RESEARCH ARTICLE

Ecotoxicological effects and accumulation of ciprofloxacin in *Eichhornia crassipes* under hydroponic conditions

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Abstract



Antibiotic residues pose a threat to the health of aquatic organisms. The effects and accumulation of antibiotic ciprofloxacin (CIP) in a floating macrophyte (*Eichhornia crassipes*) under hydroponic conditions were investigated. It was found that *E. crassipes* exposure to CIP (< 1000 μ g L⁻¹) could maintain a stable photosynthesis efficiency. In response to CIP stress, catalase and peroxidase activities of leaves were 7.24–37.51 nmol min⁻¹ g⁻¹ and 98.46–173.16 U g⁻¹, respectively. The presence of CIP did not inhibit the growth of the plant. After 14 days of exposure, tender leaves became white and withered, ascribed to the decline of chlorophyll content and chlorophyll fluorescence parameters. The CIP concentrations, absorbed by *E. crassipes*, were highest in the roots, followed by white aerial parts and green aerial parts at CIP concentrations of 100 and 1000 μ g L⁻¹. These findings demonstrated that *E. crassipes* could absorb and tolerate CIP in a limited time-scale and imply an alternative solution for phytoremediation in water bodies contaminated with antibiotics.

Keywords Antibiotic · Eichhornia crassipes · Phytoremediation · Ciprofloxacin · Tolerance · Hydroponic

Introduction

Antibiotics are extensively used to prevent infectious diseases and as growth promoters in aquaculture and livestock farming (Kummerer, 2009; Chuang et al. 2015). Most antibiotics are poorly absorbed or metabolized by living organisms, and a considerable fraction are discharged into the natural environment (Sarmah et al. 2006). Aquatic environments are especially prone to antibiotic pollution, and antibiotics are frequently found in the oceans (Du et al. 2017), lakes (Xu et al. 2014), wastewater (Yang et al. 2017a, b), and drinking water (Odendaal et al. 2015). The concentrations of antibiotic in aquatic ecosystems, including fish and shrimp farms, range

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Guoxiang Wang wangguoxiang@njnu.edu.cn from μ g L⁻¹ to mg L⁻¹ in water (Kümmerer 2009; Thuy et al. 2011), and μ g kg⁻¹ to mg kg⁻¹ in sediment (Thuy et al. 2011; Xu et al. 2014). Antibiotic ciprofloxacin (CIP), one of the most commonly used antibiotics, is frequently detected in aquatic environments, due to its high stability and resistance to degradation (Maul et al. 2006; Picó and Andreu 2007). Its residues not only pose a threat to living organisms but also accumulate in their bodies (Jia et al. 2017; Song et al. 2017), and the induced antibiotic resistance genes in contaminated matrices harm human health (Yan et al. 2013; Yang et al. 2017a, b). Therefore, the effective removal of CIP deserves to receive more attentions.

Recently, advanced technologies, including advanced oxidative processes, activated carbon adsorption, and membrane filtration, have been successfully applied with high removal efficiencies (Elmolla and Chaudhuri 2010; Fu et al. 2017; Sharma et al. 2017). However, they are not widely employed in full-scale due to high cost and secondary pollution. Phytoremediation is one of the most effective, low-cost, and ecologically safe technologies available to remove antibiotics in contaminated water via uptake, transformation, assimilation, or degradation (Gujarathi et al. 2005; Michelini et al. 2012; Thuy et al. 2013; Dan et al. 2013). For example, it has been shown that up to 99.5% sulfonamides can be removed by

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three kinds of Italian ryegrass (*Lolium multiflorum Lam.*), including *Dryan*, *Tachimasari*, and *Waseyutaka* in swine wastewater (Xian et al. 2010). Removal percentages of 73.1–74.8% for sulfamethoxazole by different plant species were also observed in constructed wetlands (Liang et al. 2018). Therefore, antibiotics can be effectively removed via phytoremediation.

