Enantioselective accumulation, metabolism and phytoremediation of lactofen by aquatic macrophyte *Lemna minor*

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ABSTRACT

Pesticides are frequently detected in water bodies due to the agricultural application, which may pose impacts on aquatic organisms. The enantioselective bioaccumulation and metabolism of the herbicide lactofen in aquatic floating macrophyte *Lemna minor* (*L. minor*) were studied and the potential *L. minor* phytoremediation was investigated. Ultra-high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS-MS) analysis for lactofen and its two known metabolites in *L. minor* was performed. The initial concentrations of racemic lactofen, R-lactofen and S-lactofen were all $30\,\mu g\,L^{-1}$ in the growth solution. The distribution of lactofen and its metabolites in growth solution and *L. minor* was determined throughout a 5-d laboratory trial. It was observed that S-lactofen was preferentially taken up and metabolized in *L. minor*. After rac-lactofen exposure, the accumulation amount of S-lactofen was approximately 3-fold more than that of R-lactofen in *L. minor* and the metabolism rate of S-lactofen ($T_{1/2} = 0.92$ d) was significantly faster than R-lactofen ($T_{1/2} = 1.55$ d). *L. minor* could only slightly accelerate the metabolism and removal of lactofen in the growth solution. As for the metabolites, desethyl lactofen was found to be the major metabolite in *L. minor* and the growth solution, whereas the metabolite acifluorfene was undetectable. No interconversion of the two enantiomers was observed after individual enantiomer exposure, indicating they were configurationally stable. The findings of this work represented that the accumulation and metabolism of lactofen in *L. minor* were enantioselective, and *L. minor* had limited capacity for the removal of lactofen and its metabolite in water.

1. Introduction

The widespread use of pesticides for agricultural application has contributed to the contamination of the environment. Pesticides have been detected worldwide in water due to the surface runoff and leaching (Ruff et al., 2015). Water contamination may affect human health via drinking water or food and pose influence on aquatic organisms directly. So development of cheap and effective methods to decrease or remove pollutants from water is encouraged. Among the techniques used for removal, phytoremediation has emerged as a cost-effective technology which only uses living plants for in situ cleanup of a variety of inorganic and organic pollutants from water (Ziegler et al., 2016).

Lemnaceae family is a common free-floating macrophyte that generally grows in quiescent waters. As a major producer, duckweed is the first organism exposed to pollutants in the aquatic environment. Therefore, duckweed have functioned as a useful biomonitor of contaminants in ecotoxicological research (Appenroth et al., 2010). On the other hand, plants are contributed to the bioaccumulation,

biotransformation and removal of xenobiotics from contaminated water. In fact, duckweed have been served as a good option for phytoremediation because of its fast growth rate and easy harvest. Previous research has shown that duckweed plays an important role in the removal of pharmaceuticals (Garcia-Rodriguez et al., 2015), pesticides (Olette et al., 2008), dyes (Khataee et al., 2012), petroleum hydrocarbons (Kösesakal et al., 2016), cytotoxins (Kaminski et al., 2014), heavy metals (Sasmaz et al., 2015) and minerals (Tatar and Obek, 2014). *L. minor*, one of the most common species of duckweed, has been found to have superior potential to remove xenobiotics compounds from water. For instance, among five aquatic macrophytes (*L. minor, Spirodela polyrhiza, Cabomba aquatica, Callitriche palustris* and *Elodea canadensis*), *L. minor* showed the best removal efficiency for dimethomorph and pyrimethanil from contaminated water (Dosnon-Olette et al., 2009). Moreover, Török et al. reported that *L. minor* possessed higher heavy metal phytoremediation capacity than *Elodea canadensis* and *Salvinia natans* (Török et al., 2015). *L. minor* also has been identified as an efficient arsenic (As) accumulator, which could accumulate 735 mg/kg of As in the leaves (Singh et al., 2016).
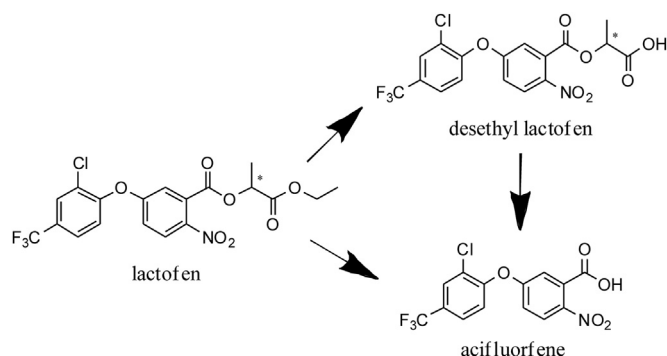
Fig. 1. Chemical structures of lactofen and the two metabolites.

Some current-use chiral pesticides are composed of one or more pairs of mirror enantiomers which have almost the same physicochemical properties and abiotic degradation rates. However, the toxicity and biodegradation rates of the enantiomers may be significantly different to each other because of their interaction with natural chiral molecules, such as biological receptors (Wong, 2006). For instance, the insecticidally active R-fipronil showed lower toxicity to aquatic organism *Anodonta woodiana* (*A. woodiana*) and faster degradation than S-fipronil in *A. woodiana* (Qu et al., 2016). Therefore, evaluating environmental impacts of individual enantiomers is required for comprehensive risk assessments.

