



Effects of humic acid and suspended soils on adsorption and photo-degradation of microcystin-LR onto samples from Taiwan reservoirs and rivers

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ABSTRACT

This article covers the adsorption capacity of microcystin-LR (MC-LR) onto natural organic matter (NOM) or suspended solids of water samples from reservoirs (Emerald and Jade reservoirs) and rivers (Dongshan, Erhjen and Wukai rivers) in Taiwan to determine the fate, transport behavior and photo-degradation of microcystins in natural water systems. Langmuir adsorption and photo-degradation studies were carried out and the capability of samples for MC-LR adsorption was confirmed. Among these, samples collected from reservoir showed enhanced MC-LR adsorption than that of river samples and the greater adsorption behavior was always favored by larger content of organic matter and suspended particles in the system. It is obvious from the experimental results that the adsorption of MC-LR was influenced by suspended particles (turbidity), humic acid (HA), organic matter content and other pollutants. The effective photo-degradation of MC-LR was achieved using higher energy, lower wavelength (254 nm) UV light within 60 min. The presence of humic acid and turbidity affected the photo-degradation process. These data provide important information that may be applied to management strategies for improvement of water quality in reservoirs and rivers and other water bodies in Taiwan.

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1. Introduction

The photoautotrophic prokaryotic cyanobacteria organisms commonly grow in eutrophic lakes, reservoirs and rivers. Massive development of cyanobacteria and the formation of so-called water blooms have negative effects on water quality and represent also human health hazards because of toxin production [1,2]. Among cyanobacterial toxins (cyanotoxins), microcystins (MCs) are probably seen to occur most frequently. MCs are hepatotoxic cyclic heptapeptides most of which have the general structure [3] cyclo(-D-Ala-X-D-erythro-β-methylAsp-Z-Adda-D-Glu-N-methyldehydroAla), where X and Z represent variable L-amino acids and Adda is the β-C20 amino acid 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. One of the most commonly occurring microcystins is the highly toxic microcystin-LR in which the two variable amino acids are leucine and arginine and they have adverse effects on animal and human health [4]. In addition to the toxicity, high chemical and biological stability is another hazardous property of these peptide toxins. These toxins are crucial components for assimilation, retention and transformation of dissolved and particulate organic material in the aquatic environment [5]. MCs are neutral or anionic in

environmental waters [6] and MCs achieve extremely high concentrations in aquatic water bodies, particularly at the end of the vegetation season when the cyanobacterial blooms collapse. The ambient concentration of dissolved microcystin is a function of several factors [3] such as dilution, photodegradation, adsorption onto particles and biodegradation. In order to evaluate the environmental risk of microcystin and assess its public health implications, it is important to trace the fate of microcystins in the aquatic environment [7]. However, there have already been studies on the fate of microcystins in water columns and aquatic organisms [8], the environmental fate of dissolved MCs is not well described. The relative importance of the processes influencing the dissolved microcystin concentration during and after a cyanobacterial bloom is difficult to estimate. Hence, to assess the actual implications of MCs exposure, it is crucial to pursue MCs under field conditions, which necessarily suggested the need for laboratory studies. Both planktonic and benthic microorganisms can participate in the sorption and degradation of high MCs amounts. While bacterial degradation of MCs can occur, in general, there is a lag period of several days or weeks [9]. Microbial degradation may be a major pathway for the elimination of microcystins, although a lag phase seems to be required in some cases [10–13]. While UV light near the absorption maximum of the MCs (238 nm) can lead to the degradation of MCs, solar irradiation in the absence of other substrates has a minimal effect on the fate and decomposition of MCs [14,15]. However, in the presence of pigments or humic substances (HS), photosensitized reactions

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appear to play an important role in the environmental transformation of MCs and may be applicable to specific water treatment [16–20].

Although there were studies focused on the interaction of water with MCs [21–23], the fate and the kinetics of toxin degradation in natural water system is not fully understood. Despite many other microcystins are known, MC-LR is the most commonly found microcystin, present almost in all natural water systems in considerable amount. However, as they are all cyclic heptapeptides containing an unusual β -amino acid, it seems likely that their degradative behavior would be similar. Hence, our study examined the MC-LR degradation under simulated conditions and investigated the factors influencing the adsorption and photo-degradation of MC-LR. In the present study, a systematic investigation was performed to survey the fate of MC-LR in the water column of Taiwan reservoirs and rivers. The objective of this study was to evaluate the potential adsorption and degradation of MC-LR by water samples collected from the reservoirs and rivers, in Taiwan based on toxin distribution and bioaccumulation. In addition, this study traces the fate of MC-LR in the presence of suspended soils and humic acid in water system as both soils and humic acid associated with water influence the assessment of MC-LR under study.

2. Experimental

2.1. Chemicals and devices

Standard microcystin-LR (MC-LR) ($C_{49}H_{74}N_{10}O_{12}$, FW = 995.2, 95% purity) was purchased for analysis from Yu-technology Company, Taiwan. Deionized distilled water was used to prepare all solutions and suspensions. HPLC grade solvents were used for extraction and HPLC analysis. All other chemicals used in this study were of analytical grade. A LM-595R rotary incubator (YIH DER Company) was used in the experiments. The pH was determined using a Jenco 6171 microcomputer-based bench pH meter. HERMLE Model Z323K freeze dryer was used. HPILIPS brand UV lamp ($\lambda = 365$ nm, 15 W, 270 lx) and SYLVANIA brand UV lamp ($\lambda = 254$ nm, 15 W, 115 lx) were used for irradiation.

