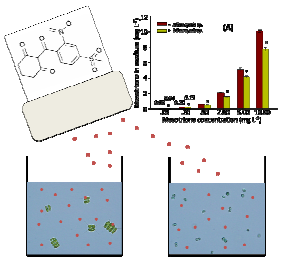
Environmental Science
Processes & Impacts

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

rsc.li/process-impacts



Photosynthetic responses and accumulation of mesotrione in *Microcystis* sp. and *Scenedesmus quadricauda* were investigated by PAM fluorometry, HPLC and SDAPCI-MS.

Environmental impact

The dispersion of organic xenobiotics and their residues in aquatic ecosystems is of major public concern due to most of applied herbicides did not reach to their targets but rather went into adjacent aquatic environment and may have negative impact on aquatic organisms. Mesotrione is one of them. The toxic effects and fate of mesotrione on freshwater algae are still not well understood. In this study, photosynthetic responses and accumulation of mesotrione in two freshwater algae were investigated. The results suggest that mesotrione led to a greater adverse effect on photosystem of *S. quadricauda* than *Microcystis* sp. and *Microcystis* sp. was a superior competitor in mesotrione stress. Mesotrione was shown to be readily accumulated by both species and may allow us to develop alga-based clearing up systems for bioremediation of herbicide contamination in aquatic environments.

Journal Name

RSCPublishing

ARTICLE

Photosynthetic responses and accumulation of mesotrione in two freshwater algae

Cite this: DOI: 10.1039/xoxxoooooox

Yan Ni,[a] Jinhu Lai,[a] Jinbao Wan[a]*and Lianshui Chen[b]

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/xoxxoooooox

www.rsc.org/

Mesotrione is a herbicide used for killing annual grasses and broad-leaved weeds in maize. Recent investigation shows that mesotrione has been detected as an organic contaminant in aquatic environments and may have negative impact on aquatic organisms. To evaluate the eco-toxicity of mesotrione to algae, experiments focusing on photosynthetic responses and mesotrione accumulation in *Microcystis* sp. and *Scenedesmus quadricauda* were carried out. Both algae treated with mesotrione at 0.05-10 mg L^{-1} for 7 d reduced photosynthetic capacity. Fluorescence of chlorophyll a, maximal PSII activity (F_v/F_m), and the parameters (I_k, α and ETR_{max}) of rapid light curves (RLCs) in both algae were decreased under mesotrione exposure. The 96 h EC_{50} value for mesotrione on *S. quadricauda* and *Microcystis* sp. were 4.41 and 6.19 mg L^{-1}, respectively. The latter is more tolerance to mesotrione. Mesotrione was shown to be readily accumulated by both species. Such uptake of mesotrione led to the rapid removal of mesotrione from medium. Overall, this study represents the initial comprehensive analyses of *Microcystis* sp. and *S. quadricauda* in adaptation to the mesotrione contaminated aquatic ecosystems.

Introduction

The dispersion of organic xenobiotics such as pesticides and their residues in ecosystems is of major public concern. Over the last decades, accumulation of the artifically synthetic chemicals in ecosystems has increased substantially. Most of applied pesticides did not reach to their targets but rather went into adjacent aquatic ecosystems through spray drift, surface runoff, and infiltration.[1] As a consequence, residual pesticides are often detected as pollutants in aquatic ecosystems including underground streams, lakes, rivers or even costal sea waters.[2-4] Mesotrione (2-[4-methylsulfonyl-2-nitrobenzoyl]-1,3-cyclohexanedione) belongs to the triketone family and has been widely utilized as a controller of a wide range of annual grasses and broad-leaved weeds in maize. It is chemically derived from a natural phytotoxin obtained from the Californian bottlebrush plant, *Callistemon citrinus*. This compound prevents carotenoid synthesis and acts by competitive inhibition of the enzyme 4-hydroxy-phenyl-pyruvate-dioxygenase (HPPD), a component of the biochemical pathway that converts tyrosine to plastoquinone and α-tocopherol.[5] Mesotrione is moderately soluble in water (0.16 mg mL^{-1}, 20℃). This physicochemical property is implicated in its mobility in soil and it is relatively easy to pollute the surface- or ground-waters. With increasing mesotrione was used,[6] it may bring about high risks not only to crop production but also to aquatic organisms. The biological effects of mesotrione include inhibition of carotenoid biosynthesis of perennial ryegrass (*Lolium perenne L.*), changes in activity of non-specific esterases of *Tetrahymena pyriformis* and metabolism of *Vibrio fischeri*.[7, 8]

Algae, the major primary producers in aquatic environment, have been extensively used in the toxicity test on organic xenobiotics. Generally, cell density, biomass, pigments, nutrient uptake rate and enzyme activity were taken to evaluate environmental toxicity.[9, 10] However, studies on responses of photosynthetic activities, such as maximal PSII activity, chlorophyll a fluorescence and rapid light curves, suggested those were much more sensitive.[11-13] Besides, most herbicides

choose photosynthetic apparatus as one of the primary and effective target sites,[14] mesotrione is one of them. To date, very few studies have been reported on the effects of mesotrione on algae.[15] Photosynthetic responses of algae under mesotrione exposure are even poorly understood. Pulse Amplitude Modulation (PAM) fluorometry was first described by Schreiber *et al.* in 1986,[16] which has been confirmed to be sensitive and efficient in toxic tests in algae recently.[13, 17] In this study, PAM fluorometry was employed to investigate the toxic effects of mesotrione on the photosynthetic apparatus of algae. The potential adverse effects are most likely the result of unspecific uptake or accumulation of herbicides by algae. Accumulation by algae can remove toxic organic compounds from contaminated sites. Hence, algae could potentially be used in mesotrione remediation due to their high surface area to volume ratio. Up to now, the ecological fate of mesotrione in the environment is not completely elucidated. This compound is stable and is not sensitive to solar light by direct photolysis.[18] MNBA (4-(methylsulfonyl)-2-nitrobenzoic acid) and AMBA (4-(methylsulfonyl)-2-aminobenzoic acid), were identified as two main metabolites of a pure bacterial strain (*Bacillus* sp. 3B6) in soil.[19] Therefore, accumulation and degradation of mesotrione in algae are largely unclear and merits further investigation with efforts.

