and four possible metabolic pathways of enrofloxacin in *E. crassipes* were described.

Keywords Emerging contaminants · Wetlands · Depuration · Macrophytes · Nature-based solutions · Enrofloxacin

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Introduction

The global extensive use of antibiotics is a cause for concern due to their numerous negative effects on the environment (Adachi et al. 2013; Riaz et al. 2018; de Ilurdoz et al. 2022; Manoharan et al. 2022). Antibiotics and their degradation products have been repeatedly found in water courses, such as rivers, lakes, and groundwater and, worryingly, in drinking water in populated areas (Bedoya-Ríos et al. 2018; Zhang et al. 2018a, 2019). These compounds occur through different pathways: excretion from animals and humans (since most of them are not fully metabolized in the body), the direct release from pharmaceutical manufacturing facilities, and/or hospital wastewater, among other effluents (de Ilurdoz et al. 2022).

Enrofloxacin (ENR) is a broad-spectrum antibiotic belonging to the family of fluoroquinolones (FQ); it inhibits the bacterial DNA gyrase activity (Van Bambeke et al. 2005) and is used to treat a wide range of bacterial infections (de Ilurdoz et al. 2022). In Argentina, ENR is widely used in veterinary medicine. In fact, it has been found in poultry litter (Teglia et al. 2017) and in wastewater and river water

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(Alcaraz et al. 2016; Teglia et al. 2019) at concentrations ranging from 0.81 to 1.73 μg g^{-1} and 0.53 to 11.9 μg L^{-1}, respectively. These previous studies reporting the prevalence of ENR in the Argentine environment suggest the possible presence of this compound in drinking water. ENR resists both biotic and abiotic degradation and has a long half-life in the environment (up to 72 days) (Knapp et al. 2005; Walters et al. 2010).

Ciprofloxacin (CIP) is the major active metabolite of ENR. CIP is obtained by deethylation of the ethyl on the ENR piperazine ring and keeps the antimicrobial effects of ENR (Trouchon and Lefebvre 2016). CIP can be naturally produced from ENR by aquatic microorganisms, animals and plants (Trouchon and Lefebvre 2016; Gomes et al. 2019). Moreover, because CIP has a strong bactericidal effect, it is often used to treat infections (Chen et al. 2020); hence, it is frequently detected in various watercourses (Alcaraz et al. 2016; Teglia et al. 2019; Kovalakova et al. 2020).

Drinking water treatment system do not include the removal of antibiotics and many other contaminants of emerging concern (ADX et al. 2020, de Ilurdoz et al. 2022; Lu et al. 2022). In this scenario, nature-based systems (NBS) appear as a sustainable and cost-effective strategy to reduce the impacts of emerging contaminants on the environment (Biswal et al. 2022). Moreover, constructed wetlands (CWs) are NBS that could be used for the bioremediation of wastewaters contaminated with emerging pollutants. Although CWs have been shown to be efficient in removing antibiotics, the processes involved in antibiotic removal are poorly understood (Choi et al. 2016; Zhang et al. 2018b; Chen et al. 2019; Liu et al. 2019; Li et al. 2021; Du et al. 2022). While it is known that antibiotics can accumulate in the sediment and plants, further studies should evaluate bioaccumulation in vegetation to elucidate the full cycling of these contaminants in CWs (Gomes et al. 2019; Maldonado et al. 2022). In this sense, the ability of some plants to bioaccumulate FQs in roots and distribute these analytes to other organs has been previously demonstrated (Migliore et al. 2003; Zhao et al. 2019).

The free-floating macrophyte *Eichhornia crassipes* is well adapted to diverse environmental conditions. This species has been frequently used in phytoremediation studies, such as studies on the removal of triazine (Wang et al. 2021) and metals (Maine et al. 2017; Barya et al. 2022; Gomes et al. 2022; Sayago and Castro 2022) from wastewaters or drinking water.

In this work, the efficiency of free-water surface wetlands (FWSWs) for the removal of ENR using *E. crassipes* in microcosms was evaluated. We also investigated the behavior of ENR in the system and described its accumulation and distribution in the plant, its detoxification mechanisms, and potential effects of the emergent contaminant on plant growth.

Material and methods

Experimental design

The experiment was conducted in the facilities of the Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santa Fe, Argentina, in November 2021. Healthy plants of *E. crassipes* were collected from an uncontaminated pond in the floodplain of the Paraná River in Argentina. The collected plants were washed and acclimated in plastic reactors. The reactors were randomly placed in a greenhouse under a naturally lit semi-transparent plastic roof; plants of similar size and weight were selected for the experiment. The reactors consisted of 12-L plastic containing 5 L of dechlorinated tap water, ENR (1.00 mg L^{-1}), sediment (2 kg), and/or fresh plant material (100 g) in the combinations described below. Water pH and conductivity were 7.9 and 256 μS cm^{-1}, respectively, and mean temperature ranged from 24 to 28 °C. The systems and controls were as follows (see Fig. 1):

Water and plant sampling

Water samples (1.00 mL) were collected from each experimental plastic reactor at 0, 2, 8, 24, 48, 72, 96, 120, 144, 168, 192, 216, 264, 288, and 360 h. At the end of the experiment, the total plant biomass was collected; separated into roots, petioles, and leaves; and dried at 60 °C to constant weight. Subsequently, the dried biomass was used for ENR and degradation products quantitation in each tissue.