2.2. Study area

Jade reservoir, Emerald reservoir, and three rivers such as Dongshan river, Erhjen river and Wukai river were selected as sampling areas for simulation studies. Both the reservoirs and rivers provide water for drinking, agriculture, aquaculture and industrial uses. Along with the prompt development of the economy and the intensive use of water resources, the water is highly polluted. Heavy blue-green algae, which have previously occurred in warm seasons, are becoming increasingly frequent.

2.2.1. Sampling

Water samples were collected at different sites of each reservoirs and rivers. Samples were collected in axenic plastic containers, which were labeled with, at a minimum, date, time and sample location placed on ice and transported to the laboratory as soon as possible at 4 °C. All samples were stored at 0–4 °C in a refrigerator in the dark before use. To prevent contamination equipment was thoroughly cleaned between sites. Some basic analyses, including pH, organic matter content (Shimadzu TOC-5050 analyzer), total hardness (titrating with EDTA using Eriochrome black T as indicator), total alkalinity (titrating with 0.02 M HCl), effect of suspended particles and humic acid were carried out.

2.3. Adsorption (dissolution) of MC-LR onto water samples

Adsorption of MC-LR onto water samples is dependent on the presence of suspended particles and humic acid. So, the simulation experiments were carried out as follows.

2.3.1. Suspended particles (soils) and water system

To prepare the suspended particles, appropriate amount of inorganic soils kaolin [$(Al_2Si_2O_5(OH)_4)$] (0.1–4 μ m particle size, 25.07 m²/g surface area and pH 3.5–5) and montmorillonite (monte) [$(Na,Ca)_{0.33}(Al,Mg)_2(Si_4O_{10})(OH)_2 \cdot nH_2O$] (<1 μ m particle size, 286.82 m²/g surface area and pH 3–5) was added into 1 L of deionized water and stirred for 1 h in such a way to produce 1000 and 5000 NTU turbid solution. The turbidity of the system was measured using nephelometer. Turbidity is more difficult to master a certain value, so advance preparation of a large number of suspension solution per batch adsorption experiments was thus fixed. Then upper suspension sample was collected. To 20 mL of this suspension, MC-LR solution was added, then mixed and the mixture was covered with aluminum foil. 20 mL of each turbidity system contains (1.6, 1.2, 0.8, 0.4, 0.2, 0.1) μ g/mL MC-LR. The reaction mixture was then shaken horizontally for 24 h at 150 rpm in a Lab-line orbit environ shaker at 28 °C. After the mixture was evenly mixed, the resulting suspensions were centrifuged (4500 rpm) for 5 min and the upper fluid was drawn up and filtered through 0.22 μ m filter paper. The filtrate was subjected to high-performance liquid chromatography (HPLC) analysis to determine the concentration of MC-LR.

2.3.2. Humic acid and water system

Appropriate amount of humic acid was dissolved in 100 mL of deionized water. To this humic acid solution, MC-LR solution was added and the experiment was carried out as above.

2.3.3. Natural water systems

To study the effect of externally added humic acid in the presence of soils on adsorption behavior in natural water systems, the humic acid–soil mixture was added to each 20 mL of sample and spiked with MC-LR, which was then subjected to the same procedure as above. Experiments were also carried out using collected samples in absence of humic acid–soil mixture.

2.4. Photodegradation experiments

The radiation of sunlight would definitely affect the fate and of MC-LR in natural water. Considering this factor, we have carried out the photo-degradation (UV irradiation) of MC-LR under two different wavelengths ($\lambda = 254$ nm and $\lambda = 365$ nm) of UV light as follows.

For each treatment the mixture containing samples and 1.6 μ g/mL of MC-LR was placed in a pyrex reactor vessel under stirring (also clear solution of MC-LR without HA and SS). The samples were stirred and illuminated at pH 3 for 1 h (in the case of 254 nm) and 5–6 days (in the case of 365 nm), and the resulting suspensions were collected at regular time interval (each 5 min in the case of 254 nm and each day in the case of 365 nm), centrifuged (4000 rpm) for 10 min and the supernatant liquid was collected. The collected samples were preconditioned with C18 cartridges and then eluted with 10 mL of 90% methanol (v/v) solution. After the eluates were concentrated using a rotary evaporator, the residues were re-dissolved with 0.2 mL of 20% methanol, filtered through 0.22 μ m filter paper and stored at –20 °C until HPLC analysis. The eluted methanol solutions were evaporated prior to being re-dissolved in

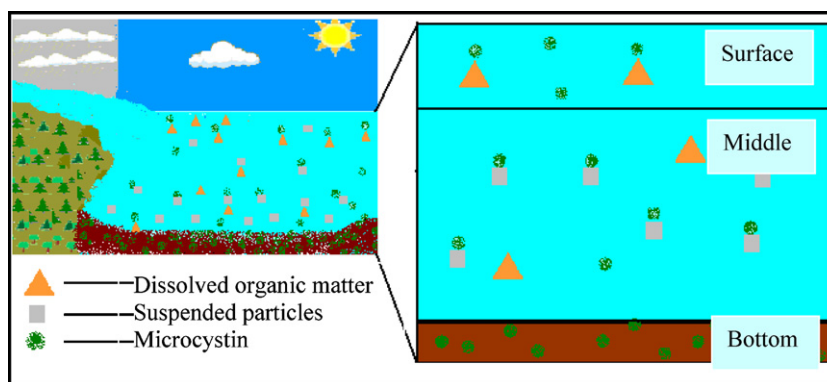


Fig. 1. Simulation diagram of the transport of microcystin in natural water systems.

1 mL of distilled water and then analyzed using HPLC. This shows that effective recovery of MC-LR can be achieved easily [24].