Before using phytoremediation, it is necessary to ascertain the antibiotic effects on aquatic plants during the phytoremediation processes. Till date, only few studies have reported that elevated CIP concentration found in the surface water has adverse effects on the biomass of Lemna minor at 499 μ g L⁻¹ (Ebert et al. 2011), and on the chlorophyll fluorescence of Azolla filiculoides at 3.05 mg L^{-1} (Gomes et al. 2018). In order to find suitable aquatic macrophytes for antibiotic removal of contaminated water bodies, it is necessary to first clarify the effects and accumulation processes of potential species. Antibiotic toxicity can be reflected by the physiological and biochemical responses of plants (Gomes et al. 2019), and in some cases, organic chemical pollutants not only induce stress signals to plants but also inhibit their accumulation (Susarla et al. 2002; Madikizela et al. 2018). Therefore, phytotoxic studies are crucial in the assessment of appropriateness of phytoremediation for specific plant species.

The floating macrophyte *E. crassipes* possesses most of the characteristics required for application in phytoremediation, including high production of renewable energy and spread root apparatus (Rezania et al. 2015; Guna et al. 2017). In this study, the possibility of employing *E. crassipes* in phytoremediation for CIP removal was investigated. Its tolerance to CIP was explored, reflected via chlorophyll content, chlorophyll fluorescence parameter, antioxidative enzymes, and root activity. Additionally, the bioaccumulation and translocation of CIP in *E. crassipes* were evaluated. These findings will provide a deep understanding and alternative solutions with phytoremediation in antibiotic-contaminated water bodies.

Materials and methods

Chemicals

Ciprofloxacin (98%, pure grade, CAS No.86393-32-0) was used in this study (Shanghai Macklin Biochemistry Co., Ltd, China). Acetonitrile and methanol (HPLC grade) was obtained from Tedia Company (Fairfield, OH, USA). All other reagents are of analytical reagent grade. Milli-Q water was purchased from a Milli-Q water gradient system (Millipore, Bedford, MA, USA).

Experimental design and setup

The experiment was carried out in the Experimental Platform for Ecological Remediation at Nanjing Normal University (32° 6' 27 N, 118° 54' 19" E), inside a glass greenhouse with abundant supply of light. E. crassipes was transplanted from an uncontaminated pond located in the campus of Nanjing Normal University. Prior to the experiment, ciprofloxacin was not detected in the plants that were cultured for 1 week in 1/2 modified Hoagland nutrient solution for acclimatization. The composition of nutrient solution (mg L^{-1}) is as follows: MgSO₄·7H₂O, 490; KNO₃, 510; Ca(NO₃)₂·4H₂O, 1180; KH₂PO₄, 140; Fe-citrate, 0.02; MnCl₂·4H₂O, 1.81; ZnSO₄· 7H₂O, 0.22; CuSO₄·5H₂O, 0.08; HBO₃, 2.86; and H₂MoO· 4H₂O, 0.09. The pH of nutrient solution was adjusted to 6.0 with 0.05 mol L^{-1} HCl. E. crassipes was washed thoroughly with tap water followed by deionized water, before being transferred to plastic barrel (0.58 m height \times 0.5 m diameter) filled with 100 L of water at pH 6.71. They were of approximately equal weights: 75.17 g of fresh plants with average root length 16.87 cm and leaf width 9.38 cm was planted in each pot. Each plastic barrel contained three plants, constituting three replicates and irrigated with per liter of 1/2 Hoagland nutrient solution. The surface of each container was covered with silver paper to prevent photochemical degradation of antibiotics: a complete randomized block design in triplicate with three barrels along with the concentrations of CIP at 0 (control), 10, 100, and 1000 μ g L⁻¹. During the experiment, the average water temperature was 25.1 °C, and the photoperiod was 12 h. Deionized water was added to balance water volume. Chlorophyll content, chlorophyll fluorescence parameter, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) were measured on the 7th and 14th day. CIP distributions in both aerial parts and roots of the plants on the 14th day were measured and compared. In addition, the aerial parts were divided into green and white parts to evaluate their differences.