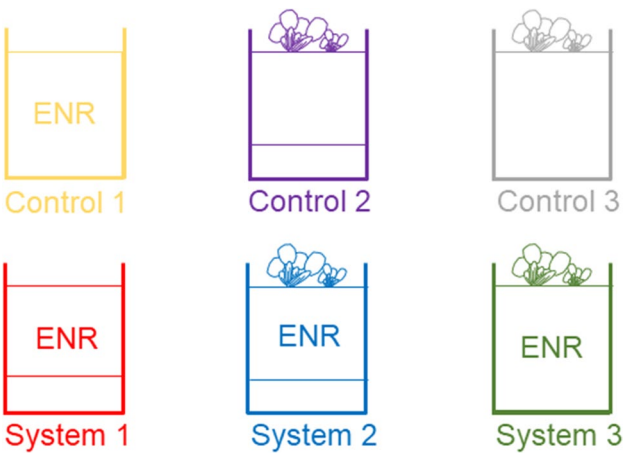


Fig. 1 Experimental design scheme. Control 1: 1.00 mg L^{-1} of enrofloxacin in 5 L of water; control 2: 100 g of plants and 2 kg of sediment in 5 L of water; control 3: 100 g of plants in 5 L of water; system 1: 1.00 mg L^{-1} of enrofloxacin and 2 kg of sediment in 5 L of water; system 2: 1.00 mg L^{-1} of enrofloxacin, 100 g of plants and 2 kg of sediment in 5 L of water; and system 3: 1.00 mg L^{-1} of enrofloxacin and 100 g of plants in 5 L of water

Chemicals and reagents

ENR and CIP were purchased from Fluka (Buchs, Switzerland). Acetonitrile (ACN), methanol (MeOH), and formic acid of HPLC grade were purchased from Merck (Darmstadt, Germany). Milli-Q water was purchased from a Millipore system (Bedford, MA, USA). Sodium acetate trihydrate (NaAc) and glacial acetic acid (HAc) were acquired from Anedra (La Plata, Argentina).

An acetic acid-acetate buffer solution (0.02 mol L^{-1} pH 4.00) was prepared by mixing a mass of NaAc and a volume of commercial glacial HAc in Milli Q water.

Stock solutions were prepared using 10.0 mg of ENR or CIP standard and dissolving it in 10.00 mL of MeOH to give a concentration of 1.00 mg mL^{-1}. These solutions were stored in light-resistant containers at 4 °C; before use, they were allowed to reach room temperature. The working standard solutions were prepared by diluting the stock solutions in the previously described buffer solution. According to the experimental design, an appropriate volume of the ENR stock solution was added to 5 L dechlorinated tap water in some reactors to reach the final concentration of 1.00 mg L^{-1}.

Equipment and software

Chromatographic separations were performed in an Agilent 1260 Infinity Ultra HPLC (UHPLC) system (Waldbronn, Germany). The mobile phase consisted of a mixture of the acetic acid-acetate buffer solution and ACN (85:15), in isocratic mode at a flow rate of 1.50 mL min^{-1} and 45 °C. Chromatographic data were recorded at 450 nm (emission wavelength), using 280 nm as excitation wavelength.

A time-of-flight (TOF) mass spectrometer (Agilent MSD TOF 6230, Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray (ESI) interface operating in positive ion mode was used for the degradation product analysis. The MS operating parameters used for the determination of the mass were capillary voltage, 4000 V; nebulizer pressure, 40 psi; drying gas, 9 L min^{-1}; gas temperature, 300 °C; skimmer voltage, 60 V; octapole DC 1, 37.5 V; octapole RF, 250 V. Mass spectra were recorded across the range m/z 50–1000 with accurate measurement of all peaks. Accurate mass measurements of ions corresponding to each peak from the total ion chromatograms were taken using an automated calibrant system that provides mass correction. The instrument performed the internal mass calibration automatically using a dual-atomizer ESI source and an automatic calibration system that introduced flow from the output of the LC system along with a small flow of calibration solution containing the compounds that provided the internal reference ions.

The structure of the ENR degradation products was analyzed in isocratic mode at a flow rate 0.40 mL min^{-1} and 40 °C. The mobile phase consisted of a mixture of 0.5% formic acid and ACN (75:25).

In both cases, the chromatographic separation was performed in a Zorbax Eclipse XDB-C18 column (4.6 × 75 mm, 3.5 µm particle size; Agilent Technologies).

Determination of ENR and degradation products

The presence of ENR, CIP, and/or their degradation products in water and tissues was investigated using UHPLC-FSFD and LC-TOF. Mass spectrometric analysis was performed to determine the structure of the degradation products.

The dried tissues and sediment were processed as follows: 1.00 mL of MeOH was added to 100.0 mg of sample; then, the solution was vortexed for 1 min, sonicated for 15 min, and centrifuged at 3000 rpm for 10 min. The supernatant was collected in a glass tube, and then, the extraction was repeated. The supernatant was dried under a stream of nitrogen. Then, 0.5 mL of buffer solution was added to the pellet, vortexed for 1 min, sonicated for 15 min, and centrifuged at 3000 rpm for 10 min. This solution was used to resuspend the extract obtained after evaporation of the supernatant. Finally, these solutions were filtered through 0.22-µm nylon filters and injected into the chromatographic systems.

To determine the structure of the degradation products, two websites were used: https://xenosite.org/ and https://smartcyp.sund.ku.dk/mol_to_som. The procedure for the analysis is as follows: the EAWAG-BBD Pathway Prediction System predicts microbial degradation reactions using a substructure search, rules database, and atom-to-atom mapping. Secondly, XenoSite (Zaretzki et al. 2013), a tool for predicting the atomic sites at which xenobiotics are metabolically modified by cytochrome P450 enzymes, combines the calculation of multiple quantitative descriptions of molecules, including topological and quantum chemical descriptions, as well as robust atomic site reactivity descriptions generated by the SmartCyp software (Rydberg et al. 2010).