2.5. Analytical procedure

High-performance liquid chromatography (HPLC) was employed to determine the concentrations of MC-LR in samples from the laboratory studies during adsorption and photo-degradation. HPLC analysis (Agilent HPLC/DAD230-P230 instrument, 230 Detector, photodiode array detector) was performed using and LC column (C18, 5 μm , 4.6 mm \times 150 mm) using HPLC-grade acetonitrile and 0.01 M ammonium acetate (30:70) as the mobile phase with a flow rate of 1 mL/min, injection volume of 100 μL , at room temperature. The detector uses a fully enclosed spectrophotometric system to ensure the high spectral resolution and wavelength accuracy. The detection limit is 0.007 $\mu\text{g/mL}$ of MC-LR concentration (S/N ratio is 6.1). The adsorption of MC-LR from the aqueous solutions was studied (see Fig. S1). After the desired reaction period for each batch, the aqueous phases were separated from the materials and the concentration of MC-LR was measured.

The amount of MC-LR was calculated using the following equation;

$$q_e = \frac{[(C_0 - C) \cdot V]}{M}$$

where q_e is the amount of MC-LR adsorbed per unit amount of the adsorbent ($\mu\text{g/g}$); C_0 and C are the concentrations of MC-LR in the initial solution ($\mu\text{g/mL}$) and after adsorption respectively; V is the volume of the adsorption medium (mL); and M is the amount of the adsorbent (g). q_{max} was calculated from q_e using Langmuir linear isotherms and the corresponding values were obtained by a linear method.

3. Results and discussion

3.1. Langmuir adsorption isotherms of MC-LR

The fate and transport behavior of MCs in natural water bodies will vary at the surface, middle and bottom water layers as shown in Fig. 1. Natural organic matter (NOM) is a complex mixture of organic substances. In most aquatic system, the most abundant dissolved NOM [25] is fulvic and humic acids. In general the complexity of water due to the presence of suspended soils, humic substances and other substances in different proportion will have potential contribution to MCs adsorption in natural water systems. The fate of MCs in the aquatic environment is mainly of four types [24] such as cell bound toxins, dissolved toxins, bioaccumulated toxins and conjunct toxins with sediment or suspended substances.

In this laboratory study it is going to be dissolved toxins as we are carrying out the simulation studies by adding MC-LR into the samples. The adsorption studies of samples collected from different sampling areas conveyed that the binding of MC-LR for river samples is comparatively lower than that of reservoir samples. The adsorption onto the sample (12.95 $\mu\text{g/g}$ MC-LR) from Jade reservoir is significantly higher relative to that of sample (6.98 $\mu\text{g/g}$ MC-LR) from Emerald reservoir. The adsorption capacities of MC-LR for samples collected from Erhjen, Dongshan and Wukai rivers were 2.83, 3.52, 2.54 $\mu\text{g/g}$ respectively. The results indicated that the binding of MC-LR for river samples is comparatively lower than that of reservoir samples. This may be due to the dissolved organic matter, soils, pollution, and long-term erosion, which significantly differ from reservoir and river environment. The physico-chemical properties of the samples are given in Table 1 and these properties definitely vary from each sample. It could be seen from the results that in all cases, the calculated R^2 value ranged from 0.98 to 0.99 indicating that the Langmuir isotherm model fitted well in all cases. In this study, natural water system is divided into two parts respectively, water system/water-soil saturated system, to explore the effects of suspended particles and humic acid solution on the fate and transport behavior of MC-LR. The existence of organic carbons in natural water accounts for the presence of humic substances and soil organic matter. Assuming that different natural water systems contain different amount of organic matter on seasonal variation, we have used high humic acid (HA) concentrations, to study the effect of the HA even in high concentration in natural water systems. The experimental simulation by Total Organic Analyzer (TOC) carried out in the humic acid (HA) solution (40 and 80 mg/L) at pH 6.63, quantified the organic matter content of the solution as 20.85 mg/L and 40.35 mg/L respectively. This simulation study clearly claims the presence of humic acid in the highest proportion in natural water system.

Fig. 2 shows the Langmuir adsorption isotherms of MC-LR adsorption by humic acid solution with increasing concentration of MC-LR. The weak dissociation of humic acid in water creates negative charge on surface. Since both toxins and humic acid (HA) solution are negatively charged, the adsorption was carried out almost in the neutral range (pH = 6.67) which probably facilitated the availability of humic acid for the distribution of microcystins in the hydrophobic environment and caused for the adsorption of microcystins onto humic acid (HA). When the water contains the humic acid solution it will have the following conditions; (i) the adsorbed microcystins on humic acid solution increases the distribution of microcystins in water body and thereby increases the mobility of microcystins in natural water system; (ii) when the water contains humic acid solution and soil/water-saturated systems, both will definitely have an impact on adsorption of microcystin. The environment of natural water body is primarily due

Table 1
Physico-chemical properties of the samples.