Two freshwater algae were employed to perform a comprehensive analysis on its photosynthetic responses to mesotrione toxicity as well as accumulation and degradation of mesotrione from medium. *Microcystis* sp. (unicellular), which often exists in the beginning of algae bloom recovery, is a desirable species for studying biological responses due to its ability to acclimate rapidly to the unfavorable environments. *Scenedesmus quadricauda*, a widely distributed green algal species in freshwater lakes and rivers,[20] is also frequently used for studying toxicology and tolerance to various abiotic stresses. Thus, the purposes of this study were to: (1) assess the effect of mesotrione on the photosynthetic responses of *Microcystis* sp. and *S. quadricauda*; and (2) test the potential for both algae accumulation and degradation of mesotrione in the aquatic environments. We determined chlorophyll fluorescence properties of algae under mesotrione exposure, dynamic accumulation of mesotrione and degradation of cellular adsorbed mesotrione. The outcome of this study will help to understand the biological mechanisms for mesotrione-induced toxicity in both algae and to assess the capability of both algae to accumulation and degradation of mesotrione.

Materials and methods

Growth conditions and treatments

The herbicide mesotrione (CAS 104206-82-8, purty 99.0%) was purchased from Dr.Ehrenstorfer, Germany. *S. quadricauda* (Chlorophyceae, Scenedesmaceae, culture collection no. FACHB-1297) and *Microcystis* sp. (Cyanophyceae, Chroococcacaea, culture collection no. FACHB-562) were obtained from the Institute of Hydrobiology, the Chinese Academic of Sciences, China. Both species were precultured in 500 mL Erlenmeyer flasks containing 200 mL of axenic BG-11 medium.[21, 22] The BG-11 medium contains $NaNO_3$ 1.5g, K_2HPO_4 0.04g, $MgSO_4 \cdot 7H_2O$ 0.075g, $CaCl_2 \cdot 2H_2O$ 0.036g, Citric acid 0.006g, Ferric ammonium citrate 0.006g, $EDTANa_2$ 0.001g, Na_2CO_3 0.02g and 1 mL of trace metal solution per litre, pH 7.1. The trace metal solution contains H_3BO_3 2.86g, $MnCl_2 \cdot 4H_2O$ 1.86g, $ZnSO_4 \cdot 7H_2O$ 0.22g, $Na_2MoO_4 \cdot 2H_2O$ 0.39g, $CuSO_4 \cdot 5H_2O$ 0.08g, $Co(NO_3)_2 \cdot 6H_2O$ 0.05g per liter.[23] Cultures under the routine condition of 12 h photoperiod at 80 μmol photons m^{-2} s^{-1} and temperature of 25±1□ for 1-7 d, both algae were harvested in the log or late log growth phase.[24] The vessels were manually shaken three times per day. Experiments were performed with exponential growth phase of both species. Log-phase cultures of approximately 10×10^6 μm^3 mL^{-1} of both species were inoculated into series dilutions of mesotrione to meet the final concentrations of 0.05, 0.2, 0.5, 2, 5, 10 mg L^{-1}, respectively. No mesotrione was added to the control. Each test was performed in triplicate. All treatments and controls were run at the same time.

Chlorophyll fluorescence parameters determination

Maximal PSII activity (F_v/F_m), fluorescence of chlorophyll a and rapid light curves (RLCs) were determined using a pulse amplitude modulated fluorescence system (PAM, Walz, Effeltrich, Germany). After 10 min dark adaptation, fluorescence of chlorophyll a, minimum fluorescence (F_0) and maximal fluorescence (F_m) were determined, fluorescence of chlorophyll a was taken as the algal growth indicator and F_v/F_m was calculated as $(F_m\text{-}F_0)/F_m$.[25] RLCs based on measurements of relative electron transport rates were derived from estimates the photosynthetic capacity of algae, its light adaptation state and capacity to tolerate short-term changes in light.[26] After the RLCs mode was turned on, the actinic light was applied for 20s to each of a series of increasing intensity (16 to 512 μmol photons PAR $m^{-2}s^{-1}$). The initial slope of RLCs of *ETR* (α), the maximal electron transport rates in PSII (ETR_{max}) and the index of light adaptation of PSII (I_k, ETR_{max}/α) were derived from the RLCs, which were automatically calculated by the PhytoWin software (version 1.46).[27]

Accumulation of mesotrione in algae

Residual mesotrione in medium was determined by high-performance liquid chromatography (HPLC, Agilent 1260, Agilent Technologies, USA) with ultraviolet detector at wavelength of 254 nm. Mobile phase was 0.5% H_3PO_4–acetonitrile (v/v 2:3, 1 mL min^{-1}). Injection volume was 5μL. Column temperature was kept at 20□. *S. quadricauda* and *Microcystis* sp. were treated with mesotrione at 0, 0.05, 0.2, 0.5, 2, 5 and 10 mg L^{-1} culture for 4 d or incubated with culture containing 5 mg L^{-1} mesotrione for 0, 1, 2, 3, 4 and 5 d. Culture solution was harvested by centrifugation at 4000g for 15 min individually. The supernatant was subjected to mesotrione analyses. A solid phase extraction column (LC-C_{18}) was activated separately by eluting 10 mL methanol and 10 mL water. The supernatant was passed through the SPE column at 2

mL min^{-1}. The remaining section in the column was eluted with 10 mL methanol at a flow rate of 1 mL min^{-1}. The eluting solution was concentrated to dryness in a rotary-vacuum evaporator.[28, 29] The residue was dissolved in 1 mL methanol and quantified by HPLC.