Analytical methods for the determination of ENR and CIP

A validation step was performed in which the parameters linearity, limits, repeatability, and recovery were defined according to the IUPAC and EMEA guidelines (Danzer and Currie 1998; EMEA 2012).

To study the linearity, a calibration set of eight standard solutions was prepared ($n = 3$) by transferring appropriate aliquots of ENR stock solution to 5.00-mL volumetric flasks to the mark with water to obtain concentrations ranging from 0.048 to 0.969 µg mL^{-1}. For CIP, five standard solutions were prepared ($n = 3$) by transferring appropriate aliquots of

the stock of CIP to 5.00 mL volumetric flasks and completing to the mark with water to obtain concentrations ranging from 0.050 to 0.900 µg mL^{-1}. The quality of the curve was analyzed by taking into account the value of the linear regression (R^2) and by comparing the variance of the lack of fit with the pure error variance as recommended by González and Herrador (2007). Moreover, to verify the goodness of the calibration, the analytical figures of merit (AFOM) were calculated following the recommendations of Gegenschatz et al. (2021), i.e., sensitivity, analytical sensitivity, and limits of detection (LOD) and quantification (LOQ).

Precision and recovery tests for ENR were performed by triplicate analysis of blank samples of leaves and roots spiked at three concentrations (0.145, 0.303, and 0.605 µg mL^{-1}). Then, the samples were processed as described in "Determination of ENR and degradation products."

Statistical analysis

Data were analyzed using a *t*-test at a 5% level of probability. The results are presented as the average of the replicates. The statistical analysis was performed using the Origin® 6.1 (Northampton, USA) and Minitab® 16.2.4 (Pennsylvania, USA) software.

Results and discussion

Analytical performance

Assessment of linearity and calculation of LOD and LOQ (Table SI1) showed a LOD of 0.03 µg mL^{-1} and a LOQ of 0.09 µg mL^{-1} for ENR and CIP, respectively. Moreover, the calibration model was considered suitable due to the F_{exp} was lower than the one-tailed tabulated value of 2.40 (see Table SM1). The result of the precision and recovery tests demonstrate the good performance of the method (see Table SI2).

Water removal efficiency

Figure 2 shows the ENR removal rate over the experimental period. No significant variation in ENR concentration was observed in control 1, showing that there was no loss or conversion of ENR by light degradation during the experimental period. This result is consistent with the behavior described by Knapp (2005) and Walters et al. (2010).

In contrast, the removal rate observed for the other systems showed that the addition of sediment, plants, or both had a positive effect on the removal of ENR from the water. To go further into the analysis, Boltzmann modelling (Table 1) was performed using the following equation (Aguiar et al. 2003):

$$y = A_2 + \frac{A_1 - A_2}{1 + e^{\frac{x - x_0}{dx}}} \quad (1)$$

where x is the independent variable, A_1 and A_2 are the upper and lower limits of the sigmoid, respectively, x_0 is the midpoint of the sigmoid, and dx is directly related to the range of the independent variable within which the abrupt change in the dependent variable occurs. Thus, the behavior of each studied was defined.

The results show that the three systems were able to remove ENR (Fig. 2). Systems 2 and 3 (systems containing plants) exhibited the highest removal rates in the first 100 h. The lack of significant differences between the removal rates of these two systems (Table 1) suggests that *E. crassipes* has the ability to accumulate ENR over a short period at high removal rates. Furthermore, the differences in the removal rates in the first 100 h between system 1 (sediment only) and system 2 (sediment + plants) confirm the capacity of *E. crassipes* to uptake ENR (Fig. 2). The lack of significant differences (p-value = 0.217) between the presence and absence of sediment (system 2 *vs.* system 3) demonstrates the important role of *E. crassipes* in the rapid and efficient removal of ENR.

At the end of the experiment, the removal efficiency showed significant differences (p-value = 0.021) between system 1 (using only sediment) and system 2 (sediment + plants), indicating that removal by the sediment is

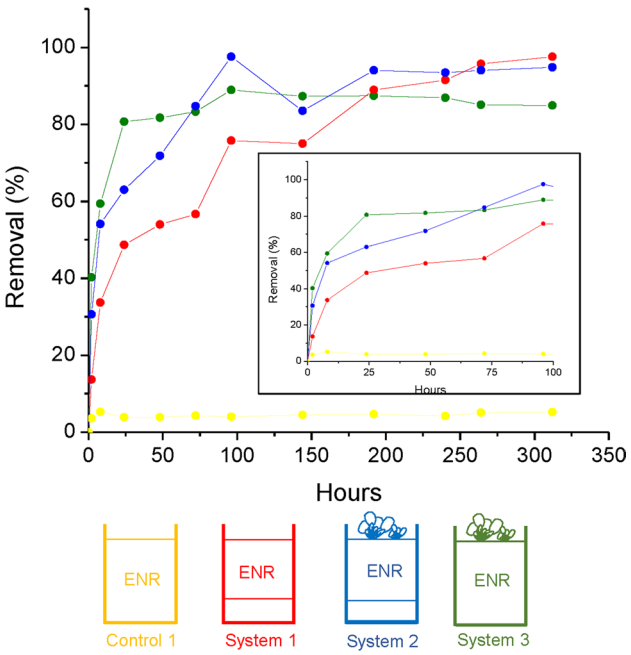


Fig. 2 Comparison of enrofloxacin removal rate between control 1 (yellow dots), system 1 (red dots), system 2 (blue dots), and system 3 (green dots) over the experimental period. The insert shows the first 100 h