Samples	Dissolved organic matter content (%)	pH of the sediment system	Total alkalinity (mg/L)	Total hardness (mg/L)
Emerald reservoir	8.81	5.5	55	50
Jade reservoir	10.48	5.1	50	52
Dongshan river	7.15	6.8	60	44
Erhjen river	5.67	7.7	68	40
Wukai river	4.83	7.8	71	38

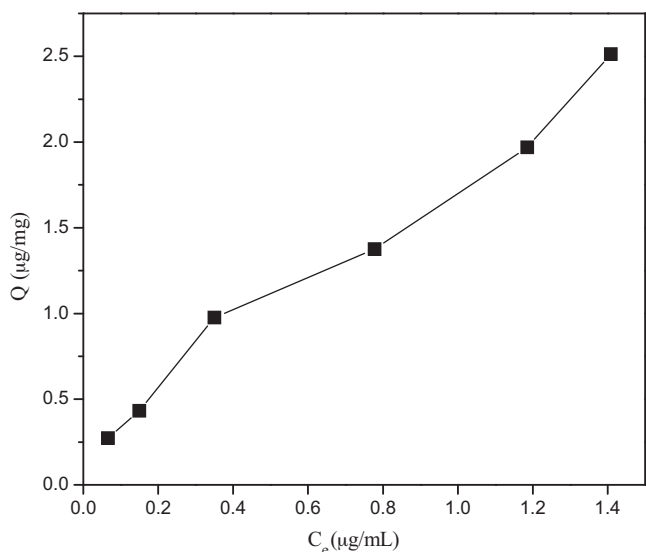


Fig. 2. Adsorption of MC-LR onto humic acid (HA) solution.

to the existence of organic matter, inorganic soils and humic substances. Among them, the humic substances provide hydrophobic environment for MC-LR. Hence obviously if humic acid and organic matter content increases in water, the tendency of MC-LR adsorption would also be influenced in soil/water saturated system. Hence to understand the impact on fate and transport of MC-LR, the knowledge of presence of organic matter and humic substances is necessary. In this study, the adsorption of microtoxin poisoning was carried out by adding the humic acid in the presence of soils to the samples collected from the sampling areas (reservoirs and rivers). The samples were stirred and filtered prior to HPLC analysis. For example, Fig. 3 shows the linear adsorption isotherms of MC-LR onto Jade reservoir sample containing dissolved organic matter due to the addition of 40 mg/L and 80 mg/L of humic acid solution. The measured TOC values are 20.85 mg/L for the addition

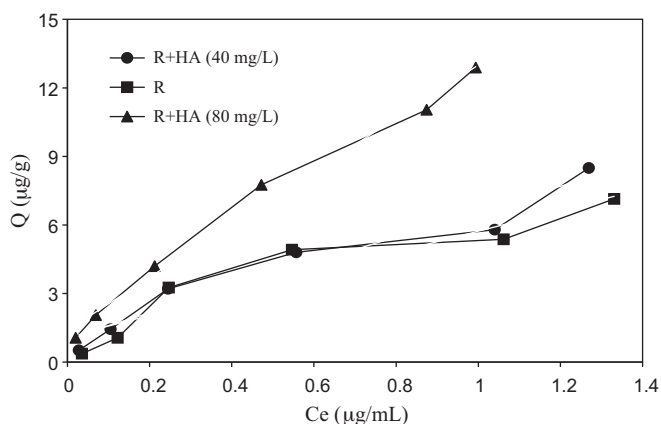


Fig. 3. Adsorption of MC-LR onto Jade reservoir sample both in absence and presence of humic acid-soil mixture.

of 40 mg/L of humic acid and 40.53 mg/L for the addition of 80 mg/L of humic acid. The adsorption of MC-LR poisoning in the presence of humic acid is tabulated in Table 2. From the Table 2, it is seen that the adsorption increases as the concentration of humic acid increases in the samples due to the increase in slope of adsorption isotherm (k_d) [26]. The slope of adsorption isotherm (k_d) is greater in the presence of humic acid in the system when compared to the system without humic acid as shown in Table 2. This strengthened adsorption mechanism in this hydrophobic environment of system is clearly due to the combined fact that hydrogen bonding and van der Waals forces are dominant and more significant in the case of hydrophobic Adda unit of microcystin rather than repulsive forces. This experimental result suggested that the presence of humic acid significantly increases the adsorption capacity of toxic MC-LR in water systems.

The distribution of soil in soil/water saturated environment addresses the dominant role of the soil in controlling the adsorption of microcystins. The structure of the soils that occupied the water molecules along with the organic matter content would promisingly alter the adsorption of microcystins into water sample. In order to simulate the environment of suspended particles in water [26], inorganic soils kaolin [$(\text{Al}_2\text{Si}_2\text{O}_5(\text{OH})_4$] and montmorillonite (monte) [$(\text{Na},\text{Ca})_{0.33}(\text{Al},\text{Mg})_2(\text{Si}_4\text{O}_{10})(\text{OH})_2 \cdot n\text{H}_2\text{O}$] were uniformly mixed with deionized water. As it is difficult to quantify SS in low turbid solution, total suspended particulate (SS) measurements in this study were carried out in two specified high turbid solutions (5000 NTU and 1000 NTU). Even in such high turbid solutions, the measured SS content of these two turbidities was only 0.018 g/mL and 0.011 g/mL and hence, it was unavoidable to use such high turbid solution. The presence of such high particles content may sometime unfortunately affect the HPLC peak of MC-LR (see Fig. S2). The experimental studies carried out on different turbidity systems conveyed that higher the turbidity, the higher is MC-LR adsorption as shown in Fig. 4. This clearly corroborates that the adsorption of microcystins in natural water systems is definitely altered by the presence of suspended particles (turbidity). As the depth of the water surface increases, turbidity also increases due to the settled suspensions. If the reservoirs and rivers contain large quantities of organic matters and suspended particles, then the adsorption of toxic microcystin is enhanced regardless the depth of the water sources. The adsorption studies carried out using inorganic soils kaolin and montmorillonite (monte) with the samples collected from the Emerald reservoir clearly depicted that the adsorption of MC-LR into soil/water system is affected by the suspended soils in addition to the organic matter content in water system. The organic matters induced a more negative charge, due to the presence of uncomplexed carboxylic and phenolic hydroxyl groups. Among the two inorganic soils, the adsorption of MC-LR onto kaolin soil/water

Table 2
Adsorption of MC-LR onto water samples in the presence of humic acid (HA).

