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RESEARCH ARTICLE

Physiological responses and phytoremediation capacity of floating and submerged aquatic macrophytes exposed to ciprofloxacin

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Abstract

Ciprofloxacin (Cipro) water contamination is a global concern, having reached disturbing concentrations and threatening the aquatic ecosystems. We investigated the physiological responses and Cipro-phytoremediation capacity of one floating (*Salvinia molesta* D.S. Mitchell) and one submerged (*Egeria densa* Planch.) species of aquatic macrophytes. The plants were exposed to increased concentrations of Cipro (0, 1, 10, and 100 µg.Cipro.L^{-1}) in artificially contaminated water for 96 and 168 h. Although the antibiotic affected the activities of mitochondrial electron transport chain enzymes, the resulting increases in H_2O_2 concentrations were not associated with oxidative damage or growth reductions, mainly due to the activation of antioxidant systems for both species. In addition to being tolerant to Cipro, after only 96 h, plants were able to reclaim more than 58% of that from the media. The phytoremediation capacity did not differ between the species, however, while *S. molesta* bioaccumulate, *E. densa* appears to metabolize Cipro in their tissues. Both macrophytes are indicated for Cipro-phytoremediation projects.

Keywords Bioremediation · Nature-based solution · Pharmaceuticals · Freshwater toxicology · Antibiotics

Introduction

One of the pharmaceutical classes that has stood out in recent years in ecotoxicological studies are antibiotics widely used in human and veterinary medicine (de Assis 2021; Kelly and Brooks 2018; Rocha et al. 2021a). Those drugs have greatly contributed to the economic growth of sectors such as agriculture, aquaculture, apiculture, and livestock husbandry, where they are used as growth promoters and to combat diseases (Kelly and Brooks 2018; Liu et al. 2018; Rocha et al. 2021a). The uncontrolled use of those antibiotics and the lack of appropriate waste treatments, however, have contributed to their being increasingly found in bodies of water (Gothwal and Shashidhar 2015; Liu et al. 2018; Kelly and Brooks 2018), where they contribute to one of the most serious problems of twenty-first century—the selection for (and spread of) antibiotic-resistant microorganisms (Kelly and Brooks 2018).

Among antibiotics, particular attention must be given to the fluoroquinolone class. Treated animals and humans do not usually completely metabolize those drugs (Kelly and Brooks 2018), and they are consequently released into the environment, where they can induce bacterial resistance and can cause negative impacts to non-target species (Kelly and Brooks 2018). The presence of fluoride in their chemical composition makes fluoroquinolones stable and persistent in the environment, stimulating a growing interest in their ecotoxicological impacts (Janecko et al. 2016; Riaz et al. 2017). Among the antibiotics belonging to this

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class of drugs, ciprofloxacin (Cipro) has gained prominent use (Kelly and Brooks 2018; Kovalakova et al. 2020), and has been identified in bodies of water in different regions around the world, but mainly in the developed countries of Europe, Asia, South America, North America, and in Australia, at concentrations varying from 18 ng.L^{-1} to 8 mg.L^{-1} (Frade et al. 2014; Janecko et al. 2016; Quadra et al. 2017; Riaz et al. 2017; Gomes et al. 2022a). Cipro has been detected in rivers in India at concentrations varying from 1 to 10 µg.L^{-1} (Mutiyar and Mittal 2014), in hospital wastewater at concentrations varying from 1100 to 44000 ng.L^{-1} in Vietnam (Duong et al. 2008), and 388 to 578 ng.L^{-1} in Malaysia (Thai et al. 2018). It was detected in Pakistan at concentrations from 0.35 to 2.210 µg.L^{-1} in residual waters, and from 83 to 341 µg.L^{-1} in effluents of pharmaceutical industries (Riaz et al. 2017). Cipro concentrations up to 15000 ng.L^{-1} were found in surface waters in South Africa (Agunbiade and Moodley 2014), and at concentrations of from 0.41 to > 4482 ng.L^{-1} in rivers in Brazil (Quadra et al. 2017; Beatriz et al. 2020; Gomes et al. 2022a).

The deleterious effects of environmentally relevant concentrations of Cipro on aquatic organisms have been described, with oxidative stress as well as histopathology being evidenced in fish such as *Cirrhinus mrigala* when exposed to 1 and 1.5 µg.L^{-1} (Ramesh et al. 2021) and *Rhamdia quelen* when exposed to 10 and 100 µg.L^{-1} (Kitamura et al. 2022). Toxic effects have also been reported in photoautotrophic organisms such as microalgae (at exposures ranging from 10 to 100 mg.L^{-1}) (Xiong et al. 2017), cyanobacteria (1.50 to 17.24 µg.L^{-1}) (Azevedo et al. 2019), as well as macrophytes such as *Ricciocarpus natans* (0, 0.75, 1.05, and 2.25 mg.L^{-1}) (Gomes et al. 2018), *Lemna minor* L., and *L. gibba* L. (5, 31, 78, and 195 µg.L^{-1}) (Nunes et al. 2019). Negative impacts on photosynthesis and respiration in those species and the generation of reactive oxygen species was observed (Azevedo et al. 2019; Gomes et al. 2018; Liu et al. 2018; Rocha et al. 2021a; Xiong et al. 2017). There is therefore a great need for monitoring and mitigating the effects of that antibiotic on aquatic ecosystems throughout the world (Gothwal and Shashidhar 2015; Nunes et al. 2019).

Conventional water treatment systems are not efficient at removing antibiotics (O'Flaherty and Cummins 2017), so that phytoremediation appears as an emerging technological alternative for water decontamination (Kurade et al. 2021). Phytoremediation consists of the use of plants to metabolize, stabilize, and/or accumulate contaminants and pollutants in their biomasses (Ansari et al. 2020). The use of aquatic macrophytes to reclaim contaminants from water is a Nature-Based Solution (NBS), being considered an efficient sustainable technique (Song et al. 2019; Fletcher et al. 2020), and has emerged as a promising alternative for depuration of antibiotic-contaminated waters (Rocha et al. 2021b; Yan et al. 2019a). Although several aquatic macrophytes species have been indicated for antibiotic-phytoremediation, few studies have associated their remediation capacities with their respective biotypes (submerged and floating) (Rocha et al. 2021b). For instance, submerged macrophytes (*Myriophyllum aquaticum* Vell. Verdc. and *Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne) were more effective in the removal of erythromycin from water than floating ones (*Salvinia molesta* DS. Mitch and *L. minor*). Biological characteristics related to phytoremediation capacity, such as the growth rate, contact surface, and intrinsic tolerance vary between species and biotype (Rocha et al. 2021a). Therefore, understanding the differences in tolerance and removal efficiency between different morphotypes of aquatic macrophytes may help to better indicate species with greater performance for phytoremediation programs (Fletcher et al. 2020).

Between the candidate species, aquatic macrophytes of the genus *Salvinia* have shown prominence for water decontamination, mainly due to their rapid growth and their ability to reclaim contaminants (Wolff et al. 2012; Schwantes et al. 2019; Praveen and Pandey 2020). *S. molesta*, for instance, has been found to be efficient for treating industrial effluents and is considered as having a great potential for post-treatments of contaminated waters (Ng and Chan 2017; Schwantes et al. 2019). *S. molesta* is a Brazilian native floating macrophyte belonging to the Salviniaceae family (Coetzee and Hill 2020) that demonstrates fast growth and a high absorption capacity for different xenobiotics, including phosphate, nitrate, nitrite, ammoniacal nitrogen, glyphosate, and aminomethylphosphonic acidlead, mercury, arsenic and nanoparticles, mainly in tropical regions (Mendes et al. 2021; Mustafa and Hayder 2021; Ng and Chan 2017). Similarly, the submerged macrophyte *Egeria densa* Planch. has gained attention in phytoremediation programs, due to its potential to reclaim organic (saflufenacil, oxytetracycline) (Vilvert et al. 2017; Pestana et al. 2018; Alonso et al. 2021) and inorganic compounds (trace elements, nanoparticles) (Pestana et al. 2018; Alonso et al. 2021). This species is native to Brazil (Yarrow et al. 2009) and has fast growth and great tolerance to different contaminants (Pestana et al. 2018; Alonso et al. 2021). To our best knowledge, however, there have been no studies testing the use of these species for phytoremediation of Cipro and comparing their phytoremediation capacity. As such, we evaluated the tolerance and the capacity of *S. molesta* and *E. densa* to remove Cipro from contaminated water by examining both the primary (photosynthesis and respiration) and oxidative metabolism of plants exposed to environmentally relevant concentrations of that antibiotic. In addition to evaluate the physiological responses and uptake capacity of plants along time, we compared the phytoremediation potential of the species aiming to identify the most appropriate biotype to reclaim Cipro from contaminated water.

Material and methods

Plant material

Salvinia molesta DS. Mitch. (Salviniaceae) specimens were collected in Barigui Park, Curitiba, Paraná State, Brazil (25° 25′ 18″ S; 49° 18′ 22″ W) and *Egeria densa* Planch. (Hydrocharitaceae) specimens were collected at Guaraguaçu river, Paraná State, Brazil (25° 40′ 19.95″ S; 48° 30′ 47.20″ W). Before initiating the experiments, the macrophytes were acclimated and depurated in a sterile reconstituted medium (SRS) (5.298 µM $CaCl_2$, 2.044 µM $MgSO_4$, 1.500 µM $NaHCO_3$, and 0.7377 µM KCl, in ultrapure water) at 25 ± 2 °C under a 10/14-h photoperiod regime of 80 µmol photons $m^2.s^{-1}$ (PPFD) for a period of 60 days.