Lactofen (Fig. 1), a broad-spectrum herbicide recommended for post-emergence use in soybeans, peanuts and rice, is a protoporphyrinogen oxidase inhibitor which causes lipid peroxidation of cell membrane (Orr and Hess, 1982). Due to surface runoff and leaching, lactofen may end up in surface water. So there is an increasing concern about the environmental fate and potential risk for non-target aquatic organisms. Research has shown that lactofen is highly toxic to aquatic organisms. The EC_{50} values for *L. minor* (Wang et al., 2016), *Scenedesmus obliquus* (Cheng et al., 2015)and *Daphnia magna* (Diao et al., 2010) are $100\,\mu g\,L^{-1}$, $0.784\,\mu g\,L^{-1}$, and $4.308\,mg\,L^{-1}$ respectively. Besides, lactofen metabolism has been investigated in primary rat hepatocytes (Wang et al., 2013), morningglory (Jeffery et al., 1988), soil (Diao et al., 2009) and sediment (Diao et al., 2010). Desethyl lactofen and acifluorfene are the most commonly detected metabolites (Diao et al., 2010; Wang et al., 2013). Nevertheless, no information is available about the metabolism of lactofen in aquatic photosynthetic plants.

This work was undertaken to study the fate of lactofen in aquatic floating macrophyte *L. minor* on an enantiomeric level and thus to assess the potential phytoremediation. *L. minor* was cultivated in the growth solution and exposed to lactofen. Racemate exposure experiment was performed to analyze the enantioselectivity of lactofen and individual enantiomer exposure was carried out to verify the enantioselective behavior of the individual enantiomer and the interconversion of the two enantiomers. The contents of lactofen and its metabolites (desethyl lactofen and acifluorfene) were measured both in *L. minor* and the growth solution by ultra-high performance liquid chromatography – tandem mass spectrometry (UHPLC-MS-MS).

2. Materials and methods

2.1. Test chemicals

Racemate of lactofen (rac-lactofen, purity 95%) and acifluorfene (purity 96%) were obtained from Qingdao Hansheng Biotechnology Incorporated Company (Qingdao, China). S-lactofen, R-lactofen (both 95%, optical purity 98%) and desethyl lactofen (purity 98%) were synthesized according to the literature (Diao et al., 2010). Working solutions of each compound were prepared in acetone just prior to the

experiment. All the reagents were HPLC-grade and purchased from Fisher Scientific (Fair Lawn, NJ). Florisil Solid Phase Exaction (SPE) cartridges (1000 mg, 6 mL) were purchased from Agilent (Palo Alto, CA, USA). Water was purified by a Milli-Q water purification system.

2.2. Plant material and pre-cultivation

Plants (*Lemna minor L.*) were cultured and gathered from an artificial fresh water pond owned by Zhuwen Gardening Company, Ningbo, Zhejiang, China. The *Lemna* species was identified through morphology observation. This plant had one, two or three green oval-shaped fronds each having a single root hanging in the water. And fronds also had three unconspicuous veins. We also studied root sheath and root apex under a microscope and discovered the sheath was not winged, and apex was mostly rounded. After being washed three times with distilled water, *Lemna minor L.* was transferred into a modified Hoagland nutrient solution containing $506\,mg\,L^{-1}$ KNO_3, $493\,mg\,L^{-1}$ $MgSO_4 \cdot 7H_2O$, $136\,mg\,L^{-1}$ KH_2PO_4, $13.9\,mg\,L^{-1}$ $FeSO_4 \cdot 7H_2O$, $18.65\,mg\,L^{-1}$ $EDTANa_2$, $0.025\,mg\,L^{-1}$ $CuSO_4 \cdot 5H_2O$, $0.25\,mg\,L^{-1}$ $Na_2MoO_4 \cdot 2H_2O$, $80\,mg\,L^{-1}$ NH_4NO_3, $0.83\,mg\,L^{-1}$ KI, $6.2\,mg\,L^{-1}$ H_3BO_3, $22.3\,mg\,L^{-1}$ $MnSO_4 \cdot 4H_2O$, $8.6\,mg\,L^{-1}$ $ZnSO_4 \cdot 7H_2O$, $0.025\,mg\,L^{-1}$ $CoCl_2 \cdot 6H_2O$, $945\,mg\,L^{-1}$ $Ca(NO_3)_2 \cdot 4H_2O$. Before the experiment, *Lemna minor L.* was cultured in a growth chamber at $25 \pm 1\,°C$ and supplied with light from fluorescent tubes at irradiance of $100\,\mu mol\,m^{-2}\,s^{-1}$ (TLD 36 W/54, Philips, China) in a 12 h light (9 a.m. to 9 p.m.)/12 h dark (9 p.m. to 9 a.m.) cycle for two weeks.

2.3. Experiment design

The aquatic plants (30 g fresh weight) were transferred in a glass container (42 cm long, 30 cm wide and 30 cm high) filled with 6 L of nutrient solution under the same condition as the pre-cultivation. This container is sufficiently large and the plant has enough room for its reproduction. Separate treatments were spiked with rac-lactofen, R-lactofen and S-lactofen work solution in acetone to obtain an initial concentration of $30\,\mu g\,L^{-1}$ in the growth solution, respectively. Controls (without *Lemna minor L.*) were also done for rac-lactofen, R-lactofen and S-lactofen at spike level of $30\,\mu g\,L^{-1}$. All the treatments and controls were triplicated. The experiment was conducted under static conditions and carried out over 5 days. Growth solution (without pesticide) was added regularly to compensate for the water lost due to evaporation. Plant and solution samples were collected after 3 h (12 a.m.), 6 h (3 p.m.), 9 h (6 p.m.), 12 h (9 p.m.), 15 h (12 a.m.), 1 d (9 a.m.), 36 h (9 p.m.), 2 d (9 a.m.), 3 d (9 a.m.) and 5 d (9 a.m.) of exposure.

2.4. Extraction and cleanup

The solution sample (5 g) was transferred to a centrifuge tube and extracted by adding 5 mL of ethyl acetate. The mixture was vortexed for 3 min and centrifuged for 5 min at 3800 rpm. The same extraction procedure was repeated, and the organic phase was combined and dried through a gentle stream of nitrogen. Finally, the residue was dissolved in 0.5 mL of methanol and then filtered through a 0.22 µm membrane before analysis.