Table 1 Boltzmann analysis of the removal rate and *t*-test for the comparison among systems

Complete experimental period						
System	Chi^2	A_1	A_2	x_0	dx	s (dx)
1	90.5	−8590.7	93.0	−316.3	67.5	0.825
2	107.0	−2725.8	87.8	−56.7	15.8	0.188
3	40.9	−6111.0	85.0	−23.5	5.4	0.446
Comparison		*t*-value		*p*-value[a]		
System 1 vs 2		86.5		0.007		
System 1 vs 3		93.7		0.007		
System 2 vs 3		30.3		0.021		
First 100 h						
System	Chi^2	A_1	A_2	x_0	dx	s (dx)
1	103.1	−2.894.6	62.9	−56.1	14.4	0.10
2	138.9	−5037.8	77.8	−29.2	6.89	0.80
3	72.2	−12481.8	83.1	−25.4	5.01	0.50
Comparison		*t*-value		*p*-value[a]		
System 1 vs 2		13.1		0.048		
System 1 vs 3		26.0		0.024		
System 2 vs 3		2.82		0.217		

[a]*p*-value lower than 0.05 shows statistically significant differences between systems

slower than by plants. System 1, for its part, had the highest average percentage of removal (97.6%). However, the presence of *E. crassipes* accelerated ENR removal. This result suggest that free-floating macrophytes accumulate pollutants in their tissues and their dense root system supports the microbial film. Sun and Zheng (2022) reported an ENR removal of 98.40% in a constructed wetland with vertical flow. These authors concluded that microbial degradation and sorption play a major and minor role, respectively, in the removal of FQs in that system. Moreover, Gorito et al. (2018) obtained a removal rate close to 100% of organic micropollutants in a microcosm system simulating a vertical subsurface flow CW planted with *Phragmites australis*. In addition, Santos et al. (2019) reported 85% ENR removal in a microcosm-scale CW planted with *P. australis*. Our results are consistent with those found in the bibliography and show that the use of *E. crassipes* had a positive effect during the removal process.

Plant tolerance

In order to determine the influence of ENR on the development of *E. crassipes*, a photo analysis was carried out during the experimental period to assess possible macroscopic changes in the plants (Fig. 3). While plant growth was normal in controls 2 and 3, chlorosis was observed in systems 2 and 3, on days 9 and 5, respectively.

To analyze the effects of ENR on the growth of *E. crassipes*, dry biomass was compared between the systems and the corresponding controls (see Table 2). In controls 2 and 3, the proportions of roots, petioles, and leaves was the same: 29, 47, and 24%, respectively. In systems 2 and 3, the proportions were different, and chlorosis was observed in both leaves and petioles (Fig. SI1).

Plant growth in the systems without sediment (control 3 and system 3) was significantly lower than in the systems with sediment, probably because the plants uptake nutrients from the sediment. Plant growth was significantly higher in the systems with ENR than in the corresponding controls (Table 2).

Chlorotic leaves were observed in systems 2 and 3 (Fig. 3; Table 2). The absence of pigmentation (completely white tissues) in above-ground tissues may be attributed to chlorosis associated with the high availability of ENR. Chlorotic leaves were detected on day 5 in system 3 and on day 9 in system 2. In system 2, the presence of sediments may have delayed chlorosis.

In summary, ENR increased the total biomass of *E. crassipes* and resulted in chlorosis (Fig. SI1).

As described by Maldonado et al. (2022), exposure of plants to antibiotics usually has negative effects, such as changes in the production of reactive oxygen species (ROS) and in the integrity of photosystem II; these changes affect the production of chlorophyll, which in turn affects the complexes II, III, and IV of mitochondria. On the other hand, several authors described hormesis (growth enhancement), a response of different plant species exposed to ENR. Migliore et al. (2003) described that the alteration of the studied species (*Cucumis sativus, Lactuca sativa*, *Phaseolus vulgaris*, and *Raphanus sativus*) could be due to the effect of ENR on DNA-topoisomerase II, an enzyme involved in the eukaryotic DNA duplication.

