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Effects of light, microorganisms, farming chemicals and water content on the degradation of microcystin-LR in agricultural soils

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ABSTRACT

An experiment was conducted to investigate the effect of farming activities on microcystin-LR (MC-LR) degradation in soils. Three farming activities were assessed: 1) fertilization via addition of different nitrogen sources and organic matter; 2) pesticide application by addition of different commercial pesticides; and 3) irrigation by addition of different amount of water. The contribution of the two major degradation processes of MC-LR in soils, photodegradation and biodegradation, were also evaluated. MC-LR was added into the soil samples to create a concentration of 500 μg kg^{-1} for each treatment. Results showed that natural degradation of MC-LR in soils was mainly by biodegradation rather than photodegradation. MC-degradation was stimulated by the addition of $NaNO_3$ and humic acid, whereas degradation was inhibited by addition of NH_4Cl, glucose, and glycine. Application of high concentrations of glyphosate and chlorothalonil significantly inhibited the degradation of MC-LR in soils and the half-life was almost twice as long as the control. No significant effect was found by addition of $CO(NH_2)_2$ and dimethoate. Both low (10%) and high water content (60%) could lead to inhibition of MC-LR degradation. Results from our study help to inform farm practices that could alleviate contamination by MC-LR in agroecosystems.

1. Introduction

Microcystins (MCs) are frequently produced by some bloom-forming cyanobacteria, mainly *Microcystis*, *Anabaena, Nostoc* and *Oscillatoria*, in eutrophic fresh water lakes (Chen et al., 2016). Owing to MCs' potential carcinogenicity, they can negatively affect both public health and fundamental ecological processes (Rastogi et al., 2014; Zhao et al., 2016). More than 200 different structural analogues of MCs, with a range of molecular weights from 882 to 1116 Da, have been identified from cyanobacterial blooms and cultures (Zastepa et al., 2015; Spoof and Catherine, 2017). Microcystin-LR is the most common and potent analogue, followed by microcystin-RR and microcystin-YR (Chen et al., 2016). The World Health Organization (WHO) proposed a safety guideline of 1.0 μg L^{-1} MC-LR for drinking water (Falconer, 1999). However, total MCs concentrations in surface waters vary from less than 1 μg L^{-1} to 29,000 μg L^{-1} (Spoof et al., 2003; Billiam et al., 2006; Nasri et al., 2008; Giannuzzi et al., 2011; Liu et al., 2011). The highest concentrations of MCs come from very dense cyanobacterial biomass, but their concentration in most of the water samples is less than 100 μg L^{-1} (Lindholm and Meriluoto, 1991; Jones and Orr, 1994; Lahti et al., 1997).

Due to their cyclic structure, MCs have a high chemical stability in the natural environment. Although MCs are produced in aquatic ecosystems, irrigation with cyanobacteria-containing water can bring MCs to agricultural soils; in addition, cyanobacterial blooms can be used as organic fertilizer after being intentionally harvested from lakes (Liu et al., 2008; Saqrane et al., 2009; Chen et al., 2010a, 2012). Once MCs are present in the soil, they can be removed according to various processes (e.g., photolysis, hydrolysis or microbial degradation) (Corbel et al., 2014a). Moreover, microbial activity is thought to be the major degradation process for MCs in soil ecosystems (Miller and Fallowfield, 2001; Chen et al., 2006; Rastogi et al., 2014). To the best of our knowledge, no previous studies concerning photodegradation of MCs in soils have been reported. Normally, MCs are stable in water under natural sunlight, but can rapidly be degraded under ultraviolet radiation (UVR). Tsuji et al. (1995) reported that about half of MC-LR was degraded after a 10 min exposure to 1.47 W m^{-2} UVR, whereas complete degradation was measured after a 10 min exposure to 25.50 W m^{-2} UVR. Moreover, MCs can be rapidly degraded following exposure to UVR near the absorption maximum (238 nm) by

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isomerisation or intramolecular reaction (photosensitized reactions) in the presence of pigments or humic substances (Tsuji et al., 1995; Kaya and Sano, 1998).

Biodegradation of MCs in the environment has been widely studied. Environmental factors, such as nutrient conditions (Chen et al., 2010b; Li et al., 2011), oxygen status (Holst et al., 2003) and temperature (Park et al., 2001; Chen et al., 2010b) are reported to affect the biodegradation rate of MCs. In aquatic systems, MCs have been shown to be readily degraded by bacteria with MC half-lives ranging from approximately 1 to 14 d (Edwards et al., 2008; Chen et al., 2008). However, studies on the biodegradation of MCs in soils are rare. Chen et al. (2006) reported that the half-life of MCs degradation in soils ranged from 6.0 to 17.1 days for MC-LR, 7.9–17.8 days for MC-RR, and 7.1–10.2 days for MC- Dha7 LR. High organic carbon content in soils favored MCs degradation (Chen et al., 2006). Miller and Fallowfield (2001) found that MC-LR could be completely degraded within 10–16 d in two of three tested soils. Soils with higher microbial activity favored the degradation of MC-LR.