Degradation of mesotrione in algae

Experiments were carried out using a commercial linear ion trap (LTQ) mass spectrometer (Finnigan, San Jose, CA, USA) installed with a surface desorption chemical ionization source (SDAPCI).[30] The SDAPCI-MS instrument was set to work in negative ion mode. The corona discharge voltage was ±3 kV with a discharge current of 1-3 mA. The temperature of the heating capillary of the SDAPCI-MS was maintained at 100□. The voltages for the heating capillary, tube lenses, conversion dynode, detectors, *etc.* were used as default values. All of the mass spectra were recorded as peak profile with an average time of 1 min and were background subtracted using the Xcalibur software of the LTQ instrument. Collision induced dissociation (CID) was performed to the precursor ions of interest, isolated with 1 *m/z* unit, with low collision energy (2-13eV). MS/MS spectra could be collected with a recording time more than 1 min if necessary. *S. quadricauda* and *Microcystis* sp. were treated with mesotrione at 5 mg L^{-1} culture for 48 and 96 h, the supernatant was harvested by centrifugation at 4000g for 15 min individually. For highly enhanced of the negative SDAPCI-mode, the basic solution (methanol/water, v/v 1:1) was added. To make a solid sample surface, the extracting solution was deposited on the surface of polytetrafluoroethylene sample plate to form a spot of about 1 cm^2. This spot was dried in air, and then analyzed directly with SDAPCI-MS.

Statistical analysis

All analyses were performed with SPSS (IBM SPSS Statistics 19). One-way analysis of variance (ANOVA) was used to determine the difference between control and treatments. Least significance difference (LSD) test at $p<0.05$ was conducted to detect statistical significance of the differences. Data presented in this study are presented as means ± standard deviation (SD). The log-logistic dose response model described by Haanstra *et al.*[31] was used to determine the 50% reduction (EC_{50}) by the single compound. Concentrations of mesotrione (mg L^{-1}) taken to calculate EC_{50} were part of the whole concentrations at the 4th day ranged from observable inhibitory concentration to total inhibition. EC_{50} was calculated as $y = c/(1+ e^{b(\log(x)-\log(a))})$, where y is the fluorescence of chlorophyll a, x is the concentration of the toxicant (mg L^{-1}), a is the EC_{50} value (mg L^{-1}), b is the slope of the curve and c is the fluorescence of chlorophyll a of the control.

Results

Effects of mesotrione on photosynthetic apparatus of *Microcystis* sp. and *S. quadricauda*

The effects of mesotrione on the fluorescence of chlorophyll a (μg L^{-1}) of both algae were expressed as the median inhibitory effect concentrations (EC_{50}) at 96 h. The 96-h EC_{50} of mesotrione on *S. quadricauda* and *Microcystis* sp. were 4.41 and 6.19 mg L^{-1}, respectively, which showed significant difference between both species ($p<0.05$). The EC_{50} value of *Microcystis* sp. was 1.40-fold higher than that of *S. quadricauda*, indicating that *Microcystis* sp. was more tolerant to mesotrione than *S. quadricauda*.

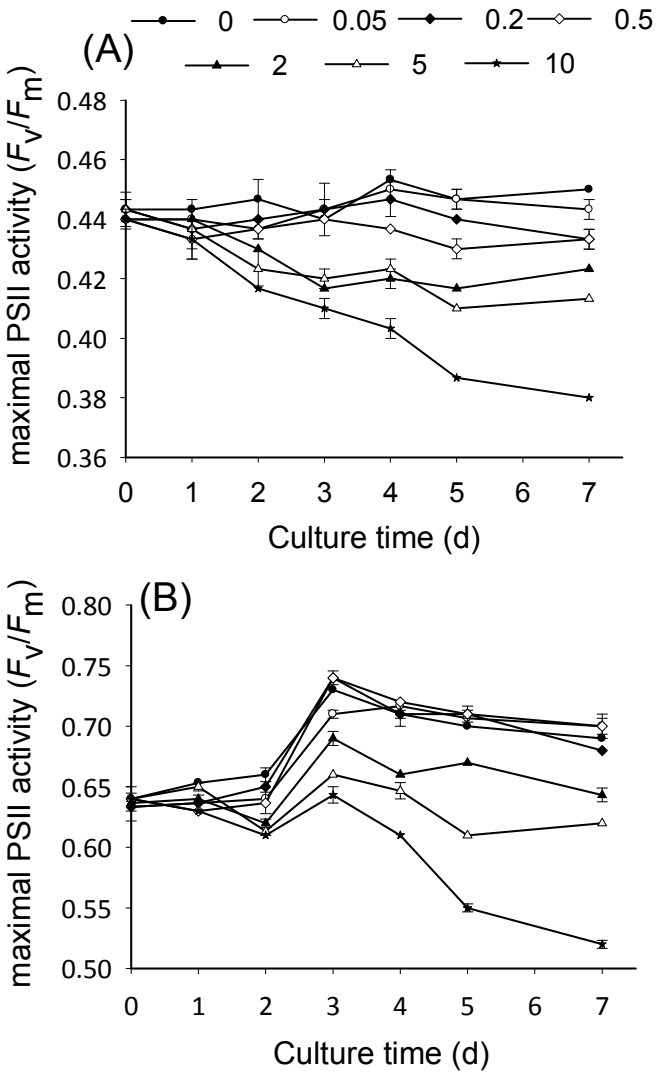


Fig. 1 Changes on maximum PS II activity (F_v/F_m) in *Microcystis* sp. (A) and *S. quadricauda* (B) under 0-10 mg L^{-1} mesotrione treatments during 7 days. Data points represent the means ± standard deviation (SD) (n = 3). Error bars not shown are smaller than symbol size.