Bioassay

The experiments were carried out in 250-mL Erlenmeyer flasks containing 100 mL of SRS. A stock solution (10 mg. L^{-1}) of Cipro was prepared in ultrapure water using analytical grade Cipro (Sigma-Aldrich, Brazil). The appropriate volumes of that stock solution were added directly to the SRS medium prior to transferring the plants to the experimental flasks. The macrophytes were exposed to four different concentrations of Cipro: 0 (control), 1, 10, and 100 µg.Cipro.L^{-1}. Those concentrations were chosen based on reports of field occurrences of the antibiotic in surface waters (Agunbiade and Moodley 2014; Frade et al. 2014; Mutiyar and Mittal 2014; Beatriz et al. 2020), effluents to sewage treatment plants (Pal et al. 2010; Frade et al. 2014; Janecko et al. 2016; Riaz et al. 2017), and effluents from hospitals and pharmaceutical industries (Duong et al. 2008; Frade et al. 2014; Mutiyar and Mittal 2014; O'Flaherty and Cummins 2017; Riaz et al. 2017; Thai et al. 2018).

The bioassays were formed in biochemical oxygen demand chambers (BOD) and the macrophytes were exposed in static tests for 96 and 168 h, at 23 ± 2 °C with a 10/14-h light/dark photoperiod at and illumination of 80 µmol photons $m^2.s^{-1}$ (OECD, 2006). Before use, plants were surface disinfected in hypochlorite solution (0.5%) for 3 min (Mendes et al. 2021) and after being thoroughly washed in ultrapure water, they were distributed into each test flask (constituting a replicate) at the density of 10 g.L^{-1}; four flasks were used in each of the treatments. The *E. densa* apices were cut from the stems of mature plants from the apex in the direction of the base to obtain the plant weighs including the apex. Four plants were harvested for analysis in each time of evaluation (96 h and 168 h of exposure), constituting than four replicates for each treatment in a factorial combination time × Cipro concentrations. Water samples were collected for chemical analyses at each evaluation time, as well as at the initial time (0 day). Simultaneous tests were carried out in flasks without plants ($n = 4$), under the same condition of temperature and illumination used for plant cultivation, to study the natural (light, temperature, and hydrolysis) degradation of ciprofloxacin.

Photosynthesis and relative growth rates

Photosynthesis evaluations were conducted using whole macrophytes, employing an open-infrared gas system (CI-340 Photosynthesis System; CID Bio-science, Inc, USA) coupled to a chlorophyll CI-510CF fluorescence module. The net rates of photosynthesis (P_N) were measured three times/plant (80 nmol of photons m^2 s^{-1}) during each evaluation. Minimum (F_0) and maximum (Fm) fluorescence were measured, and the maximum quantum yields of photosystem II (Fv/Fm) were calculated according to Kitajima and Butler (1975). For relative growth rate evaluations, the plants were centrifuged at 3000 rpm for 10 min. at room temperature (in centrifuge tubes with small holes to remove surface water) and weighed to determine their fresh weights (OECD 2006). The relative growth rates were calculated as the difference between final and initial fresh weights divided by the time of exposure.

Biochemical analyses

Freshly collected plants were flash-frozen in liquid nitrogen and then stored at − 80 °C in aluminum foil until analyzed. Photosynthetic pigment assays were conducted using 0.1 g of fresh leaves; extraction was performed in 80% acetone, and the concentrations of chlorophyll-*a*, -*b*, and carotenoids were assessed following Lichtentauler and Wellburn (1983).

Antioxidant enzyme activities, as well as hydrogen peroxide (H_2O_2) concentrations (Velikova et al. 2000), and lipid peroxidation (MDA) (Hodges et al. 1999) were determined using 0.1 g of plants (leaves + roots). The enzymes were extracted in 1 mL of phosphate buffer (pH 7.8) containing 100 mM EDTA, 1 mL of *L*-ascorbic acid, and a 2% polyvinylpyrrolidone solution (PVP m/v) (Gomes et al. 2016). The activities of ascorbate peroxidase (APx) (Nakano and Asada 1981) and catalase (CAT) (Aebi 1984) were assessed after determining the total protein concentrations (Bradford 1976).

The effects of Cipro on the activities of mitochondrial electron transport chain-related enzymes were determined using a spectrophotometer. The analyzes were performed with intact mitochondria (Howell et al. (2006), with modifications proposed by Murcha and Whelan (2015) using 100 µg.of $protein^{-1}$ (Bradford 1976). The activities of complex I (NADH: ubiquinone oxireductase), complex II (succinate dehydrogenase) (Estornell et al. 1993), complex III

(ubiquinol-cytochrome c reductase) (Birch-Machin et al. 1993), and complex IV (cytochrome c oxidase) (Birch-Machin et al. 1993) were evaluated.

Chemical analyses and phytoremediation potential

The detection and quantification of Cipro in water and plants were performed by high performance liquid chromatography (Waters 2695 HPLC), coupled to a fluorometric detector (FD Waters multi-fluorescence detector 2475) following Shi et al. (2009), with modifications of the mobile phase. The fluorescence wavelengths evaluated were 278 nm for excitation and 453 nm for emission. The solvents used as the mobile phase were: triethylamine 0.4% (v/v), as phase A; methanol as phase B; and acetonitrile as phase C. Cipro was extracted from the macrophytes according to the method proposed by Zhao et al. (2007), with modifications. Analytical grade Cipro (United States Pharmacopeia, Rockville, MD, USA) was used to establish the calibration curves. The curves were composed of six points, and demonstrated good linearity for the analyte ($r^2=0.999$; $p<0.0001$) and Cipro concentrations in the water and in macrophytes were calculated using the linear equation ($y=11800\times—6572.7$, where: $y=$Cipro concentration and; $x=$area). Each batch of samples included three blanks, three standards, and three fortified samples. Recovery rates were 94.4%. The LOD and LOQ were 0.3 and 1.00 µg.Cipro.L^{-1} respectively.

After the quantification of the antibiotic in plants, the Cipro bioconcentration factor (BCF) was calculated according to Jayanpathi et al.(2019) (Eq. 1) and the phytoremediation efficiency was calculated following Gomes et al. (2020b) (Eq. 2 and 3):

$$\mathrm{BCF}=\frac{c}{cw} \tag{1}$$

where c is the Cipro concentration in the tissue plants (µg. kg^{-1}) and cw is the Cipro concentration in water (µg.L^{-1}).

$$\mathrm{Degradation}(\%)=100-\left(\frac{c2\ \text{without plants}}{c_{1\ \text{without plants}}}\times 100\right) \tag{2}$$

$$\text{Phytoremedation efficiency }(\%)=100-\left(\frac{c2\ \text{with plants}}{c_{1\ \text{with plants}}}\times 100\right)-\text{Degradation} \tag{3}$$

where c_1 is the initial Cipro concentration in the water; c_2 is the final Cipro concentration in the water.

Data analyses

The data were tested for normality (Shapiro–Wilk) and homoscedasticity (Levene), and evaluated using two-way ANOVA. Interactions between Cipro concentrations (0, 1, and 100 µg. Cipro.L^{-1}) and time (96 and 168 h) or between Cipro and species (*S. molesta* and *E. densa*) were included in the model and when differences were detected by ANOVA, the means were compared using the Tukey test, at a 0.05% level of significance. The efficiency of phytoremediation was compared between species (*S. molesta* and *E. densa*) by *T* test. The results were expressed as the means of four replicates. The data were statistical analyzed using R software (R.3.2.2, Team 2015). The graphs were prepared using PRISM, version 7.01 software.

Results

Cipro effects on photosynthesis and pigments

S. molesta

Significant interactions were observed in terms of the maximum quantum yields of PSII (Fv/Fm) (Table 1). Decreased Fv/Fm was observed in plants exposed to 100 µg.Cipro.L^{-1} after 168 h, in relation to the control (Fig. 1A). Fv/Fm also decreased over time, regardless of the antibiotic treatment (Fig. 1A). Chlorophyll and carotenoid concentrations were not significantly affected ($P<0.05$) by Cipro concentration or by the time of exposure (Fig. 1B and C, Table 1).

E. densa

Decreased Fv/Fm was observed in plants exposed to 10 and 100 µg.Cipro.L^{-1} at 96 h, and to µg.Cipro.L^{-1} after 168 h, in relation to control (Table 2, Fig. 1A). Regardless the time of evaluation, when exposed to 10 and 100 µg.L^{-1} of Cipro, decreased chlorophyll-*a* concentration was observed in plants when compared to control (Table 2, Fig. 1B). Significant interaction between Cipro concentrations and times of exposure was observed for chlorophyll-*b* and carotenoid concentrations (Table 2). At 96 h, the concentration of chlorophyll-*b* (Fig. 2C) and carotenoids (Fig. 2D) were lower in plants exposed to Cipro, while after 168 h, this effect was observed only in plants exposed to 100 µg.Cipro.L^{-1} (Table 2, Fig. 2).