The plant samples (2g, fresh weight) were ground with a ball grinder for 3 min and extracted twice with 5 mL of ethyl acetate with vortex for 3 min and centrifugation at 3800 rpm for 5 min. The supernatants were collected and concentrated. Then the extract was purified by Solid Phase Exaction (SPE) using Florisil column. Different purification methods were used to elute lactofen and its metabolites separately. Lactofen was eluted with 7 mL of n-hexane: ethyl acetate: trifluoroacetic acid=19/1/0.1, and metabolites were eluted with 7 mL of n-hexane: ethyl acetate: trifluoroacetic acid=3/1/0.1. The eluted extracts were both evaporated under a gentle stream of nitrogen and dissolved in 0.2 mL of methanol. The solution was filtered with a filtration
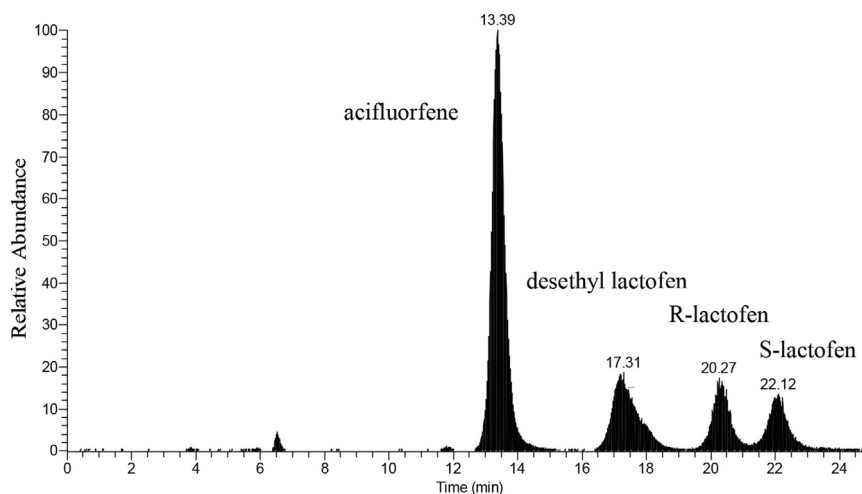
Fig. 2. Total ion chromatogram of lactofen, desethyl lactofen and acifluorfene in the extract of *L. minor*.

membrane of 0.22 µm and stored at −20 °C until being analyzed.

2.5. Chromatographic conditions

The contents of lactofen and its metabolites were determined using UHPLC UltiMate 3000 system coupled to a TSQ Quantum Access Max (Thermo Scientific, San Jose, CA, USA) in the selected reaction monitoring (SRM) scan mode. Negative ion mode was used except for lactofen analyzed in the positive ion mode. The following conditions were used: spray voltage at 3500 V for positive polarity and 3000 V for the negative polarity, capillary temperature at 350 °C, vaporizer temperature at 300 °C, sheath gas pressure at 40 psi, aux valve flow at 3.3 L/min, Q2 collision gas pressure at 1.5 mTorr. Lactofen and its metabolites were separated with a Lux Cellulose-1 chiral column (250 × 4.6 mm, 5 µm, coated with tris (3, 5-dimethylphenylcarbamate) cellulose, manufactured by Phenomenex, Torrance, CA). The mobile phase consisted of 88/12 (v/v) methanol/water (containing 0.1% formic acid). The flow rate was 500 µL min^{-1} and the injection volume was 5 µL.

2.6. Method validation

Recovery test was carried out by simultaneously adding lactofen, desethyl lactofen and acifluorfene into blank sample at three concentration levels. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on a signal-to-noise ratio of 3:1 and 10:1, respectively. Linear curve was generated by plotting peak area versus the concentration over the range of 2–200 µg L^{-1} in the growth solution and 5–2000 µg L^{-1} in the *L. minor*. The precision of the method for all chemicals was measured by three replicates.

2.7. Data calculation

Metabolism of pesticides generally fitted first-order reaction kinetics. The corresponding degradation rate constant k and half-life $T_{1/2}$ were calculated using the following equations:

$$C_t = C_0 e^{-kt}$$

$$T_{1/2} = \ln 2/k$$

Where C_t is the concentration at time t (day); C_0 is the maximum concentration; k is the degradation rate constant; t is the treatment time (day) and $T_{1/2}$ is the elimination half-life. All data were analyzed and fitted using Microsoft Excel.

The enantiomeric fraction (EF) value was calculated using the following equation to study the enantioselective behavior of lactofen in growth solution and in *L. minor*.

EF = peak area of *R*−enantiomer/(*R*−+*S*−enantiomer)

The EF value ranges from 0 to 1, and 0.5 indicates racemate.

3. Results and discussion

3.1. Analysis of lactofen enantiomers and the metabolites

The typical chromatographic separation of lactofen and its metabolites was shown in Fig. 2, the four peaks (two enantiomers of lactofen and two metabolites) were baseline separated and there was no interference peak. The total chromatographic run time was 25 min and the retention times of acifluorfene, desethyl lactofen, R-lactofen and S-lactofen were 13.4, 17.3, 20.3 and 22.1 min, respectively.

3.2. Method validation

As shown in Tables A.1-A.2, The mean recoveries of lactofen enantiomer and the metabolites from *L. minor* and growth solution ranged from 82.2% to 105.0% in growth solution and from 72.9% to 112.3% in *L. minor* with RSD below 15%. Good linearities were obtained over the range of 2–200 µg L^{-1} in the growth solution and 5–2000 µg kg^{-1} in *L. minor* with the correlation coefficient (R^2) of all the compounds higher than 0.994. The LOQs for each chemical were found to be 0.5 µg L^{-1} in growth solution and 1 µg kg^{-1} in *L. minor*, and the LODs for each chemical were 0.2 µg L^{-1} in growth solution and 0.4 µg kg^{-1} in *L. minor*, respectively.

