

Sorption, degradation and mobility of microcystins in Chinese agriculture soils: Risk assessment for groundwater protection

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The use of cyanobacterial blooms as plant fertilizer is likely to be unsafe in soils.

Abstract

In the present paper, sorption, persistence, and leaching behavior of three microcystin variants in Chinese agriculture soils were examined. Based on this study, the values of capacity factor and slope for three MCs variants in three soils ranged from 0.69 to 6.00, and 1.01 to 1.54, respectively. The adsorption of MCs in the soils decreased in the following order: RR>Dha⁷ LR>LR. Furthermore, for each MC variant in the three soils, the adsorption rate in the soils decreased in the following order: soil A>soil C>soil B. The calculated half-time ranged between 7.9 and 17.8 days for MC-RR, 6.0–17.1 days for MC-LR, and 7.1–10.2 days for MC-Dha⁷ LR. Results from leaching experiments demonstrated that recoveries of toxins in leachates ranged from 0–16.7% for RR, 73.2–88.9% for LR, and 8.9–73.1% for Dha⁷ LR. The GUS value ranged from 1.48 to 2.06 for RR, 1.82–2.88 for LR, and 1.76–2.09 for Dha⁷ LR. Results demonstrated the use of cyanobacterial collections as plant fertilizer is likely to be unsafe in soils.

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Keywords: Microcystin; Soils; Leaching behavior; Persistence; Sorption; Plant fertilizer; Groundwater protection; Risk assessment

1. Introduction

Microcystins (MCs), a family of hepatotoxins produced by freshwater cyanobacterial blooms, are responsible for liver failure of wild animals, livestock and aquatic life (Carmichael, 1992, 1994; Sivonen and Jones, 1999), as well as human illnesses and even death due to the exposure to hepatotoxin contaminated water (Azevedo et al., 2002). Over 70 structurally different MCs have been discovered in the past century (Harada et al., 1990; Sivonen and Jones, 1999). More specifically, MC-RR, LR, and Dha⁷ LR are commonly found in freshwater cyanobacterial blooms in China. In recent few years, cyanobacterial blooms occurred in many large freshwater lakes such as Lake Dianchi (the 6th largest freshwater lake

in China) and Lake Taihu (the 3rd largest freshwater lake in China) with increasing frequency and intensity (Li et al., 2001; Song et al., 1998). As a result, toxic cyanobacterial blooms and MCs have received more attention following the report by Jochimsen and his colleagues (Jochimsen et al., 1998) regarding the deaths of 50 patients exposed to MC-contaminated water during a dialysis treatment. Reduction of threats to human health and aquatic life involves toxic cyanobacteria blooms and/or microcystins to be removed from water columns, especially water supplies (Miller et al., 2001). Many strategies for the removal of cyanobacterial toxins and/or cyanobacterial blooms have been investigated all over the world. In Australia, riverbank filtration and clay adsorption and other strategies for the removal of cyanobacterial toxins had been studied based on low-cost soil (Morris et al., 2000; Miller et al., 2001). In China, thousands of tons of cyanobacterial blooms had been taken out of Lake Dianchi and Taihu in

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the past three years by mechanical collection to reduce contamination. Some of this collection was directly discharged as organic fertilizers to leek and tobacco farms. Therefore, there is a possibility that soils in those regions might be contaminated with microcystins.

Once toxic cyanobacterial collections are put into soil, MCs will be released and migrate from the surface to the deeper layers of the soil after precipitation events. This may cause MCs to leach into groundwater supplies and further degrade drinking water quality. In 2000, it was reported that soil was unable to protect groundwater from toxins that originated from rivers and lakes around Riga during algal blooming periods and eventually led to groundwater contamination (Eynard et al., 2000).