Physiological parameters of E. crassipes

Chlorophyll contents (Chl a, Chl b, and total Chl) of leaves were measured according to the method of Huang et al. (2004). Plant leaves were weighed to 0.1 g, cut into chips, and incubated in 80% (v/v) aqueous acetone for 24 h in dark. Absorbance of solution was measured using spectrophotometer (UV-2500, Shimadzu, Japan) at 663 and 645 nm, and the total chlorophyll content was calculated. Chlorophyll fluorescence parameters were measured using a plant efficiency analyzer (Hansatech Co., UK). All the parameters including baseline (F_0), maximum (F_m), photochemical efficiency of photosystem II (F_v/F_m), and potential photochemical efficiency (F_v/F_0) were measured after 20 min of dark adaptation. SOD, POD, and CAT activities were measured according to Xu et al. (2010). Plant root activity was analyzed by the triphenyl tetrazolium chloride

(TTC) method Li (2000). Roots were weighed to 0.5 g and placed into tubes filled with 5 mL of 0.4% TTC and 5 mL of phosphate buffer (0.06 mol 1⁻¹, pH 7.0). The tubes were incubated at 37 °C for 3 h. The chemical reaction was stopped with the addition of 2 mL of 1 mol L^{-1} sulfuric acid in the tubes. This step was followed by extraction with triphenyl formazan (TPF). The roots were then transferred to a mortar and ground with a pestle. Four milliliters of ethylacetate and little amount of quartz sand were added to the mortar. After grinding, the extraction was deposited at room temperature for half an hour. The liquid phase was put in the test tube. Ethylacetate was added up to 10 mL, and optical density was recorded using a UV-VIS recording spectrophotometer at 485 nm. The optical density was used to calculate equivalent TPF concentrations that determined the root activity for each of the fresh root mass, and the root activity was expressed in μg (TPF) g^{-1} (FM) h^{-1} .

Antibiotic analysis of plant samples

High-performance liquid chromatography (HPLC) was used to analyze the CIP concentrations of plant samples. The aerial parts and roots of plants were frozen at -18 °C, and then freeze-dried for 72 h and finally weighed to 1.0 g and ground with a sterile pestle. Plant samples were added into centrifuge tubes with 20 mL Na₂ EDTA-Mellvaine buffer (pH = 3.0). The tubes were shaken using vortex generator for 30 s, sonicated for 10 min, and centrifuged at 8000 r min⁻¹ for 10 min. The extraction process of each sample was repeated three times in a constant volume of Milli-Q water up to 200 mL. The samples of 200 mL were extracted using Waters Oasis HLB extraction cartridge (500 mg, Waters, Milford, MA). The extraction cartridge was sequentially pre-conditioned with 6.0 mL methanol, 6.0 mL Milli-Q water, and 6.0 mL 10 mM L⁻¹ Na₂ EDTA- Environ Sci Pollut Res (2019) 26:30348-30355

Mellvaine buffer (pH = 3.0). The extraction rate was 5 mL min⁻¹. Subsequently, the cartridge was rinsed with 10 mL Milli-Q water and the cartridge was eluted with 6.0 mL methanol. Finally, the target fraction was dissolved in 40% methanol solution to make the volume up to 1.0 mL. The target antibiotics were analyzed using AgilentTM 1100 series HPLC equipped with DAD operated at a wavelength of 278 nm and a Zorbax 300SB-C18 column (4.6 mm \times 150 mm, 5 μ m). The mobile phase used acetonitrile and 0.025 mol L^{-1} phosphoric acid solution (15:85, v/v) at the flow rate of 1.0 mL min⁻¹. The column oven temperature was set at 30 °C, and the injection volume was 20 µL. Quantification of target analyte was based on external calibration curves, and correlation coefficients (R^2) of the calibration curves used were 0.999. The recovery efficiencies were 74.8%. Limits of quantification (LOO) of the antibiotics were calculated with signal/noise ratios of 10. LOQ of the samples were 35.5 ng L^{-1} . Bioconcentration factors were calculated using the formula:

leaf bioconcentration factors (LCF)

 $= C_1/C_w$, root bioconcentration factors (RCF) $= C_r/C_w$

where C_1 and C_r are average CIP concentrations in the aerial parts and roots of *Eichhornia crassipes*, respectively, and C_w is the CIP concentration in the water.