It is suggested that the adsorption of MCs in soils is generally low, which can potentially result in higher bioavailability in plant-soil systems (Machado et al., 2017). Moreover, Eynard et al. (2000) indicated that soil was unable to protect groundwater from surface water that contains cyanotoxins. Consequently, degradation of MCs in soils is very important to protect plant-soil system and groundwater from MCs contamination. Farming activities, such as fertilizing, pesticide spraying and irrigation can have a significant impact on the soil microbial community (Atlas et al., 1978; Bollen, 2003; Paul et al., 2003; Dai, 2007; Tejada et al., 2014). However, to the best of our knowledge, no previous work has assessed the effect of different farming activities on MCs degradation in soils. In the present study, first we evaluated the contribution of photodegradation and biodegradation in the MC-degradation process. Then we assessed the effect of three different farming practices on MC-LR degradation. 1) the impact of fertilizers by addition of carbamide, ammonia chloride, sodium nitrate, glucose, glycine and humic acid at different concentrations; 2) the impact of pesticide application by addition of dimethoate, glyphosate and chlorothalonil at different concentrations; and 3) the impact of water content by incubation of soils under different water content (10%, 20%, 30% and 60%). Results from this study provide insights into the impact of farming activities on MC-LR degradation, and provide guidance regarding the alleviation of MC-LR contamination in agricultural soils.

2. Materials and methods

2.1. Toxins and chemicals

The MC-LR standard used in the present study was purchased from Taiwan Algal Science Inc. (≥ 95% purity, Lot No.: L1101003, CAS No.: 101043–37-2) and stored at − 25 °C. HPLC-grade methanol was used as the mobile phase in HPLC analysis. Technical grade pesticides were used in the present study (dimethoate, purity ≥ 97.5%; glyphosate, purity ≥ 98%; chlorothalonil, purity ≥ 99.7%). All other chemicals were of analytical grade.

2.2. Soil collection and characterization

Farmland soils (0–15 cm) that were not previously exposed to MCs were collected from the lakeside of Lake Taihu at Suzhou. After being taken back to the laboratory, the soil samples were homogenized and passed through a 2-mm sieve to sift out roots and other large debris. A portion of each soil sample was used for physico-chemical analysis after being air-dried at 25 °C for 48 h (Sparks et al., 1996). Physicochemical properties included the following: 27.9% sand, 31.0% silt, 41.2% clay, pH 7.46, 9.91 g organic matter kg^{-1} soil, 0.78 g total nitrogen kg^{-1} soil, 15.4 mg available P kg^{-1} soil, and 150 mg available K kg^{-1} soil. The remaining soil sample was kept fresh at 4 °C for degradation experiments.

2.3. Degradation study for MC-LR

2.3.1. Impact of light and microorganisms

MC-LR degradation experiments were conducted in a series of 100-mL pots. Each of the pots was filled with 50 g soil. Four treatments were applied: (1) MC-LR application and incubation in the light (Light treatment); (2) MC-LR application and incubation in the dark (Dark treatment); (3) MC-LR application, 2% azide (w/v) solution, which has been shown previously to kill soil microorganisms (Miller and Fallowfield, 2001), and incubated in the light (Sterile light treatment); (4) MC-LR application, 2% azide (w/v) solution, and incubation in the dark (Sterile dark treatment).

Soil samples (4 kg) were artificially contaminated by spiking the MC-LR solution to give a final concentration of 500 μg kg^{-1}. After thoroughly mixed with a rotary mixer (Philips handmixer, HR1570) to assure uniform MC-LR distribution, the soil sample was subjected to different treatments (the same below in 2.3.2, 2.3.3 and 2.3.4). Soil samples in the light treatment were incubated in a growth chamber under natural sunlight (temperature: 30 °C; relative humidity: 70%). All other samples were incubated under the same conditions in the dark. To avoid excessive water evaporation from soil, pots were covered with porous plastic film. All experiments were carried out in duplicate for 18 days.

The moisture content of soil was brought to 60% of the maximum water holding capacity (WHC) in treatments described in Sections 2.3.1, 2.3.2 and 2.3.3, whereas the water content of soils described in Section 2.3.4 was set as needed. The water content in all treatments was kept constant by amending as needed after daily weighing. For each treatment, 4 g soil was sampled every three days and stored at − 40 °C for MC-LR analysis.

The specific design of the experiments was showed in the diagram (Fig. S1).