System (R = Jade reservoir sample)	Adsorption of MC-LR (q_{max} , $\mu\text{g/g}$)	k_d
R	11.86	1.011
R + HA (40 mg/L)	12.95	1.308
R + HA (80 mg/L)	18.28	1.873

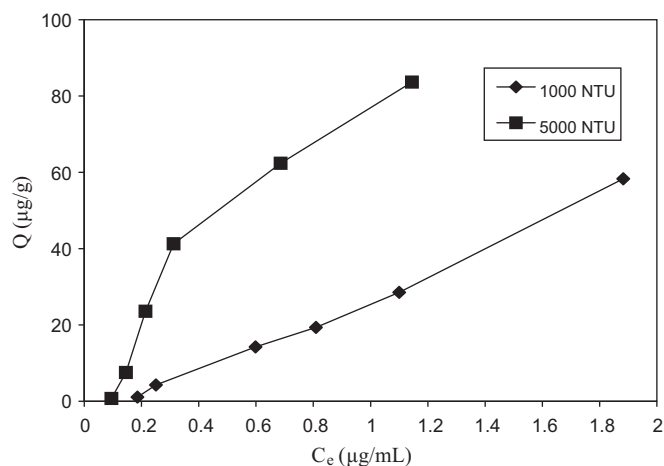


Fig. 4. Adsorption isotherms of MC-LR onto two different turbid solutions of monte soil.

system is higher than that of monte soil/water as shown in Fig. 5, despite monte soil has the high surface area when compared to kaolin soil. Based on this interesting observation, it was presumed that the adsorption mechanism in soil/water system is not primarily due to the large specific area but due to some other factors such as the charge gap between the clay layer of organic pollutant and soils [26]. When compared to monte, the layer of kaolin soil and clay is too close due to small charge gap [27] which in turn reduces the negative charge repulsion of MC-LR towards kaolin and thus enhanced adsorption of MC-LR into kaolin/water system. Due to weak dissociation of inorganic soil, there is negative charge repulsion between soil and MC-LR which is higher in the case of monte soil when compared to kaolin as the charge gap between clay and soil in the case of monte is more and hence organic and inorganic layers became less interactive. So, the high persistence of negative surfaces would tend to repel MC-LR rather to adsorb.

3.2. Photo-degradation studies

It is known that MCs isomerize when exposed to UV light. Hence the removal of the physical adsorption of the microcystins on the water sample is an indicative of the essential role of the UV irradiation in achieving degradation. The structure of MC-LR contains Adda constituents which comprises unsaturated double

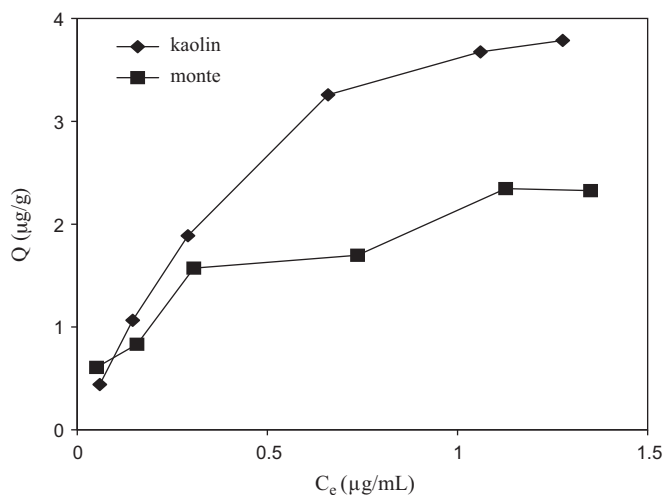


Fig. 5. Adsorption of MC-LR onto Emerald reservoir sample containing kaolin and monte soils (1000 NTU).

bonds. These double bonds are degraded by light causing for structural damage of MC-LR. These unsaturated α,β -conjugated double bonds of MC-LR structure are susceptible for photodegradation, oxidation, biological degradation resulting into structural change in MC-LR to reduce or even disappear. The UV radiation caused the geometrical isomerization [6] of microcystin, converting the (4E), (6E) Adda configuration to (4E), (6Z) or (4Z), (6E) Adda configurations. The (4E), (6E) Adda configuration is the geometry that is considered essential for the biological activity, and the two isomers do not show toxicity. The hydroxyl radical attack then destroyed the conjugated diene structure to form dihydroxylated products, and the last mechanism was the oxidation cleavage of the hydroxylated 4–5 and/or 6–7 bond of Adda. The degradation of MCs in natural water systems is dependent on several factors including the intensity of sunlight, water depth, season and thickness of ozone layer. To understand the impact on toxic microcystin degradation factors in the environment through different wavelengths of ultraviolet light and in different environmental conditions (turbidity and dissolved organic matter), we have carried out simulation studies on photo-degradation of adsorbed MC-LR onto water samples under two different wavelengths of UV light and discussed the experimental results to further understand the fate of microcystin toxin in the natural water environment. The susceptible destruction of unsaturated α,β -conjugated double bonds of MC-LR structure (Adda side chain) under light paved the mechanism [28] for photo-degradation. To understand the impact on degradation under two wavelengths (365 and 254 nm) of UV light, the same concentration (1.6 $\mu\text{g/mL}$) of MC-LR was irradiated in both cases and the experimental results were compared. As shown in Fig. 6, under irradiation with 254 nm UV light, complete photo-degradation of MC-LR was obtained within 60 min where as irradiation with 365 nm required 5 days to reach the complete photo-degradation of MC-LR. This is due to the occurrence of direct photolysis via direct photoisomerization under 254 nm light [29]. But in the case of 365 nm light [14,29] the isomerization involves a photosensitized process due to the presence of NOM such as humic acid in the sediment samples. Hence under higher energy irradiation, the effective destruction of Adda side chain takes place faster and quick degradation occurs. Thus faster decomposition and isomerization rates with the formation of various new microcystin isomers were obtained with the use of UV irradiation (254 nm) near the absorption maxima of the microcystin toxins.