Statistical analysis

Data was analyzed using the SPSS package (version 19.0). One-way ANOVA was used to test the significant differences between treatments. The mean values of different treatments were compared with LSD test at the significance level of 0.05.

Fig. 1 Effects of CIP concentrations on chlorophyll content in leaves. Bars denote standard errors (n = 6). The a and b denote the significant differences among different treatments based on LSD ($p \le 0.05$). The A and B denote the significant differences among same treatments based on LSD ($p \le 0.05$). The red column represents the chlorophyll content on the 7th day; the black column represents the chlorophyll content on the 14th day



Results and discussion

Influence of CIP on leaf physiological characteristics

Measurement of chlorophyll

The chlorophyll content of plants, considered an indicator of pollutant-induced plant stress, is an important parameter for assessing photosynthetic activity (Huang et al. 2004). The total chlorophyll content on the 7th day under CIP treatments of 0, 10, 100, and 1000 μ g L⁻¹ was 3.37, 3.88, 3.36, and 3.06 mg g⁻¹, respectively (Fig. 1). The test results indicate that the total chlorophyll content was found to be increased under relatively low antibiotic concentrations, whereas high antibiotic concentrations reduced the total chlorophyll content due to the hormesis of CIP (Wan et al. 2014). According to Zhou et al., it was found that chlorophyll biosynthesis is promoted at low concentrations of antibiotics by affecting the nucleic acid and protein content in cells, and that antibiotics reduce chlorophyllase activity, which disturbed chlorophyll degradation, and increases chlorophyll content. The increased chlorophyll content in cells can serve as a protective mechanism to scavenge the accumulated reactive oxygen species in chloroplasts (Kasahara et al. 2002). However, on the 14th day, chlorophyll a and b as well as total chlorophyll content were significantly reduced. The results obtained showed that, in treated plants, antibiotics cause the tender leaves to turn from green to white in color. The effect of antibiotics on chlorophyll content was dependent upon the exposure time of antibiotics. It attributed the inhibition of CIP to chloroplast-specific enzyme activity: fluoroquinolone antibiotics mediate their action as quinone site inhibitors in photosystem II, which is a key enzyme in photosynthetic electron transport (Wall et al. 2004; Evansroberts et al. 2011).

Measurement of chlorophyll fluorescence parameters

Physiological responses in photosynthesis metabolism of E. crassipes were seen to be modulated with CIP, where it is known to inhibit photosynthesis (Wall et al. 2004), the chlorophyll fluorescence kinetics of plants were monitored to evaluate the effects of antibiotics on photosynthesis metabolism. The $F_{\rm m}$ values, an indicator of the total amount of plastoquinones, showed not significant difference between the control and CIP treatments on the 7th day (Table 1), indicating that plastoquinone biosynthesis is not a target of antibiotics at the initial cultivation. However, after 14 days of exposure, there was a decrease in F_v/F_m , representing maximal photochemical efficiency of PS II and PS II integrity (Walter et al. 2003), in E. crassipes upon CIP with long exposure time. This indicated that maximal PS II photochemical yield was disturbed and CIP had deleterious effects on its integrity (Gomes et al. 2017). All these observations demonstrated that E. crassipes cannot tolerate with longer exposure to CIP.