Oxidative stress markers

S. molesta

Significant interactions between Cipro concentrations and times of exposure were observed for APx and CAT activity as well as for H_2O_2 concentration in *S. molesta* plants (Table 1). At 96 h, APx activity was lower in plants exposed to 10 and 100 µg.Cipro.L^{-1} in relation to the control and 1 µg.Cipro.L^{-1} (with APx activities not significantly differing between those latter treatments) (Fig. 3A). After 168 h of exposure, however, the plants exposed to 100 µg.Cipro. L^{-1} evidenced the greatest APx activity. With exception of

Table 1 *F* values and two-way ANOVA results for the effects of Cipro at different concentrations (control, 1, 10, 100 µg.L^{-1}) and at two exposure times (96 and 168 h) on *Salvinia molesta* plants. Values represent the means of four replicates

ANOVA *F* values	D.F											
		Fv/Fm	Chlo-a	Chlo-b	Carotenoids	CAT	APx	H_2O_2	MDA	CI	CII	CIII	CIV
Cipro concentration	3	0.68	2.46	2.97	3.03	18.83^{***}	31.05^{***}	144.97^{***}	0.23	0.68	97.96^{***}	17.81^{**}	6.17^{**}
Time	1	64.34^{***}	2.16	0.87	0.48	145.08^{***}	5.26^{*}	126.29^{*}	1.31	0.06	0.28	1.12	1.21
Cipro concentration x Time	3	7.74^{**}	0.15	0.70	0.10	68.83^{***}	137.84^{***}	137.56^{***}	0.19	2.20	0.29	1.54	17.15^{***}
Comparison of means[$$]													
Time													
Student *t* test, $P<0.05$													
96 h		70.45^{a}	3.22^{a}	2.26^{a}	344.23^{a}	0.05^{b}	0.16^{b}	1.00^{b}	0.15^{a}	0.36^{a}	0.54^{a}	1.78^{a}	2.95^{a}
168 h		58.79^{b}	2.75^{a}	2.09^{a}	338.51^{a}	0.09^{a}	0.35^{a}	2.78^{a}	0.13^{a}	0.36^{a}	0.53^{a}	1.80^{a}	3.05^{a}
Cipro concentration `(µg L^{-1})													
Tukey Test, $P<0.05$													
	Control	66.05^{a}	3.17^{a}	2.22^{a}	332.04^{a}	0.06^{b}	0.63^{b}	0.67^{b}	0.12^{a}	0.30^{a}	1.38^{a}	2.68^{a}	2.70^{b}
	1	65.51^{a}	2.90^{a}	2.28^{a}	357.48^{a}	0.06^{b}	0.76^{b}	0.83^{ab}	0.15^{a}	0.36^{a}	0.31^{b}	1.62^{b}	3.08^{ab}
	10	63.45^{a}	2.79^{a}	2.02^{a}	323.40^{a}	0.09^{a}	0.95^{a}	2.83^{a}	0.15^{a}	0.35^{a}	0.31^{b}	1.55^{b}	2.98^{ab}
	100	63.90^{a}	3.10^{a}	2.18^{a}	352.44^{a}	0.08^{ab}	0.41^{c}	3.21^{a}	0.13^{a}	0.34^{a}	0.26^{b}	1.63^{b}	3.42^{a}

D.F, degrees of freedom; *Fv/Fm*, maximum quantum yields of photosystem II; *Chlo-a*, chlorophyll-a; *Chlo-b*, chlorophyll-b; *CAT*, catalase; *APx*, ascorbate peroxidase; H_2O_2, hydrogen peroxide; *MDA*, lipid peroxidation; *CI*, complex I (NADH:ubiquinone oxidoreductase); *CII*, complex II (succinate dehydrogenase); *CIII*, complex III (ubiquinol-cytochrome c reductase); *CIV*, complex IV (cytochrome c oxidase). *Significant $P<0.05$; **significant $P<0.01$; ***significant $P<0.001$. Treatment means from two-way ANOVA. Values followed by the same letter, within the same source of variation, are not significantly different ($P<0.05$)

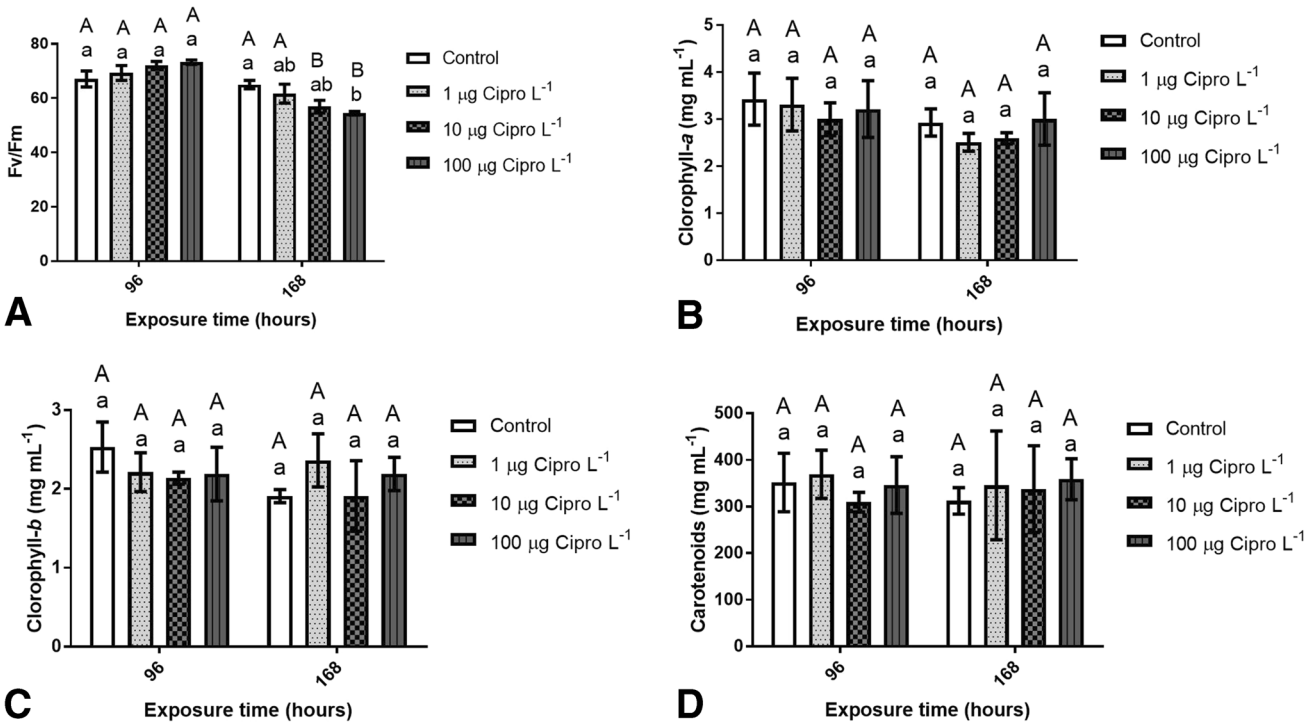

Fig. 1 Effects of Cipro concentrations and time of exposure on photosynthesis and pigments in *Salvinia molesta*. **A** Maximum quantum yields of photosystem II (Fv/Fm); **B** chlorophyll-*a*; **C** chlorophyll-*b*; **D** carotenoids. Values are represented as the mean ± standard error of four replicates. Lowercase letters indicate significant difference between Cipro concentrations at the same evaluation time; uppercase letters indicate significant differences between times within the same Cipro concentration, by the post hoc Tukey Test (considering $P < 0.05$)

the plants exposed to 100 µg.Cipro.L^{-1} (in which APx activity increased), APx activity decreased over time (Fig. 3A). Significant differences were not observed in terms of CAT activity in plants exposed to different Cipro treatments for 96 h; at 168 h, however, the plants exposed to 10 and 100 µg.Cipro.L^{-1} showed greater CAT activity than the control, while plants exposed to 1 µg.Cipro.L^{-1} showed lower CAT activity (Fig. 3B). With the exception of plants exposed to 1 µg.Cipro.L^{-1}, CAT activity increased over time (Fig. 3B).

At 96 h, the H_2O_2 concentration was greater than the control only in plants exposed to 100 µg.Cipro.L^{-1} (Fig. 3C); H_2O_2 concentrations increased over time in plants exposed to both 10 and 100 µg.Cipro.L^{-1} after 168 h (Fig. 2C). Lipid peroxidation (MDA concentration) was not significantly affected by Cipro concentrations or time of exposure (Fig. 3D, Table 1).

E. densa

Regardless the time of evaluation, APx activity (Fig. 4A) and H_2O_2 concentration increased in plants exposed to 10 and 100 µg.Cipro.L^{-1} in relation to the control (Fig. 4C). After 168 h, the amount of H_2O_2 decreased, when compared with 96 h (Table 2, Fig. 4C). Regardless the time of exposure, CAT activity increased in Cipro-exposed plants in relation to control (Table 2, Fig. 4B). MDA concentrations were not significantly affected by Cipro concentrations or time of exposure (Fig. 4D, Table 2).

Mitochondrial electron transport chain effects

S. molesta

Complex I activity was not significantly affected by Cipro or the time of exposure to it (Fig. 5A, Table 1). In contrast, the activities of complexes II and III were significantly reduced in *S. molesta* plants exposed to Cipro, regardless of the time of exposure (Fig. 5B and C, Table 1). Significant interactions between Cipro concentrations and times of exposure were observed for complex IV activity (Table 1). At 168 h of exposure, increased complex IV enzyme activity was observed in plants exposed to 100 µg.Cipro.L^{-1} in relation to the control (Fig. 5D). With exemption of plants exposed to 10 µg.Cipro.L^{-1}, complex IV activity increased over time (Fig. 5D).