3.3. Enantioselective fate of lactofen in L. minor

During the 5-day exposure, no visible symptoms of phytotoxicity were observed in *L. minor*. Concentration-time curves of lactofen and desethyl lactofen in *L. minor* were shown in Fig. 3. Lactofen and desethyl lactofen could be detected in *L. minor* soon after the exposure, while acifluorfene was undetectable at any time point. In the rac-lactofen exposure experiment, the content of rac-lactofen in *L. minor* progressively increased from 0 to 1041 µg L^{-1} during the first day, then decreased to 106 µg L^{-1} on the third day and kept at a steady level. The calculated half-life of rac-lactofen in *L. minor* was 1.28 d. In addition, an obvious difference concentration between lactofen enantiomers was observed after racemic lactofen exposure. The concentration of S-lactofen (843 µg L^{-1}) was much higher than that of R-lactofen (198 µg L^{-1}) in the first day of exposure, indicating the preferential accumulation of S-lactofen. After that, the concentration decreased to 49 µg L^{-1} (S-lactofen) and 58 µg L^{-1} (R-lactofen) on the third day. The
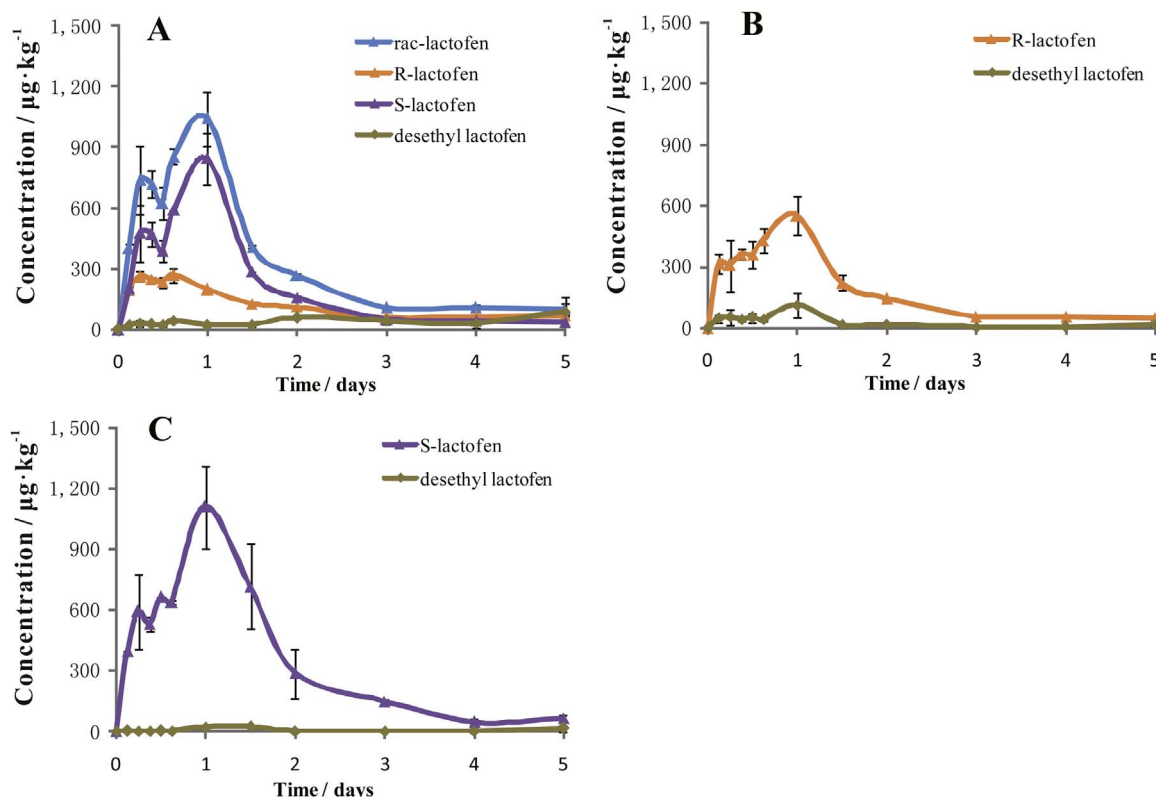
Fig. 3. Concentration-time curves of lactofen and the metabolite in *L. minor* exposed by 30 µg L^{-1} (A) racemic lactofen; (B) R-lactofen; (C) S-lactofen. Error bars represent standard deviation of the mean (*n* = 3).

half-lives of R- and S-lactofen were 1.55 d and 0.92 d, indicating the preferential metabolism of S-lactofen. As shown in Fig. 5A, the enantiomeric fraction (EF) value in *L. minor* decreased from 0.50 to 0.19 after one day of racemic lactofen exposure due to the preferential accumulation of S-lactofen, then the EF value gradually enhanced to 0.65 in the fifth day of exposure, which might be caused by the preferential metabolism of S-lactofen.

In order to further elucidate the enantioselective accumulation, metabolism and chiral stability of the enantiomers, exposure of individual R- and S-lactofen was also conducted. As shown in Fig. 3B and C, both enantiomers also reached to a maximum level in the first day and decreased after that. It was also found more S-enantiomer was accumulated than R-enantiomer. S-enantiomer was metabolized faster by *L. minor* than R-enantiomer, with T$_{1/2}$ of 0.89 and 1.22 d, respectively. The metabolism rate of R-lactofen and S-lactofen in individual enantiomer exposure was faster compared to the racemate exposure. No interconversion of the two enantiomers of lactofen was found in *L. minor* after individual enantiomer exposure, indicating the enantiomers were configurationally stable.