2.3.2. Impact of fertilizers

Fifty grams of soils were placed in a series of pots (same as above). Each soil sample was further enriched by spiking with different fertilizers to give a final concentration as needed. The recommended application rate for N fertilizer in a vegetable field near Taihu Lake is about 60 kg ha^{-1}. Assuming that the soil weight is 1.5 × 105 kg ha^{-1} at the effective soil depth of 10 cm (GB/T 31270.1-2014), 60 kg ha^{-1} corresponds to 400 mg N kg^{-1} dry soil, so the three different levels of N fertilizer in the present study represent 0.5, 1 and 2 times the concentration of the recommended rate, 200, 400 and 800 mg N kg^{-1} dry soil, respectively. The levels of organic fertilizer were set similarly to N fertilizer. The impact of different fertilizers on MC-LR degradation was investigated by addition of $CO(NH_2)_2$ (0, 200, 400, 800 mg N kg^{-1} dry soil), NH_4Cl (0, 200, 400, 800 mg N kg^{-1} dry soil), $NaNO_3$ (0, 200, 400, 800 mg N kg^{-1} dry soil), glucose (0, 200, 400, 800 mg kg^{-1} dry soil), glycine (0, 200, 400, 800 mg kg^{-1} dry soil) and humic acid (0, 200, 400, 800 mg kg^{-1} dry soil). The dark treatment described in Section 2.3.1 also served as the control treatment for this fertilizer experiment.

2.3.3. Impact of pesticides

The impact of different pesticides on MC-LR degradation was investigated by addition of dimethoate, (50, 100, 200 mg kg^{-1} dry soil), glyphosate (0, 25, 50, 100 mg kg^{-1} dry soil), and chlorothalonil (0, 5, 10, 20 mg kg^{-1} dry soil) to pots containing 50 g of soil. The rates represent 0.5, 1 and 2 times the concentration of the highest recommended rate. The dark treatment described in Section 2.3.1 also served as the control treatment for this pesticide experiment.

2.3.4. Impact of water content

The impact of water content on MC-LR degradation was studied by incubation of pots containing 50 g of soil with 10%, 20%, 30% and 60%

water content. 60% soil water content created a flooded condition.

2.4. HPLC analysis for MC-LR

MC-LR in soils was determined as described by Chen et al. (2006). Briefly, lyophilized soil samples were extracted 3 times with 30 mL of 0.1 M EDTA-0.1 M $Na_4P_2O_7$ with an ultrasonic bath (400 W, 10 min) after being ground with a mortar and pestle. The homogenate was then centrifuged at 4000 g for 10 min. After modifying the pH of the supernatant to 3 with TFA, the solution was centrifuged again under the same conditions. The aqueous extractions were then applied to a Sep-pak ODS cartridge (500 mg, 6 mL, Waters). The cartridge with toxin was rinsed with 15 mL of 20% (v/v) aqueous methanol, and eluted with 10 mL 90% aqueous methanol. The eluant was evaporated to dryness and the residue was dissolved in 0.1 mL 100% aqueous methanol. The toxin solutions obtained were stored at − 40 °C for High-Performance Liquid Chromatography (HPLC) analysis.

2.5. Statistical analysis

SPSS software (SPSS 22.0, Inc., 2013) was used for statistical analyses. Results were examined with One-Way Repeated-Measures ANOVA to determine if there were significant differences between the treatments of different factor levels. A further means comparison (Tukey method) was conducted when necessary. Differences were considered significant if $P < 0.05$.

First order kinetics were applied to calculate the half-lives of MC-LR in different treatments, using OriginPro 8.5 software (OriginLab corporation, MA, USA). The kinetic model is represented by the following equation: $C_t = C_0 e^{-kt}$,

where C_t is the concentration of MC-LR at the instant t; C_0 is the initial concentration of MC-LR; e is the natural constant; k is the rate constant; t is the degradation time. Half-lives were calculated as: $t_{1/2} = \ln 2/k$.

3. Results and discussion

3.1. Effect of light and sterilization on MC-LR degradation

Four treatments (light; dark; sterile light; sterile dark) were set up to investigate the effect of light and microorganisms on MC-LR degradation in soils. No significant reduction was observed in either sterilization treatment (Fig. 1). In contrast, MC-LR was rapidly degraded in both non-sterile treatments. The half-lives of the non-sterile control were 5.2 d with light and 5.0 d in the dark (Table 1).

MCs have been widely studied in waters due to their stable chemical structure and high toxicity. However, little attention has been paid to their degradation, especially in soils. Of the studies that have been conducted, microbially-mediated degradation appears to be the main pathway (Miller and Fallowfield, 2001; Chen et al., 2006). Manage et al. (2009) isolated some MCs-degrading bacteria (*Rhodococcus* sp., *Brevibacterium* sp. and *Arthrobacter* sp.), which can be commonly found in soils. However, different results were obtained by Corbel et al. (2014b), who reported that microbial mineralization of cyanotoxin was very weak in silty sand soil, and consequently large portions of the toxin remained available in the ecosystem.