E. densa

Significant interactions between Cipro concentrations and times of exposure were observed for the activities of

Table 2 *F* values and two-way ANOVA results for the effects of Cipro at different concentrations (control, 1, 10, 100 µg.L^{-1}) and at two exposure times (96 and 168 h) on *Egeria densa* plants. Values represent the means of four replicates

ANOVA *F* values	D.F											
		Fv/Fm	Chlo-a	Chlo-b	Carotenoids	CAT	APx	H_2O_2	MDA	CI	CII	CIII	CIV
Cipro concentration	3	5.58**	59.36***	16.01***	35.14***	61.87***	26.75***	27.78***	0.74	145.5***	60.17***	87.19***	542.1***
Time	1	0.02	0.01	0.27	8.11*	0.69	3.88	27.01*	0.02	12.85*	0.49*	0.10	6.26
Cipro concentration × time	3	2.12	0.82	4.74*	5.71**	7.24	1.05	0.32	1.53	5.22**	4.23*	0.36	3.36
Comparison of means$^{\$\$}$													
Time													
Student *t* test, $P<0.05$													
96 h		64.88^{a}	3.36^{a}	1.75^{a}	430.59^{a}	0.14^{a}	2.06^{a}	0.37^{a}	0.11^{a}	0.49^{a}	1.79^{a}	2.38^{a}	3.11^{a}
168 h		65.03^{a}	3.37^{a}	1.69^{a}	390.91^{b}	0.14^{a}	1.96^{a}	0.31^{b}	0.11^{a}	0.46^{b}	1.88^{b}	2.40^{a}	3.02^{a}
Cipro concentration (µg.L^{-1})													
Tukey test, $P<0.05$													
	Control	73.26^{a}	4.19^{a}	2.15^{a}	497.70^{a}	0.09^{a}	1.39^{a}	0.28^{a}	0.11^{a}	0.58^{a}	2.06^{a}	3.11^{a}	4.15^{a}
	1	66.85^{ab}	3.74^{ab}	1.78^{b}	445.80^{a}	0.14^{b}	1.61^{ab}	0.31^{ab}	0.11^{a}	0.53^{ab}	1.86^{ab}	2.30^{b}	3.28^{b}
	10	60.43^{b}	2.89^{b}	1.74^{b}	395.70^{bc}	0.15^{b}	2.36^{b}	0.37^{b}	0.12^{a}	0.41^{b}	1.70^{b}	2.13^{bc}	2.78^{c}
	100	59.25^{b}	2.63^{b}	1.21b	303.80^{c}	0.17^{b}	2.49^{b}	0.40^{b}	0.12^{a}	0.36^{b}	1.59^{b}	2.03^{c}	2.06^{d}

D.F, degrees of freedom; *Fv/Fm*, maximum quantum yields of photosystem II; *Chlo-a*, chlorophyll-a; *Chlo-b*, chlorophyll-b; *CAT*, catalase; *APx*, ascorbate peroxidase; H_2O_2, hydrogen peroxide; *MDA*, lipid peroxidation; *CI*, complex I (NADH:ubiquinone oxidoreductase); CII, complex II (succinate dehydrogenase); CIII, complex III (ubiquinol-cytochrome c reductase); *CIV*, complex IV (cytochrome c oxidase). $^{\$}$*Significant $P<0.05$; **significant $P<0.01$; ***significant $P<0.001$. Treatment means from two-way ANOVA. Values followed by the same letter, within the same source of variation, are not significantly different ($P<0.05$)

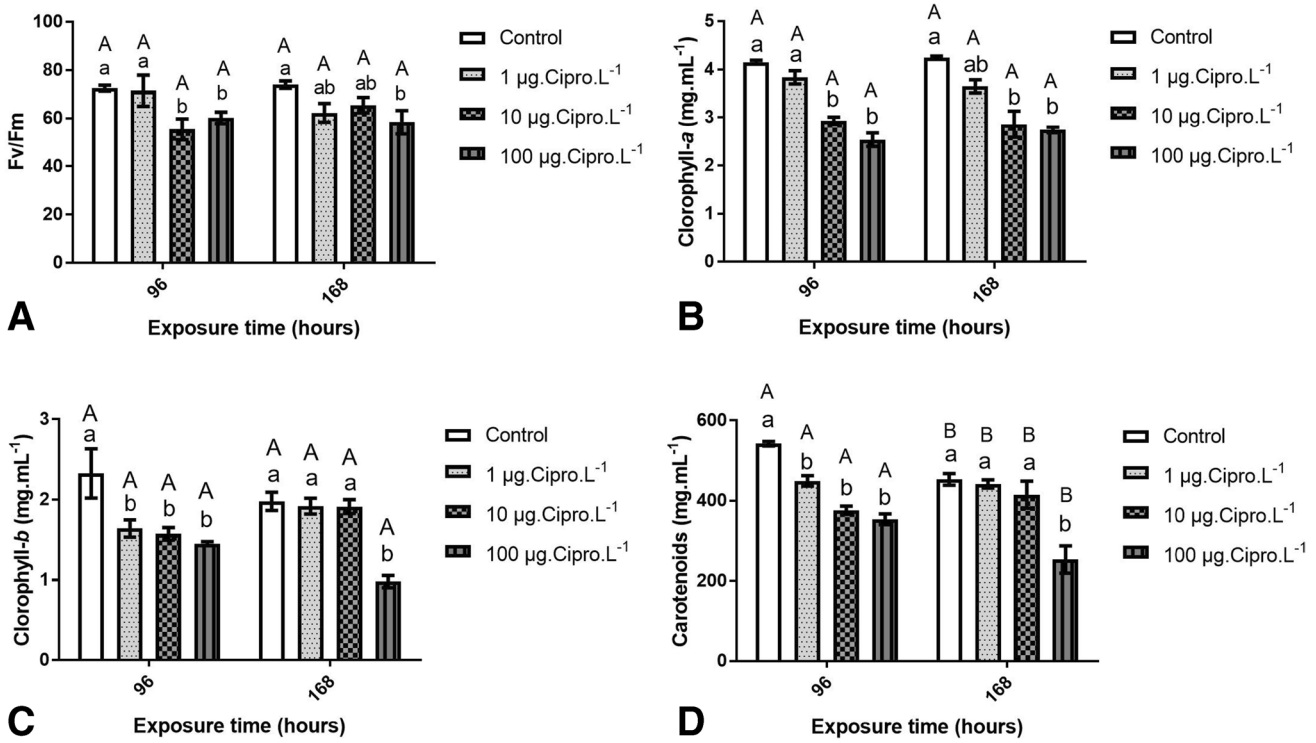

Fig. 2 Effects of Cipro concentrations and time of exposure on photosynthesis and pigments in *Egeria densa*. **A** Maximum quantum yields of photosystem II (Fv/Fm); **B** chlorophyll-*a*; **C** chlorophyll-*b*; **D** carotenoids. Values are represented as the mean ± standard error of four replicates. Lowercase letters indicate significant difference between Cipro concentrations at the same evaluation time; uppercase letters indicate significant differences between times within the same Cipro concentration, by the post hoc Tukey Test (considering $P < 0.05$)

complexes I and II in *E. densa* plants (Table 2). At 96 h, CI and CII activity was lower in plants exposed to 10 and 100 µg.Cipro.L^{-1} in relation to the control and 1 µg.Cipro.L^{-1} (Fig. 6A and B). After 168 h of exposure, the activities of CI and CII decreased in plants exposed to Cipro when compared to control (Table 2, Fig. 6A and B). In Cipro-exposed plants, the activity of CI and CII decreased over time (Fig. 6A and B). Regardless of the time of exposure, the activities of complexes III and IV were significantly reduced in *E. densa* plants exposed to Cipro concentrations in relation to control (Fig. 6C and D, Table 2).

Phytoremediation potential

S. molesta

Cipro was not found in water samples of the control treatment (Table 3). Cipro degradation in flasks without *S. molesta* plants increased as its concentrations increased ($P_{96} = 0.001$; $P_{168} < 0.001$). Regardless of the treatment or the time of evaluation, lower Cipro concentrations were observed in flasks with plants in relation to flasks without plants ($P < 0.001$) (Table 3). As such, the phytoremediation efficiency of plants treated with 1 µg.Cipro.L^{-1} was the lowest at 96 h of exposure, but that efficiency became the greatest among Cipro-treated plants at 168 h (Table 3).

Cipro concentrations in plant tissues ranged from 6.41 to 114.35 µg.g.DW^{-1} at 96 h and from 7.60 to 145.90 µg.g.DW^{-1} at 168 h. Significant interactions between Cipro concentrations were observed in terms of its concentrations in plant tissues (Table 4). Regardless of the time of exposure, greater concentrations of Cipro were observed in plants exposed to 100 µg.Cipro.L^{-1} (Table 2). Within the same Cipro treatment, however, plant tissue Cipro concentrations did not significantly differ over time (Table 4).

After 96 h of exposure, bioconcentration factors (BCF) decreased as Cipro concentrations in the water media increased (Table 4). After 168 h of exposure, the BCF was only greater for plants exposed to 1 µg.Cipro.L^{-1}, not differing among the other treatments. The BCF did not significantly differ over time within the same Cipro treatment (Table 4).