concentations F_0 F_m $F_{\sqrt{F_m}}$ $F_{\sqrt{F_0}}$ F_0 F_m $F_{\sqrt{F_m}}$ F_{F_m	a a sa a su tura ti a sa a	•				¢			
Control 178.25 ± 22.48^{aA} 502.00 ± 105.28^{aA} 0.63 ± 0.04^{aA} 1.79 ± 0.34^{aA} 162.00 ± 21.46^{aA} 659.62 ± 120.69^{aA} 0.75 ± 0.01^{aB} $3.05 \pm 10.02 \pm 120.61^{aA}$ 10 235.71 ± 26.74^{bA} 661.28 ± 97.16^{abA} 0.64 ± 0.04^{aA} 1.81 ± 0.39^{aA} 32.44 ± 13.35^{bB} 39.88 ± 20.47^{bB} 0.14 ± 0.10^{bB} 0.18 ± 0.18^{bB}	concenuauons (µg L ⁻¹)	F_0	$F_{ m m}$	$F_{\rm V}/F_{ m m}$	$F_{\rm v}/F_0$	F_0	$F_{ m m}$	$F_{ m v}/F_{ m m}$	$F_{\rm v}\!/F_0$
$10 \qquad 235.71 \pm 26.74^{bA} \qquad 661.28 \pm 97.16^{abA} \qquad 0.64 \pm 0.04^{aA} \qquad 1.81 \pm 0.39^{aA} \qquad 32.44 \pm 13.35^{bB} \qquad 39.88 \pm 20.47^{bB} \qquad 0.14 \pm 0.10^{bB} \qquad 0.18 \pm 0.18^{bB} \qquad 0.18 \pm 0.10^{bB} \qquad 0.18 \pm 0.18^{bB} \qquad 0.18 \pm 0.10^{bB} \qquad 0.18 \pm 0.10^{b$	Control	178.25 ± 22.48^{aA}	502.00 ± 105.28^{aA}	0.63 ± 0.04^{aA}	1.79 ± 0.34^{aA}	$162.00 \pm 21.46^{\rm aA}$	$659.62 \pm 120.69^{\mathrm{aA}}$	0.75 ± 0.01^{aB}	$3.05\pm0.28^{\mathrm{aF}}$
	10	235.71 ± 26.74^{bA}	661.28 ± 97.16^{abA}	$0.64\pm0.04^{\rm aA}$	1.81 ± 0.39^{aA}	32.44 ± 13.35^{bB}	$39.88\pm20.47^{\rm bB}$	$0.14\pm0.10^{\rm bB}$	$0.18\pm0.15^{\rm bI}$
$100 \qquad 214.57 \pm 25.20^{abA} \qquad 739.00 \pm 159.44^{bA} \qquad 0.70 \pm 0.05^{aA} \qquad 2.44 \pm 0.63^{aA} \qquad 22.15 \pm 4.88^{bB} \qquad 24.35 \pm 5.20^{bB} \qquad 0.09 \pm 0.05^{bB} \qquad 0.10 \pm 0.05^{bB} \qquad 0.10 \pm 0.05^{bB} \qquad 0.10 \pm 0.05^{bB} \qquad 0.10 \pm 0.05^{bB} \qquad 0.00 \pm 0.05^{b$	100	214.57 ± 25.20^{abA}	$739.00 \pm 159.44^{\rm bA}$	0.70 ± 0.05^{aA}	2.44 ± 0.63^{aA}	22.15 ± 4.88^{bB}	24.35 ± 5.20^{bB}	$0.09\pm0.05^{\rm bB}$	$0.10\pm0.61^{\rm bI}$
$1000 206.8 \pm 48.44^{abA} 585.20 \pm 137.94^{abA} 0.65 \pm 0.05^{aA} 1.85 \pm 0.53^{aA} 19.66 \pm 3.04^{bB} 22.11 \pm 3.37^{bB} 0.11 \pm 0.03^{bB} 0.12 \pm 0.12^{bB} 0.1$	1000	206.8 ± 48.44^{abA}	585.20 ± 137.94^{abA}	$0.65\pm0.05^{\rm aA}$	$1.85\pm0.53^{\rm aA}$	$19.66\pm3.04^{\rm bB}$	$22.11\pm3.37^{\rm bB}$	$0.11\pm0.03^{\rm bB}$	$0.12 \pm 0.45^{\rm bI}$

Effects of CIP on chlorophyll fluorescence parameters

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m ≤ 0.05). The A and different treatments based on LSD (p The a and b denote the significant differences among Bars denote standard errors (n = 6). treatments based on LSD ($p \le 0.05$)

