ELSEVIER

Journal of Environmental Management 86 (2008) 566–574

Journal of Environmental Management

www.elsevier.com/locate/jenvman

A laboratory study on risk assessment of microcystin-RR in cropland ☆

Liu Bibo[a,b], Gong Yan[a,b], Xiao Bangding[a,b], Liu Jiantong[a,*], Liu Yongding[a]

[a]*Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan, Hubei Province 430072, China*
[b]*Graduate School of the Chinese Academy of Sciences, Beijing 100039, China*

Received 14 March 2006; received in revised form 11 September 2006; accepted 12 December 2006
Available online 27 April 2007

Abstract

The persistence time and risk of microcystin-RR (MC-RR) in cropland via irrigation were investigated under laboratory conditions. In order to evaluate the efficiency of the potential adsorption and biodegradation of MC-RR in cropland and the persistence time of MC-RR for crop irrigation, high performance liquid chromatography (HPLC) was used to quantify the amount of MC-RR in solutions. Our study indicated that MC-RR could be adsorbed and biodegraded in cropland soils. MC-RR at 6.5 mg/L could be completely degraded within 6 days with a lag phase of 1–2 days. In the presence of humic acid, the same amount of MC-RR could be degraded within 4 days without a lag phase. Accordingly, the persistence time of MC-RR in cropland soils should be about 6 days. This result also suggested the beneficial effects of the organic fertilizer utilization for the biodegradation of MC-RR in cropland soils. Our studies also demonstrated that MC-RR at low concentration ($<10\,\mu g/L$) could accelerate the growth of plants, while high concentration of MC-RR ($>100\,\mu g/L$) significantly inhibited the growth of plants. High sensitivity of the sprouting stage plants to MC-RR treatments as well as the strong inhibitory effects resulting from prolonged irrigation further indicated that this MC-RR growth-inhibition may vary with the duration of irrigation and life stage of the plants.

Keywords: Microcystin-RR; Environmental behavior; Cropland soils; Adsorption; Biodegradation; Persistence time; Crop plants

1. Introduction

Microcystins (MCs) are a group of heptaptide hepatotoxins produced by cyanobacteria in eutrophic freshwater (Carmichael, 1996; Carmichael and Falconer, 1993). Many of them are known for their tumor promoting activity (Falconer, 1991), especially as risk factors for liver cancer (Harada, 1996; Ueno et al., 1996; Yu, 1995). MCs normally exist inside cyanobacterial cells and enter the surrounding water after cell lysis (Watanabe et al., 1992). When cyanobacteria-containing water is used as an irrigation source, it is possible for a large amount of MCs being released into the cropland.

The growth inhibitory phenomenon of MCs was first observed in white mustard (*S. alba*) seedlings (Kós et al., 1995). MCs were also found to inhibit photosynthesis of *Phaseolus* vulgaris primary leaves (Abe et al., 1996). By using radioactive cyanotoxin, a large amount of MCs (5.3 mg toxin/kg) has been observed to accumulate in plant tissues (Kurki-Helasmo and Meriluoto, 1998). MCs had been detected in the tissues of toxin exposed plants using a commercialized ELISA kit, suggesting that the uptake of these toxins by edible plants may have significant implications to human health (McElhiney et al., 2001). MC-LR could exist as a glutathione conjugate formed enzymatically via soluble glutathione S-transferase in various aquatic organisms including plants (*Ceratophyllum demersum*), invertebrates (*Dreissena polymorpha*, *Daphnia magna*), and fish (*Danio rerio* Pflugmacher et al., 1998). This formation of this conjugate appears to be the first step in the detoxication of cyanobacterial toxin in aquatic organisms. The toxin was found to have little effect on growth for up to 18 days, but impaired the development of the roots of exposed plants, causing them to take up approximately 30% less growth medium than those grown in the absence of toxin. Hamvas et al. (2002) described the inhibitory effects of MC-LR on growth, lateral root

☆ No studies involving humans or experimental animals in this study.

*Corresponding author. Tel.: +86 02768780712; fax: +86 02768780712.
E-mail address: Jtliu@ihb.ac.cn (L. Jiantong).

0301-4797/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.jenvman.2006.12.040

formation, development, and anthocyanin content of mustard seedlings (a model system) and proved that MC-LR induces necrosis on mustard seedlings, in particular on the cotyledons and in tissues with high ssDNase activity. During exposure of *C. demersum* to the cyanobacterial toxin MC-LR at a concentration of 5.0 mg/L, an elevation of microsomal and cytosolic glutathione S-transferase was observed, indicating the initiation of the glutathione-toxin conjugate formation. The SOD as well as the increased hydrogen peroxide levels in parallel provide the evidence for oxidative stress in the aquatic plant. Other reactive oxygen detoxifiying enzymes might also be elevated. In addition, the glutathione pool is affected, represented by reduced glutathione and glutathione disulfide concentration (Pflugmacher, 2004). Chen et al. (2004) also suggested significant growth inhibition effect of MCs to rice and rape. While SOD and peroxidase (POD) may take place in plant stress response process, the increase of ROS contents in plant cells by MC-RR has been reported recently (Yin et al., 2005a). These observations suggested a phenomenon that the exposure of MC-RR would cause a oxidative stress in plant cells in addition to its known toxic components, which is also manifested as an oxidative stress, contributing to its deleterious effects. On the other hand, evidence also indicated that plant cells would improve their antioxidant abilities to combat MC-RR induced oxidative injuries simultaneously. Furthermore, in *Vallisneria natans* seedlings, MC-RR could accumulate differentially in the roots and leaves. Such toxin accumulation in the roots and leaves were time- and dose-dependent, with a high uptake detected in the roots. It was also suggested that MC-RR could be up-taken by *V. natans*, and result in developmental retardation (Yin et al., 2005b).