In our study, microorganisms played an important role in MC-LR degradation. Both sterile treatments showed no significant degradation of MC-LR, whereas MC-LR was degraded within 18 d in both non-sterilized treatments. This result indicated that in our soils, photodegradation of MC-LR under natural sunlight was negligible in soils and biodegradation by soil microorganisms was the main removal process. Chen et al. (2006) reported half-lives for MC-LR of 6.0, 6.1 and 12.9 d

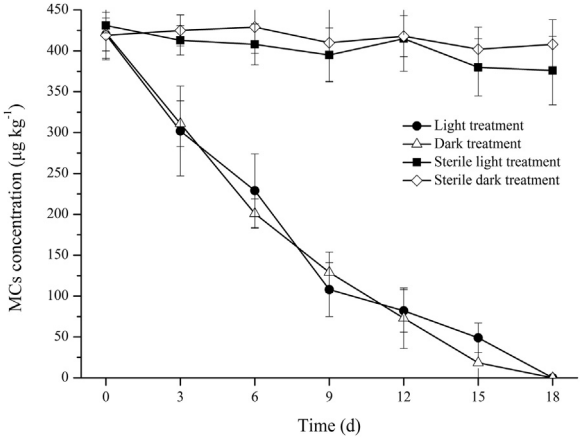

Fig. 1. Degradation curves of MC-LR in soils with or without sterilization. Error bars represent the range of duplicate microcosms.

Table 1
Kinetics of MC-LR degradation in soils submitted to different treatment.

Treatment	Equation	R^2	K/d^{-1}	$t_{1/2}$/d
Light treatment	$C_t = 422e^{-0.132t}$	0.972	0.132	5.2
Dark treatment	$C_t = 420e^{-0.139t}$	0.974	0.139	5.0
$CO(NH_2)_2$				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
200	$C_t = 424e^{-0.151t}$	0.959	0.151	4.6
400	$C_t = 422e^{-0.111t}$	0.935	0.111	6.2
800	$C_t = 428e^{-0.124t}$	0.969	0.124	5.6
$(NH_4)Cl$				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
200	$C_t = 429e^{-0.148t}$	0.966	0.148	4.7
400	$C_t = 424e^{-0.126t}$	0.953	0.126	5.5
800	$C_t = 424e^{-0.076t}$	0.951	0.076	9.1
$NaNO_3$				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
200	$C_t = 419e^{-0.165t}$	0.965	0.165	4.2
400	$C_t = 425e^{-0.192t}$	0.970	0.192	3.6
800	$C_t = 421e^{-0.231t}$	0.985	0.231	3.0
Glucose				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
200	$C_t = 428e^{-0.141t}$	0.952	0.141	4.9
400	$C_t = 428e^{-0.084t}$	0.947	0.084	8.3
800	$C_t = 419e^{-0.081t}$	0.951	0.081	8.6
Glycine				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
200	$C_t = 416e^{-0.122t}$	0.918	0.122	5.7
400	$C_t = 420e^{-0.107t}$	0.954	0.107	6.5
800	$C_t = 426e^{-0.089t}$	0.947	0.089	7.8
Humic acid				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
200	$C_t = 425e^{-0.145t}$	0.957	0.145	4.8
400	$C_t = 430e^{-0.179t}$	0.986	0.179	3.9
800	$C_t = 415e^{-0.211t}$	0.981	0.211	3.3
Dimethoate				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
50	$C_t = 412e^{-0.132t}$	0.973	0.132	5.2
100	$C_t = 420e^{-0.140t}$	0.930	0.140	4.9
200	$C_t = 417e^{-0.121t}$	0.932	0.121	5.6
Glyphosate				
0	$C_t = 422e^{-0.139t}$	0.972	0.139	5.0
25	$C_t = 421e^{-0.133t}$	0.972	0.133	5.2
50	$C_t = 419e^{-0.088t}$	0.949	0.088	7.9
100	$C_t = 425e^{-0.077t}$	0.957	0.077	9.0
Chlorothalonil				
0	$C_t = 422e^{-0.139t}$	0.974	0.139	5.0
5	$C_t = 424e^{-0.140t}$	0.958	0.140	4.9
10	$C_t = 418e^{-0.122t}$	0.924	0.122	5.7
20	$C_t = 420e^{-0.080t}$	0.942	0.080	8.7
Water content				
10%	$C_t = 415e^{-0.083t}$	0.902	0.083	8.4
20%	$C_t = 421e^{-0.125t}$	0.948	0.125	5.6
30%	$C_t = 423e^{-0.127t}$	0.960	0.127	5.5
60%	$C_t = 424e^{-0.096t}$	0.867	0.096	7.2