E. densa

Regardless of the treatment or the time of evaluation, the presence of plants contributes to lowering Cipro concentrations in

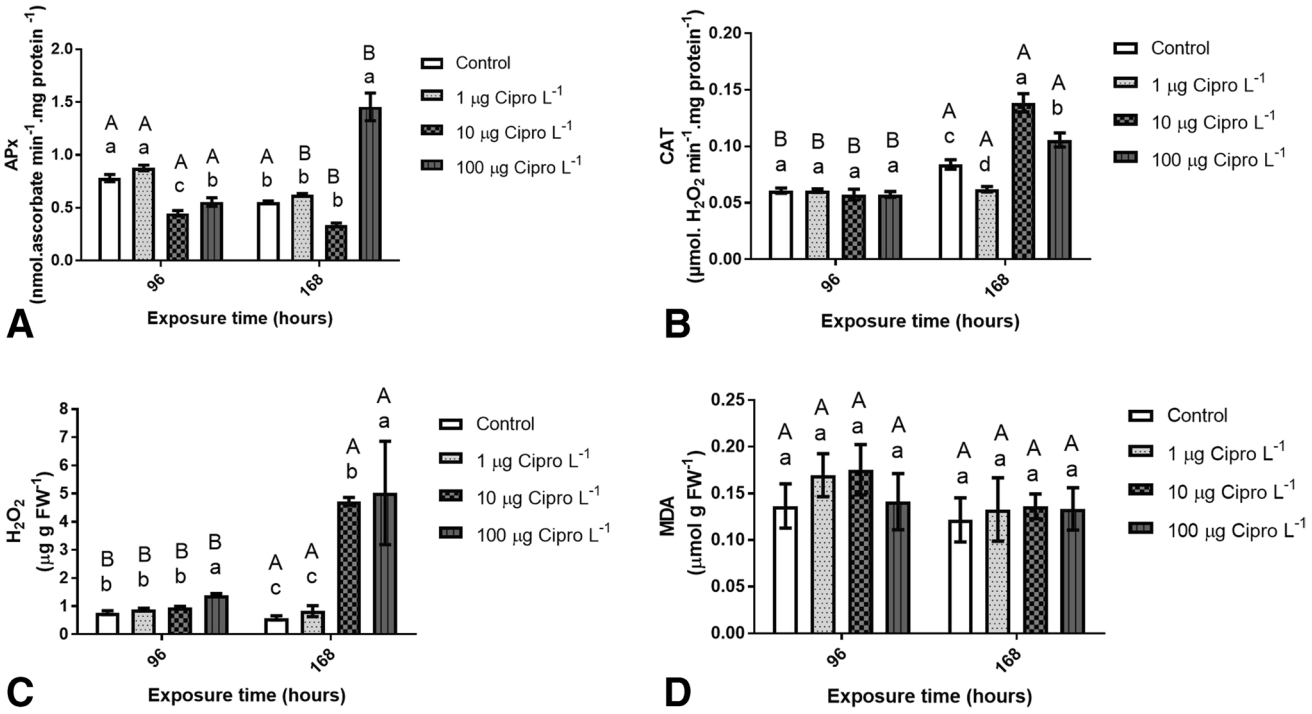

Fig. 3 Effects of Cipro and time of exposure on oxidative stress markers in *Salvinia molesta*: **A** ascorbate peroxidase activity (APx); **B** catalase activity (CAT); **C** hydrogen peroxide concentration (H_2O_2); **D** lipid peroxidation (MDA concentration). Values are presented as the mean ± standard error of four replicates. Lowercase letters indicate significant differences among Cipro concentrations within the same evaluation time, while uppercase letters indicate significant differences between times within the same Cipro concentration, by the post hoc Tukey test (considering $P < 0.05$)

relation to flasks without plants ($P < 0.001$) (Table 4) and the highest phytoremediation efficiency was observed in plants treated with 1 µg.Cipro.L^{-1} ($P < 0.0001$, Table 3).

The Cipro concentrations in plant tissues ranged from 1.78 to 69.46 µg.g.DW^{-1} at 96 h and from 2.39 to 119.6 µg.g.DW^{-1} at 168 h. Regardless of the time of evaluation, Cipro concentration in plant tissues increased with the antibiotic addition to the water (Table 4). BCF increased over time in *E. densa* plants, being < 0.84 at 96 h and > 1.14 after 168 h of exposure (Table 4).

S. molesta vs. *E. densa*

Significant interactions between Cipro and species were observed for the relative growth rate (RGR, Table 4). *S. molesta* plants exposed to 100 µg.Cipro.L^{-1} showed the highest RGR at both times of evaluation, while the same was observed for *E. densa* plants only at 96 h of exposure, when compared to the other treatments (Table 2). Regardless of the time of evaluation and Cipro treatment, *S. molesta* presented higher RGR than *E. densa* (Table 4).

Regardless of the time of evaluation and Cipro treatment, the Cipro concentrations in *S. molesta* plant tissues were higher than those observed for *E. densa* (Table 4; $P < 0.0001$). This was also reflected in the higher BCF observed for *S. molesta* in relation to *E. densa* for all Cipro treatments at 96 h, and in plants exposed to 1 and 10 µg. Cipro.L^{-1} after 168 h of exposure (Table 4).

Discussion

The comprehension of plant mechanisms of tolerance to environmental contaminants is important for choosing species for phytoremediation programs (Carvalho et al. 2014; Gomes et al. 2022b). We investigated the physiological responses and the ability of *S. molesta* (floating) and *E. densa* (submerged) plants to remove Cipro from contaminated water to evaluate it for use in phytoremediation programs. Overall, the plants showed efficient phytoremediation and high tolerance to that antibiotic, with no observed mortality or visual damage to the plants, even when submitted to the highest Cipro concentration investigated.

The tolerance of plants to aquatic contaminants has been related to their great ability to cope with oxidative stress (Praveen and Pandey 2020), with that tolerance allowing plant survival and their antioxidant activity being closely related to their remediation capacities (Gomes et al. 2020a, 2022b). The exposure of plants to antibiotics such as Cipro increases the generation of reactive oxygen species (ROS) through their

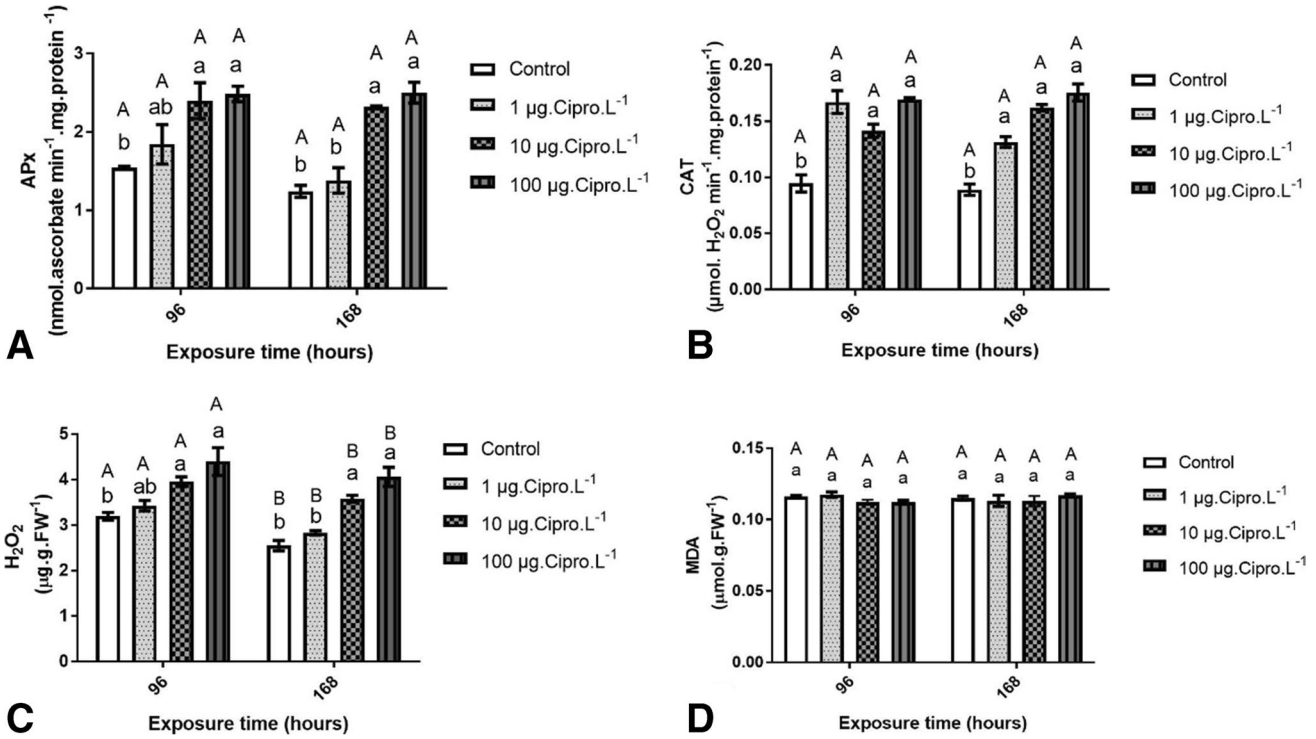

Fig. 4 Effects of Cipro and time of exposure on oxidative stress markers in *Egeria densa*: **A** ascorbate peroxidase activity (APx); **B** catalase activity (CAT); **C** hydrogen peroxide concentration (H_2O_2); **D** lipid peroxidation (MDA concentration). Values are presented as the mean ± standard error of four replicates. Lowercase letters indicate significant differences among Cipro concentrations within the same evaluation time, while uppercase letters indicate significant differences between times within the same Cipro concentration, by the post hoc Tukey Test (considering $P < 0.05$)