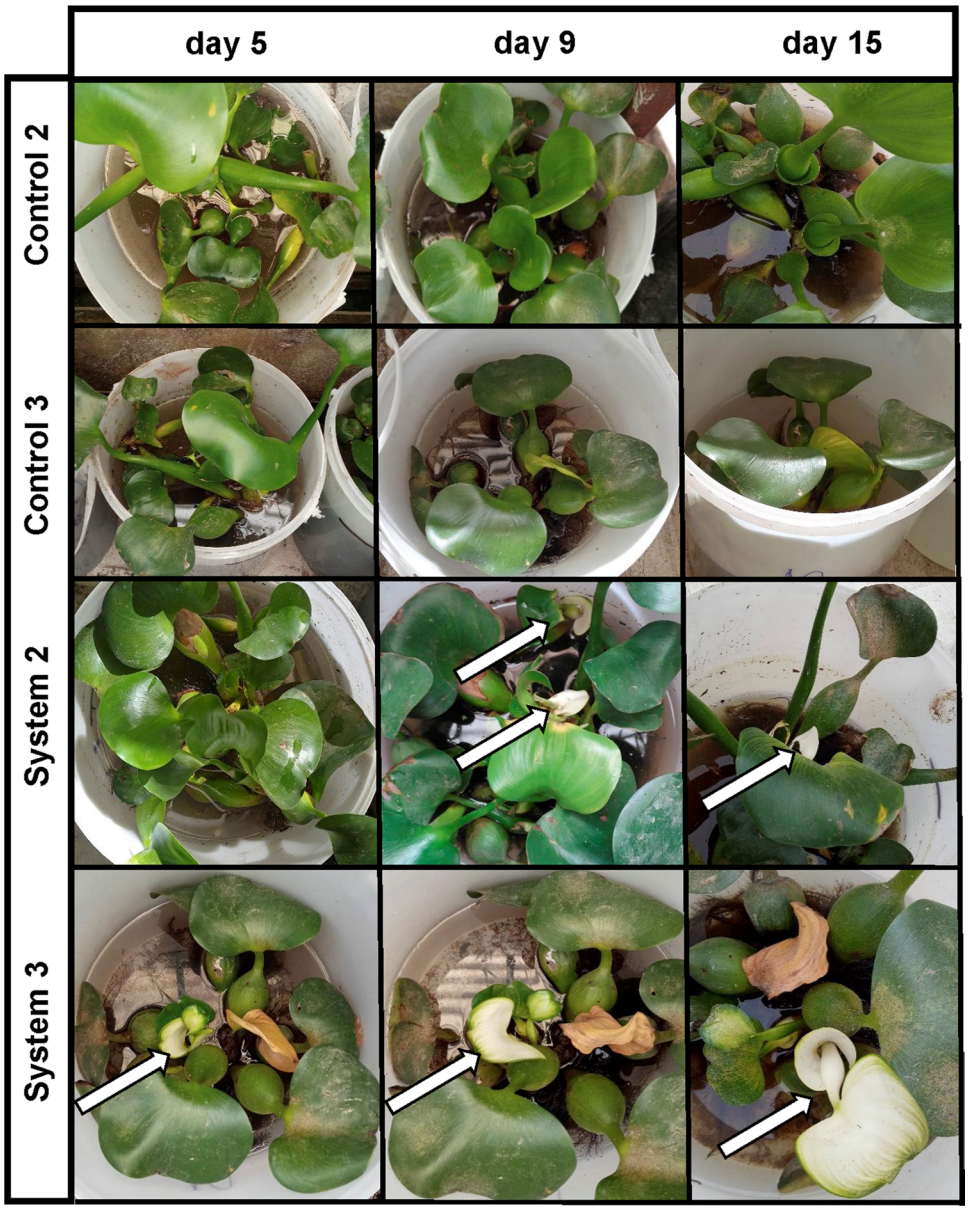


Fig. 3 Photographic analysis. The first signs of chlorosis are observed on day 5 in system 3 and on day 9 in system 2. The arrow indicates the parts where chlorosis occur

Table 2 Total biomass and percentage of each tissue in the *E. crassipes* plant at the end of the experimental period

	Total biomass (g)	Percentages					*p*-value[a]
		Root	Leaf		Petiole		
			Healthy	Chlorotic	Healthy	Chlorotic	
Control 2	6.035	29.0	24.3		46.7		
System 2	7.871	36.6	26.0	0.7	33.1	3.5	0.026
Control 3	3.914	29.0	26.0		46.7		
System 3	5.089	44.4	22.5	2.0	29.0	2.1	0.028

[a] *p*-value lower than 0.05 shows statistically significant differences between systems

Similarly, changes of respiratory and photosynthesis pathways by ENR resulted in hormesis and toxic effects on *Medicago sativa* (Vilca et al. 2022). In addition, Ramdat et al. (2022) found an increase in total mass at the end of an experiment involving floating treatment wetlands planted with *Iris pseudacorus*.

On the other hand, chlorosis was described in the macroalga *Ulva rigida* after 96 h of ENR exposure (Rosa