There is much research on the fate of MCs in water columns and aquatic organisms (Ishii et al., 2004; Lahti et al., 1997; Pflugmacher et al., 1998). However, to our knowledge, very little information is available on the fate of MCs in soils (Morris et al., 2000; Tsuji et al., 2001). Therefore, in order to evaluate the risk of groundwater contamination, more knowledge about the fate and transport characteristics of microcystins in soils is absolutely critical.

The objectives of this study are: (1) characterize the adsorption of three MC variants in three different soils and identify the main factors influencing this process; (2) determine the persistence of MCs in soils (expressed as DT₅₀, half time for degradation) in order to define their leaching potential according to Gustafson (1989); and (3) investigate the leaching behavior of MCs in Chinese agriculture soils. The results of this study will lead to a better understanding of the leaching potential of MCs in soils and the risks associated with the use of toxic cyanobacterial blooms as plant fertilizers.

2. Materials and methods

2.1. Toxins and reagents

Microcystin-RR, LR and Dha⁷ LR was isolated from a laboratory mass culture of *Microcystis aeruginosa* PCC 7806 and cyanobacterial blooms collected from Lake Dianchi using an improved Ramanan method (Ramanan et al., 2000; Chen et al., 2004). The *Microcystis* cell extracts were sequentially applied to an ODS column, preparative high-performance liquid chromatography (HPLC) (Waters 590), thin layer chromatography (TLC) and Sep-pak plus PS-2 cartridges. The purity of the purified MCs exceeded 95% according to HPLC-DAD, LC/MS and HPTLC (Pelander et al., 2000; Chen et al., 2004). The ODS and PS-2 Sep-pak cartridges were obtained from Waters (Part No. Wat051910 and JJAN20131). The microcystin standards for HPLC analysis were obtained from Kanto Reagents (Japan). The ODS silica gel and TLC plates were purchased from Merck.

2.2. Soils

All studies were performed with non-sterile soil materials hereby mimicking near-natural conditions. Three unpolluted

fresh soils (0–15 cm) were collected from Wuhan and Kunming in China; the properties of test soils are shown in Table 1. For sorption and leaching experiments, the soils were air dried without being exposed to light, ground, sieved through 2 mm mesh sieve and stored at 4 °C; Collected fresh soils without any treatment were stored at 4 °C for persistence study use.

2.3. Adsorption studies

Sorption isotherms were conducted by the standard batch equilibration method (OECD, 2000). As there were minor differences between the amounts adsorbed after 4 and 24 h, respectively, 24 h was found to be a sufficient equilibration time. Adsorption experiments were carried out in duplicate. MC-RR, LR and Dha⁷ LR solutions (20 ml) containing 0.5, 1, 2, 4, 6, 8 and 10 µg/ml were taken in the 30 ml capacity glass joint test tubes and 4 g air-dry soil was added. The tubes were sealed and shaken for 24 h at 25 °C. After equilibrium was achieved, the tubes were centrifuged at 2000 rpm for 10 min and clear supernatant from each was extracted using a pipette and analyzed by HPLC.

2.4. Persistence studies

The persistence studies were performed according to SETAC procedures (Lynch, 1995), in two replicates. The persistence of microcystin variants and the toxins contained in cyanobacterial blooms were examined using the three soils. Toxic cyanobacterium used in persistence studies were both from mass cultures of *Microcystis aeruginosa* DS and from the collection of cyanobacterial blooms in Lake Dianchi. The two cyanobacteria were mixed in a ratio of 1:1.6 so that the concentrations of the three MC variants together totaled approximately 1 mg g⁻¹ (dry weight algae). The microcystin toxins of interest were applied at rates of 15 µg/g (toxin/soil), and the cyanobacterial blooms at rates of 15 mg/g (algae/soil). The soils containing toxins or cyanobacterial blooms were incubated at 25 °C in the dark, under constant soil moisture content. For each experiment, samples were taken 3 times a week and lyophilized by frozen vacuum drier. 2 g of the dried soil sample was extracted with 0.1 M EDTA – 0.1 M Na₄P₂O₇ – 0.1% TFA and ultrasonicated (400 W, 10 min) (Chen et al., 2006), the resulting suspensions were centrifuged