It is known that MCs could be adsorbed into soils and lake sediments (Miller et al., 2001; Jin et al., 2001). The biodegradation (BD) of MCs in lake water and lake sediments has been reported previously (Holst et al., 2003; Christffersen et al., 2002; Takenaka and Watanabe, 1997; Jin et al., 2001). However, the environmental fate and effect of MCs in cropland remains unknown. It is also intriguing to know the toxicological effects of MCs for different life stages of crop, since most of the previous reports of MCs' plant growth inhibitory effects were made with the sprouting plants only. (Kós et al., 1995; Kurki-Helasmo and Meriluoto, 1998; McElhiney et al., 2001; Chen et al., 2004).

In our present study, the adsorption and BD of MC-RR in cropland soils were investigated under laboratory conditions. The persistence time of MC-RR in cropland and its influences of MC-RR on the growth of rape and cabbage were also studied.

2. Material and methods

2.1. Material and reagents

The soil was collected from a leek cropland being irrigated with the water of Lake Dianchi. Lake Dianchi is the largest lake in Yunnan province, and the sixth largest fresh water lake in China (longitude 102°29′–103°01′ east, latitude 24°29′–25°28′ north). Since the 1980s, eutrophication of the lake water has increased steadily and heavy cyanobacterial blooms (mainly composed of *Microcystis* spp. and *Anabaena* sp.) occurs in each warm season. MC-RR was reported as the main variant of MCs in the lake (Chen et al., 2004), with its concentrations higher than other forms of MCs by several folds. Water from the lake has been used as drinking and irrigation source for the area.

Soils were dried, ground and saved under room temperature. MC-RR was extracted from cyanobacterial powder of Lake Dianchi with 75% methanol (Chen et al., 2005). After evaporation of methanol, the extract of MC-RR was diluted with distilled water. To imitate the natural condition, no purification process was carried out.

All reagents used in the experiment were of the analytical pure grade. Humic acid (HA) was purchased from Sigma (Aldrich chemical company, Sigma). Seeds of rape (*Brassica napus* L., Zhong You 821, Institute of Oil Crop Research, Chinese Academy of Agriculture Sciences) and cabbage (*Brassica chinensis* L., Si ji zhong qi xiao bai caiy, Wuhan Jiu Tou Niao Seeds) were purchased from local seed market.

2.2. Experiment method

2.2.1. The adsorption of MC-RR onto cropland soils

Different amounts of soil (0, 1, 2, 3, 4, 5, 6, 7, 8 g) were added into nine 50 ml conical flasks containing 30 ml MC-RR solution (1 mg/L) to evaluated the adsorption of MC-RR into cropland soil. Sodium azide (NaN_3) (0.002% w/v) was added into these conical flasks to prevent MC-RR from BD (Ding and Wu, 1997). All the conical flasks were placed on an orbital shaker (HZQ-F, China) and shake at 110 rpm (Miller et al., 2001) at 25 °C for 24 h.

2.2.2. Biodegradation in sediment

A 1.0 g soil was added into three conical flasks containing 30 ml MC-RR solution. In order to investigate the influence of organic materials on the BD, HA was added into another three flasks with same MC-RR solution and soil mixture. Three more flasks of MC solution with NaN_3 were used as controls. All the conical flasks were placed on an orbital shaker (HZQ-F, China) and mixed at 110 rpm and 25 °C for 24 h (Miller et al., 2001). Samples were kept at the same condition for another seven days, or until all the MC-RR were degraded. The amount of toxin remaining in solution was monitored so as to evaluate the amount of adsorption and BD. All the samples were filtered through a 0.45 μm filter, and 20 μl of these solutions were injected into the High performance liquid chromatography (HPLC) system for detection.

2.2.3. The dosage effects of MC-RR on the sprout of rape and cabbage

The germination test was mainly developed based on the method described previously by Chen et al. (2004). The concentrations of MC-RR extract was 0, 1, 10, 100, and 1000 μg/L. Before the germination experiments, seeds were treated in various concentrations of the extract for 24 h. The germination test was carried out in the culture with four layers of pledget in petridishes (Φ90 mm). The pledget was saturated with 30 ml of MC-RR extract or distilled water as control group. The amount of 100 seeds were placed in each dish. Three replicate dishes were used for every concentration of MCs. The dishes were placed at 25 °C, and illuminated by fluorescent lights, with a daylight photon flux density of 150 mmol/m^2 s in the center, which maintained a 12 h photoperiod. During germination, 15 ml of the extract at designed concentration was added to prevent dryness of the culture.

The percentage of sprout was observed everyday, and the lengths and weights of plants were measured on the 7 day post-treatment.

2.2.4. The influence of different irrigation durations on the growth of rape

The MC-RR concentration was set at 1000 μg/L (the same in Section 2.2.5). Each group was irrigated with MC-RR solution for 0, 2, 4, 6, and 8 days, and followed with distilled water.

Fresh weight, plant length, SOD, TTC and chlorophyll were measured on the 10 day post-treatment.

2.2.5. The growth inhibitory effects of irrigation on different life stages of the rape

According to the persistence time, the duration of the irrigation time was set at 7 days. The irrigation treatments began before sprouting, or at the end of germination, or on the fifth day after germination. The control was irrigated with distilled water.

Fresh weight, plant length, SOD, TTC and chlorophyll were monitored on 20 and 30 day post-treatments.

2.3. Analysis method

SOD activity detection was based on the method described by Giannopotitis and Ries (1977). About 0.2 g of plant leaf tissue was ground into slurry with a mortar and pestle in 2 ml of phosphate buffer (pH 7.8) in an ice bath. The homogenates were centrifuged at 12,000 *g* at 4 °C for 10 min, and the supernatants were kept at 4 °C prior to use. One unit of the enzyme activity was defined as the amount of enzyme required to result in a 50% inhibition of the rate of nitro blue tetrazolium reduction measured at 560 nm (Yin et al., 2005a).

TTC (2, 3, 5-triphenyl tetrazolium chloride): The viability test was carried out according to the method of Towill and Mazur (1975). The absorbance was measured at 485 nm.