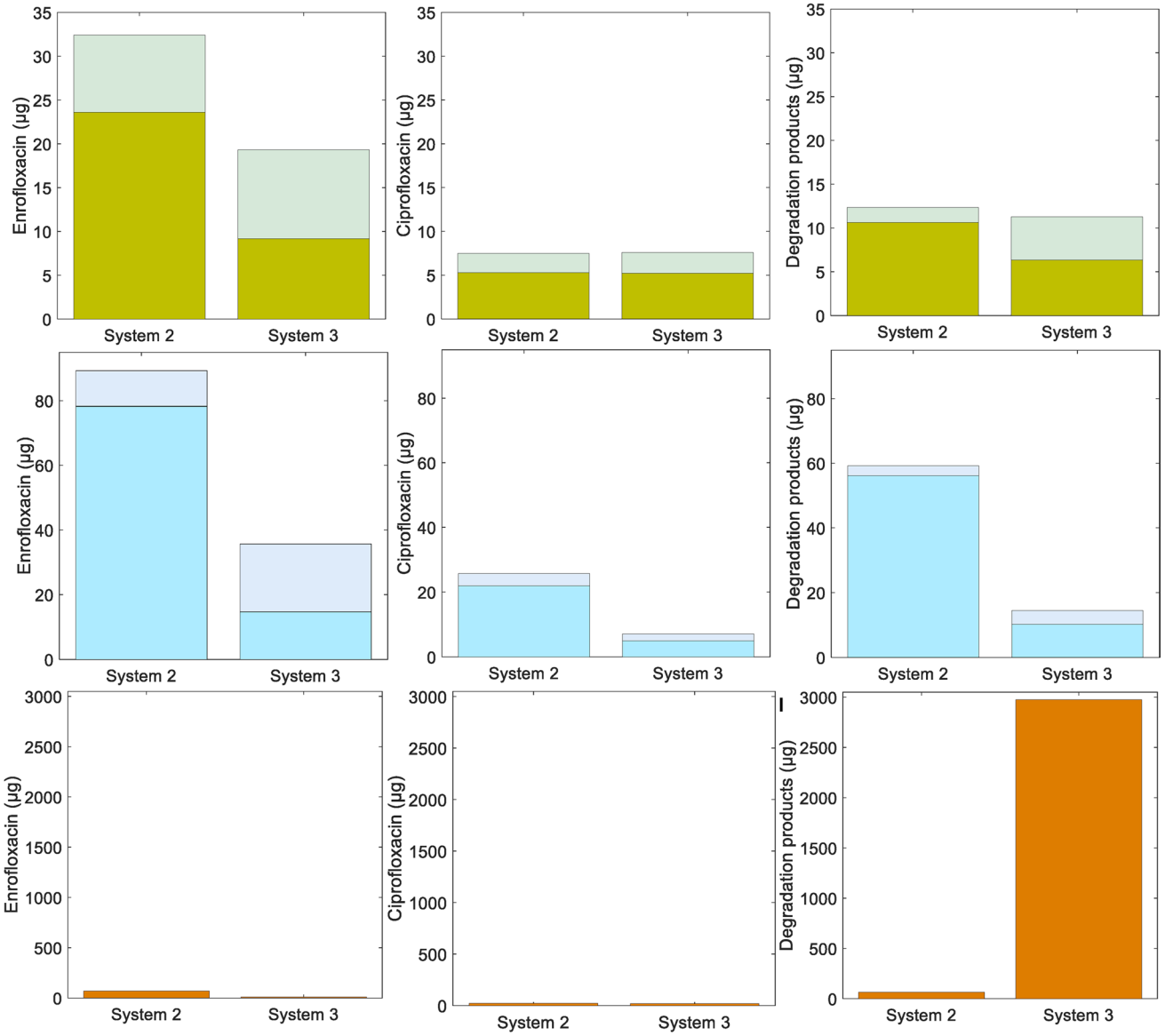


Fig. 4 Enrofloxacin, ciprofloxacin, and degradation products quantified in tissues of *E. crassipes*: leaves (green), petioles (blue), and roots (brown). Within each tissue, the light color corresponds to the values found in the chlorotic part

et al. 2020), ultimately leading to plant death. Similarly, a decrease in the photosynthetic pigments was observed in the alga *Scenedesmus obliquus* (Qin et al. 2012); the authors proposed chlorophyll concentration as an excellent biomarker to analyze the presence of ENR in aquatic systems. They concluded that ENR had toxic effects on *S. obliquus*, mainly regulated by the generation of ROS, causing lipid peroxidation of membranes and other damages to biological macromolecules, eventually leading to cell death. In addition, plants of *Juncus* spp. and *Salicornia europea* exposed to ENR and other pharmaceutical compounds appeared slightly yellow and greyish, respectively (Barreales-Suárez et al. 2021). Similarly, CIP was found to reduce the content of pigments such as chlorophyll *a* and *b*, total chlorophyll, and carotenoids in *Lemma minor* and *L. gibba*, affecting photosynthesis and leading to chlorosis (Nunes et al. 2019). In addition, water lettuce (*Pistia stratiotes*) was found to develop chlorosis and necrosis at high CIP concentrations (higher than 10 mg L^{-1}), probably because plants become toxic from the absorbed fluorine (Masiyambiri et al. 2023). As Masiyambiri et al. (2023) described, since fluorine is present in the structure of ENR and CIP, the plants sensitive to fluorine are susceptible to injury and chlorosis.

In agreement with the results found in the bibliography, plant uptake of ENR during the experimental period had a negative effect on the metabolic process, due to the presence

of chlorotic leaves on different days, due to a possible effect of ENR in the chlorophyll pathway. Moreover, further studies will be conducted by our research group to determine if respiratory and photosynthetic pathways, levels of ROS, and associated enzymes are altered in *E. crassipes* exposed to ENR.

Presence of ENR and degradation products in tissues

To test the ability of *E. crassipes* to bioaccumulate ENR, the plants were processed as described in "Material and methods" and the determination of ENR and their degradation products was carried out.

In this sense, evidence suggests that in the first step of ENR metabolism in plants, cytochrome P450 plays a primary role in detoxification by converting ENR to CIP (Gomes et al. 2019). For this reason, a calibration curve for CIP was established during the analysis to obtain the final concentration of the main degradation product of ENR (i.e., CIP) after root uptake from the water used for experimental and plant metabolism. During the analysis of the samples, other degradation products were found that could be identified and whose description will be discussed later.