Table 1
properties of test soils used in the investigation

Items	Soil A	Soil B	Soil C
Sample location	Wuhan	Wuhan	Kunming
	N 30°29.976'	N 30°31.371'	N 24°56.437'
	E 114°13.904'	E 114°23.068'	E 102°42.499'
Organic matter (g kg ⁻¹)	13.56	17.76	22.31
pH (1:5 H ₂ O)	6.5	7.1	6.7
Particle size fraction (g kg ⁻¹)			
Sand	325	643	497
Silt	423	250	265
Clay	252	107	238

at $4000 \times g$ for 8 min, and the residues were extracted for two times. For each sample, the three supernatants were applied to preconditioned Sep-pak ODS cartridges and the eluted methanol solutions were reduced to 1 ml under nitrogen flow, and then analyzed using an HPLC.

2.5. Leaching studies

All tests were performed according to the OECD guideline (OECD, 2003). The air-dried and sieved soils (<2 mm mesh) were packed in glass columns to a height of approximately 30 cm. To obtain uniform packing, the soil was added in small portions under gentle vibration of the column. Subsequently, the soils were saturated with artificial rain (CaCl_2 at 0.01 mol/l) to their maximal water holding capacity according to OECD. The test substances were applied on the top of the soil columns as aqueous solutions at a concentration level of 10 $\mu\text{g/g}$ soil (dry weight). All tests were performed in darkness at a temperature of 20 ± 2 °C. A total amount of 393 ml artificial rain (0.01 mol/l CaCl_2) corresponding to 200 mm rainfall was added drop wise within 24 h on each soil column. Glass sinter disks on top of the columns ensured an even distribution of the artificial rain.

At the end of the experiment, leachates were concentrated using 1 g ODS cartridges and eluted with 15 ml methanol. The soil columns were then frozen under -20 °C, broken, and the remaining soil samples were equally cut into 12 pieces. The toxins in each sample piece were then extracted and analyzed by HPLC.

2.6. Determination of microcystins

HPLC was employed to determine the concentrations of microcystins in extracted solutions (Chen et al., 2005; Lawton et al., 1994). Briefly, The Shimadzu LC-10A system included two LC-10A pumps and a UV detector. Eluted samples were passed through a Shimadzu shim-pack column (CLO-ODS 4.6×150 mm) with 60% solution A (100% methanol) and 40% solution B (0.05 mol/L K_2PO_4 , pH 3) over 20 min. The flow rate was 1 ml min^{-1} . HPLC columns were maintained at 40 °C and the solution injection volume was 10 μl .

2.7. Data analysis

The amount adsorbed on soil (C_s , $\mu\text{g g}^{-1}$) was calculated as the difference between the initial aqueous phase concentration and the equilibrium concentration (C_e , $\mu\text{g ml}^{-1}$). The distribution coefficient (K_d) was calculated as the ratio of the adsorbed concentration in soil and its equilibrium concentration in solution. The adsorption data taken during the adsorption tests under various concentrations were fitted to the logarithmic form of the Freundlich equation: $\log_{10} C_s = 1/n \log C_e + \log k_f$ (OECD, 2000). This is equivalent to the linear regression equation ($Y = a + bX$). The data was subjected to regression analysis and fitness of data to Freundlich equation was confirmed by calculation of correlation coefficient and determining its statistical significance. In the Freundlich equation,

K_f is the Freundlich distribution coefficient and $1/n$ is the slope. For K_{OC} (the normalized distribution coefficient with respect to the organic content (OC) (%) of the soils) was calculated according to: $K_{OC} = K_d \times 100/\text{OC}$. The half-life ($t_{1/2}$) values were calculated based on first order dissipation kinetics: $C_t = C_0 \times e^{-Kt}$, where C_t = concentration after time t , C_0 = apparent initial concentration and K = rate constant. This first order equation resolves into the straight-line equation: $\log C_t = \log C_0 - K \times t/2.303$. The dissipation constant (K) was calculated from slope (b) of the regression equation. The half-life values were calculated from dissipation constant ($t_{1/2} = 0.693/K$).