Resistance to antioxidant enzymes

Photosynthetic organisms counteract antibiotic toxicity induced by accumulated reactive oxygen species in cells by changing their antioxidative enzymes' defense system (Xiong et al. 2017). SOD is viewed as the first line of defense against reactive oxygen species damage. It catalyzes the dismutation of the superoxide radicals to H₂O₂ and O₂, keeping low the levels of superoxide radicals in cells. Herein, the SOD activities on the 7th day were found to be lower than the control, 20.81, 42.39, and 46.1 U g^{-1} under CIP concentrations of 10, 100, and 1000 μ g L⁻¹, respectively (Fig. 2a). It was found to decline along with the increasing antibiotic concentrations, in agreement with previous observations of Phragmites australis exposed to CIP (Liu et al. 2013). But there was no significant difference between treatments, since the binding of fluoroquinolones probably induced structural change in SOD instead of its activity (Cao et al. 2015). However, the SOD activities on exposure to CIP on the 14th day were found to be significantly lower than those on the 7th day due to the oxidative stress by CIP with exposure time (Carreras et al. 2004; Xu et al. 2010; Talla and Veerareddy 2011). CAT is an enzyme that decompose H_2O_2 into H_2O and O₂ (Gomes et al. 2017). In this study, CAT activities increased

Fig. 2 Enzyme activity of SOD, CAT, and POD in leaves. Bars denote standard errors (n = 6). The different lowercase letters denote the significant differences among different treatments based on LSD ($p \le 0.05$). The capital letters denote the significant differences among same treatments with different times based on LSD ($p \le 0.05$)

along with CIP concentrations at the beginning of cultivation (Fig. 2b), which attributed to the better acclimation of plants. However, after 14 days of exposure, CAT was found to be lower on the 7the day. CAT is a photosensitizer, and it is likely inactive in white leaf cells because of the fewer photosynthetic pigments (Smirnoff 1995). POD activities gradually increased with CIP concentrations during cultivation (Fig. 2c). After 14 days of exposure, they were significantly higher than those on the 7th day with their values of 21.55, 258.73, and 251.02 U g^{-1} under CIP concentrations 10, 100, and 1000 µg L⁻¹, respectively. Due to the spatial distribution of POD at cytosol, vacuole, and extra-cellular components of plant tissues (Liu et al. 2013), POD activities increased to keep the balance of H₂O₂, produced by the action of SOD on superoxide radicals and the generation of OH via a Haber-Weiss reaction during the process of photorespiration. Therefore, the different SOD, CAT, and POD activities may be related to the potential enzyme toxicity of CIP to antioxidative enzymes.

Response of root activity to CIP

Root activity is regarded as an indicator to assess the growth of plant roots (Liu et al. 2013). When compared with the control, the root activity with 10 μ g L⁻¹ of CIP was



Antibiotics concentrations in solution ($\mu g L^{-1}$)



Fig. 3 Root activity of *Eichhornia crassipes* with four treatments. Bars denote standard errors (n = 3). The a and b denote the significant differences among different treatments based on LSD ($p \le 0.05$)

significantly higher (Fig. 3), while it decreased by 77.58 and 177.71 μ g g⁻¹ at 100 and 1000 μ g L⁻¹. Similar to other studies (Li et al. 2011; Liu et al. 2013), root activity declined with high antibiotic concentrations, because antibiotics influenced folic acid synthesis and catalyzed ATP-dependent DNA supercoiling to prevent the growth of new cells in plant roots (Wall et al. 2004; Aristilde et al. 2014). However, the division of cells in maize roots was not disrupted by CIP (Gomes et al. 2019) due to the difference of different plant species.