The enantioselective metabolism of lactofen has been investigated in soil (Diao et al., 2009), sediment (Diao et al., 2010) and primary rat hepatocytes (Wang et al., 2013). The calculated T$_{1/2}$ values of lactofen ranged from 9 to 50 days in soil, from 1.1 to 2.4 days in sediment and from 166 to 181 min in primary rat hepatocytes. A preferential metabolism of S-lactofen and configuration stability of lactofen enantiomer was observed in all these matrixes, which was consistent with our result. Besides, desethyl lactofen and acifluorfene were the most commonly detected metabolites. Generally, lactofen was metabolized to desethyl lactofen and acifluorfene, and desethyl lactofen could also be metabolized to acifluorfene. However, in our study, though lactofen degraded rapidly in *L. minor*, low level of desethyl lactofen and undetectable acifluorfene appeared. This was consistent with the previous research in primary rat hepatocytes (Wang et al., 2013).

3.4. Enantioselective fate of lactofen in growth solution

Fig. 4 displayed the concentrations of target compounds in the growth solution throughout the experiment. In the *L. minor* growth solution, as in Fig. 4A, the half-life of rac-lactofen was 0.96 d. More than 68% was metabolized in the first day and the residue was 3.5 µg L^{-1} after 5 days. Similar as in *L. minor* the metabolite desethyl lactofen could be detected in the *L. minor* growth solution, while acifluorfene was also not observed. An obvious increase of desethyl lactofen was observed soon after the exposure and the concentration reached to the maximum level of 9.7 µg L^{-1} at 15 h, and then progressively declined. The residue level kept 2.5 µg L^{-1} at the end of exposure. In the blank growth solution (without *L. minor*), as shown in Fig. 4B, rac-lactofen gradually decreased during the 5-day exposure, with T$_{1/2}$ being 0.99 d, also showing a fast degradation. About 52% of rac-lactofen was degraded after one day, and the residue was 4.3 µg L^{-1} after 5 days. Desethyl lactofen appeared in growth solution after 3 h, and progressively increased to 3.9 µg L^{-1} after 3 days and then kept at a steady level. Acifluorfene was not observed either. From Fig. 4A and B, it was found *L. minor* could slightly accelerate the metabolism and removal of lactofen, indicating limited ability of phytoremediation for both lactofen and its metabolite.

In the racemate exposure group, a slight different degradation of the enantiomers was observed in the *L. minor* growth solution (Fig. 5B) with EF values always higher than 0.5, showing the enrichment in R-enantiomer, which might result from the preferential accumulation of S-enantiomer by *L. minor*. The half-lives of R- and S-lactofen in *L. minor* growth solution were 0.98 d and 0.94 d, respectively. While in the blank growth solution, the EF values (Fig. 5B) were always around 0.5, indicating no obvious enantioselectivity. The half-lives of R- and S-lactofen in control solution were both 0.99 d. Individual enantiomer exposure group were carried out in order to verify the metabolism behavior of the individual enantiomers and the chiral stability. As shown in Table 1, the half-lives of S- and R-lactofen in the growth
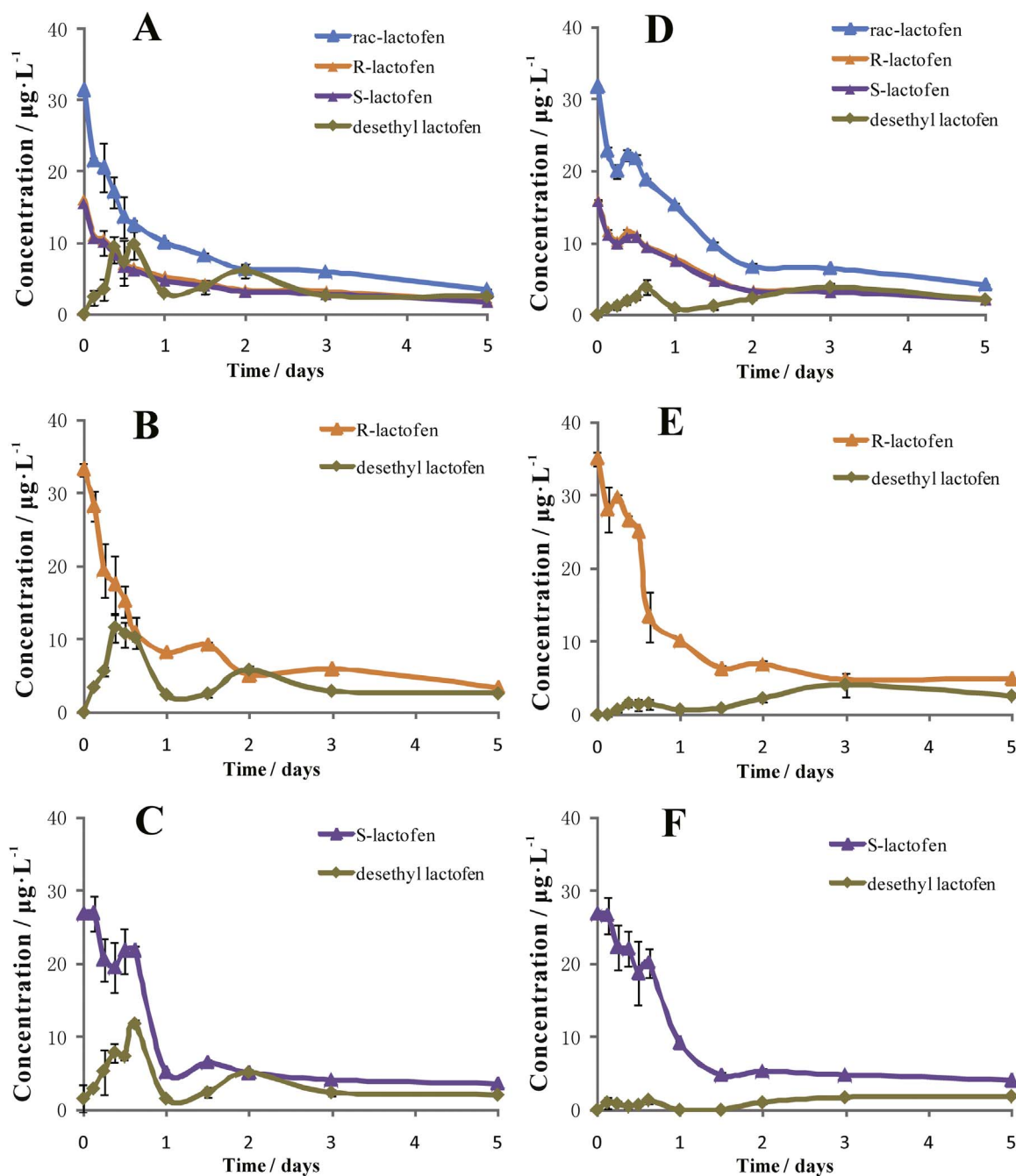
Fig. 4. Concentration-time curves of lactofen and the metabolite in *L. minor* growth solution exposed by 30 µg L^{-1} (A) racemic lactofen, (B) R-lactofen, and (C) S-lactofen, and blank growth solution (without *L. minor*) exposed by 30 µg L^{-1} (D) racemic lactofen, (E) R-lactofen and (F) S-lactofen. Error bars represent standard deviation of the mean ($n=3$).

