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Simultaneous elimination of cyanotoxins and PCBs *via* mechanical collection of cyanobacterial blooms: An application of "green-bioadsorption concept"

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ABSTRACT

In this study, the distribution, transfer and fate of both polychlorinated biphenyls (PCBs) and cyanotoxins *via* phytoplankton routes were systematically investigated in two Chinese lakes. Results indicated that PCB adsorption/bioaccumulation dynamics has significantly positive correlations with the biomass of green alga and diatoms. Total lipid content of phytoplankton is the major factor that influences PCB adsorption/bioaccumulation. Cyanobacterial blooms with relatively lower lipid content could also absorb high amount of PCBs due to their high cell density in the water columns, and this process was proposed as major route for the transfer of PCBs in Chinese eutrophic freshwater. According to these findings, a novel route on fates of PCBs *via* phytoplankton and a green bioadsorption concept were proposed and confirmed. In the practice of mechanical collections of bloom biomass from Lake Taihu, cyanotoxin/cyanobacteria and PCBs were found to be removed simultaneously very efficiently followed this theory.

Introduction

The occurrence of toxic cyanobacterial blooms in eutrophic freshwaters has become a worldwide problem (Carmichael 1992; Song et al., 2007; Chen et al., 2009a). Some of the cyanobacterial genera such as *Microcystis*, *Dolichospermum*, *Nostoc*, and *Aphanizomenon* can produce a wide range of potent toxins, including a family of hepatotoxins called microcystins (MCs) that are the most frequently encountered cyanotoxins in freshwater (Chorus and Bartram, 1999; Akcaalan et al., 2006). Exposure to these hepatotoxic compounds can lead to liver failure in wild animals, livestock and aquatic life (Carmichael 1992; Smith et al., 2008), as well as cause human illnesses and death (Azevedo et al., 2002). Some reports suggest that cases of human primary liver cancer in the eastern region of China are related to the presence of MCs found in the drinking water (Yu, 1989). Due to rapid economical development and the intensive use of water resources, many large and shallow freshwater lakes, including Lake Taihu and Lake Chaohu, are becoming more seriously polluted (Song et al., 2007). In late May, 2007, a serious drinking water crisis caused by a cyanobacterial bloom in Lake Taihu took place in the city of Wuxi (Yang et al., 2008), which left approximately two million people without drinking water for at least a week due to harmful off-flavor metabolites released by the bloom (Yang et al., 2008). To avoid/reduce the risks associated with the occurrences of toxic cyanobacterial blooms and release of intracellular toxins, many strategies, including the use of chemical algaecides, physical adsorption and removal, biological control, as

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well as mechanical collection of biomass were implemented in these lakes. Among the strategies, mechanical collection of cynobacterial bloom biomass proved to be the most effective and was widely used as the emergency response in many Chinese freshwater lakes during bloom seasons. After the "2007 Wuxi drinking water crisis", over 1000 tons of fresh cyanoacterial blooms was harvested daily (May to August) from Meiliang Bay and Gonghu Bay, Lake Taihu by Wuxi Water Authorities in the past several years (Chen et al., 2012).

Lake Taihu (119°54′–120°36′ N, 30°56′–31°33′ E), the third largest freshwater lake in China, is located in the highly developed and densely populated Yangtze Delta. Its water depth ranges from 1 to 2.5 m (average 1.89 m) with a total water surface area of about 2338 km^2, and a mean water volume of approximately 4.43×10^{12} L. The lake serves as an important resource for drinking water, irrigation, aquaculture and industrial waters, in addition to being a popular recreational and tourist attraction. Lake Chaohu, the fourth largest freshwater lake in China, is located between 30°25′–31°43′ N and 117°16′–117°51′ E. It has a water area of 780 km^2 with an average depth of approximately 5.0 m. Two big cities in Anhui Province, named Hefei and Chaohu, are located on the northwest and eastern shore of the lake, respectively. Due to rapid economical development and the intensive use of water resources, the water qualities are becoming increasingly more deteriorated (Song et al., 2007). Detailed characteristics and limnological variables of the studied lakes are listed in Table 1.

The mechanically collected blooms usually contained over 90% of moisture, therefore the preparation of dry biomass and utilization of the toxic blooms tends to be energy-intensive and expensive. In addition to the lack of batch dewatering techniques, to treat huge amounts of harvested blooms daily, some of the collected blooms were discharged directly into croplands, irrigation canals, and forest land near the lakeshore as plant fertilizer, without any treatment. Some other harvested blooms were deposited in artificial ponds near the lakeshores in order to receive further treatment with new soil-based technologies. In China, the soil-based technologies for the treatment of mechanically collected blooms have experienced two generations of technology in the past 10 years. For the 1st generation technology, the blooms were discharged into an artificial pond directly without any further treatment. After the bloom layer dropped due to evaporation of the pond water over time or it leaching into shallow groundwater, fresh blooms are then discharged into the pond again. With the increase in the amount of the collected blooms in recent years, the 1st generation technology could not dispose of the huge amounts of biomass collected daily. To enhance treatment capacity the 2nd generation technology was designed. With the 2nd generation technology, known as the, "three-level bloom treatment system", cyanobacterial blooms were separated gradually with soil filtration and water containing toxins due to the blooms were used as plant fertilizer for crops or forest irrigation with help of pump stations. The filtered clean water would flow back into Lake Taihu, and in this way, the treatment of collected blooms could be conducted continuously (Chen et al., 2012).