interference with energy metabolism (Gomes et al. 2017a, b). The increased H_2O_2 concentrations observed in *S. molesta* plants exposed to 100 µg.Cipro.L^{-1} (Figs. 3C and 4C) and in *E. densa* at 10 and 100 µg.Cipro.L^{-1} after 96 h of exposure, indicated that Cipro concentrations > 10 µg.Cipro.L^{-1} can induce physiological disruption and ROS accumulation after short exposures. Despite H_2O_2 accumulation in the plants, no increased lipid peroxidation was observed for both species, regardless of the time of exposure (Figs. 3D and 4D). The role of antioxidant systems in avoiding oxidative stress was evidenced by increased APx and CAT activities after 168 h of exposure, with the plants showing increased H_2O_2 but not MDA concentrations (Fig. 3 and 4). Similar results were reported by Gomes et al. (2017a, b) in *L. minor* plants exposed to Cipro, with antibiotic tolerance in that species being related to increased CAT and APx activities. Similarly, by using specific inhibitors of H_2O_2-scavenging enzymes, Gomes et al. (2022b) observed the central role of APx and CAT in the tolerance and remediation capacity of Cipro, amoxicillin, and erythromycin by *L. minor* plants. In addition to reinforce that antioxidant enzyme activity is related to Cipro tolerance, our results also evidenced that Cipro effects on plant physiology are time dependent, which must be considered when evaluating the toxicological effects of that antibiotic.

Although Cipro exposure did not result in detectable oxidative damages, we were interested in better understanding how Cipro induced plant H_2O_2 accumulations. According to Gomes et al. (2018), as photosynthesis and respiration are the major sources of ROS in plants, H_2O_2 accumulation must be related to antibiotic interference with that energy metabolism. We therefore investigated chlorophyll-*a* fluorescence in *S. molesta* and *E. densa* plants. Fv/Fm is a proxy of PSII integrity and is very sensitive to ROS accumulations (Gomes et al. 2017a, b). In *S. molesta*, that parameter was only affected in plants by treatments with 100 µg.Cipro.L^{-1} after 168 h of exposure (Fig. 1A)—indicating interference with the photosynthetic apparatus when plants are exposed for long periods to high antibiotic concentrations. Those negative effects were not related to pigment composition, however, as the plants' photosynthetic pigment concentrations were not affected by Cipro (Fig. 1). In contrast, in *E. densa*, significant reduction on pigment concentration was observed in plants showing decreased Fv/Fm. Decreased Fv/Fm indicates photochemical disruptions that ultimately may contribute to ROS accumulations (Gomes et al. 2017a, b), and it has been reported that fluoroquinolones can act as quinone inhibitors in photosystem II, disrupting the chloroplast electron transport chain (Evans-Roberts et al. 2016). The

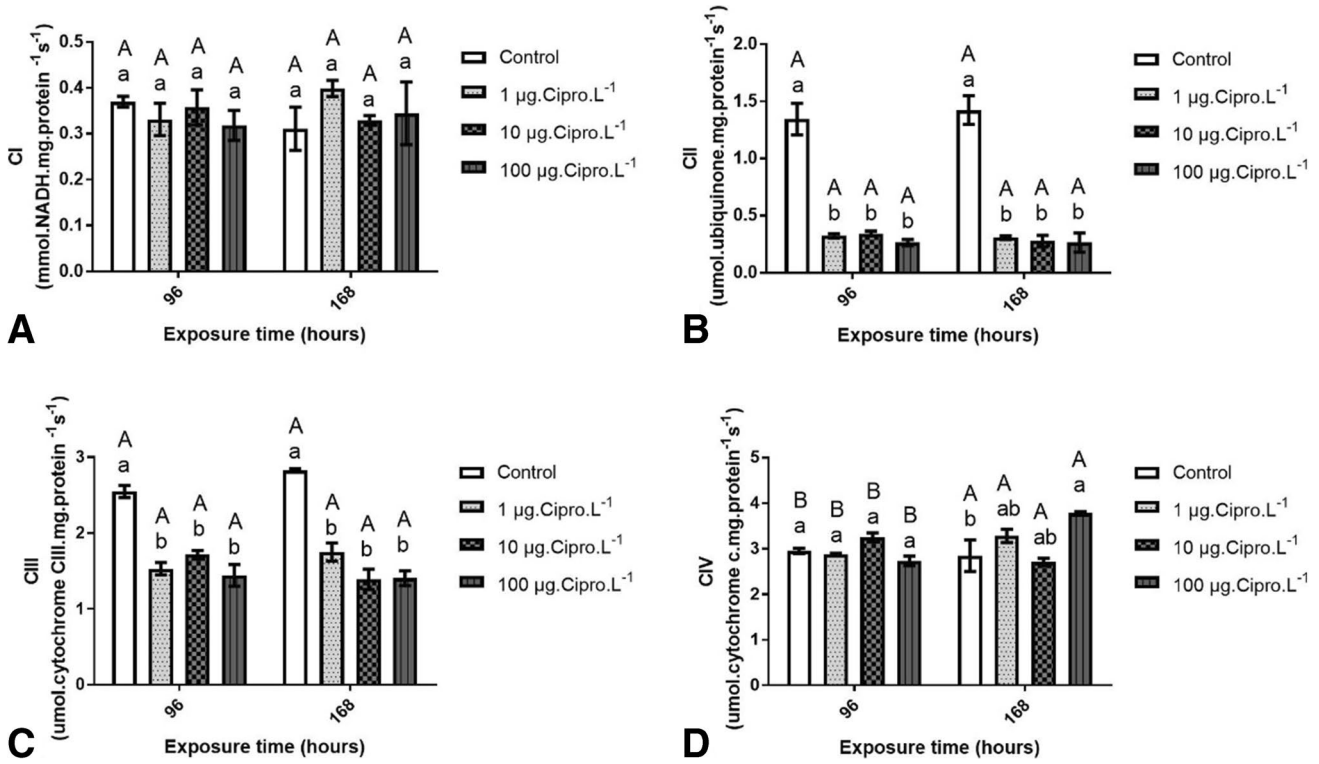

Fig. 5 Effects of Cipro concentrations and times of exposure on the activities of enzymes associated with the mitochondria electron transport chain in *Salvinia molesta*. **A** Ubiquinone oxireductase (CI); **B** succinate dehydrogenase (CII); **C** ubiquinol-cytochrome *c* reductase (CIII); **D** cytochrome *c* oxidase (CIV). Values presented as the mean ± standard error of four replicates. Lowercase letters indicate significant differences among Cipro concentrations within the same evaluation time, while uppercase letters indicate significant differences between times within the same Cipro concentration, by the post hoc Tukey Test (considering $P < 0.05$)

observed decrease in Fv/Fm must be a result in place of the cause of ROS accumulation. In excess, ROS have negative effects on the PSII apparatus by affecting the repair and synthesis of PSII-associated proteins (Gomes et al. 2017a, b), which can justify the results observed for *S. molesta*. In the case of *E. densa*, in addition to ROS formation, the reduction on pigment concentrations may contribute to decreased Fv/Fm (Fig. 2). Chlorophyll biosynthesis, likewise, is sensitive to cellular ROS content (Stenbaek and Jensen 2010), which can also induce the pigment degradation (Gomes et al. 2016). However, it is interesting to note that the decreased Fv/Fm in both plant species exposed to the highest concentration of Cipro was not followed by decreases on their relative growth rates (Table 4). In contrast, after 7 days of exposure, at 100 µg.Cipro.L^{-1}, Cipro increased fresh weigh production in *S. molesta* and did not affect RGR in *E. densa* plants. The decrease on pigment concentrations in *E. densa* (which can be reflected in decreased Fv/Fm), can be a tolerant mechanism of plants aiming to reduce photooxidation due to negative effects of ROS on photosynthesis apparatuses. This may allow lower production of ROS (by photochemistry) assuring lower ROS accumulation and no oxidative damages (as observed here). The stimulator effect of Cipro on *S. molesta* fresh weight production can be associated to greater negative effect of the antibiotic on the catabolic metabolism (respiration) than in photochemistry, assuring greater carbon fixation than consumption. However, this must be better investigated by evaluating net photosynthesis and respiration in Cipro-exposed plants—which were not performed here. Moreover, stimulation on the growth rate in plants exposed to pharmaceutical drugs (Pomati et al. 2004; Rocha et al. 2021b; Wan et al. 2015) has been associated with signaling between ROS and plant hormones (Gomes et al. 2019)—an interesting topic for further investigations.