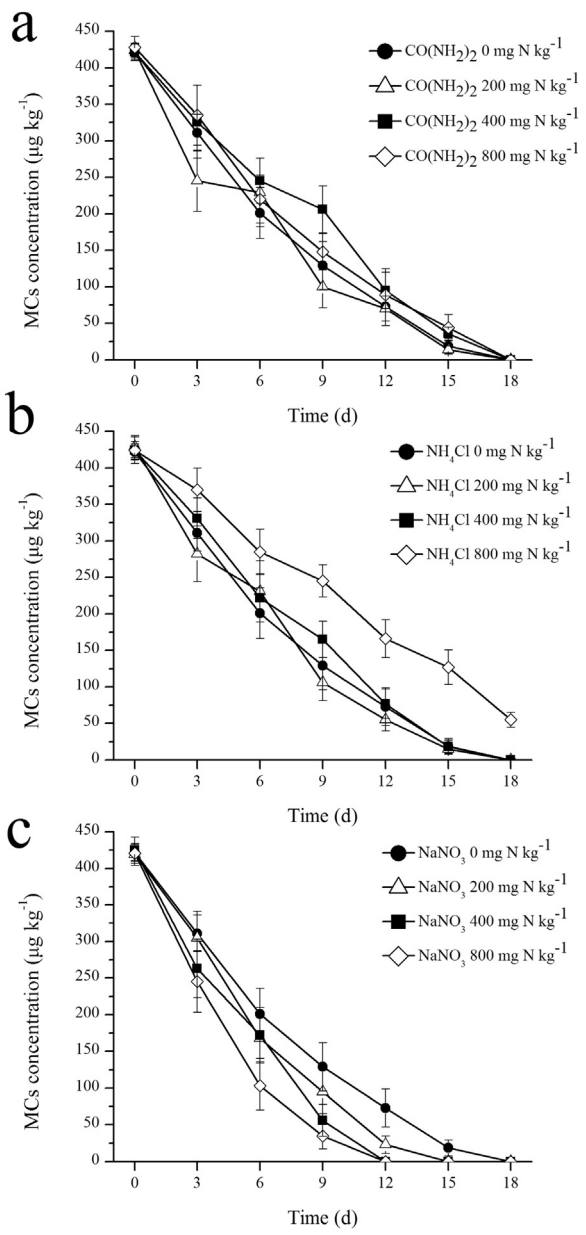

Fig. 2. Effect of different N fertilizer on the degradation of MC-LR in soils. Error bars represent the range of duplicate microcosms. (a) $CO(NH_2)_2$; (b) NH_4Cl; (c) $NaNO_3$.

in 3 types of Chinese agriculture soils. Hence, degradation of MC-LR in soils appears to be affected by soil type, due to different physico-chemical properties, as well as microbial properties.

In aquatic systems, photodegradation of MC-LR is expected only in shallow systems (Wörmer et al., 2010), where light can penetrate. Because of limited light penetration in soils, photodegradation of MC-LR was expected to take place only on the soil surface, as noted for other organic pollutants (Konstantinou et al., 2001; Katagi, 2004; Quan et al., 2005). In the present study, light had no significant effect on MC-LR degradation, regardless of whether or not the soil was sterilized. Hence, we recommend studies to isolate and identify the soil bacteria that may be responsible for MCs degradation in agricultural systems.

3.2. Effect of different nitrogen fertilizer on MC-LR degradation

Three different nitrogen fertilizers were used to investigate the effect of nitrogen application on MC-LR degradation. Addition of $CO(NH_2)_2$ at all levels had no significant effect on MC-LR degradation (Fig. 2A). The longest half-life was observed in the 400 mg N kg^{-1} treatment, 6.2 d (Table 1). Low levels of NH_4Cl (200 and 400 mg N kg^{-1}) had no impact on MC-LR degradation compared to the control, whereas addition of 800 mg N kg^{-1} NH_4Cl significantly inhibited MC-LR degradation in soils, resulting in a half-life of 9.1 d (Fig. 2B; Table 1). In contrast, all $NaNO_3$ additions significantly increased degradation rates compared to the control (Fig. 2C; Table 1), and addition of 800 mg N kg^{-1} $NaNO_3$ significantly increased MC-LR degradation. All the MC-LR was degraded within 12 d in the 400 and 800 mg N kg^{-1} $NaNO_3$ treatments, with half-lives of 3.6 d and 3.0 d, respectively, compared to 5.0 d in the control (Table 1).

The N sources used in our study to assess MC-LR degradation yielded variable results. No significant effect was found with $CO(NH_2)_2$ addition, whereas inhibition was found only at high concentrations of NH_4Cl, and $NaNO_3$ addition stimulated degradation at all concentrations. These results suggest that MC-LR degradation is influenced more by the type of N source as opposed to absolute N concentration, even though MC-LR can be used as the sole nitrogen source, as reported by Valeria et al. (2006). A similar result to our study was obtained by Li et al. (2011), who found MC-LR degradation was inhibited with NH_4Cl and stimulated with nitrate in water; they attributed the different response to bacterial community structure, as determined by mlrA gene abundance. One possible reason for inhibition by NH_4Cl in soils was that high concentrations of NH_4Cl can lead to declines in soil pH, which can regulate soil microorganisms (Staddon et al., 1998; Fu et al., 2012). Similar to our results, Chen et al. (2010b) found that addition of 1000 mg L^{-1} NH_4Cl had a negative effect on MC-LR degradation in sediments under anoxic conditions. However, no mechanism was proposed for this result in their study. Although $CO(NH_2)_2$ can also be hydrolysed to NH_4-N by urease, the rate of the process and the extent to which it occurs depends on the urease activity of the soil (Nkrumah et al., 1989). Therefore, the slight change of pH caused by $CO(NH_2)_2$ may have no impact on MC-LR degradation.