The leaching potentials of the examined toxins were calculated using the following equation:

$$\text{GUS index} = \log(DT_{50}) \times (4 - \log K_{oc}) \quad (\text{Gustafson, 1989}).$$

All data in the current studies were calculated using Microsoft Excel 2000, statistical analyses were performed by Origin software.

3. Results and discussion

3.1. Sorption behavior of microcystins in agricultural soils

The adsorption isotherms were different for each MC variant in three different soils studied (Fig. 1). Adsorption parameters (K_d , K_f and n_f) calculated from linear and non-linear methods, equations are provided in Table 2. The experimental data fitted to Freundlich isotherms indicated a statistically significant ($P < 0.05$) correlation coefficient values ($r_f > 0.97$).

The values of capacity factor (K_f) and slope (n_f) for three MC variants in three soils ranged from 0.69 to 6.00 and 1.01 to 1.54, respectively. As is evident in Fig. 1 and Table 2, the adsorption of MCs in any of the soils decreased in the following order: RR > Dha⁷ LR > LR, furthermore, for each MC variant in the three soils, the adsorption in the soils decreased in the following order: soil A > soil C > soil B. In numerous cases, soil organic matter contents have been shown to affect the organic pollutants adsorption in soils (Stevenson, 1972; Worrall et al., 2001; Spark and Swift, 2002), however, in the present study the sorption behavior did not depend on the total organic matter contents (Table 1), but on the content of clay in the soils. That is to say, the soil type and the soil clay content are the main factors influencing the adsorption of MCs in soils. Our results are partially consistent with Miller's investigation (Miller et al., 2001), in which he proposed high clay contents would have the higher nodularin adsorption. According to the existing literature (Chen et al., 2005) and our recent results (data not shown), we proposed the adsorption mechanism of MCs in soils is not only physical sorption, but also chemical binding with the metal ions on the surfaces of soil particles. Due to the nitrogen and oxygen atoms in the toxin structures, MCs can chelate with the metal ions in clay. Therefore the clay content is more important than the content of organic compounds which influencing the toxin adsorption. Of the