Aiming to better understand how Cipro induces ROS generation in plants, we investigated mitochondrial metabolism. While in *S. molesta* Cipro decreased the activities of mitochondrial complexes II and III at both exposure times (Fig. 5B and C), in *E. densa*, the activity of all the mitochondrial complexes was affected, demonstrating major interference of Cipro in the energetic metabolism of *E. densa* than *S. molesta*. Complexes I, II, and III have been identified as the major source of ROS in mitochondria (O'Leary and Plaxton 2016; Huang et al. 2019), and the inhibition on those complexes causes electron

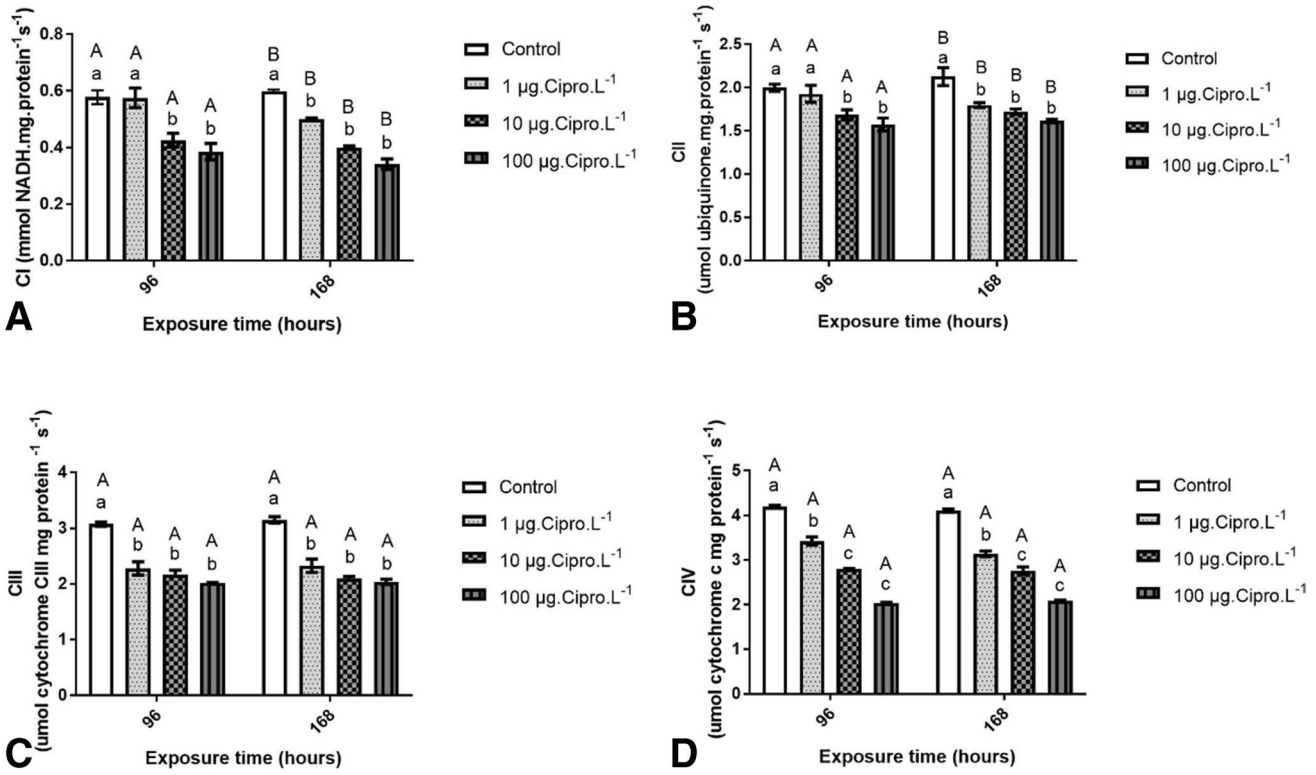

Fig. 6 Effects of Cipro concentrations and times of exposure on the activities of enzymes associated with the mitochondria electron transport chain in *Egeria densa*. **A** Ubiquinone oxireductase (CI); **B** succinate dehydrogenase (CII); **C** ubiquinol-cytochrome *c* reductase (CIII); **D** cytochrome *c* oxidase (CIV). Values presented as the mean ± standard error of four replicates. Lowercase letters indicate significant differences among Cipro concentrations within the same evaluation time, while uppercase letters indicate significant differences between times within the same Cipro concentration, by the post hoc Tukey test (considering $P < 0.05$)

transport chain imbalances, leading to ROS formation, as was observed in our study. Similar results were reported by Gomes et al. (2018) while investigating the effects of Cipro and temperature on *Ricciocarpus natans* (L.) Corda. Those authors demonstrated that Cipro acts as an inhibitor of the ubiquinone reaction site (Q_o site) of complex III, blocking quinol oxidation and leading to the accumulation of unstable semiquinones at the Q_o site—which results in increased ROS production. The quantities of antibiotics inside the plants (which increased with increased Cipro concentrations in media) and its effects on mitochondria may be related to ROS accumulation, which would help explain the absence of H_2O_2 accumulation in plants exposed to only 1 µg.Cipro.L^{-1}.

Interestingly, we observed increased complex IV activity in *S. molesta* plants exposed to 100 µg.Cipro.L^{-1} after 168 h (Fig. 3D). According to Buchanan et al. (2015), complex IV (together with complex III) acts in proton export to the outside of the mitochondrial matrix to assure the ionic and functional balance of the electron transport chain. As reductions in complex III activities were observed here, the increased complex IV activity may have been acting as a mechanism to control the ionic balance and represented attempts to optimize H^+ proton pumping (which would accumulate in the mitochondrial matrix when complex III activity is disrupted). As a result, the production of ATP via mitochondrial ATP synthase will be guaranteed, supplying energy for the plant's physiological demands. This may represent the intrinsic tolerance mechanism of *S. molesta* to Cipro, as reductions in complex IV activity have been reported in other species exposed to that antibiotic (Gomes et al. 2017a, b).

Time of exposure is an important factor to be considered in phytoremediation programs using aquatic plants as the plant tolerance and remediation capacity can be altered over time, affecting their phytoremediation capacity (Carvalho et al. 2014; Adesanya et al. 2021; Park and Son 2022). After some time of exposure, plants can be saturated by the contaminants, reducing their uptake, since the rate of degradation cannot follow the rate of uptake, or due to the saturation of accumulation sites for the contaminants. However, it was not seen in the present study, since the time of exposure did not significantly affect the phytoremediation efficiency of plants. In another way, the increase on antioxidant responses over time, indicate the tolerance of plants in avoiding negative effects of Cipro as a result of its accumulation in plant

Table 3 Ciprofloxacin concentration in water, degradation and phytoremediation efficiency of *Salvinia molesta* and *Egeria densa* (means ± standard error of four replicates)

System	Treatments ($\mu g.L^{-1}$)	Cipro concentration in water			Degradation (%)		Phytoremediation efficiency (%)	
		Initial (T0)	96 h	168 h	96 h	168 h	96 h	168 h
Salvinia molesta								
− Plants	0	n.d	n.d	n.d	n.d	n.d	-	-
	1	1.39 ± 0.01^{a}	1.24 ± 0.02^{a}	1.10 ± 0.04^{a}	2.16 ± 0.09^{a}	6.25 ± 2.42^{b}	-	-
	10	7.16 ± 0.95^{a}	5.59 ± 0.26^{a}	5.34 ± 0.15^{a}	14.67 ± 3.29^{a}	21.15 ± 10.47^{b}	-	-
	100	100.63 ± 9.58^{a}	74.01 ± 5.81^{b}	64.44 ± 2.07^{b}	21.70 ± 5.48^{a}	29.01 ± 4.00^{b}	-	-
+ Plants	0	n.d	n.d	$n.d^{b}$	-	-	-	
	1	1.39 ± 0.01^{a}	$0.57 \pm 0.00^{b*}$	$n.d^{b}$	-	-	63.75 ± 1.68^{aA}	93.74 ± 2.42^{bA}
	10	7.16 ± 0.95^{a}	$1.01 \pm 0.08^{b*}$	$n.d^{b}$	-	-	72.15 ± 1.30^{aB}	78.85 ± 6.04^{aB}
	100	100.63 ± 9.58^{a}	$2.47 \pm 0.17^{b*}$	$1.51 \pm 0.18^{b*}$	-	-	76.61 ± 3.21^{aB}	69.39 ± 2.44^{aB}
Egeria densa								
− Plants	0	n.d	n.d	n.d	n.d	n.d	-	-
	1	1.01 ± 0.51^{a}	0.97 ± 0.04^{a}	0.93 ± 0.04^{a}	3.12 ± 0.92^{a}	7.75 ± 0.64^{b}	-	-
	10	10.81 ± 0.05^{a}	9.16 ± 0.39^{a}	8.38 ± 0.43^{b}	15.14 ± 0.86^{a}	22.47 ± 0.92^{b}	-	-
	100	106.00 ± 3.91^{a}	85.75 ± 4.37^{a}	72.69 ± 1.69^{b}	$19.18 \pm 1,57^{a}$	31.24 ± 2.04^{b}	-	-
+ Plants	0	n.d	n.d	n.d	-	-	-	
	1	1.01 ± 0.51^{a}	$0.17 \pm 0.03^{b*}$	$0.01 \pm 0.01^{b*}$	-	-	75.03 ± 4.33^{bA}	90.36 ± 1.29^{bA}
	10	10.81 ± 0.05^{a}	$2.44 \pm 0.26^{b*}$	$n.d^{b*}$	-	-	$58.26 \pm 2.01^{aB\$}$	77.53 ± 0.92^{aB}
	100	106.00 ± 3.91^{a}	$12.08 \pm 1.33^{b*}$	$0.50 \pm 0.24^{b*}$	-	-	$66.61 \pm 3.01^{aB\$}$	68.08 ± 1.74^{aB}

Lowercase letters indicate significant differences between times of exposure within the same Cipro concentration and treatment systems ($P < 0.05$); a single asterisk (*) indicates significant differences between systems with (+ Plants) and without plants (− Plants) within the same Cipro concentration and time of evaluation ($P < 0.05$); uppercase letters indicate significant difference among Cipro concentrations within the same evaluation times ($P < 0.05$). A single dollar sign ($^{\$}$) indicates significant difference between phytoremediation efficiency of macrophytes by T test. n.d: not detected

tissues. Both, the capacity to tolerate and the ability to reclaim contaminants over time must be considered when selecting plants to reclaim contaminants (Adesanya et al. 2021).