Holst et al. (2003) also found a stimulatory effect of $NaNO_3$ on MC-LR degradation in sediments under anoxic conditions. They suggested that NO_3^- might stimulate metabolic activity, including microcystin degradation, as an alternative electron acceptor. However, Chen et al. (2010a, 2010b) reported an inhibition effect of nitrate on MC-LR degradation in sediments under anoxic conditions, which may have been a result of increasing redox potential by nitrate addition. In contrast to our study, both of the forementioned studies were conducted under anoxic conditions, so the mechanism for the effect of nitrogen fertilizer on MC-LR degradation in all soil systems needs further study.

3.3. Effect of different types of organic matter on MC-LR degradation

Glucose, glycine and humic acid were used to investigate the effect of organic matter application on MC-LR degradation in soils. Low levels of glucose (200 mg kg^{-1}) had no significant effect on MC-LR degradation, whereas additions of 400 mg kg^{-1} and 800 mg kg^{-1} significantly reduced the degradation of MC-LR, resulting in half-lives of 8.3 d and 8.6 d, respectively (Fig. 3A; Table 1). A significant reduction of MC-LR degradation was also observed at high levels of glycine addition (400 and 800 mg kg^{-1}) (Fig. 3B). The half-life in the 800 mg kg^{-1} treatment was lengthened by 2.8 d, compared to the control (Table 1). Unlike the other two types of organic matter, humic acid stimulated MC-LR degradation at all tested concentrations (Fig. 3C). MC-LR degradation at 800 mg kg^{-1} treatment was significantly quicker than that of the control treatment. The half-life at 800 mg kg^{-1} treatment was shortened by 1.7 d compared to the control (Table 1).

We included organic matter in our study because of its low cost and ability to provide multiple nutrients and improve soil quality in agroecosystems. As with N, we observed variable effects of organic matter type and concentration on MC-LR degradation. Contradictory effects of glucose have been noted in previous studies. Park et al. (2001) claimed that an isolated MC-degrading bacterium could use MCs as a

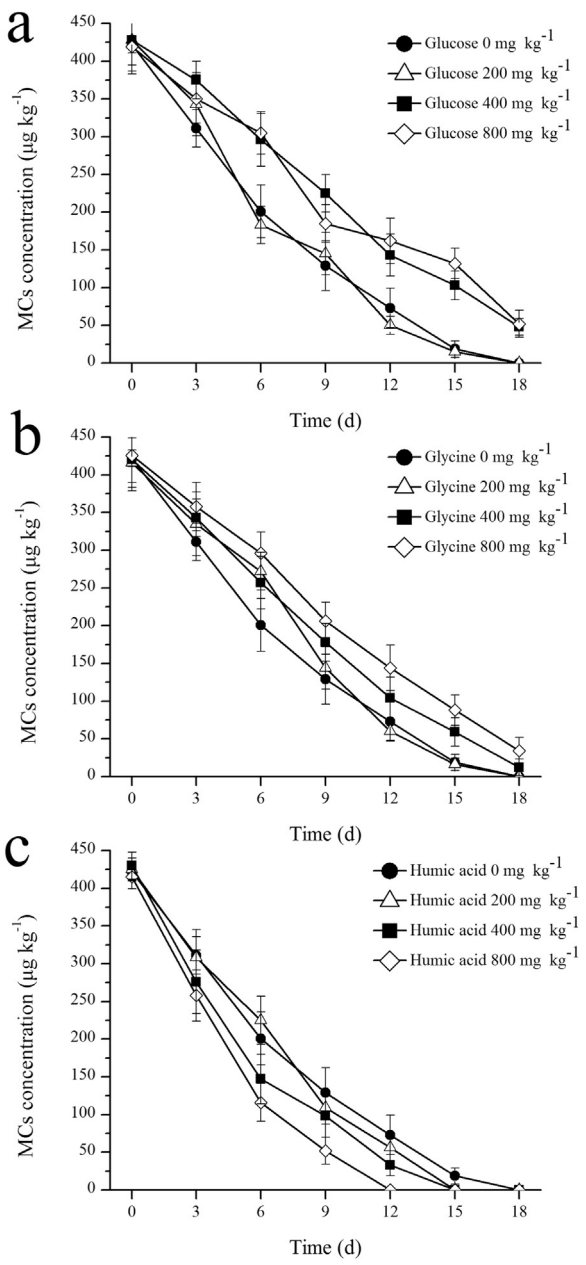

Fig. 3. Effect of different organic fertilizer on the degradation of MC-LR in soils. Error bars represent the range of duplicate microcosms. (a) Glucose; (b) Glycine; (c) Humic acid.