F_v/F_m ratios were measured to assess the activity of the PSII of chloroplasts. The F_v/F_m ratios remained high and did not show any significant change in both algae under low dose (0.05-0.5 mg L^{-1}) mesotrione treatments until day 7 (Fig.1A, Fig.1B). However, The F_v/F_m ratios were significantly lower in both species at higher (2-10 mg L^{-1}) mesotrione exposure, and proportional to the concentration of mesotrione. The significant

inhibitory concentration of mesotrione ranged from 5 to 10 mg L^{-1} and 2 to 10 mg L^{-1} in *S. quadricauda* and *Microcystis* sp., respectively ($p < 0.05$). At the 7th d with 5 and 10 mg L^{-1} mesotrione treatments, 1.11-fold and 1.33-fold were detected notably in control compared treatments in *S. quadricauda* and 1.09-fold and 1.22-fold in *Microcystis* sp., respectively. Compared with *Microcystis* sp., *S. quadricauda* appeared more sensetive to mesotrione.

Table 1 Descriptive parameters of the light response reaction derived from the rapid light curves (RLCs) in *S. quadricauda* and *Microcystis* sp. measured after exposure to various concentrations of mesotrione for 48h. α, the initial slope of RLC, which reflected the photochemical efficiency ; ETR_{max}, the maximal electron transport rates in PS Ⅱ; I_k, the index of light adaptation of PS Ⅱ, was calculated as ETR_{max}/α. Values are the means ± standard deviation (SD) (n = 3). * is significantly different at $p < 0.05$.

Mesotrione (mg L^{-1})	Parameters of RLCs in *S. quadricauda*			Parameters of RLCs in *Microcystis* sp.		
	I_k(μmol photon m^{-2} s^{-1})	α (e^- photon^{-1})	ETR_{max}(μmol e^- m^{-2} s^{-1})	I_k(μmol photon m^{-2} s^{-1})	α (e^- photon^{-1})	ETR_{max}(μmol e^- m^{-2} s^{-1})
0	119.35±5.30	0.31±0.002	37.00±1.81	85.20±0.35	0.21±0.001	17.37±0.35
0.05	114.70±2.09	0.30±0.002*	33.93±0.45	81.13±4.72	0.20±0.012	16.50±0.50
0.2	113.33±2.74	0.30±0.003*	33.63±1.23	79.47±2.49	0.20±0.007	16.20±0.26
0.5	112.97±1.04	0.30±0.001*	33.6±1.75	78.67±3.42	0.20±0.013	16.20±0.30
2	106.27±0.64*	0.29±0.002*	31.17±1.22*	78.17±1.96	0.20±0.002	15.47±0.12*
5	84.23±0.96*	0.29±0.002*	24.77±0.32*	77.67±8.18	0.19±0.006	14.73±0.45*
10	79.07±2.72*	0.29±0.001*	22.93±1.01*	69.13±5.05*	0.19±0.008	13.20±0.87*

The informations about the RLCs in *S. quadricauda* and *Microcystis* sp. could be derived from the descriptive parameters (Table 1). I_k, α and ETR_{max} of the RLCs in *S. quadricauda* decreased with increasing mesotrione concentration and significantly decreased when the cells were treated with more than 2, 0.05 and 2 mg L^{-1} mesotrione, respectively ($p < 0.05$). α of the RLCs in *Microcystis* sp. did not show significant difference between different treatments and control. Whereas I_k and ETR_{max} of RLCs in *Microcystis* sp. decreased with increasing concentration of mesotrione after 48 h of treatment. I_k was significantly decreased at 10 mg L^{-1} mesotrione, while ETR_{max} showed significantly decrease for treatments with more than 2 mg L^{-1} mesotrione ($p < 0.05$). Comparison between both species showed that the rate of reduction of parameters of RLCs in *S. quadricauda* was higher than in *Microcystis* sp., indicating mesotrione was lead to a greater adverse effect on *S. quadricauda* than *Microcystis* sp. ($p < 0.05$).

Accumulation of mesotrione in *Microcystis* sp. and *S. quadricauda*

Residual mesotrione was quantified in algae-incubated medium and algae-free medium (control). Accumulation amount of mesotrione by both algae was proportional to mesotrione concentration in the medium (Fig.2A, Fig.2B). After 4 d of 5 and 10 mg L^{-1} mesotrione treatments, approximate 18.3% and 23.3% decay and 15.2% and 20.5% decay were measured in *Microcystis* sp. and *S. quadricauda*, respectively. From these data it appears that accumulation capability of *Microcystis* sp. was higher than that of *S. quadricauda*.