3.3. Factors affecting the photo-degradation

The presence of suspended particles and humic acid has major contribution to the photo-degradation mechanism. The turbidity of the system due to suspended particles would definitely disturb the photo-degradation due to masking the system to be degraded. Hence, these factors may slow down the photo-degradation or sometimes provoke the usage of strong experimental conditions. Since the photo-degradation rate is slowed down, it is necessary to alter the sampling conditions in natural water systems such as the volume and depth of the samples to reduce the aerosol masking effect of suspended particles on the light penetrating into the water system. Hence the analog experimental conditions should be balanced in the photo-degradation simulation process based on the suspended particles and the equivalent amount of light required for degradation. In such cases two hypotheses are necessarily presumed assuming first the total amount of MC-LR in water system containing suspended particles (MC-LR adsorbed onto NOM and suspended particles) and secondarily the desorption of MC-LR both from NOM and suspended particles by degradation of required amount of light. But still, this is really a complicated one to understand the intrinsic rate of photo-degradation of MC-LR adsorbed onto NOM as well as suspended particles. As shown in Fig. 6, the

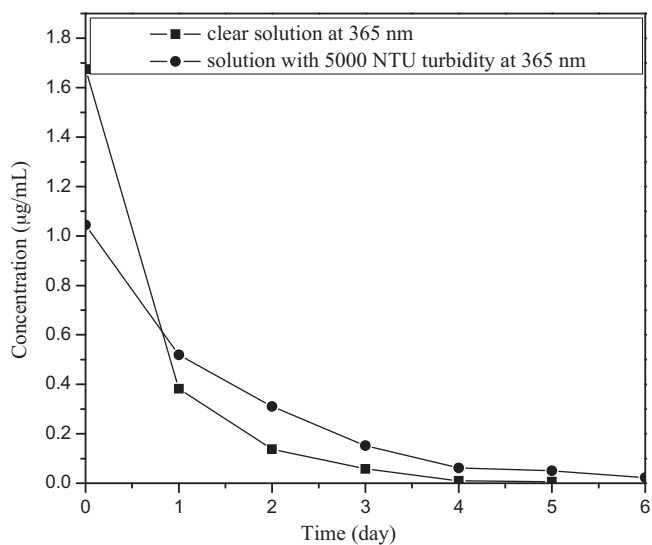
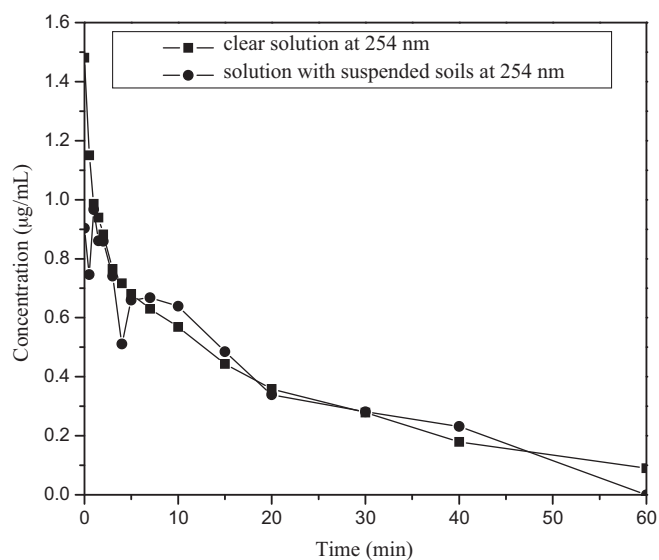


Fig. 6. Photo-degradation of MC-LR with and without suspended soils.

photo-degradation of water with suspended particles with two different wave lengths indicated that under irradiation with 254 nm UV light, the photo-degradation curve is not smooth and highly trodden up due to the disturbance created by the imbalance in the system. The pattern of photo-degradation curve in the case of 365 nm is smooth and not disturbed by the system but photo-degradation rate diminishes. This is again due to the reason that the higher energy of shorter wave length (254 nm) requires rapid degradation with shorter time where no sufficient time is available to reach the equilibrium due to the perturbation in the system which causes for rapid degradation with distracted light waves when compared with lower energy of longer wave length (365 nm) where the equilibrium reaches slowly with longer time interval and hence causes for smooth and slow prolonged degradation. The experimental studies clearly demonstrated that the low and high turbidity due to suspended particles at 254 nm has impact only on the pattern of the photo-degradation curves and not on the degradation time and hence their effect of turbidity on photo-degradation may be considered as insignificant, i.e., the photo-degradation rate of clear solution is similar to that of the system containing turbidity regardless the presence of suspended particles. However, suspended particle at high turbidity