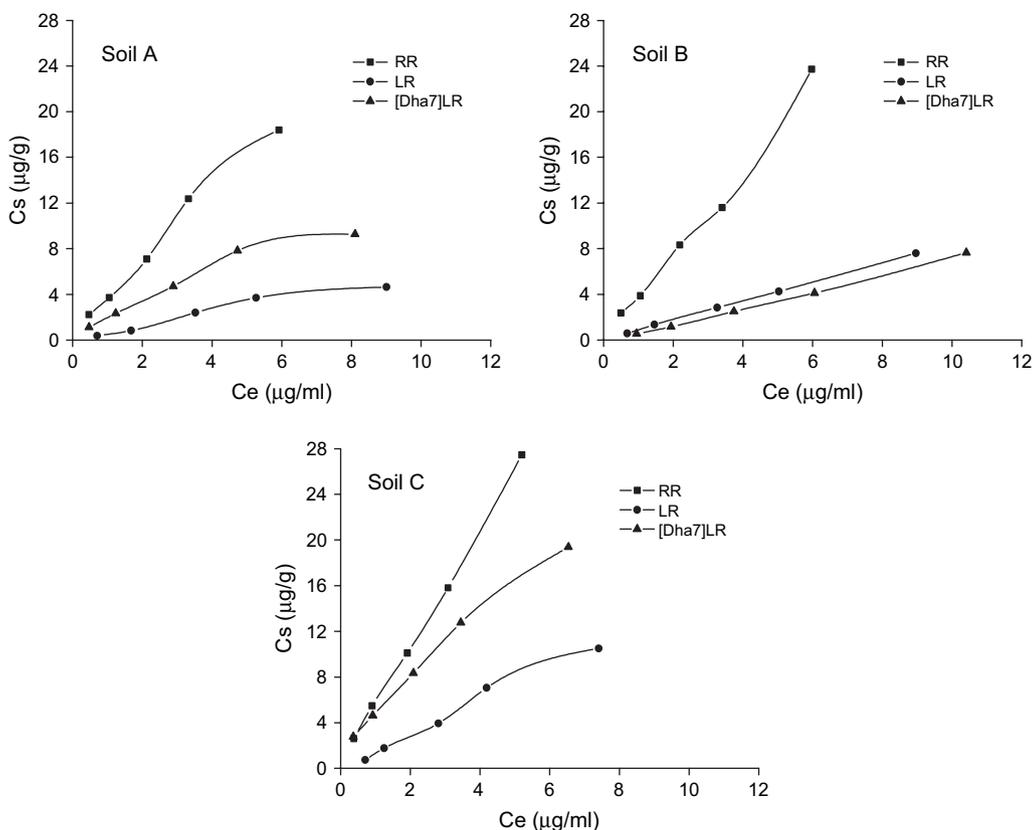


Fig. 1. Sorption isotherms of three microcystin variants (RR, LR, [Dha⁷] LR) binding to three agriculture soils (Soil A, Soil B and Soil C).

three microcystin variants used in this study, RR is more strongly absorbed on the soils than other two MC variants (Fig. 1 and Table 2). This also can be explained by the chemical structures of the toxins studied, in microcystin structures, MC-RR has three more nitrogen atoms than MC-LR, which may provide additional binding positions compared to the other two toxins studied. Therefore, MC-RR would interact more strongly with metal ions in soils.

3.2. Persistence of MCs in agriculture soils

Persistence of microcystins in soils under laboratory conditions was performed using three MC variants and toxic cyanobacterium in three different soils under imitated field moisture

Table 2
Adsorption parameters (K_d , K_f and n_f) for MCs in three Chinese agriculture soils, calculated from linear and non-linear methods

Microcystin	Test soils	K_d	K_f	n_f	r_f
Microcystin RR	Soil A	3.06	3.96	1.16	0.975**
	Soil B	3.86	1.61	1.22	0.990**
	Soil C	5.11	2.04	1.29	0.974**
Microcystin LR	Soil A	0.586	4.08	1.08	0.984**
	Soil B	0.841	0.692	1.54	0.981**
	Soil C	1.08	3.31	1.02	0.997**
Dha ⁷ microcystin LR	Soil A	1.11	6.00	1.21	0.999**
	Soil B	1.55	1.25	1.01	0.976**
	Soil C	1.90	5.25	1.46	0.998**

**Significance at $P < 0.05$.

regime. The dissipation of residues in different treatments followed first order kinetics. The calculated half-time (expressed as DT_{50}) based on first order kinetics ranged from 7.9 to 17.8 d for MC-RR, 6.0 to 17.1 d for MC-LR and 7.1 to 10.2 d for MC-Dha⁷ LR (Table 3). As is indicated in Table 3, the half-time of the examined microcystins in the three soils decreased in the following order: soil A > soil B > soil C, and in each kind of soil, the toxins are more persistent in the treatments with cyanobacteria added than those with toxin standards added. Under imitated field conditions, it seems that the major dissipation process is mainly due to microbial degradation (Gupta and Gajbhiye, 2004). High organic carbon content will be propitious to growth of the bacterium and lead to more intensive microbial population, therefore toxins in soil B and C yielded faster degradation than in soil A. The microbial degradation process of the toxins in the treatment with cyanobacteria added can be divided into two parts: the cell break and toxin release process, and the toxin degradation process. When cyanobacteria cells was added to soils, it would take more time to break the cells and release the toxins into soils, giving an explanation for the stronger persistence of toxins in cyanobacterium-contaminated soils.