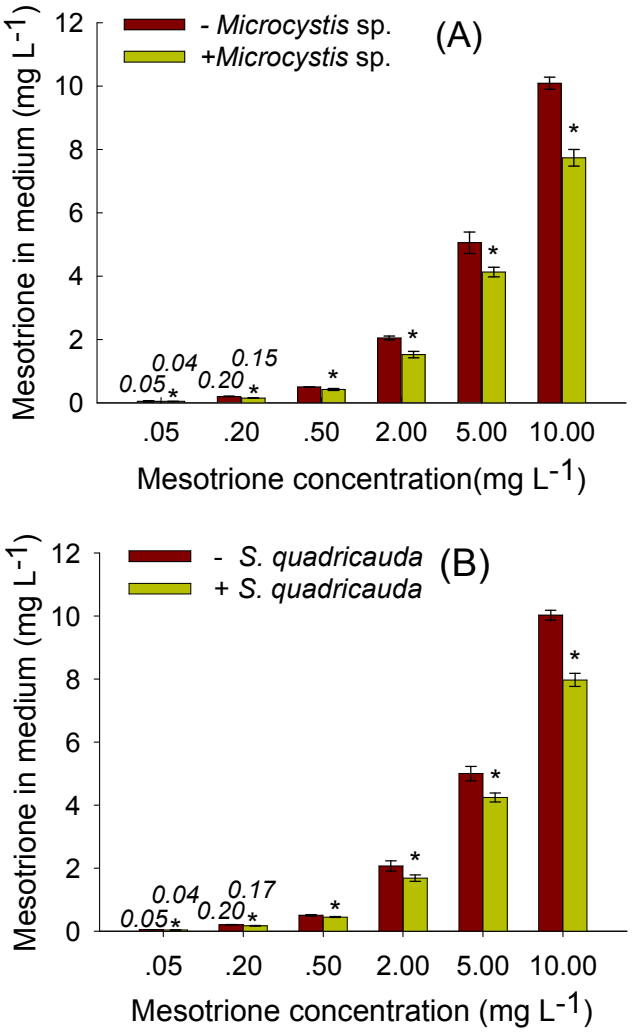


Fig. 2 Residual mesotrione in algae-free (-*S. quadricauda*, -*Microcystis* sp.) and algae-incubated (+*S. quadricauda*, +*Microcystis* sp.) medium (A and B). Both algae were treated with mesotrione at 0.05, 0.2, 0.5, 2.0, 5.0 and 10.0 mg L^{-1} culture, after 4 d, mesotrione in medium were quantified by HPLC. Values are

the means ± standard deviation (SD) (n = 3). * is significantly different at $p < 0.05$.

In the time-course experiment, the concentrations of mesotrione residues in the medium with *S. quadricauda* and *Microcystis* sp. were progressively reduced over the time (Fig.3A, Fig.3B). The significant decrease of mesotrione concentrations in medium occurred following the first day of experiment ($p<0.05$). On day 3 and 5, 88.73% and 79.42% mesotrione and 84.80% and 76.39% mesotrione remained in *S. quadricauda*-incubated and *Microcystis* sp.-incubated medium, respectively. In contrast, the concentration of mesotrione in the control medium did not show significant change.

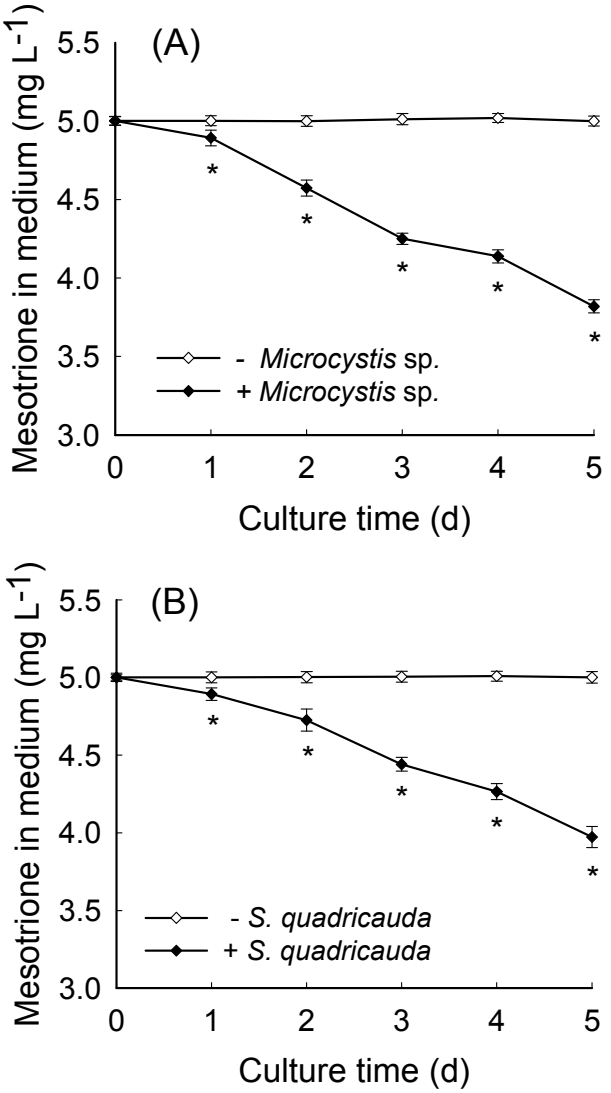


Fig. 3 Both algae were incubated with culture containing 5 mg L^{-1} mesotrione for 0, 1, 2, 3, 4 and 5 d, a time-dependant change in residual mesotrione concentrations in medium where algae (*+S. quadricauda*, *+Microcystis* sp.) or no algae (*-S. quadricauda*, *-Microcystis* sp.) were added (A and B). Values are the means ± standard deviation (SD) (n = 3). * is significantly different at $p < 0.05$.

Degradation of mesotrione in *Microcystis* sp. and *S. quadricauda*

The concentrations of mesotrione residues in the medium with algae progressively were reduced with the time. To confirm whether the concomitant biodegradation occurred during the process of its accumulation, SDAPCI-MS experiments were carried out. In negative mode, mesotrione was detected as deprotonated molecules (*m/z* 338) in *Microcystis* sp.-incubated medium (Fig.4A) and *S. quadricauda*-incubated medium (Fig.4B) after 48 h under 5 mg L^{-1} mesotrione exposure, indicating that mesotrione in medium were not absorbed completely by algae. From the CID spectra (insets in Fig.4A), the spectrum showed ions at *m/z* 291 and 212 corresponds to the fragments $[M\text{-}HNO_2]^-$ and $[M\text{-}HNO_2\text{-}SO_2CH_3]^-$, respectively. It is evident that *m/z* 338 was deprotonated molecules of mesotrione. However, mesotrione is easier to deprotonate the acidic proton rather than an aromatic hydrogen in the negative mode.