For analysis of chlorophyll content, each plant was extracted in 0.5 ml of 90% acetone with mortars and pestles kept on ice. The absorbance was measured at 664 and 647 nm. The amounts of chlorophylls a and b (mg) per mg of wet weight of cells were calculated using the equations below, which were described by Geider and Osbome (1992):

$$\text{Chl a (mg/dm)} = 11.93 \times \text{abs } 664 - 1.93 \times \text{abs } 647,$$

$$\text{Chl b (mg/dm)} = 20.36 \times \text{abs } 647 - 5.5 \times \text{abs } 664.$$

MC-RR analysis: The HPLC-system consisted of a waters-600 controller, 600 pump, 600 dual λ absorbance detector (monitoring between 200 and 300 nm) and column (BDS Hypersil C18 $250 * 4.6$ mm i.d.). The column temperature was kept at 25 °C and with the carriage gas (helium) flow rate at 30 mL/min. The mobile phase (methanol: water (MilliQ) with 0.1% TFA = 62:38) flow rate was set to 1 mL/min and injection volume of sample was 20 μL. Concentration of MC-RR was determined according to the MC-RR standard (obtained from Sigma).

All experiments were repeated at least twice. Significant differences between each treatment groups were determined with student *t*-test at $p<0.05$.

3. Results

3.1. The adsorption of MC-RR into cropland soils

For this study, it has to be pointed out that the concentration and volume of MC-RR was set, while the dose of soil varied from 0 to 8.0 g. The toxin: soil ratio measured varied from 3.75 to 30 μg/g. As shown in Fig. 1, the MC-RR could be adsorbed into the cropland soil. With the increasing dose of soil, the ratio between the final concentration of MC-RR in the solution and that in the soil significantly decreased. The highest adsorption capacity of soil to MC-RR in this experiment was 9.94 μg/g, when the final concentration of MC-RR was 0.93 mg/L in final solution after the treatment.

3.2. The biodegradation of MC-RR in cropland soils

As shown in Fig. 2, being mixed with cropland soils, MC-RR (6.5 mg/L) could be completely biodegraded within 6 days. With the addition of HA, the BD time could be further reduced to 4 days without any lag phase.

The loss rate of MC-RR in this experiment is shown in Fig. 3. On the first day, the loss of MC-RR should be mainly contributed to adsorption, the addition of HA made the HA group (BD with addition of HA) lose more MC-RR than that of the BD group. From the second day on, the speed of both groups increased with the duration of the treatment. The loss rate of MC-RR in HA group was faster than that of BD groups. By the fifth day, all the MC-RR in HA group was consumed, while the rate of BD group remained unchanged.

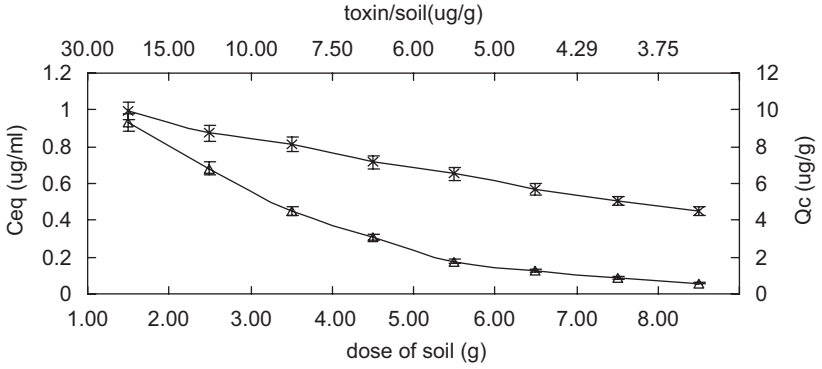

Fig. 1. The adsorption of MC-RR onto cropland soils. (×) C_{eq} is the quantity of MC-RR adsorption onto per unit soils; (△) Q_c is the final concentration of MC-RR in solution. Toxin/soil is the ratio between the concentration of MC-RR and the dose of soil in per unit solution.

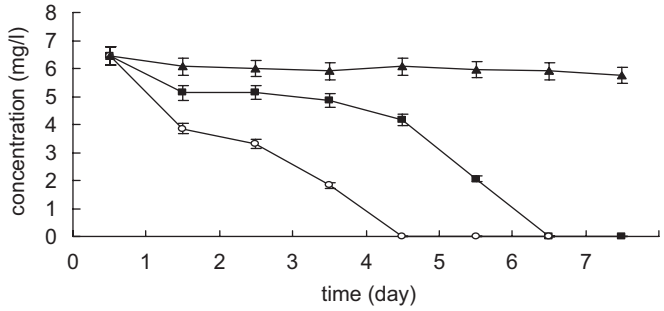

Fig. 2. The biodegradation of MC-RR in cropland soils. (■) MC-RR biodegradation in cropland soils; (○) MC-RR biodegradation in cropland soils with the presence of humic acid; (▲) the control with NaN_3.

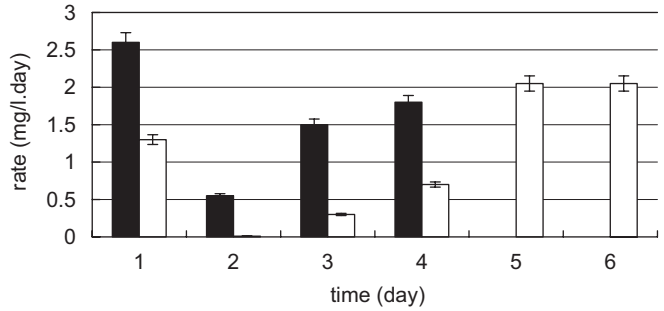

Fig. 3. The biodegradation rate of MC-RR in cropland soil with and without HA; (□) MC-RR biodegradation in cropland soils; (■) MC-RR biodegradation in cropland soils with the presence of humic acid.