solution were 0.72 and 0.75 d in the presence of *L. minor* and were 0.79 and 0.80 d in the absence of *L. minor*, respectively. In comparison with the racemate exposure groups, an accelerated metabolism of R- and S-lactofen was observed in the growth solution with the individual enantiomer exposure. No interconversion of the two enantiomers of lactofen was found in the growth solution, showing the enantiomers were configurationally stable.

It was reported that the EC$_{50}$ values of lactofen and desethyl lactofen were 0.790, 0.821 µg L^{-1} for *Scenedesmus obliquus*, 4.308, 13.684 mg L^{-1} for *Daphnia magna* and 100, 80 µg L^{-1} for *L. minor*, indicating desethyl lactofen was more toxic to *L. minor* but less toxic to *Scenedesmus obliquus* and *Daphnia magna* than lactofen. In our work, *L. minor* could slightly accelerate the removal of lactofen but significantly increase the concentration of desethyl lactofen in the growth solution.

There may be synergy or antagonism effect between lactofen and desethyl lactofen when organisms were exposed to a combination of both. Therefore, further research is needed to examining the joint toxicity of lactofen and its metabolite for predicting the environment risk.

There have been some reports on the bioaccumulation and metabolism of xenobiotics in aquatic plants duckweed. For example, *Lemna gibba* accumulated sulfamethoxazole up to 0.08 µg g^{-1} of plant tissue from a 100 µg L^{-1} sulfamethoxazole solution (Brain et al., 2008). *L. minor* and *Spirodela polyrhiza* accumulated fungicide dimethomorph up to 3 and 2 µg g^{-1} fresh weight, respectively, when exposed to 25 µg L^{-1} dimethomorph (Dosnon-Olette et al., 2010). The accumulation of herbicide isoproturon by *L. minor* from a 58 µg L^{-1} isoproturon solution was 1 µg g^{-1} fresh weight (Böttcher and Schroll, 2007). In this
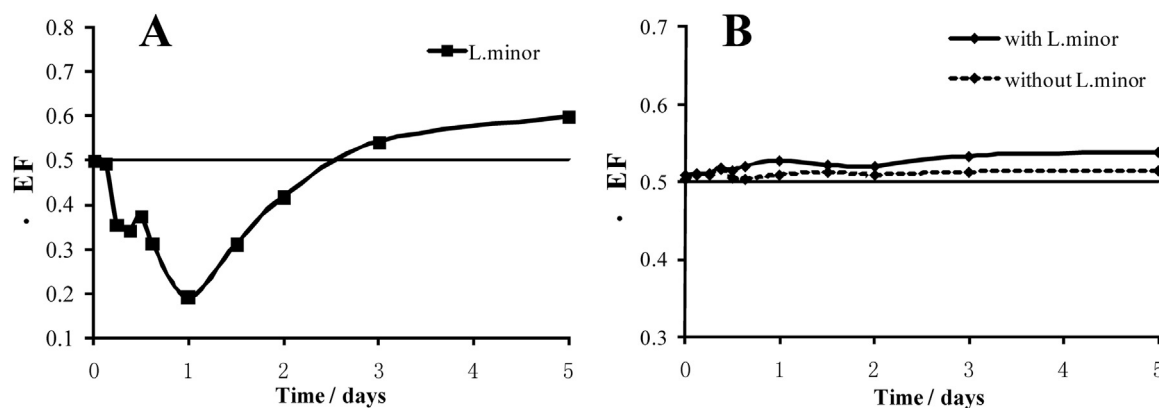
Fig. 5. EF values of lactofen in (A) *L. minor* and (B) growth solution after racemic lactofen exposure.

Table 1
The degradation equations of lactofen and its enantiomers in growth solution and *L. minor*.