Table 3 ENR intermediates identified in the samples using LC-TOF

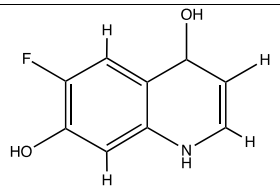
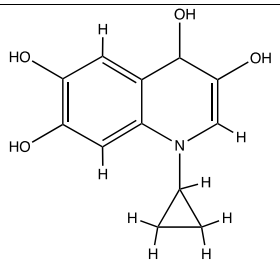
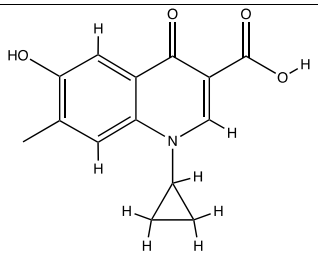
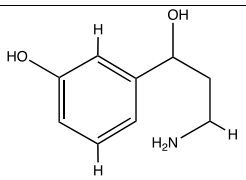
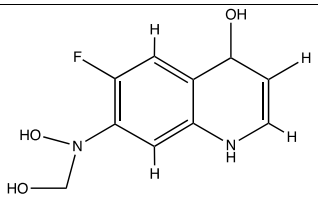
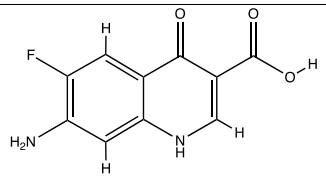
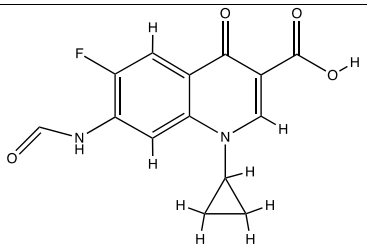
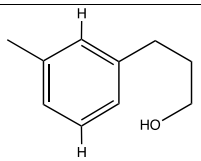
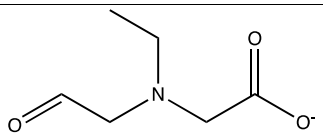
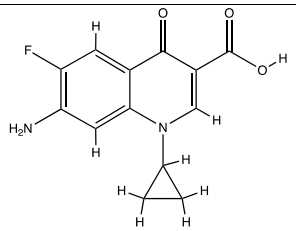
Retention time	Measured mass (m/z)	Height	%Vol	Exact mass $[M+H]^+$ (m/z)	Structure	Ref.
3.19	182.0897	9883	0.11	182.0617		P4 in Zhao et al. (2021)
3.196	236.1494	13570	0.17	236.0922		P2 in Zhao et al. (2021) and E236 in online webservers
3.24	260.1444	5839	0.07	260.0922		P1 in Zhao et al. (2021)
3.362	168.058	8907	0.11	168.1024		P6 in Zhao et al. (2021)
4.303	227.1513	5606	0.05	227.0831		P3 in Zhao et al. (2021)

Table 3 (continued)

7.407	223.1086	19007	0.28	223.0518		
7.714	291.0439	13201	0.13	291.0781		DP290 in Lu et al. (2022) and E291 in online webservers
7.837	150.0946	7762	0.11	150.1044		P7 in Zhao et al. (2021)
8.805	145.0425	34531	0.53	145.0738		
8.853	263.0582	437657	13.14	263.0831		DP262 in Lu et al. (2022) and E263 in online webservers

% Vol volume percentageBased on the available literature, chemical libraries, and in silico metabolic prediction tools, four potential metabolic pathways of ENR can be proposed (Fig. 5)

The levels of ENR, CIP, and degradation products determined in the different plant tissues are listed in Table SI3 and shown in Fig. 4.

ENR was poorly degraded in leaves and petioles of systems 2 and 3. Contrarily, degradation products were observed in the roots of system 3, which only included the presence of plants. In the case of system 2, the sediment decreased the effect of enrofloxacin in plants. According to Maldonado et al. (2022), the degradation capacity of plants is mediated by a variety of mechanisms that occur in roots directly exposed to the experimental solution. Therefore, we hypothesize that ENR would be taken by the roots and then converted to other compounds, possibly as a mechanism to reduce its toxicity. Furthermore, as plants come into contact with a high concentration of ENR, they transport this compound to the aerial parts. This high ENR concentration negatively affected photosynthesis and contributed to the chlorosis, i.e., the absence of chlorophyll, observed in petioles and leaves.

Figure SI2 shows the chromatographic profile of the standard solution of ENR, CIP, and the profile of a root sample from system 3, in which the presence of a predominant degradation product was observed. In addition to CIP, others eight degradation products were detected by mass spectrometry (Table 3).

In the first route (R1 in Fig. 5), P1 was produced by C–N bond cleavage induced by hydroxylation and methylation, which was further hydroxylated to P2. In route 2 (R2 in Fig. 5), ENR was cleaved by ring cleavage, hydroxylation, and decarboxylation to form P3, which was further hydroxylated to form P4. In both routes, both P2 and P4 compounds can be dehydroxylated and methylated to generate an