carbon source, although they are not a preferred substrate. As a consequence, in the presence of alternative organic carbon sources, MCs degradation was much slower than in the organic-free medium. Holst et al. (2003) observed a stimulatory effect on MC-LR degradation by glucose addition under anoxic conditions, and attributed this effect to the stimulation of bacterial activity by glucose. Other studies have also shown that MC-degrading bacteria may choose glucose over MCs as a carbon source (Park et al., 2001; Valeria et al., 2006), as labile carbon sources can stimulate bacterial activity (Christoffersen et al., 2002; Holst et al., 2003). We speculate that microbial preference for glucose and glycine over MC-LR in our study resulted in the inhibition of MC-LR degradation in the glycine addition treatment. However, this explanation does not apply to the results obtained by Li et al. (2011), who reported a similar inhibitive effect of MC-LR degradation by addition of both peptone and glucose. In their study, the enhanced inhibition in MC-LR-degradation was correlated to the increased inhibition in proliferation of MC-degrading bacteria, as concentration of nutrients increased.

Humic acid is a main component of humus with a high molecular weight. As shown by Visser (1985), there are no indications that humic additives were used as a metabolite or cometabolite by the microorganisms. The stimulatory effect of humic acid on MC-LR degradation may be the result of its surface-active properties combined with its ability to cross cell membranes, which will lead to utilization of substances that they cannot normally utilize (Visser, 1985). Moreover, some of the MC-LR was adsorbed onto soil particles after entering the soil; this adsorption was affected by soil characteristics (e.g. texture and organic matter content). Natural organic matter in soils, like humic acids, can increase the adsorption of MCs onto soils. (Wu et al., 2011; Thirumavalavan et al., 2012; Corbel et al., 2014b). Hence, adding humic acid to soils may lead to increased adsorption of MC-LR onto soils, resulting in less extractable MC-LR.

3.4. Effect of different pesticides on MC-LR degradation

Dimethoate, glyphosate and chlorothalonil were used to investigate the effect of different pesticides application on MC-LR degradation. No significant difference was observed between all dimethoate treatments and control treatment (Fig. 4A). However, high levels of glyphosate (50 and 100 mg kg^{-1}) significantly reduced MC-LR degradation compared to the control (Fig. 4B), with half-lives of 7.9 d and 9.0 d, respectively (Table 1). MC-LR was degraded to below the detection limit within 18 days in the 5 and 10 mg kg^{-1} treatments of chlorothalonil (Fig. 4C). Addition of 20 mg kg^{-1} treatment significantly inhibited MC-LR degradation with a half-life of 8.7 d.

To our knowledge, this is the first report of pesticide impact on MC-LR degradation. In general, the effects of pesticides on microorganisms will vary depending on the chemical dosage, the properties of the soil, and various environmental factors (Ecobichon, 1991). Results obtained from our study suggest that high concentrations of glyphosate and chlorothalonil decreased the microorganism community and/or the activity of MC-degrading bacteria. Consequently, we recommend that farmers wait for a period of 2–3 weeks after irrigating with cyanobacterial-contaminated water before applying pesticides, to allow MC-degrading bacteria to have an effect.

3.5. Effect of water content on MC-LR degradation

Four levels of water content (10%, 20%, 30% and 60%) were used to investigate the effect of water content on MC-LR degradation. No significant effect was observed between the 20% and 30% treatments, with half-lives of 5.6 d and 5.5 d, respectively (Fig. 5; Table 1). Degradation of MC-LR in the 60% treatment was relatively slow in the first 6 d, and increased over time (Fig. 5). All the MC-LR was degraded within 18 d in the 20%, 30% and 60% treatments. Degradation of MC-LR in the 10% treatment was relatively slow, with a half-life of 8.4 d (Table 1). Statistical analysis showed significant faster degradation in 20% and 30% treatments, compared to 10% and 60% treatments.

Water in soils plays an important role in transport of substrates, hydrolysis processes, and redox state. Therefore, soil water content affects microbial activity and influences rates of mineralization (Paul et al., 2003). Given that O_2 diffusion in water is much lower (about 104 times) than in air, excess soil water content will reduce the activity of aerobic microorganisms (Skopp et al., 1990), but could increase the activities of anaerobes. In our study, degradation of MC-LR with 10% and 60% water content is slower than that with 20% and 30%. Water content of 60% created an anaerobic condition, which is not favorable for MC-LR degradation (Holst et al., 2003). However, Chen et al. (2010b) and Wu et al. (2015) found rapid degradation of MC-LR in sediments under anoxic conditions. These different results may be due to different bacteria in the tested soils/sediments. Our results suggest that a water content of 20–30% is favorable for MC degradation, as well