Matrix	Exposure	Compound	Regressive functions	R^2	$T_{1/2}$ (days)
L. minor	rac-lactofen	rac-lactofen	$y = 1002.8e^{-0.543x}$	0.7815	1.28
		R-lactofen	$y = 291.5e^{-0.447x}$	0.8795	1.55
		S-lactofen	$y = 937.3e^{-0.753x}$	0.8439	0.92
	R-lactofen	R-lactofen	$y = 560.1e^{-0.569x}$	0.8138	1.22
	S-lactofen	S-lactofen	$y = 1839.1e^{-0.777x}$	0.8795	0.89
Growth solution (with *L. minor*)	rac-lactofen	rac-lactofen	$y = 23.5e^{-0.722x}$	0.9075	0.96
		R-lactofen	$y = 12.0e^{-0.708x}$	0.9103	0.98
		S-lactofen	$y = 11.5e^{-0.736x}$	0.8955	0.94
	R-lactofen	R-lactofen	$y = 25.9e^{-0.921x}$	0.8928	0.75
	S-lactofen	S-lactofen	$y = 28.2e^{-0.943x}$	0.7788	0.72
Growth solution (without *L. minor*)	rac-lactofen	rac-lactofen	$y = 28.3e^{-0.700x}$	0.9578	0.99
		R-lactofen	$y = 14.3e^{-0.697x}$	0.9592	0.99
		S-lactofen	$y = 13.9e^{-0.703x}$	0.9558	0.99
	R-lactofen	R-lactofen	$y = 32.5e^{-0.863x}$	0.8742	0.80
	S-lactofen	S-lactofen	$y = 28.7e^{-0.872x}$	0.9471	0.79

The regressive functions were obtained based on the mean values of three replicates.

study, the maximum bioaccumulation of rac-lactofen by *L. minor* was up to 1 µg g^{-1} fresh weight from a 30 µg L^{-1} rac-lactofen solution. The lactofen accumulation ability in the *L.minor* is relatively moderate compared to the xenobiotics mentioned above. Herbicide propanil can be taken up and transformed to its metabolite by *L. minor* (Mitsou et al., 2006). The fate of antimicrobial trimethoprim in *L.minor* wastewater treatment system was studied and the result showed that the demethylation occurred only in absence of *L. minor* (Iatrou et al., 2017). Some ibuprofen metabolites were detected in the growth solution only in the presence of *L.minor* (Pietrini et al., 2015). In this study, lactofen metabolite desethyl lactofen was significantly higher in the growth solution in the presence of *L. minor*. This result was similar to a study on phytotransformation of ibuprofen by *Typha angustifolia*, in which the amount of ibuprofen metabolites in growth solution of plant was significantly higher (Li et al., 2016).

As a matter of fact, the plant metabolism of pollutants depends on the physicochemical property of xenobiotics (Iatrou et al., 2017), the species of plant (Gao et al., 2000) and rhizosphere-associated microorganisms (Anudechakul et al., 2015; Barac et al., 2004). It has been reported that the microorganisms play very important role in the metabolism and mineralization of xenobiotics (Stottmeister et al., 2003). For instance, rhizosphere-associated bacteria of *Spirodela polyrhiza* enhanced the removal of phenol (Toyama et al., 2006). Chlorpyrifos removal was obviously facilitated in the presence of a root-associated bacteria (Anudechakul et al., 2015) and isoproturon metabolism by *L. minor* was also associated with rhizosphere microorganisms (Böttcher and Schroll, 2007). Therefore, *L. minor* metabolism of lactofen may be facilitated with the presence of rhizosphere-associated

microorganisms.

In this study, obviously enantioselective bioaccumulation and metabolism were observed in *L. minor*, with S-lactofen preferentially accumulated and metabolized. Similar results have been reported on *Eichhornia crassipes* exposed to fipronil (Lu et al., 2010), with S-fipronil preferentially uptaken and metabolized. The EF values showed no obvious enantioselective degradation of rac-lactofen occurred in the control growth solution, while slight enantioselective metabolism was found in *L. minor* growth solution.

4. Conclusion

As a crucial functional and structural element of aquatic communities, duckweed plays an important role in chemical cycles. The enantioselective uptake and metabolism of lactofen in the aquatic macrophyte *L. minor* was reported in this work. Identification of lactofen enantiomers and its degradation products in growth solution and *L. minor* was performed on UHPLC-MS-MS. Racemic lactofen dissipated very fast in *L. minor* and the growth solution with half-lives less than 1.5 days. The metabolite desethyl lactofen could be detected in both *L. minor* and growth solution with the degradation of lactofen, while acifluorfene was not found. Obvious enantioselective bioaccumulation and degradation of lactofen occurred in *L. minor,* with preference of S-enantiomer. The enantiomers were confirmed to be configurationally stable in *L. minor* and growth solution by the individual enantiomer. It was found that the removal of lactofen in the growth solution was slightly accelerated by the presence of *L. minor*. Further research is needed to examine the joint toxicity of lactofen and

its metabolite for predicting the environment risk. The findings of this work indicated that *L. minor* could accumulate and metabolize lactofen enantioselectively, but has limited potential for phytoremediation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2017.04.051.

References

Anudechakul, C., et al., 2015. Removal of chlorpyrifos by water hyacinth (*Eichhornia crassipes*) and the role of a plant-associated bacterium. Int. J. Phytoremediat. 17, 678–685.

Appenroth, K.J., et al., 2010. Effects of nickel on the chloroplasts of the duckweeds *Spirodela polyrhiza* and *Lemna minor* and their possible use in biomonitoring and phytoremediation. Chemosphere 78, 216–223.

Barac, T., et al., 2004. Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat. Biotechnol. 22, 583–588.

Böttcher, T., Schroll, R., 2007. The fate of isoproturon in a freshwater microcosm with *Lemna minor* as a model organism. Chemosphere 66, 684–689.

Brain, R.A., et al., 2008. Herbicidal effects of sulfamethoxazole in *Lemna gibba*: using p-aminobenzoic acid as a biomarker of effect. Environ. Sci. Technol. 42, 8965–8970.

Cheng, C., et al., 2015. Enantioselective toxicity of lactofen and its metabolites in *Scenedesmus obliquus*. Algal Res. 10, 72–79.