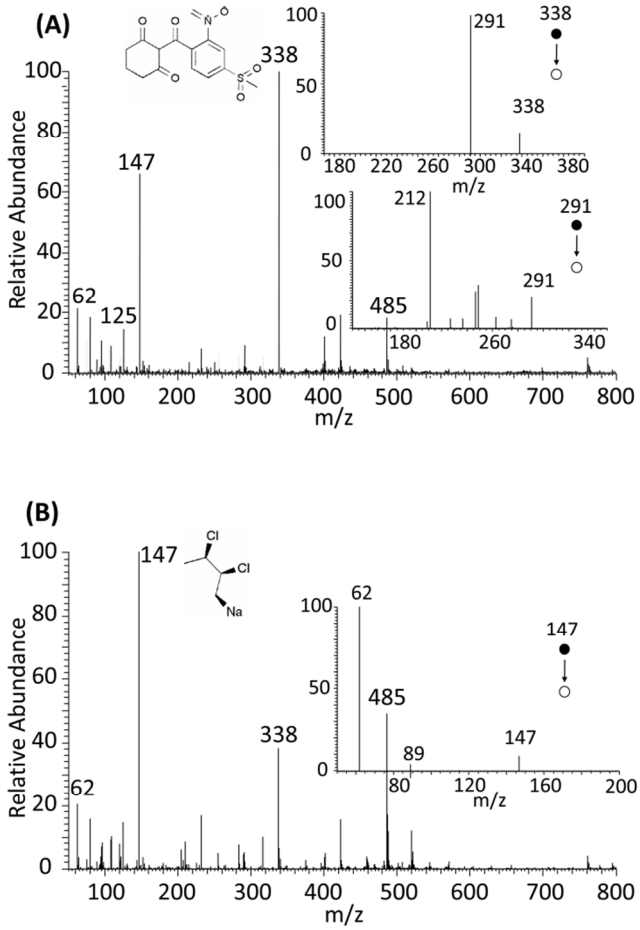


Fig. 4 SDAPCI-MS spectra of *Microcystis* sp.-incubated medium and CID spectra of *m/z* 338 and *m/z* 291 (A) and SDAPCI-MS spectra of *S. quadricauda*-incubated medium and MS/MS spectra of 147 (B) recorded after 48 h of culture in negative ion detection mode

From the SDAPCI-MS and MS/MS spectra (Fig.4A, Fig.4B), the importance of the chlorine and sodium adducts were observed, $[M+Cl]^-$, $[2M+2Cl]^-$ and $[2M+2Cl+Na]^-$ ions, at *m/z* 62, 125 and 147, respectively. The $[2M+2Cl]^-$ ion was

possible unstable intermediate phase. The chlorine ion and sodium ion are nutrients of culture medium. Based on its fragmentation patterns observed by the spectra, another compound in medium was tentatively assigned to ethylene. The ethylene may be released from algae under mesotrione stress. The peak at *m*/*z* 485 was also observed in the mass spectrum. This adduct may result from mesotrione linked to 1,2-dichloro-4-sodium butane. However, the characteristic fragmentations of MNBA and AMBA at *m*/*z* 200 and 170 were not observed in the spectra, indicating that there was not presumed metabolites of mesotrione in the medium. Compared to 48 and 96 h of culture, SDAPCI-MS and CID spectra did not show difference (data not shown).

Discussion

Microalgae species tested in the present study were differentially sensitive to herbicide exposure, showing differences in relation to parameters such as fluorescence of chlorophyll a, F_v/F_m and RLCs of photosynthetic apparatus. One of the most commonly used parameters in toxic assay is the median effective concentration (EC_{50}), which represents the concentration of toxicants causing a 50% reduction.[32, 33] Based on the values of chlorophyll a fluorescence (μg L^{-1}), the EC_{50} results showed high variability in the impact of mesotrione on *Microcystis* sp. and *S. quadricauda*. Mesotrione showed higher toxicity for *S. quadricauda* than *Microcystis* sp.. A previous study on the acute toxicity of mesotrione to the green alga *Raphidocelis subcapitata* was 3.5 mg L^{-1}(acute 72 h),[34] which was less than the EC_{50} of mesotrione in this study, suggesting that *Microcystis* sp. is more tolerant to mesotrione than *S. quadricauda* and *Raphidocelis subcapitata*. Similar results were found in a competition between green algae and blue-green algae under metribuzin exposure and allelochemicals treatments.[35,36] The observed differences in sensitivity to mesotrione may be associated with the adaptive ability of algae and cyanobacteria to cope with high excitation pressure.

F_v/F_m represent chlorophyll fluorescence emission of photosynthetic active organisms, which originates mainly from chlorophyll a molecules of PS Ⅱ and is often used as an indicator of environmental stress.[37] Mesotrione affected the photosynthetic capacity of both species, as indicated by a change in the maximum PS Ⅱ activity (F_v/F_m). Rapid light curves (RLCs) commonly used to assess the current photosynthetic capacity of PS Ⅱ as a function of irradiance with increasing levels of PAR.[38] The RLCs revealed ecophysiological differences between *Microcystis* sp. and *S. quadricauda*. It was shown by the bigger change of amplitude of the RLCs in *S. quadricauda* and the slight decrease of RLCs in *Microcystis* sp. as mesotrione concentration increased. The decrease of I_k, α and ETR_{max} of *S. quadricauda* showed that mesotrione cause more serious inhibition of the efficiency of PS Ⅱ, indicating that *Microcystis* sp. was a superior competitor in mesotrione stress in comparison to *S. quadricauda*. Previous studies have indicated that photosynthetic capacity of algae lower under herbicides exposure, for instance, isoproturon induced inhibition of PS Ⅱ activity in *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*,[39,40] atrazine affected the electron transport, primary production and photoregulation processes of *Ankistrodesmus falcatus*, *Chlamydomonas snowii*, *Microcystis flos-aquae* and *Aphanizomenon flos-aquae*.[41]