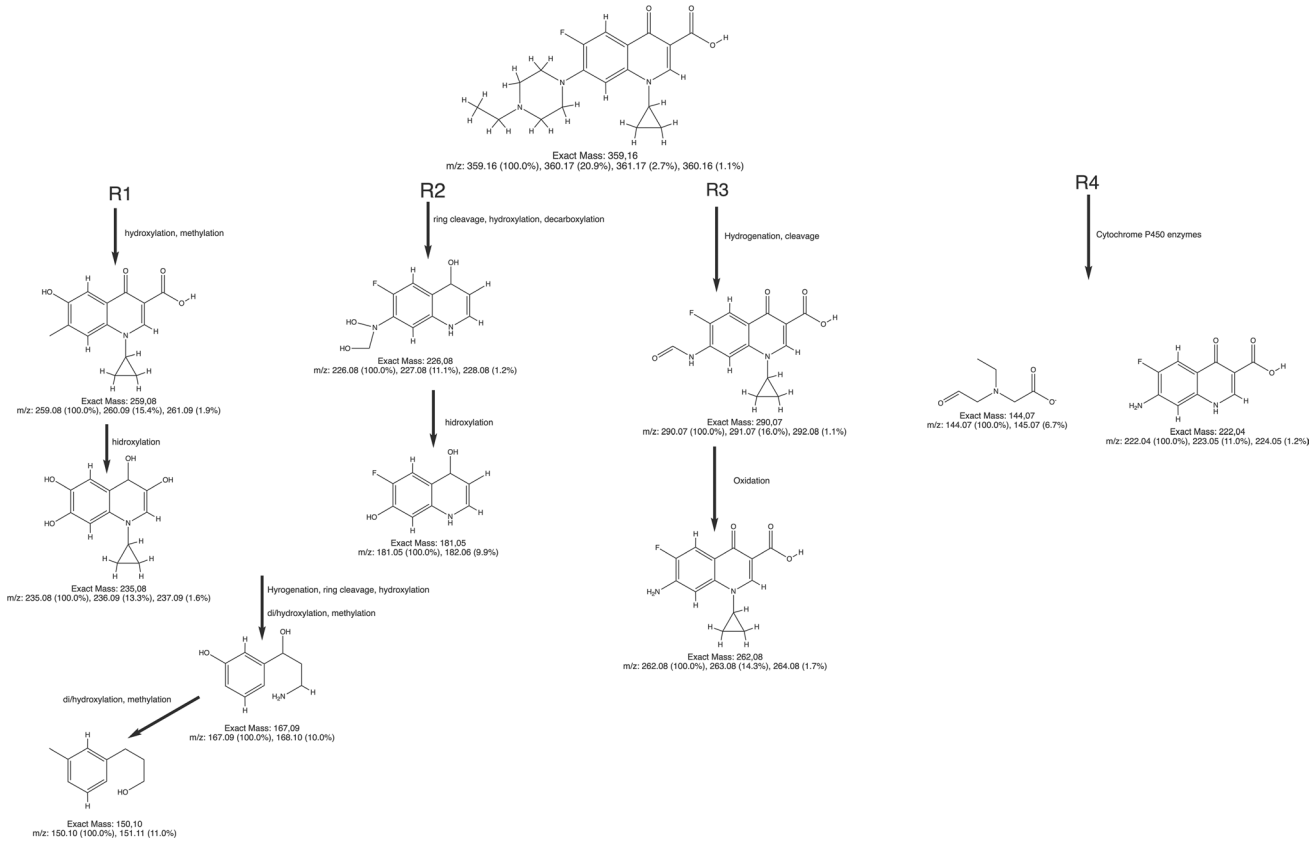


Fig. 5 Degradation pathways for enrofloxacin proposed according to the LC-TOF analysis

intermediate compound. This phenomenon, which was not observed in this study, was described by Zhao et al. (2021) for the metabolic pathway of ENR in *Lolium perenne*. This intermediate compound is rapidly hydrogenated and hydroxylated to induce ring cleavage, producing P6. Finally, P7 was formed by di/hydroxylation and methylation of P6. All these six compounds are consistent with the compounds proposed by Zhao et al. (2021).

A third pathway (R3 in Fig. 5) proposed here is consistent with that reported by Lu et al. (2022). This is a photocatalytic pathway, and both end products, DP290 and DP262, were found in the present work. These intermediates were not found in the mass spectrometry analysis, but it is known that the hydrogenation and cleavage process is rapid in a photocatalytic route.

Finally, a series of masses corresponding to degradation compounds were detected using two online webservers, revealing another metabolic route (R4) for the degradation of ENR in plant. The following compounds were identified: E144, E291 (equal to DP290), E263 (equal to DP262), E236 (equal to P2), and E222. Compound E263 (or DP262) is the one with the highest volume fraction (% Vol); this result is reasonable because E263 is the end product of the R3 metabolic pathway.

After analysis, our result showed that *E. crassipes* has five forms to metabolize ENR: (1) conversion to CIP; (2) R1, with the final conversion to P2; (3) R2, with the final conversion to P7; (4) R3, with the final conversion to DP262; and (5) R4. Comparison with the bibliography shows the agreement of Gomes et al. (2019) with pathway (1), Zhao et al. (2021) with pathway (2), Zhao et al. (2021) with pathway (3) and Lu et al. (2022) with pathway (4), but in this work, pathway (5) appears as a new pathway to metabolize ENR by plants. These results show that *E. crassipes* is able to take up ENR and that the posterior forms metabolize the compound as it´ interferes with normal plant development and causes chlorosis.

Conclusions

In the present work, the ability of *E. crassipes* to accumulate ENR was demonstrated. As a result, the plant achieved removal rates above 80% in the first 100 h in systems with

and without sediment. In this sense, ENR was first taken up by *E. crassipes* during the first 100 h and then accumulated in the sediment. Remarkably, removal rates higher than 94% were achieved in systems with sediment and sediment + *E. crassipes*. Furthermore, the presence of sediment not only favored the growth of *E. crassipes* but also delayed the onset of chlorosis because ENR accumulated in the sediment and in the plant.

In addition, the presence of ENR in the different tissues of *E. crassipes* indicated the movement of the compound within the plant. In the other hand, it can be inferred that the presence of degradation products indicates the defence mechanisms employed by the plant to eliminate the ENR that enters the tissues. Finally, five possible metabolic pathways of ENR are proposed, i.e., the conversion of ENR to CIP and four pathways involving other degradation products.

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Declarations

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Consent for publication Not applicable.

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