3.3. Leach behavior of MCs in chinese agriculture soils

The leaching potential of organic pollutants such as pesticides are usually assessed by the normalized distribution of the soil organic carbon partition coefficient (K_{oc}) (Wauchope

Table 3
Regression equation, DT₅₀, K_{oc}, and GUS for examined toxins in Chinese agriculture soils

Substances	Regression equation	DT ₅₀ (days)	K _{oc}	GUS ^a
MB ^b in soil A	$y_{RR} = -0.0461x + 3.2024$	17.8	224.0	2.06
MC ^c in soil A	$y_{RR} = -0.0826x + 2.2108$	11.0	224.0	1.72
MB in soil B	$y_{RR} = -0.1102x + 3.4909$	10.0	224.0	1.65
MC in soil B	$y_{RR} = -0.1367x + 2.2364$	7.9	224.0	1.48
MB in soil C	$y_{RR} = -0.0886x + 3.0918$	11.3	224.0	1.74
MC in soil C	$y_{RR} = -0.1785x + 2.1066$	7.9	224.0	1.48
MB in soil A	$y_{LR} = -0.0488x + 2.7973$	17.1	46.3	2.88
MC in soil A	$y_{LR} = -0.0943x + 2.9241$	12.9	46.3	2.59
MB in soil B	$y_{LR} = -0.1732x + 3.4103$	8.5	46.3	2.17
MC in soil B	$y_{LR} = -0.2087x + 2.9088$	6.1	46.3	1.83
MB in soil C	$y_{LR} = -0.1204x + 2.7591$	9.1	46.3	2.24
MC in soil C	$y_{LR} = -0.2156x + 2.8016$	6.0	46.3	1.82
MB in soil A	$y_{DLR} = -0.0821x + 0.8334$	9.9	84.8	2.06
MC in soil A	$y_{DLR} = -0.0962x + 0.6292$	10.2	84.8	2.09
MB in soil B	$y_{DLR} = -0.1344x + 1.2691$	7.7	84.8	1.84
MC in soil B	$y_{DLR} = -0.142x + 2.6748$	7.7	84.8	1.84
MB in soil C	$y_{DLR} = -0.1207x + 0.6225$	7.9	84.8	1.86
MC in soil C	$y_{DLR} = -0.1336x + 2.3775$	7.1	84.8	1.76

^a GUS index = $\log(DT_{50}) \times (4 - \log K_{oc})$; GUS > 2.8 leacher; 1.8 < GUS < 2.8 transient GUS < 1.8 non-leacher.

^b MB denote *microcystis* bloom.

^c MC denote MC variants.

et al., 2002; Stauffer et al., 1989), GUS parameters (Gustafson, 1989) and laboratory column leaching (OECD, 2003; Oppel et al., 2004). K_{oc} values of three MC variants were calculated from their K_d values in the three soils, the average values for MC-RR, LR and Dha⁷ LR were 224, 46.3 and 84.8 respectively (Table 3). According to McCall and Helling's classifications (Table 4) (McCall et al., 1980; Helling and Turner, 1968), MC-LR belongs to a very high mobility class, Dha⁷ LR belongs to a high mobility class and RR belongs to a medium mobility class. The results in Table 3 show the GUS values range from 1.48 to 2.06 for RR, 1.82 to 2.88 for LR and 1.76 to 2.09 for Dha⁷ LR. LR has a GUS value of 2.88, which is characteristic of leaching compounds. The other two toxins show leaching potentials belonging to transition compounds. The results of the column leaching experiment for MCs in the three soils evaluated are shown in Fig. 2, a significant difference between the distribution patterns of the three MC variants in soil columns and leachates were observed among the three soils. In the soil leaching experiments,

Table 4
Classification of soil mobility potential according to McCall and Helling's investigations (McCall et al., 1980; Helling and Turner, 1968)