As a result of their high surface area to volume ratio, algae could potentially be used in organic pollutants remediation. The mechanism involved in the removal of mesotrione by microalgae is similar to that of other organic contaminants, such as isoproturon,[40] prometryne[42] and fluroxypyr.[43] Initial removal is passive physicochemical adsorption and at high levels of organic xenobiotics algae always had high-turnover of contaminants. Moreover, the magnitude of accumulation of organic toxicants in algae is species-specific. In this study, the medium to which algae were added always contained less amounts of mesotrione than the control, indicating that a certain proportion of mesotrione left in the medium was absorbed by algae. This was especially presented at higher levels (2-10 mg L^{-1}) of mesotrione. The ability of accumulation of mesotrione by *Microcystis* sp. was bigger than *S. quadricauda*. The result is in agreement with previous studies that the ratio of high surface area to biovolume of freshwater algae possesses high potential for accumulation. Accumulation capacity was associate with the size and morphology of algal cells.[44,45] With the time, residual mesotrione in the medium with both species were progressively reduced. It was possible that long exposure to mesotrione activated scavenging capability and allowed the algae to become more adaptive to the stress environment. The accumulation capacity of mesotrione by *Microcystis* sp. and *S. quadricauda* may be closely correlated with an efficient scavenging/detoxification system and the precise mechanism remains to be further investigated.

Although mesotrione was accumulated by both algae, it cannot confirm that the concomitant degradation of mesotrione occurred during the process of its accumulation. MNBA and AMBA, the presumed metabolites of mesotrione, which were from the biotransformation of mesotrione by pure bacterial strain (*Bacillus* sp. 3B6).[46] In this study, the characteristic fragmentations of MNBA and AMBA at *m*/*z* 200 and 170 were not observed in the SDAPCI-MS and MS/MS spectra, indicating that degradation of mesotrione did not occurr during the process within 96 h or biodegradation pathway of mesotrione by algae is different from that by a pure bacterial strain (*Bacillus* sp. 3B6). On the contrary, *Chlamydomonas reinhardtii* showed weak ability to degrade isoproturon accumulated in its cells.[40] It may be associate with the molecular weight, water solubility, lipophilicity of herbicide and algal species. However, it is very important to identify the metabolite products produced in the system. More studies aimed at understanding precise mechanism involved in the removal and degradation of mesotrione by algae are needed.

Conclusion

The photosynthetic responses of both algae were negatively associated with external concentrations of mesotrione. The 96 h

EC_{50} value of mesotrione on *S. quadricauda* and *Microcystis* sp. were 4.41 and 6.19 mg L^{-1}, respectively. *Microcystis* sp. was more tolerance than *S. quadricauda*. Mesotrione affected the photosynthetic capacity of both species, the sensitivity of algae to mesotrione could be also presented by the photosynthetic parameters such as F_v/F_m, I_k, α and ETR_{max} of RLCs. At moderate concentrations of mesotrione (around 5.0 mg L^{-1}), both algae had high ability to accumulate mesotrione. Degradation of mesotrione did not occurr during the incubation period of both species or degradation pathway of mesotrione by algae is different from that by a pure bacterial strain (*Bacillus* sp. 3B6). The photosynthetic responses to mesotrione will be used as biomarkers and can be interpreted as an internal tolerant mechanism to allow us to assess the toxicity of the herbicide and may allow us to develop alga-based clearing up systems for bioremediation of herbicide contamination in aquatic environments.

Acknowledgements

We acknowledge the financial support of the Key Projects in the National Science & Technology Pillar Program during the Eleventh Five-year Plan Period (2007BAB23C02) and Open Fund of Key Laboratory of Poyang Lake Environment and Resource Utilization (Nanchang University) of Ministry of Education (Z04999) for this study.

Notes and references

[a] Kay Laboratory of Poyang Lake Environment and Resource Utilization, Ministry of Education, Institute of Environmental and Chemical Engineering, Nanchang University, Jiangxi, China.

[b] Department of Biology, East China Institute of Technology, Jiangxi, China.