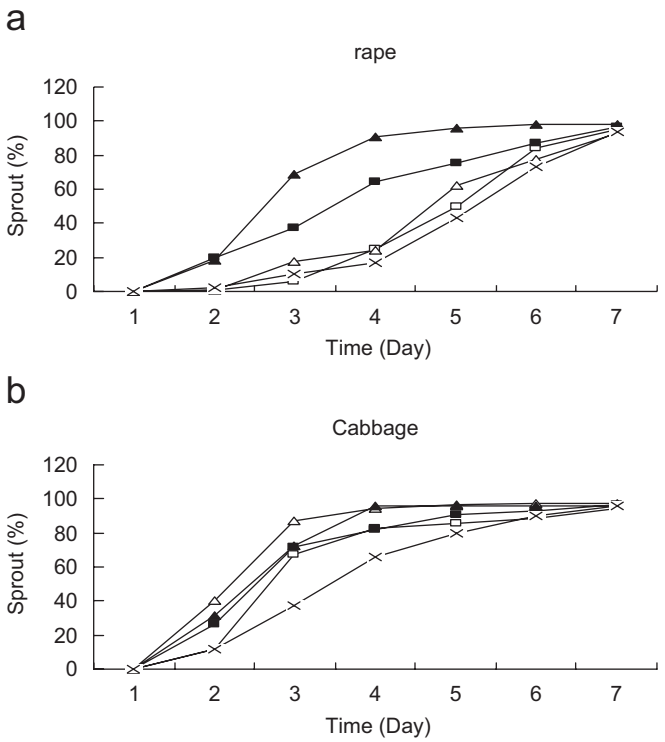

Fig. 4. Influence of MC-RR on the sprout of rape and cabbage. (□) control; (△) 1 μg/L; (▲) 10 μg/L; (■) 100 μg/L; (×) 1000 μg/L; (a) Rape. (b) Cabbage.

3.3. The dosage growth effect of MC-RR on the sprout of rape and cabbage

The sprout percentage of rape and cabbage shown in Fig. 4 suggested that the sprouts of rapes were faster with the treatment of 100, 10 and 1 μg/L MC-RR, than those of the rapes in control groups with the exception in the group treated with 1000 μg/L MC-RR. The sprouts of rapes were slower than that in control group. The trends of the sprouting speed for rape were 10 μg/L > 100 μg/L > 1 μg/L > control > 1000 μg/L (Fig. 4b), and 1 μg/L > 10 μg/L > 100 μg/L > control > 1000 μg/L for cabbage (Fig. 4a).

As shown in Fig. 5, the four treatments with extended duration caused significant changes in culture compared with the culture that had only been treated for 7 days. Obvious inhibition could be seen in the groups treated with 100 and 1000 μg/L MC-RR. The amount of roots decreased with the increasing of MC-RR concentration for both rape and cabbage seedlings. As for the treatments with MC-RR at 1000 and 100 μg/L, a lot of yellow leafs and seedlings lying on the pledgets could be seen, and severe lethal effects has been observed later in the culture (Fig. 5, I could not find this figure).

Similar trends could be found in Fig. 6a and b. Based on the measurement of the fresh crop length and weight, the

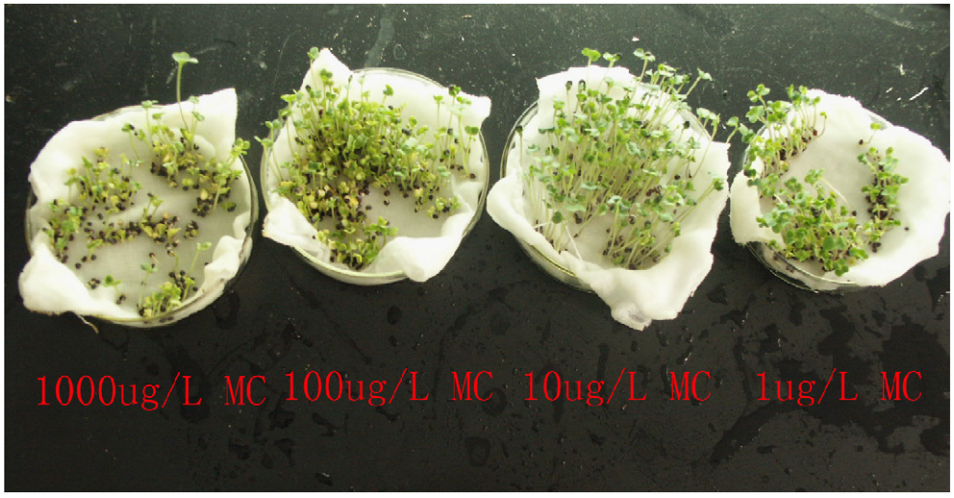

Fig. 5. Growth effects for the prolonged treatment of MC-RR for rape. For this study, additional 4 more rape irrigations were performed after 7 day treatment.

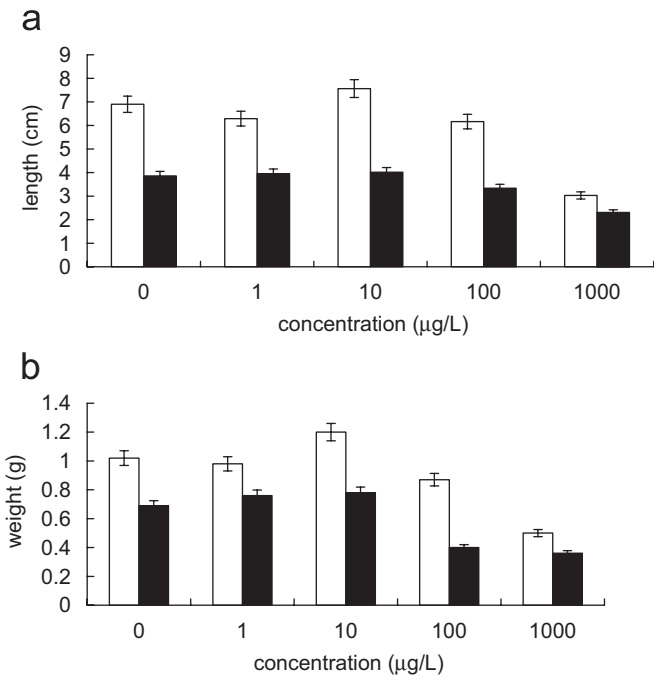

Fig. 6. influence of different dose of MC-RR on the growth of rape and cabbage. (a) (□) Rape; (■) cabbage. Mean of 20 samples, ±SE. (b) (□) Rape; (■) cabbage. Mean of 20 samples, ±SE.

growth inhibitory effects could be seen in the crop samples treated with MC-RR compared with their counterparts, the plants in control groups, with the only exception of the lowest MC-RR dosage treatment group (10 μg/L, Fig. 6).