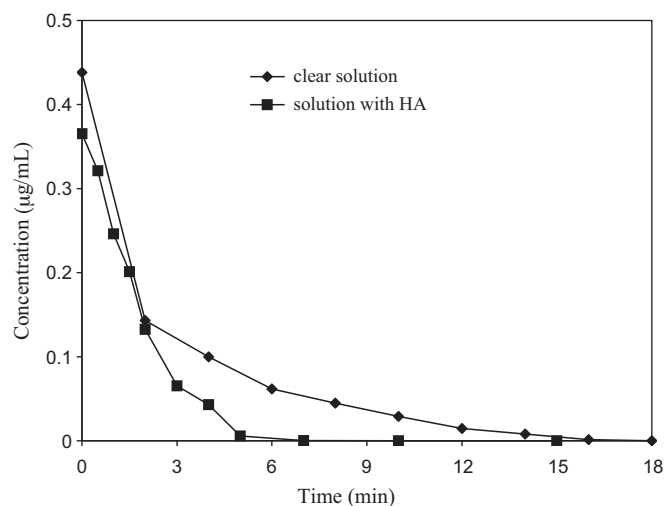


Fig. 7. Effect of HA on photo-degradation of MC-LR at 254 nm.

slows down the photo-degradation rate at 365 nm and prolonged the degradation of MC-LR from 5 days to 6 days as shown in Fig. 6.

While UV light near the absorption maximum of the MCs (238 nm) can lead to the degradation of MCs, solar irradiation in the absence of other substrates has a minimal effect on the fate and decomposition of MCs [15,29]. However, in the presence of pigments or humic substances (HS), photosensitized reactions appear to play an important role in the environmental transformation of MCs and may be applicable to specific water treatment [19]. Humic acid in water is light sensitive agent and hence it plays a major role in photo-degradation process. When water containing 40 mg/L humic acid is irradiated with UV light (254 nm), fast degradation of MC-LR occurs when compared with clear solution without humic acid as shown in Fig. 7. This is due the fact that humic acid acts as a photosensitizer producing radicals and also the organic matter in humic substances causes for higher distribution of MC-LR and the light wavelengths are not limited which accumulated more sunlight and hence the photo-degradation rate is accelerated. But in the natural environment, the system is limited to wavelengths of sunlight and the photo-degradation of MC-LR in the natural environment may take longer time, i.e., MCs decompose to a very limited extent under natural sunlight. It has been already reported that the photolysis of microcystins using sunlight resulted in relatively slow decomposition in pure solution, while the presence of algal pigments led to a photosensitization and the fast decomposition and isomerization of microcystin LR [17]. Thus, the presence of humic acid accelerates the photo-degradation of MC-LR in laboratory studies. As has been reported previously [17,30] pure photolysis can accomplish destruction of microcystins with decomposition rates dependent on the light intensity. Thus finally it could be concluded that photo-degradation rate is highly influenced by wavelength of light and humic acid and high turbidity in natural environment.

4. Conclusions

Laboratory studies demonstrated that MC-LR can be adsorbed onto NOM and suspended particles of water samples collected from Taiwan reservoirs and rivers as a route of detoxification in the natural water system. The results of batch experiments confirmed that the binding capacities of MC-LR had a correlation with turbidity, humic acid, organic matter content and pollutants of samples collected from different sites of sampling areas. The photo-degradation studies under two different irradiation of UV light emphasized that effective degradation was achieved quickly

by faster degradation within 60 min under irradiation with higher energy low wavelength (254 nm) of UV light. The turbidity of the system has effect only on the pattern of photo-degradation curves and not on rate of photo-degradation at 254 nm where as high turbidity decreases the rate of photo-degradation at 365 nm. The presence of humic acid acts as a photosensitizer and accelerated the rate of photo-degradation. The finding of this study suggested the elimination or significant reductions in toxic concentrations may be achieved through natural processes that might be associated with soil/water saturated system which requires an inexpensive and low maintenance method.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2012.03.031.