1. R. Prado, C. Rioboo, C. Herrero and A. Cid, *Chemosphere*, 2009, **76**, 1440-1444.
2. D. G. Hela, D. A. Lambropoulou, I. K. Konstantinou and T. A. Albanis, *Environ. Toxicol. Chem.*, 2005, **24**, 1548-1556.
3. M. Arias-Estévez, E. López-Periago, E. Martínez-Carballo, J. Simal-Gándara, J.-C. Mejuto and L. García-Río, *Agric. Ecosyst. Environ.*, 2008, **123**, 247-260.
4. R. Prado, R. García, C. Rioboo, C. Herrero, J. Abalde and A. Cid, *Environ. Int.*, 2009, **35**, 240-247.
5. S. R. Norris, T. R. Barrette and D. DellaPenna, *Plant Cell* 1995, **7**, 2139-2149.
6. L. G. Freitas, C. W. Götz, M. Ruff, H. P. Singer and S. R. Müller, *J. Chromatogr. A*, 2004, **1028**, 277-286.
7. J. D. McCurdy, J. S. McElroy, D. A. Kopsell, C. E. Sams and J. C. Sorochan, *J. Agric. Food. Chem.*, 2008, **56**, 9133-9139.
8. J. L. B. F. B. M. D. J. Bohatier, *Arch. Environ. Contam. Toxicol.*, 2008, **55**, 576-583.
9. M. S. Vera, E. Di Fiori, L. Lagomarsino, R. Sinistro, R. Escaray, M. M. Iummato, A. Juárez, M. d. C. R. de Molina, G. Tell and H. Pizarro, *Ecotoxicology*, 2012, **21**, 1805-1816.
10. W. Liu, Y.-B. Zhang, X. Quan, Y.-H. Jin and S. Chen, *Chemosphere*, 2009, **75**, 405-409.
11. E. Navarro, F. Piccapietra, B. Wagner, F. Marconi, R. Kaegi, N. Odzak, L. Sigg and R. Behra, *Environ. Sci. Technol.*, 2008, **42**, 8959-8964.
12. S. Knauert, B. Escher, H. Singer, J. Hollender and K. Knauer, *Environ. Sci. Technol.*, 2008, **42**, 6424-6430.
13. S. B. Sjollema, G. MartínezGarcía, H. G. van der Geest, M. H. Kraak, P. Booij, A. D. Vethaak and W. Admiraal, *Environ. Pollut.*, 2014, **187**, 106-111.
14. M. Devine, S. O. Duke and C. Fedtke, *Physiology of Herbicide Action*, PTR Prentice Hall, 1993.
15. G. B. Claire Valiente Moro, Christophe Portelli,Jacques Bohatier, *Environ. Toxicol. Chem.*, 2012, **31**, 778-786.
16. U. Schreiber, U. Schliwa and W. Bilger, *Photosynth. Res.*, 1986, **10**, 51-62.
17. O. Herlory, J.-M. Bonzom and R. Gilbin, *Aquat. Toxicol.*, 2013, **140**, 288-294.
18. A. ter Halle and C. Richard, *Environ. Sci. Technol.*, 2006, **40**, 3842-3847.
19. S. Durand, M. Sancelme, P. Besse-Hoggan and B. Combourieu, *Chemosphere*, 2010, **81**, 372-380.
20. A. Fargašová and J. Kizlink, *Ecotox Environ Safe*, 1996, **34**, 156-159.
21. R. Rippka, *Methods in Enzymology*, Academic Press, 1989.
22. R. Rippka, J. Deruelles, J. B. Waterbury, M. Herdman and R. Y. Stanier, *J. Gen. Microbiol.*, 1979, **111**, 1-61.
23. B. Cheirsilp and S. Torpee, *Bioresour. Technol*., 2012, **110**, 510-516.
24. E. J. K. Plummer J D, *Environ. Sci. Technol.*, 2001, **35**, 3661-3668.
25. M. Kitajima and W. Butler, *Biochim. Biophys. Acta*, 1975, **376**, 105-115.
26. A. J. White and C. Critchley, *Photosynth. Res.*, 1999, **59**, 63-72.
27. K. Maxwell and G. N. Johnson, *J. Exp. Bot.*, 2000, **51**, 659-668.
28. Z. P. Jin, K. Luo, S. Zhang, Q. Zheng and H. Yang, *Chemosphere*, 2012, **87**, 278～284.
29. K. Stoob, H. P. Singer, C. W. Goetz, M. Ruff and S. R. Mueller, *J. Chromatogr. A*, 2005, **1097**, 138-147.
30. H. W. Chen, J. H. Lai, Y. F. Zhou, Y. F. Huan, J. Q. Li, Z. Xie, Z. C. Wang and M. B. Luo, *Chin.J.Anal.Chem.*, 2007, **35**, 1233-1240.
31. L. Haanstra, P. Doelman and J. O. Voshaar, *Plant Soil*, 1985, **84**, 293-297.
32. C. I. Weber, *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms*, Environmental Monitoring and Support Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, 2002.
33. *OECD Guidelines for the Testing of Chemicals, Section 2: Effects on Biotic Systems Test No. 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test*, OECD Publishing, 2011.
34. J. Ma, S. Wang, P. Wang, L. Ma, X. Chen and R. Xu, *Ecotox Environ Safe*, 2006, **63**, 456-462.
35. I. R. Miquel Lürling *Chemosphere*, 2006, **65**, 618-626.
36. X. Chang, F. Eigemann and S. Hilt, *Harmful Algae*, 2012, **19**, 76-84.
37. N. Mallick and F. Mohn, *Ecotox. Environ. Safe*, 2003, **55**, 64-69.
38. U. Schreiber, R. Gademann, P. Ralph and A. Larkum, *Plant Cell Physiol.*, 1997, **38**, 945-951.
39. D. Dewez, O. Didur, J. Vincent-Héroux and R. Popovic, *Environ. Pollut.*, 2008, **151**, 93-100.

40. Y. F. Bi, S. S. Miao, Y. C. Lu, C. B. Qiu, Y. Zhou and H. Yang, *J. Hazard. Mater.*, 2012, **243**, 242-249.
41. C. P. Deblois, K. Dufresne and P. Juneau, *Aquat. Toxicol.*, 2013, **126**, 77-84.
42. Z. P. Jin, K. Luo, S. Zhang, Q. Zheng and H. Yang, *Chemosphere*, 2012, **87**, 278-284.
43. S. Zhang, C. B. Qiu, Y. Zhou, Z. P. Jin and H. Yang, *Ecotoxicology*, 2011, **20**, 337-347.
44. R. E. Lee, *Phycology*, Cambridge University Press, 2008.
45. N.-X. Wang, Y. Li, X.-H. Deng, A.-J. Miao, R. Ji and L.-Y. Yang, *Water Res.*, 2013, **47**, 2497-2506.
46. S. Durand, B. Légeret, A. S. Martin, M. Sancelme, A. M. Delort, P. Besse‐Hoggan and B. Combourieu, *Rapid commun. mass sp.*, 2006, **20**, 2603-2613.