Accumulation and translocation of CIP in E. crassipes

The level of CIP accumulated in *E. crassipes* was closely related to its concentrations. The residues in roots were 2.46, 32.79, and 1050.68 μ g g⁻¹ under CIP treatments of 10, 100, and 1000 μ g L⁻¹, respectively (Table 2). CIP migrated from the roots to aerial parts through transpiration (Dettenmaier et al. 2008). The maximal concentrations of CIP in the green and white aerial parts were 8.34 and 84.24 μ g g⁻¹, respectively. CIP accumulated the most in roots, the primary point of contact with CIP, through physicochemical absorption and biological uptake. CIP concentrations in the aerial parts were less than the roots, since antibiotics might be degraded via

photolysis in the aerial parts receiving enough light (Babić et al. 2013).

Bioconcentration factors, reflecting the bioaccumulation of organic compounds in the organisms, are more important than concentrations in plant tissues when evaluating the phytoremediation potential of a given species (Starkov and Fiskum 2001; Azanu et al. 2016). The bioconcentration factors of *E. crassipes* followed the order of RCF > LCF (white) > LCF (green) with 100 and 1000 μ g L⁻¹ of CIP. In E. crassipes, LCF of green and white aerial parts decreased with the increasing CIP concentrations, as opposed to RCF. The trend of LCF indicated a diminishing efficiency of uptake and accumulation due to physiological changes occurring at these chemical levels (Starkov and Fiskum 2001; Gomes et al. 2013). RCF was lower than LCF (green and white) with 10 µg L^{-1} of CIP, possibly related to the low biological activities of plants induced by the high CIP concentrations. Therefore, the high migration rate generally occurred at low CIP concentration.

The absorption of pharmaceutical products by aquatic plants has been well reported (Malakootian et al. 2015; Gomes et al. 2017) and has motivated investigations for using plants as potential green technologies that could purify aquatic environments. Generally, in natural aquatic environments, antibiotic concentrations are relatively low. The floating macrophyte *E. crassipes* can take up antibiotics from contaminated waters for a relatively longer period of time. However, under high antibiotic concentrations, e.g., wastewater treatment plants effluents, hospital sewage water, or pharmaceutical manufactures effluents, the growth of *E. crassipes* and the antibiotic absorption are time-limited; thus, further management of harvest is necessary for the restoration of antibiotic contaminated waters.

Conclusions

Antibiotic CIP–contaminated water did not affect the growth and survival of *E. crassipes* at the initial stage, as evidenced with the monitoring of chlorophyll content, chlorophyll fluorescence parameter, and antioxidative enzymes. However, there were morphological changes being observed during

 Table 2
 Accumulation and translocation of CIP in *Eichhornia crassipes*

	Treatment concentrations ($\mu g L^{-1}$)	Aerial parts (green) $(\mu g g^{-1})$	ACF	Aerial parts (white) $(\mu g g^{-1})$	ACF	Root ($\mu g g^{-1}$)	RCF
CIP	Control 10 100 1000	ND 5.94 ± 1.81^{a} 8.25 ± 2.85^{a} 8.34 ± 2.56^{a}	$- \\ 0.59 \pm 0.18 \\ 0.08 \pm 0.02 \\ 0.008 \pm 0.00$	$ \begin{array}{l} ND \\ 10.49 \pm 1.45^a \\ 25.59 \pm 9.19^a \\ 84.24 \pm 1.38^b \end{array} $	$- \\ 1.04 \pm 0.14 \\ 0.25 \pm 0.09 \\ 0.08 \pm 0.00$	ND 2.46 \pm 0.36 ^a 32.79 \pm 4.20 ^a 1050.68 \pm 55.65 ^b	$- \\ 0.24 \pm 0.03 \\ 0.32 \pm 0.04 \\ 1.05 \pm 0.05$

Bars denote standard errors (n = 6). The a and b denote the significant differences among different treatments based on LSD ($p \le 0.05$). ND stands for not detected

tender leaves gradually turned white after long exposure to CIP, caused by the decrease of photosynthetic pigments and antioxidative enzyme activities. In addition, CIP was absorbed with *E. crassipes* in a dose-response scenario. The initial elevated external concentrations stimulated the antibiotic absorption by *E. crassipes*. The roots, instead of leaves, were found to be the main CIP absorber. The time-scale regulation of *E. crassipes* must therefore needs be considered for the phytoremediation of antibiotic-polluted waters.

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