References

- [1] G. Liu, Y. Qian, S. Dai, N. Feng, Adsorption of microcystin LR and LW on suspended particulate matter (SPM) at different pH, *Water Air Soil Pollut.* 192 (2008) 67–76.
- [2] P. Babica, L. Bláha, B. Maršálek, Removal of microcystins by phototrophic biofilms – a microcosm study, *Environ. Sci. Pollut. Res.* 12 (6) (2005) 369–374.
- [3] P. Hyenstrand, T. Rohrlack, K.A. Beattie, J.S. Metcalf, G.A. Codd, K. Christoffersen, Laboratory studies of dissolved radiolabelled microcystin-LR in lake water, *Water Res.* 37 (2005) 3299–3306.
- [4] W. Chen, L. Li, N. Gan, L. Song, Optimization of an effective extraction procedure for the analysis of microcystins in soils and lake sediments, *Environ. Pollut.* 143 (2006) 214–246.
- [5] W.-J. Huang, B.-L. Cheng, Y.-L. Cheng, Adsorption of microcystin-LR by three types of activated carbon, *J. Hazard. Mater.* 141 (2007) 115–122.
- [6] S. Pérez, D.S. Aga, Recent advances in the sample preparation, liquid chromatography tandem mass spectrometric analysis and environmental fate of microcystins in water, *Trends Anal. Chem.* 24 (7) (2005) 658–670.
- [7] P. Babica, J. Kohoutek, L. Blaha, O. Adamovsky, B. Marsalek, Evaluation of extraction approaches linked to ELISA and HPLC for analyses of microcystin-LR, -RR and -YR in freshwater sediments with different organic material contents, *Anal. Bioanal. Chem.* 385 (2006) 1545–1551.
- [8] H. Ishii, M. Nishijima, T. Abe, Characterization of degradation process of cyanobacterial hepatotoxins by a gram-negative aerobic bacterium, *Water Res.* 38 (2004) 2667–2676.
- [9] I.T. Cousins, D.J. Bealing, H.A. James, A. Sutton, Biodegradation of microcystin-LR by indigenous mixed bacterial populations, *Water Res.* 30 (1996) 481–485.
- [10] G.J. Jones, P.T. Orr, Release and degradation of microcystin following algicidal treatment of a *Microcystis aeruginosa* bloom in a recreational lake as determined by HPLC and protein phosphatase inhibition assay, *Water Res.* 28 (1994) 871–876.
- [11] T.K. Mihali, R. Kellmann, J. Muenchhoff, K.D. Barrow, B.A. Neilan, Characterization of the gene cluster responsible for cylindrospermopsin biosynthesis, *Appl. Environ. Microbiol.* 74 (2008) 716–722.
- [12] C. Edwards, D. Graham, N. Fowler, L.A. Lawton, Biodegradation of microcystin and nodularin in freshwaters, *Chemosphere* 73 (2008) 1315–1321.
- [13] K. Lahti, J. Rapala, M. Färdig, M. Niemelä, K. Sivonen, Persistence of cyanobacterial hepatotoxin microcystin-LR in particulate material and dissolved in lake water, *Water Res.* 31 (1997) 1005–1012.
- [14] M.G. Antoniou, P.A. Nicolaou, J.A. Shoemaker, A.A. de la Cruz, D.D. Dionysiou, Impact of the morphological properties of thin TiO₂ photocatalytic films on the detoxification of water contaminated with the cyanotoxin, microcystin-LR, *Appl. Catal. B: Environ.* 91 (2009) 165–173.
- [15] B.C. Hitzfeld, S.J. Hoeger, D.R. Dietrich, Cyanobacterial toxins: removal during drinking water treatment and human risk assessment, *Environ. Health Perspect.* 108 (2000) 113–122.
- [16] K. Hayakawa, Y. Sugiyama, Spatial and seasonal variations in attenuation of solar ultraviolet radiation in lake Biwa, Japan, *J. Photochem. Photobiol. B* 97 (2008) 121–133.
- [17] K. Tsuji, S. Naito, F. Kondo, N. Ishikawa, M.F. Watanabe, M. Suzuki, K.-I. Harada, Stability of microcystins from cyanobacteria: effect of light on decomposition and isomerization, *Environ. Sci. Technol.* 28 (1994) 173–177.
- [18] P.K. Robertson, L.A. Lawton, B.J.P.A. Cornish, The involvement of phycocyanin pigment in the photodecomposition of the cyanobacterial toxin, microcystin-LR, *J. Porphyr. Phthalocyan.* 3 (1999) 544–551.
- [19] M. Welker, C. Steinberg, Rates of humic substance photosensitized degradation of microcystin-LR in natural waters, *Environ. Sci. Technol.* 34 (2000) 3415–3419.
- [20] L. Wörmer, M.H. Fontela, S. Cires, D. Carrasco, A. Quesada, Natural photodegradation of the cyanobacterial toxins microcystin and cylindrospermopsin, *Environ. Sci. Technol.* 44 (2010) 3002–3007.
- [21] H. Choi, M.G. Antoniou, M. Pelaez, A.A. de la Cruz, J.A. Shoemaker, D.D. Dionysiou, Mesoporous nitrogen-doped TiO₂ for the photocatalytic destruction of the cyanobacterial toxin microcystin-LR under visible light irradiation, *Environ. Sci. Technol.* 41 (2007) 7530–7535.
- [22] M.G. Antoniou, J.A. Shoemaker, A.A. de la Cruz, D.D. Dionysiou, LC/MS/MS structure elucidation of reaction intermediates formed during the TiO₂ photocatalysis of microcystin-LR, *Toxicol.* 51 (2008) 1103–1118.
- [23] M.G. Antoniou, J.A. Shoemaker, A.A. de la Cruz, D.D. Dionysiou, Unveiling new degradation intermediates/pathways from the photocatalytic degradation of microcystin-LR, *Environ. Sci. Technol.* 42 (2008) 8877–8883.
- [24] W. Chen, L. Song, L. Peng, N. Wan, X. Zhang, N. Gan, Reduction in microcystin concentrations in large and shallow lakes: water and sediment-interface contributions, *Water Res.* 42 (2008) 763–773.
- [25] T.P.J. Kull, O.T. Sjövall, M.K. Tammenkoski, P.H. Backlund, J.A.O. Meriluoto, Oxidation of the cyanobacterial hepatotoxin microcystin-LR by chlorine dioxide: influence of natural organic matter, *Environ. Sci. Technol.* 40 (2006) 1504–1510.
- [26] J.L. Zhou, S.J. Rowland, Evaluation of the interactions between hydrophobic organic pollutants and suspended particles in estuarine waters, *Water Res.* 31 (1997) 1708–1718.
- [27] J.L. Zhou, S. Rowland, R.F.C. Mantoura, J. Braven, The formation of humic coatings on mineral particles under simulated estuarine conditions—a mechanistic study, *Water Res.* 28 (1994) 571–579.
- [28] L. Li, W.L. Huang, P.G. Peng, G.Y. Sheng, J.M. Fu, Chemical and molecular heterogeneity of humic acids repetitively extracted from a peat, *Soil Sci. Am. J.* 67 (2003) 740–746.
- [29] W. Song, S. Bardowell, K.E. O'shea, Mechanistic study and the influence of oxygen on the photosensitized transformations of microcystins (cyanotoxins), *Environ. Sci. Technol.* 41 (2007) 5336–5341.
- [30] K. Tsuji, T. Watanuki, F. Kondo, M.F. Watanabe, S. Suzuki, H. Nakazawa, M. Suzuki, H. Uchida, K.-I. Harada, Stability of microcystins from cyanobacteria-II; effect of UV light on decomposition and isomerization, *Toxicol.* 33 (1995) 1619–1631.