3.4. The duration effects of the MC-RR irrigation on crop seedlings

As shown in Table 1, the amounts of healthy seedlings in 6 and 8 groups were remarkably lower than those in other groups. This suggested that extension of the MC-RR treatment would cause a significant detrimental effect on the growth of the rape seedlings compared with those in the groups with an less than 4 day MC-RR treatment.

Table 1
The amount of healthy seedlings in different irrigation period treatments

Treatment	Amounts of healthy seedlings
0	67±2
2	66±2
4	66±2
6	58±2
8	54±2

0: control, irrigated with water; 2: irrigated with MCs solution for 2 days; 4: irrigated with MCs solution for 4 days; 6: irrigated with MCs solution for 6 days; 8: irrigated with MCs solution for 8 days. The same in Fig. 7.

On the second day, the SOD activities increased significantly compared with those from the no MC-RR treated control group, suggesting an immediate oxidative stress response caused by the MC-RR treatment. However, according to our measurements, all the SOD activities but the 2 day treatment group decreased with the extension of the MC-RR treatment; and TTC activities decreased in all the MC-RR treatment groups (Fig. 7a). It appears that the fresh weight of rape seedlings increased with the treatment time (Fig. 7b). The chlorophyll in seedling leafs slightly decreased following the prolonged treatment (Fig. 7c).

Plant length collected from the groups of 6 day and 8 day treatment demonstrated a statistically significant increase compared with those from the other groups in student *t*-test.

3.5. The sensitivities of different life stage of rape to the growth inhibitory effects of MC-RR

In this experiment, the amounts of healthy seedlings in FB (from beginning) and ES (end of sprouting) groups were remarkably lower than those of CK (control) and AS (after sprouting) groups.

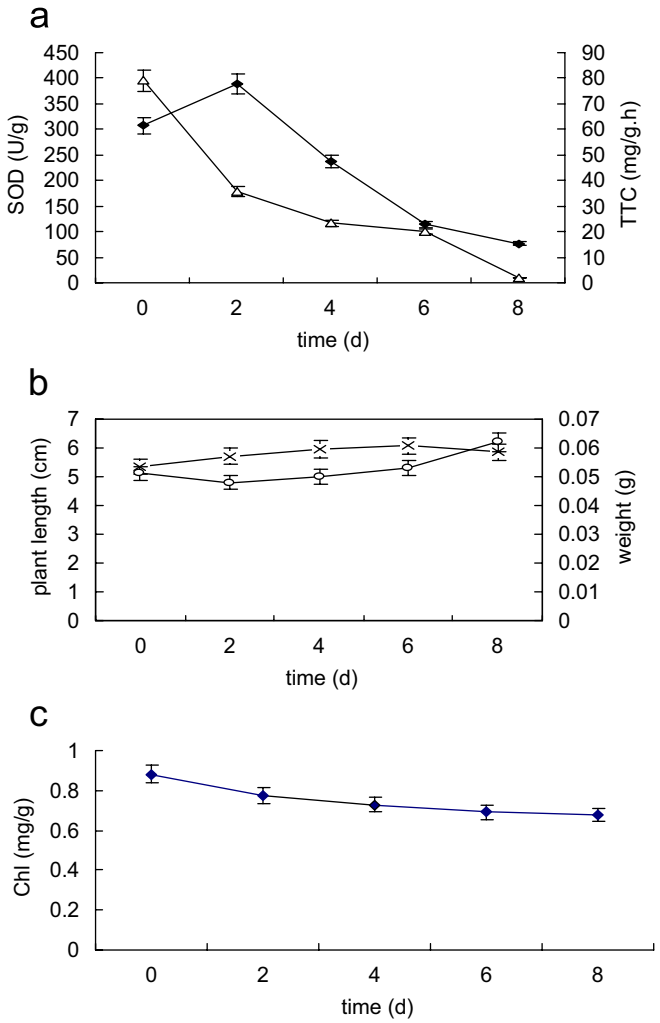

Fig. 7. Influence of different irrigation period on the growth of rape. 0: control, irrigated with water; 2: irrigated with MCs solution for 2 days; 4: irrigated with MCs solution for 4 days; 6: irrigated with MCs solution for 6 days; 8: irrigated with MCs solution for 8 days. (a) (□) SOD; (□) TTC. (b) (○) Fresh weight; (□) plant length. (c) (●) Chlorophyll.

After 20 days treatment, the activities of SOD, TTC (Fig. 8a) and chlorophyll (Fig. 8b) measured in FB and ES groups decreased following the MC-RR treatment. This indicated that the early stage of the rape was sensitive to the inhibitory effects from MC-RR irrigation. In terms of the overall growth aspect, only the fresh length from FB group demonstrated a significantly lower length ($P < 0.01$) compared with those from their control groups. No significant difference could be found among other groups.

4. Discussions

4.1. The persistence time of MC-RR in cropland

In the present study, sodium azide, as an inhibitor of aerobic microbial metabolism, can potentially prevent the BD of MCs under aerobic conditions. While, according to the study of Holst et al. (2003), MCs are susceptible to BD by facultatively anaerobic bacteria under anaerobic conditions. That is to say, both adsorption and anaerobic BD may cause the loss of MC-RR in the presence of sodium azide. However, we have measured the MC-RR concentrations versus time in sterile soil. The result was the same to that of normal soil with the presence of sodium azide, which suggested that no anaerobic BD happened.

It has been suggested that MC-RR could be adsorbed into fine-grained natural clay particles (Morris et al., 2000). The study of Miller et al. (2001) also indicated that MC-RR could be adsorbed into field soil, with various adsorption capacities depending on the pH, ions strength and many other factors. In the previous studies from our laboratory (Jin et al., 2001), it is also recognized that MCs could be adsorbed into the sediments from Lake Dianchi. These results clearly demonstrated that MCs could be adsorbed into natural particles, soils and lake sediments. So it is reasonable that cropland soils have the ability to adsorb MC-RR, and it is possible for MC-RR to persist in cropland soils for a certain period.