Diao, J., et al., 2009. Influence of soil properties on the enantioselective dissipation of the herbicide lactofen in soils. J. Agric. Food Chem. 57, 5865–5871.

Diao, J., et al., 2010. Enantioselective degradation in sediment and aquatic toxicity to *Daphnia magna* of the herbicide lactofen enantiomers. J. Agric. Food Chem. 58, 2439–2445.

Dosnon-Olette, R., et al., 2009. Phytoremediation of fungicides by aquatic macrophytes: toxicity and removal rate. Ecotoxicol. Environ. Saf. 72, 2096–2101.

Dosnon-Olette, R., et al., 2010. Influence of initial pesticide concentrations and plant population density on dimethomorph toxicity and removal by two duckweed species. Sci. Total Environ. 408, 2254–2259.

Gao, J., et al., 2000. Uptake and phytotransformation of organophosphorus pesticides by axenically cultivated aquatic plants. J. Agric. Food Chem. 48, 6114–6120.

Garcia-Rodriguez, A., et al., 2015. The influence of *Lemna sp.* and *Spirogyra sp.* on the removal of pharmaceuticals and endocrine disruptors in treated wastewaters. Int. J. Environ. Sci. Technol. 12, 2327–2338.

Iatrou, E.I., et al., 2017. Fate of antimicrobials in duckweed *Lemna minor* wastewater treatment systems. J. Hazard. Mater. 330, 116–126.

Jeffery, M.H., et al., 1988. Absorption, translocation, and metabolism of acifluorfen and lactofen in pitted morningglory (*Ipomoea lacunosa*) and ivyleaf morningglory (*Ipomoea hederacea*). Weed Sci. 36, 141–145.

Kaminski, A., et al., 2014. Phytoremediation of anatoxin-a by aquatic macrophyte *Lemna trisulca L*. Chemosphere 112, 305–310.

Khataee, A.R., et al., 2012. Phytoremediation potential of duckweed (*Lemna minor L.*) in degradation of C.I. Acid Blue 92: artificial neural network modeling. Ecotoxicol. Environ. Saf. 80, 291–298.

Kösesakal, T., et al., 2016. Phytoremediation of petroleum hydrocarbons by using a freshwater fern species *Azolla filiculoides Lam*. Int. J. Phytoremediat. 18, 467–476.

Li, Y., et al., 2016. Phytoextraction, phytotransformation and rhizodegradation of ibuprofen associated with *Typha angustifolia* in a horizontal subsurface flow constructed wetland. Water Res. 102, 294–304.

Lu, D., et al., 2010. Stereoselective metabolism of fipronil in water hyacinth (*Eichhornia crassipes*). Pestic. Biochem. Physiol. 97, 289–293.

Mitsou, K., et al., 2006. Growth rate effects, responses of antioxidant enzymes and metabolic fate of the herbicide Propanil in the aquatic plant *Lemna minor*. Chemosphere 62, 275–284.

Olette, R., et al., 2008. Toxicity and removal of pesticides by selected aquatic plants. Chemosphere 70, 1414–1421.

Orr, G.L., Hess, F.D., 1982. Mechanism of action of the diphenyl ether herbicide acifluorfen-methyl in excised cucumber (*Cucumis sativus L.*) cotyledons: light activation and the subsequent formation of lipophilic free radicals. Plant Physiol. 69, 502–507.

Pietrini, F., et al., 2015. Ibuprofen exposure in *Lemna gibba L.*: evaluation of growth and phytotoxic indicators, detection of ibuprofen and identification of its metabolites in plant and in the medium. J. Hazard. Mater. 300, 189–193.

Qu, H., et al., 2016. The toxicity, bioaccumulation, elimination, conversion of the enantiomers of fipronil in *Anodonta woodiana*. J. Hazard. Mater. 312, 169–174.

Ruff, M., et al., 2015. Quantitative target and systematic non-target analysis of polar organic micro-pollutants along the river Rhine using high-resolution mass-spectrometry – identification of unknown sources and compounds. Water Res. 87, 145–154.

Sasmaz, M., et al., 2015. The potential of *Lemna gibba L.* and *Lemna minor L.* to remove Cu, Pb, Zn, and As in gallery water in a mining area in Keban, Turkey. J. Environ. Manag. 163, 246–253.

Singh, N.K., et al., 2016. Arsenic and other heavy metal accumulation in plants and algae growing naturally in contaminated area of West Bengal, India. Ecotoxicol. Environ. Saf. 130, 224–233.

Stottmeister, U., et al., 2003. Effects of plants and microorganisms in constructed wetlands for wastewater treatment. Biotechnol. Adv. 22, 93–117.

Tatar, S.Y., Obek, E., 2014. Potential of *Lemna gibba L.* and *Lemna minor L.* for accumulation of Boron from secondary effluents. Ecol. Eng. 70, 332–336.

Török, A., et al., 2015. Phytoremediation capacity of aquatic plants is associated with the degree of phytochelatin polymerization. J. Hazard. Mater. 299, 371–378.

Toyama, T., et al., 2006. Accelerated aromatic compounds degradation in aquatic environment by use of interaction between *Spirodela polyrrhiza* and bacteria in its rhizosphere. J. Biosci. Bioeng. 101, 346–353.

Wang, F., et al., 2016. A full evaluation for the enantiomeric impacts of lactofen and its metabolites on aquatic macrophyte *Lemna minor*. Water Res. 101, 55–63.

Wang, X., et al., 2013. Stereoselective toxicity and metabolism of lactofen in primary hepatocytes from rat. Chirality 25, 743–750.

Wong, C.S., 2006. Environmental fate processes and biochemical transformations of chiral emerging organic pollutants. Anal. Bioanal. Chem. 386, 544–558.

Ziegler, P., et al., 2016. Duckweeds for water remediation and toxicity testing. Toxicol. Environ. Chem. 98, 1127–1154.