K _{oc}	Mobility class	Helling's Classification Scheme
0–50	Very high	5
50–150	High	4
150–500	Medium	3
500–2000	Low	2
2000–5000	Slight	1
≥5000	Immobile	1

recoveries of the toxins in leachates ranged from 0–16.7% for RR, 73.2–88.9% for LR and 8.9–73.1% for Dha⁷ LR, respectively. LR and Dha⁷ LR were mobile in all the soils, and RR was not detected in the leachate of soil A. The mobilities of the three toxins in the soil evaluated followed an increasing order: soil A < soil C < soil B. Investigations on the adsorption characteristics of toxins from raw water to soils and toxin extraction from sediment have demonstrated that a large mass of toxins in water will be adsorbed and stay in soils or lake sediments (Miller et al., 2001; Morris et al., 2000; Tsuji et al., 2001). However, in our column leaching experiments, the highest concentrations of the added MC-LR and Dha⁷ LR were found in the leachate, and a significant amount of MC-RR also migrated to the bottom of the columns in soils B and C. The reasons that the high mobility of MCs in soil columns may have been caused by the use of CaCl₂ in the leaching experiments. From the adsorption results, the chelating with metal ions on the soil surface was the main adsorption process (Chen et al., 2005). The addition of CaCl₂ to maintain the ionic strength in leaching tests could have led to the competition of MCs adsorption with the metal ions on the soil surface, producing lower than expected adsorption results.

As is evident in numerous studies, both clay and organic carbon are important in the binding of organic compounds in soils (Hance, 1988; Worrall et al., 2001; Spark and Swift, 2002). This was supported by the Cox investigation (Cox et al., 1997), which reported that clay was essential for enhanced adsorption of various polar herbicides. The adsorption and leaching results in the present studies also demonstrated this point. However, in the leaching experiment, as is indicated in Fig. 2, illustrates a significant difference between the leaching behavior of soils A and C, both of which have similar clay contents. There is some evidence that, in the presence of soils, an increase in the concentration of dissolved organic matter results in an increase in the mobility of hydrophilic and of some hydrophobic organic chemicals such as atrazine, 2,4-D and DDT (Gao et al., 1997; Kan and Tomson, 1990; Fitch and Du, 1996). The content of total organic carbon in soil C is nearly two times than that in soil A, therefore the significant differences in leaching behavior between the two soils may be the result of processes such as: the formation of soluble complexes with soil solution components such as dissolved organic matter; or the incomplete interaction of these toxins with the solid state organic or inorganic matter in the soil (Baskaran et al., 1996b). MCs can be degraded within 4 weeks in surface water (Ishii et al., 2004; Lahti et al., 1997); however, they can persist for 100 days in groundwater with their concentration not significantly decreased due to lack bacterium (Holst et al., 2003). Therefore, during the course of eliminating surface water contamination, a strong groundwater pollution prevention strategy should be practiced as well.

4. Conclusion

These results of the present study demonstrate that there is a positive relationship between soil clay content and MC adsorption capacity. In the tested soils, adsorption capacity