Previously, it had been reported that the BD times of MC-LR varied from 8 to 49 days (Holst et al., 2003; Christffersen et al., 2002; Takenaka and Watanabe, 1997). An exceptional case reported by Ishii et al. (2004) suggested a quick degradation of MC-LR in 4 days at 6 mg/L initial concentration. However, MC-RR has been suggested to be easily degraded in sediment of Lake Dianchi, with the degradation time varying from 3 to 9 days (Jin et al., 2001).

The observation of MC-RR BD could be supported by the study of Miller et al. (2001), which demonstrated that loss of MC in different soils was controlled by a fast adsorption to soil particles and a slower microbial degradation. Soils with a high content of organic matter and clay were most efficient in removing MC, probably due to a higher binding capacity for organic substances as well as a large surface area for bacterial attachment. Organic substance has been indicated as a carbon source at anoxic condition (Holst et al., 2003). These results suggested that the addition of organic materials would accelerate the BD of MC-RR. The results show that the use of organic fertilizer will accelerate the process of MC-RR BD in cropland soils.

In present study, only the MC-RR remaining in solution was detected, it is still unknown whether there will remain as toxins in soil. However, some minor physiological effects observed with the use of such high MC-RR concentrations in the experiment highly suggested that bacteria in cropland could have degraded MCs of certain concentration within 6 days.

It is well known that photolysis is another important path for the degradation of MCs under natural conditions. However, only adsorption and BD were investigated in this study. So under field conditions, the degradation of MC-RR could be more rapid than what we have seen in this laboratory study. Therefore, a reasonable speculated persistence time of MC-RR in cropland might be even less than 6 days.

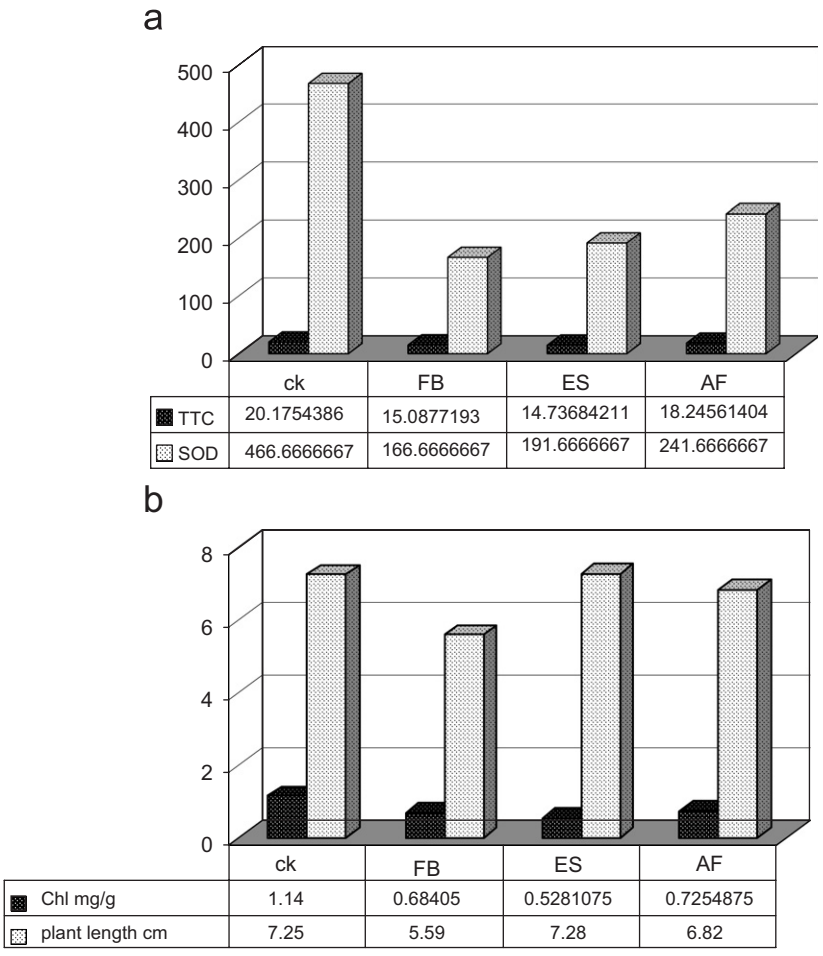

Fig. 8. The influence of different irrigation time on the growth of rape after 20 days treatment. CK: always irrigated with water; FB (from the beginning): irrigated with MCs solution within the first 7 days; ES (the end of sprout): irrigated with MCs solution at the end of sprouting; AS (after sprout): irrigated with MCs solution 3 days after the sprouting. (a) (□) SOD; (■) TTC. (b) (■) Chlorophyll; (□) plant length.

According to the persistence time observed in present studies, crops with low irrigation frequency would suffer less damage from MC-RR than aquatic crops and crops with high irrigation frequency. The use of organic fertilizer could accelerate the degradation of MC-RR in cropland. It is also strongly suggested that time of chronic toxicity studies should be based on the persistence time of the toxin and the life behavior of crops.

4.2. The influence of MC-RR on plant growth

Our present germination test demonstrated that high concentration of MC-RR (>100 μg/L) could significantly inhibit the growth of rape and cabbage. At the same time, MC-RR at low concentration could even accelerate the growth of rape (10 μg/L MC-RR) and cabbage (1 μg/L MC-RR). Similar results could be found in the study of Yin et al. (2005b), where growth and development observations revealed that *V. natans* was relatively insensitive to MC-RR at concentrations ranging from 0.0001 to 0.01 mg/L. However, when the toxin concentration was more than 0.01 mg/L, both the fresh weight and the longest leaf length of seedlings were significantly reduced after a 30-day treatment. The root and leaf numbers were significantly decreased when the plant was treated with 10 mg/L of toxin.