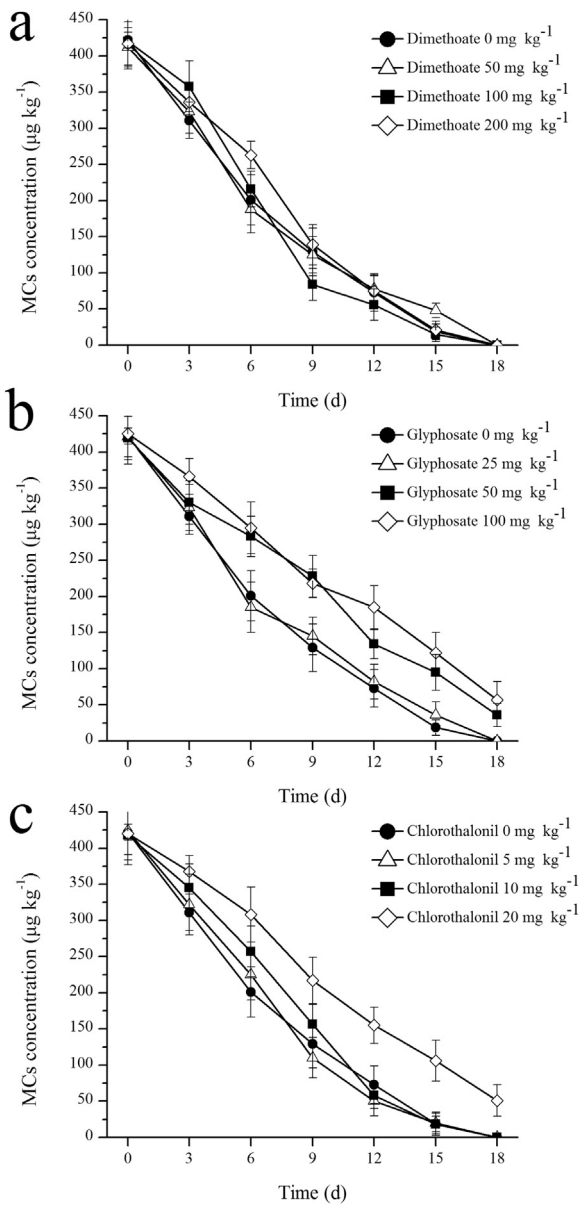

Fig. 4. Effect of different pesticides on the degradation of MC-LR in soils. Error bars represent the range of duplicate microcosms. (a) Dimethoate; (b) Glyphosate; (c) Chlorothalonil.

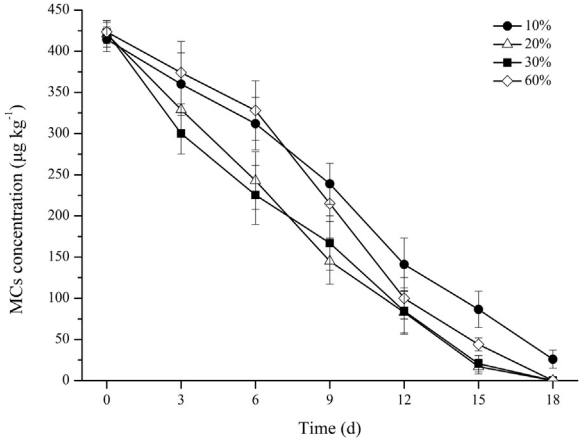

Fig. 5. Effect of different water content on the degradation of MC-LR in soils. Error bars represent the range of duplicate microcosms.

as being generally more favorable for plant growth.

3.6. Possible study limitation and prospect

A possible limitation to our research was the lack of plants. Normally the farming activities, such as fertilization, spraying pesticides and irrigation apply to soils with crops cultivated. Various effects of plant roots on other soil pollutants have been well documented: improvement of pollutant bioavailability by modifying soil conditions (Ouvrard et al., 2006; Joner et al., 2002), promotion of the development and activity of soil microorganisms (Harvey et al., 2002; Yoshitomi and Shann, 2001), and transformation/degradation of the pollutants with root exuded organic acids (activation of abiotic oxidants) (Gramss, 2000) and root-driven extracellular enzymes (oxidation of pollutants) (Schnoor et al., 1995; Siciliano et al., 1998). Consequently, the degradation of MCs in agricultural soils under natural condition may be stimulated by plants. To the best of our knowledge, no previous studies concerning degradation of MCs in plant-soil system have been reported, which should be well explored in the future.

4. Conclusion

In the present study, the effect of several farm activities, including N fertilization, pesticide application, and irrigation, on MC-LR degradation in soils was investigated. The main conclusions drawn from this study are as follows:

(1) Compared to photodegradation, biodegradation plays a dominant role in MC-LR degradation in our soils.
(2) For rapid degradation of MC-LR, nitrogen fertilizer is suggested in the following order: NO_3-N > NH_2-N > NH_4-N.
(3) Among organic fertilizers, humic fertilizer is recommended for MC-LR degradation in soils.
(4) Soil water content of 20–30% was more favorable for MC-LR degradation.

Given that MC-LR degradation in these agricultural soils appears to be strongly influenced by microbial community structure, we recommend that future studies focus on the isolation and identification of the taxa specifically responsible for degradation, and the mechanistic pathways by which degradation takes place.

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Conflict of interest

The authors declare that they have no conflict of interest.

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.2018.03.030.

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