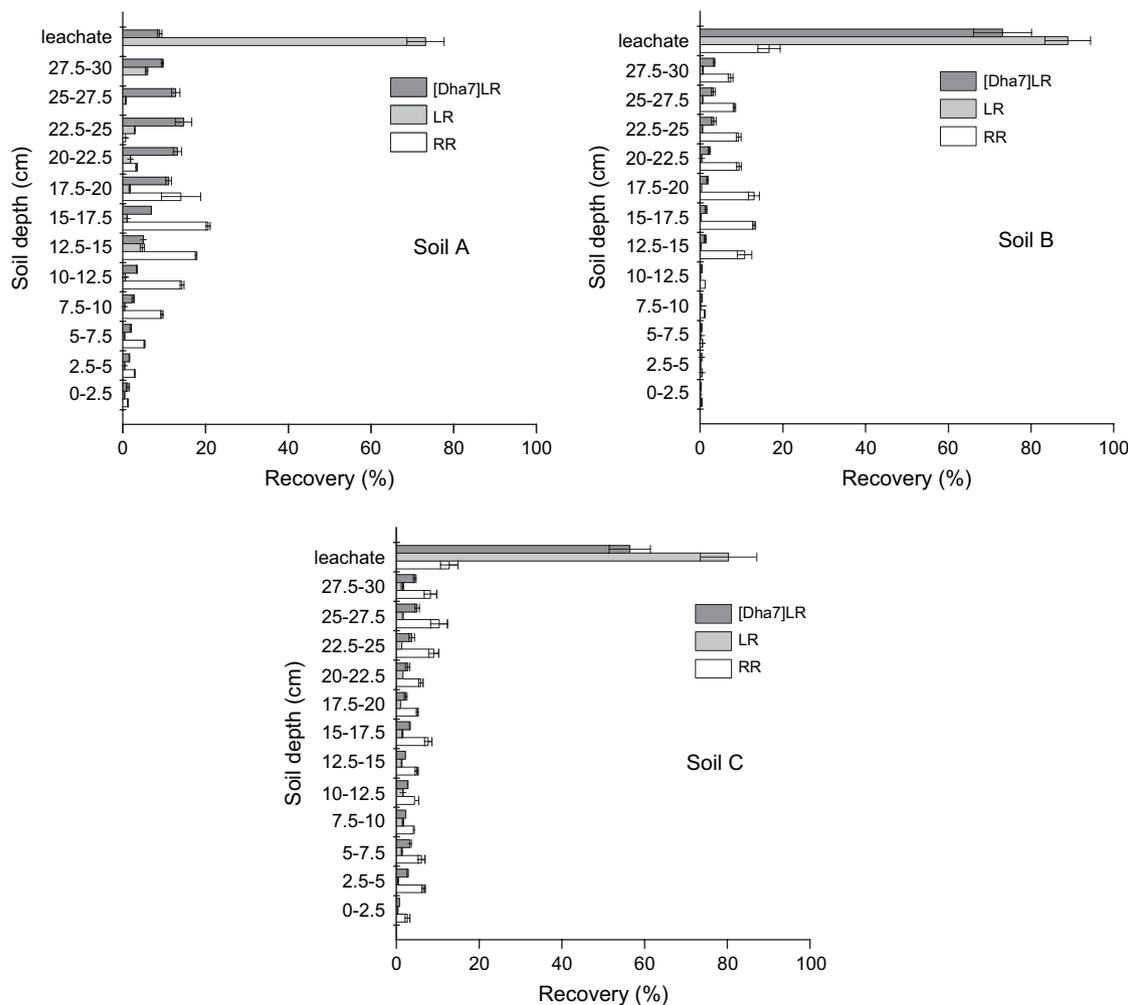


Fig. 2. Leaching behavior of three microcystin variants (RR, LR, [Dha⁷]LR) in Chinese agricultural soils (Soil A, Soil B and Soil C). Recovery rate in % of applied tested microcystins in soil and leachate.

decreased in the following order: RR > Dha⁷ LR > LR. From the results of the laboratory leaching study and leaching potential assessment using K_{oc} and GUS parameters, it was concluded that MCs belong to high mobility pollutants in soil and their mobility increased with decreasing soil clay content. The half-life of degradation in soils ranged from 7.9 to 17.8 days for MC-RR, 6.0 to 17.1 days for MC-LR and 7.1 to 10.2 days for MC- Dha⁷ LR. The dissipation of MCs was relatively faster in soils with high content organic carbon.

Thus, the use of harvested cyanobacterial blooms as plant fertilizer in agriculture soils in rainy seasons is likely to be unsafe because the toxins such as MC-LR and other toxin variants may be persistent and in certain cases leach and cause groundwater contamination.

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