Such low dose stimulation and high dose inhibition phenomenon provides another example for the concept of hormesis, which is a dosage–response phenomenon of plants with some mechanistic, physiological and evolutionary explanations. Two theories of hormesis could be used to explain this phenomenon such as the overcompensation explanation (Calabrese and Baldwin, 1999) and the over-correction theory (Stebbing, 2002). The disulfide-thiol interchange protein/NADH oxidase protein has been suggested as the molecular target of the biological effects involved in the stimulation of plant growth after low levels of toxin exposure (hormesis, Morré, 1998). Hormetic effects have been reported in a highly diverse array of biological models, for numerous organs and endpoints and chemical/physical stressors and it is evident that no single mechanism can account for these phenomena (Calabrese, 2005).

For the studies on the plant growth inhibitory effect of MC-RR, the trends of SOD, TTC and chlorophyll and

Table 2
The amount of healthy seedlings in different irrigation time treatments

Treatment	Amounts of healthy seedlings
CK	49±2
From beginning	38±2
End of sprout	37±2
After sprout	39±2

CK: always irrigated with water; from the beginning (FB): irrigated with MCs solution within the first 7 days; the end of sprout (ES): irrigated with MCs solution at the end of sprouting; after sprout (AS): irrigated with MCs solution 3 days after the sprouting. The same in Fig. 8.

plant length, fresh weight seems to differ for each one. The decreasing of SOD, TTC and chlorophyll indicated that the antioxidation and photosynthesis system were inhibited. Oxidative stress had also been reported previously in other aquatic plant (Pflugmacher, 2004), rape and rice (Chen et al., 2004) and tobacco cells (Yin et al., 2005a). The decreasing trend of TTC observed in present work also mirrored previous study on MC-LR (McElhiney et al., 2001). MC-LR was found to have little effect on growth for up to 18 days, but impaired the development of the roots of exposed plants, causing them to take up approximately 30% less growth medium than those grown in the absence of toxin (McElhiney et al., 2001). However, for the use of crude extraction toxin solutions in different treatments, and organic substance contained in the toxin solution could give the seedlings more nutrients, it is reasonable for the plant length and fresh weight of treatments to be higher than that in the control group.

Our results for the sensitivities of different stage of plant to MC-RR irrigation treatment demonstrated that MC-RR irrigation at the early life stage of the seedlings could lead to heavy growth inhibition. The data shown in Table 2 and Fig. 8 also indicated that the oxidation and photosynthesis system might be inhibited. Toxin treatments at the beginning and at the end of sprouting stage exhibited severe plant growth inhibitory effects, while the MC-RR treatment after sprout stage apparently only caused some minor defects in the crop. The data of plant length and chlorophyll indicated that the appearance of plant was not affected if the plant was irrigated after sprouting.

Based on the present studies, MC-RR at low concentration ($<10\,\mu g/L$) could not inhibit the growth of rape and cabbage while high concentration of MC-RR ($>100\,\mu g/L$) could inhibit the growth of rape and cabbage. The degree of inhibition also varied with the life stage of the plants. The severe growth inhibitory effects have been observed in the prolonged irrigation or/and irrigation at the early life stage (sprouting stage). In this way, for avoiding environmental risk, when irrigation with MCs-containing water, the frequency should be as low as possible, and the irrigation time should be several days or longer after sprouting. According to the results of Kurki-Helasmo and Meriluoto (1998) and Yin et al. (2005b), MCs could accumulate in plant tissue after exposure to extremely high concentration toxin solutions. MC levels observed in healthy-looking plants in the study of Kurki-Helasmo and Meriluoto (1998) were qualified as 5.3 mg/kg. Although, no MCs could be detected in the plant tissue in this study with the method of Xie et al. (2004), the irrigation for edible plants with MCs-containing water should be avoided.

5. Conclusions

Our results clearly show that MCs could be adsorbed and biodegraded by certain bacteria in cropland soil. Accordingly, the persistence time of MC-RR in cropland soils was about 6 days. The result also suggested the beneficial effects of the organic fertilizer utilization for the BD of MC-RR in cropland soils. Our studies also demonstrated that MC-RR at low concentration ($<10\,\mu g/L$) could accelerate the growth of plants, while high concentration of MC-RR ($>100\,\mu g/L$) significantly inhibited the growth of plants. High sensitivities of the sprouting stage plants to MC-RR treatments as well as the strong inhibitory effects resulted from prolonged irrigation observed further indicated that this MC-RR growth-inhibition may vary with the duration of the irrigation and the life stage of the plants.

Further study should identify the MCs-BD bacteria and the concentration of MCs in cropland soil. Still, long-term chronic toxic studies on different plants should be taken to evaluate the further risk of MCs in cropland.

Acknowledgments

This work was funded by Key Project of Chinese Academy of Sciences (KZCX1-351 SW-12), the National Key Project for Basic Research (2002CB412300) and the 352 National "863" High-Tech Program (2002AA601013).

References

Abe, T., Lawson, T., Weyers, J.B., Codd, G.A., 1996. Microcystin-LR inhibits photosynthesis of Phaseolus vulgaris primary leaves: implications for current spray irrigation practice. New Phytologist 133, 651–658.

Calabrese, E.J., 2005. Paradigm lost, paradigm found: the re-emergence of hormesis as a fundamental dose response model in the toxicological sciences. Environmental Pollution 138, 378–411.

Calabrese, E.J., Baldwin, L.A., 1999. The marginalization of hormesis. Toxicologic Pathology 27 (2), 187–194.

Carmichael, W.W., 1996. Toxic *microcystis* and the environment. In: Watanabe, M.F., Harada, K.-I., Carmichael, W.W., Fujiki, H. (Eds.), Toxic *Microcystis*. CRC Press, Boca Raton, pp. 1–11.

Carmichael, W.W., Falconer, I.R., 1993. Diseases related to freshwater blue-green algae toxins, and control measures. In: Falconer, I.R. (Ed.), Algal Toxins in Seafood and Drinking Water. Academic Press, London, pp. 187–209.