In addition to their Cipro tolerance, *S. molesta* and *E. densa* plants demonstrated a great ability to reclaim that antibiotic from contaminated water, removing from 69 to 93% and 68 to 90% of the antibiotic in the media after only 168 h, respectively (Table 3). Although the phytoremediation efficiency did not differ between the two macrophytes species, *S. molesta* (floating) accumulated more Cipro in their tissues when compared to *E. densa* (submerged). After the uptake, organic compounds, such as antibiotics, may undergo partial/complete degradation or being transformed into other compounds (Zhang et al. 2014). Submerged macrophytes are particularly noted for their ability to transform and/or degrade organic contaminants, being used for phytotransformation or phytodegradation programs (Alonso et al. 2021; de Morais et al. 2019). de Morais et al. (2019) observed 93% removal efficiency (phytoremediation capacity) by diclofenac by *E. densa* and *Ceratophyllum demersum* (L.); however, only 8.9% of the total amount of the drug was phytoaccumulated, suggesting that plants realized phytotransformation or phytodegradation. Similarly, Alonso et al. (2021) observed the great phytoremediation capacity but low accumulation of the herbicide saflufenacil in plant tissues of *E. densa*. According to these authors, submerged macrophytes promote physico-chemical alterations in water, such as changes in water pH, which favor the uptake of contaminants and their metabolism by biological oxidation. Our data also indicate that *E. densa* may employ the mechanism of phytotransformation or phytodegradation of Cipro, while *S. molesta* phytoaccumulate the antibiotic. This is supported by the fact that plants presented similar phytoremediation capacity (removing similar amounts of Cipro from water) but distinct Cipro concentration in their tissues. Since *S. molesta* showed greater RGR than *E. densa*, if both plants showed similar rates of Cipro metabolism, a diluting effect resulting in lower Cipro concentration in plant tissues was expected, and, in contrast, greater Cipro concentration was found in *S. molesta* plants (Table 4). It is important to note that the metabolism of organic compounds can generated toxic subproducts (Zhang et al. 2014), which could in part explain the greater negative effects of Cipro observed in *E. densa* than in *S. molesta*. This topic merits more attention.

BCF is a measure of a plant's ability to accumulate contaminants, and when that value is greater than 1, the plant is classified as a hyperaccumulator (Mishra et al. 2017;

Table 4 Relative growth rates, ciprofloxacin concentrations and bioconcentration factors (BCF) of *Salvinia molesta* and *Egeria densa* plants exposed to Cipro for 96 and 168 ho. Values presented as mean ± standard error of four replicates

Exposure time (hour)	Treatments ($\mu g.L^{-1}$)	Relative growth rates (RGR)		Cipro concentration ($\mu g.g.DW^{-1}$)		Bioconcentration factor (BCF)	
		S. molesta	*E. densa*	*S. molesta*	*E. densa*	*S. molesta*	*E. densa*
96	Control	0.082 ± 0.008^{aA}	$0.021 \pm 0.002^{aA**}$	n.d	n.d	n,d	n.d
	1	0.098 ± 0.002^{bA}	$0.042 \pm 0.017^{aA***}$	6.41 ± 0.12^{aA}	$1.78 \pm 0.23^{aA***}$	4.47 ± 0.22^{aA}	$0.35 \pm 0.21^{aA***}$
	10	0.158 ± 0.020^{abA}	$0.083 \pm 0.036^{abA**}$	60.07 ± 4.97^{bA}	$8.96 \pm 0.81^{bA***}$	8.48 ± 0.96^{bA}	$0.84 \pm 0.10^{aA***}$
	100	0.274 ± 0.049^{abA}	$0.208 \pm 0.030^{bA*}$	114.35 ± 22.07^{cA}	$69.46 \pm 14.84^{cA***}$	1.12 ± 0.17^{cA}	$0.67 \pm 0.16^{aA***}$
168	Control	0.063 ± 0.005^{aB}	$0.017 \pm 0.001^{aB***}$	n.d	n.d	n,d	n.d
	1	0.066 ± 0.011^{aB}	$0.027 \pm 0.001^{aB**}$	7.60 ± 0.91^{aA}	$2.39 \pm 0.89^{aB***}$	5.42 ± 0.36^{aA}	$1.59 \pm 0.54^{aB***}$
	10	0.104 ± 0.002^{abB}	$0.022 \pm 0.002^{aB***}$	70.92 ± 3.73^{bA}	$24.47 \pm 5.02^{bB***}$	9.95 ± 0.57^{bA}	$2.21 \pm 0.35^{aB***}$
	100	0.124 ± 0.007^{bB}	$0.031 \pm 0.003^{aB***}$	145.90 ± 32.90^{cA}	$119.6 \pm 12.84^{cB**}$	1.18 ± 0.25^{cA}	1.14 ± 0.15^{aB}
Anova *F* values							
Cipro concentration		$F = 9.673$; $P < 0.001$	$F = 12.510$; $P = 0.002$	$F = 5.990$; $P = 0.01$	$F = 131.00$; $P < 0.001$	$F = 2.36$; $P < 0.001$	$F = 1.56$; $P > 0.05$
Time		$F = 8.550$; $P = 0.01$	$F = 26.190$; $P = 0.001$	$F = 0.052$; $P > 0.05$	$F = 6.42$; $P = 0.02$	$F = 4.459$; $P > 0.05$	$F = 14.69$; $P = 0.002$
Cipro concentration × time		$F = 3.620$; $P = 0.03$	$F = 9.987$; $P = 0.006$	$F = 0.009$; $P > 0.05$	$F = 0.009$; $P > 0.05$	$F = 2.693$; $P > 0.05$	$F = 1.04$; $P > 0.05$

Lowercase letters indicate significant differences among Cipro concentrations within the same exposure time, while uppercase letters indicate significant differences between exposure times within the same Cipro concentration ($P < 0.05$). *Indicate significant differences between species (*S. molesta and E. densa*) within the same Cipro concentration and time of evaluation (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

Yan et al. 2019b). We observed decreased BCF and phytoremediation capacities by *S. molesta* as Cipro concentrations increased in the media. BCF levels generally tend to decrease when substrate contaminant levels increase (Zhao et al. 2003). According to Pence et al.(2000), decreased BCFs may result from chemical uptake saturation and/or decreased root-to-shoot transport when internal contaminant concentrations are high. Upon uptake, pharmaceutical products are mainly transported from the roots to the shoots by passive diffusion, and compounds with high molecular weights, such as Cipro, can saturate the absorption capacity of plants (Xiong et al. 2017; Adesanya et al. 2021). Although the phytoremediation capacity of *S. molesta* decreased when exposed to high Cipro concentrations, the plants showed a high removal capacity (> 69.39) in addition to a BCF > 1—regardless of the exposure time or the Cipro concentration in the medium. The lower BCF observed for *E. densa* in relation to *S. molesta* (but similar removing capacity), indicate, once more, the possible phytodegradation or phytotransformation process employed by this species. Although studies have indicated Cipro degradation by plants, studies on the mechanisms of transformation and the toxicity of Cipro by-products are claimed (Yan et al. 2020, 2021).

Conclusion

Despite the physiological alterations, both macrophyte species presented tolerance mechanisms to avoid the deleterious effects of Cipro, such as the increase of antioxidant systems to avoid oxidative damages and growth reduction. Our results indicated that *S. molesta* (floating) and *E. densa* (submerged) are candidates for Cipro removal from contaminated water. Both macrophytes species are efficient at reclaiming the antibiotic (> 60%) even when at very high concentrations, such as those found in effluents from hospitals and pharmaceutical industries (concentrations varying from 2.3 to 341 $\mu g.L^{-1}$); the species showed phytoremediation efficiency of up to 90% with Cipro concentrations commonly found in surface waters and effluent/sewage treatment plants (concentrations varying from 0.018 to 82.8 $\mu g.L^{-1}$). Although the species did not differ from their phytoremediation capacity, they might employ different strategies to reclaim Cipro from contaminated water: while the floating species accumulate high concentrations in their tissues, the submerged species appears to transform and/or degrade the antibiotic. This has important implications for phytoremediation programs aiming Cipro removal from water. In the case of areas with easy access and management of the macrophyte biomass, *S. molesta* must be a good choice. Its biomass can be recovered and then used for bioenergy through direct combustion or for biogas or bioethanol production, avoiding the return of the contaminant to the environment (Kochi et al. 2020). In contrast, *E. densa* is indicated for the removal of Cipro mainly when the management of the produced biomass is difficult, and, apparently, by the biotransformation of the antibiotic, this species may favor its permanent removal from water. It is important, however, to evaluate the toxicity to aquatic organisms of the Cipro by-product produced by plants as well as their biomagnification through the aquatic web.

Author contribution Kitamura RSA, Gomes MP and Silva de Assis CS designed the experiments, and gave technical support and conceptual advice. Gomes MP, Silva de Assis HC, Brito JCM, Kitamura RSA performed the experiments, analyzed the data, and wrote the paper. All authors have reviewed and approved the manuscript.

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Data availability Data available on request from the authors.

Declarations

Competing interests The author declare no competing interests.

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