Chen, J.Z., Song, L.R., Dai, J., Gan, N.Q., Liu, Z.L., 2004. Effects of microcystins on the growth and the activity of superoxide dismutase and peroxidase of rape (*Brassica napus L.*) and rice (*Oryzasativa L.*). Toxicon 43, 393–400.

Chen, X.G., Xiao, B.D., Liu, J.T., Fang, T., Xu, X.Q., 2005. Kinetics of the oxidation of MCRR by potassium permanganate. Toxicon 45, 911–917.

Christffersen, K., Lyck, S., Winding, A., 2002. Microbial activity and bacterial community structure during degradation of microcystins. Aquatic Microbial Ecology 27, 125–136.

Ding, J.Y., Wu, S.C., 1997. Transport of organochlorine pesticides in soil columns enhanced by dissolved organic carbon. Water Science and Technology 35, 139–145.

Falconer, I.R., 1991. Tumor promotion and liver injury caused by oral consumption of cyanobacteria. Environmental Toxicology and Water Quality 6, 177–184.

Giannopotitis, C.N., Ries, S.K., 1977. Superoxide dismutase in higher plants. Plant Physiology 59, 309–314.

Hamvas, M.M., Mathe, C., Molnar, E., Vasa, G., Grigorsky, I., Borbely, G., 2002. Microcystin-LR alters the growth, anthanocyanin content and single-stranded DNase enzyme activities in *Sinapisalba L.* seedlings. Aquatic Toxicology 61, 1–9.

Harada, K., 1996. Chemistry and detection of MCs. In: Watanabe, M.F., Harada, K., Carmichael, W.W., Fujiki, H. (Eds.), Toxic *Microcystis*. CRC Press, Boca Raton, pp. 103–148.

Holst, T., Jørfensen, N.O.G., Jørfensen, C., Johansen, A., 2003. Degradation of microcystin in sediments at oxic and anoxic denitrifying conditions. Water Research 37, 4748–4760.

Ishii, H., Nishijima, M., Abe, T., 2004. Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium. Water Research 38, 2667–2676.

Jin, L.N., Zhang, W.H., Zheng, L., Xu, X.Q., 2001. Biodegradation of microcystin in Dianchi Lake aquatic environment. China Environmental Science 22, 189–192.

Kós, P., Gorzó, G., Surányi, G., Borbély, G., 1995. Simple and efficient method for isolation and measurement of cyanobacterial hepatotoxins by plant tests (*Sinapis alba L.*). Analytical Biochemistry 225, 49–53.

Kurki-Helasmo, K., Meriluoto, J., 1998. Microcystin uptake inhibits growth and protein phosphatase activity in mustard (*Sinapis alba L.*) seedlings. Toxicon 36, 1921–1926.

McElhiney, J., Lawton, L.A., Leifert, C., 2001. Investigations into the inhibitory effects of microcystins on plant growth, and the toxicity of plant tissues following exposure. Toxicon 39, 1411–1420.

Miller, M.J., Chritchley, M.M., Hutson, J., Fallowfield, H.J., 2001. The adsorption of cyanobacterial hepatotoxins from water onto soils during batch experiments. Water Research 35, 1461–1468.

Morré, D.J., 1998. A protein disulfide-thiol interchange protein with NADH: protein disulfide reductase (NADH oxidase) activity as a molecular target for low levels of exposure to organic solvents in plant growth. Human & Experimental Toxicology 17, 272–277.

Morris, R.J., Williams, D.E., Luu, H.A., Holmes, C.F.B., Andersen, R.J., Calvert, S.E., 2000. The adsorption of microcystin-LR by natural clay particles. Toxicon 38 (2), 303–308.

Pflugmacher, S., 2004. Promotion of oxidative stress in the aquatic macrophyte Ceratophyllum demersum during biotransformation of the cyanobacterial toxin microcystin-LR. Aquatic Toxicology 70, 169–178.

Pflugmacher, S., Wiegand, C., Oberemm, A., Beattie, K.A., Krause, E., Codd, G.A., Steinberg, C., 1998. Identification of an enzymatically-formed glutathione conjugate of the cyanobacterial hepatotoxin microcystin-LR. The first step of detoxication. Biochemica Et Biophysica Acta 1425, 527–533.

Stebbing, A.R.D., 2002. Tolerance and hormesis—increased resistance to copper in hydroids linked to hormesis. Marine Environmental Research 54, 805–809.

Takenaka, S., Watanabe, M.F., 1997. Microcystin LR degradation by pseudomonas aeruginosa alkaline protease. Chemosphere 34, 749–757.

Towill, L.E., Mazur, P., 1975. Studies on the reduction of 2, 3, 5-triphenyl tetrazolium chloride as a viability assay for plant tissue cultures. Canadian Journal of Botany 53, 1097–1102.

Ueno, Y., Nagata, S., Tsutsimi, T., Hasegawa, A., Watanabe, M.F., Park, H.D., Chen, G.C., Chen, G., Yu, S.Z., 1996. Detection of MCs, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. Carcinogenesis 17, 1317–1321.

Watanabe, M.F., Tsuji, K., Watanabe, Y., Harada, K., Suzuki, M., 1992. Release of heptapeptide toxin (MC-RR) during the decomposition process of *Microcystis* aeruginosa. Natural Toxins 1, 48–53.

Xie, L.Q., Xie, P., Ozawa, K., Honma, T., Yokoyama, A., Park, H.D., 2004. Dynamics of microcystins-LR and -RR in the phytoplanktivorous sliver carp in a sub-chronic toxicity experiment. Environmental Pollution 127, 431–439.

Yin, L., Huang, J., Li, D., Liu, Y., 2005a. Microcystin-RR-induced accumulation of reactive oxygen species and alteration of antioxidant systems in tobacco BY-2 cells. Toxicon 42, 507–512.

Yin, L., Huang, J., Li, D., Liu, Y., 2005b. Microcystin-RR uptake and its effects on the growth of submerged macrophyte *Vallisneria natans* (lour) hara. Environmental Toxicology 20 (3), 308–313.

Yu, S.Z., 1995. Primary prevention of hepatocellular carcinoma. Journal of Gasteroenterology and Hepatology 10, 674–682.