Contamination caused by hydrophobic persistent organic pollutants (POPs, e.g., polychlorinated biphenyls (PCBs)) also caused another major issue due to the accelerating pace of industrialization and urbanization in the cities around these lakes (Xing et al., 2005; Wan et al., 2011). POPs are long-lasting in the environment and can migrate long distances from their source due to exhaust emissions, atmospheric fallout, sewage discharge and bioaccumulation (Berglund et al., 2001; Fitzgerald and Steuer, 2006; Weber et al., 2011). Since the restrictions against the use of PCBs in most developed countries, the major input of these toxic compounds into aquatic ecosystems is through atmospheric fallout and accumulated in the sediments after being adsorbed on suspended particles in the water column (Weber et al., 2011). However, in China, the introduction of PCBs and other POPs into the aquatic ecosystem through industrial wastewater is still a major route (Wan et al., 2011). In aquatic environments, dominating factor influencing the speciation and transport of POPs is phytoplankton (Fitzgerald and Steuer, 2006; Gerofke et al., 2005). PCB accumulations in phytoplankton were reported to be significantly affected by lake nutritional status (Berglund et al., 2001; Gerofke et al., 2005) and other impact factors were also described in several publications (Fitzgerald and Steuer, 2006; Gerofke et al., 2005). Yet few studies experimentally address PCB accumulations in relation to the phytoplankton biomass and species, especially in presence of high concentration of cyanobacterial biomass. In addition, more intensive investigations regarding the annual variation and dynamics of PCB concentrations in phytoplankton are still to be understood in order to manage the potential environmental/health risks associated with PCB bioaccumulations and transport *via* the phytoplankton route in aquatic ecosystems. As many POPs including PCBs are hydrophobic contaminants, they may be more readily absorbed into cyanobacterial cells due to the hydrophobic membrane lipids around the cells. Thus, the understanding of possibilities associated with simultaneous eliminations of the intracellular toxins contained in the huge amount of bloom biomass and adsorbed POPs will be very significant to the management and risk evaluation in these eutrophicated lakes.

The aim of the present study was to understand the fate of PCB *via* phytoplankton, especially *via* cyanobacterial routes, and the mechanisms that underline PCB adsorption/bioaccumulation in highly eutrophicated lakes. Moreover, the

Table 1 – Characteristics and limnological variables of the studied lakes.

Lake	Area (km^2)	Depth (m)	Tot-P (μg/L)	Tot-N (μg/L)	Chl a (μg/L)	Biomass (mg/L)	Ext-Mcs (μg/L)
Taihu	2427.8	3.34	0.10–0.26	1.6–3.6	14.1–212.0	0.1–374.2	0.5–2.5
Chaohu	760	5	0.14–0.23	2.2–6.9	17.5–186.2	1.0–102.1	0.3–2.4

Tot-P: total phosphorus; Tot-N: total nitrogen; Ext-Mcs: extracellular microcystins.

present study was designed to understand the possibilities of simultaneous removal of PCBs and cyanotoxins, and further evaluate the removal efficiency and annual removal amount in the course of mechanical collections of cyanobacterial bloom biomass in Lake Taihu and Chaohu.

1. Material and methods

1.1. Standards and reagents

MC standards (Microcystin-RR (MC-RR), Microcystin-YR (MC-YR), Microcystin-LR (MC-LR)) were obtained from Pure Chemical Industries (Osaka, Japan), and ODS Sep-pak cartridges were manufactured by Waters (Milford, MA, USA, Part No. Wat. 051910). PCB standards (PCB 28, 52, 101, 118, 138, 153, 180) and Amberlite® XAD absorbent for PCB sample preparations were purchased from Sigma. Methanol (high performance liquid chromatography (HPLC) grade) used as the HPLC mobile phase and for extractions was purchased from Fisher (Loughborough, UK). Other HPLC grade solvents such as acetone, hexane, toluene, benzene for PCB extractions and sample preparations were from Merck or Fisher. All other chemicals used in the study were of analytical grade and were purchased from standard sources.

1.2. Sampling and phytoplankton species analysis

Bloom and water samples were collected monthly from three sites in each of the two lakes. Bloom samples were collected from the surface of the lake water column using a phytoplankton net (40 μm mesh), and stored in 250 mL polyethylene bottles. In the laboratory, the cyanobacterial bloom samples were allowed to settle overnight in a refrigerator at 4°C to separate the zooplankton according to method described by Utermöhl (1958). Sampling for phytoplankton species was conducted each month from August 2009 to July 2010 at each site at the investigated lakes, at zero (surface) to 0.5-m depth, using a vertical sampler. Fixed phytoplankton was identified and enumerated by light microscope, according to commonly used monographs on phytoplankton (Song et al., 2007). For PCB analysis, 5 L of lake water was sampled from zero (surface) to 0.5-m depth and filled into a glass container. The detailed GPS information for the sampling sites were listed as the follows: Taihu N1(31° 30′ 49″ N, 120° 11′ 35″ E), Taihu W2 (31° 23′ 51″ N, 120° 01′ 55″ E), Taihu S2 (31° 02′ 16″ N, 120° 03′ 35″ E), Chaohu W1 (31° 41.790′ N, 120° 31.747′ E).

1.3. Sample preparation for toxin, PCB and total lipid analysis

For toxin analysis, bloom samples were lyophilized and extracted in a solution of 5% (V/V) acetic acid and 80% (V/V) aqueous methanol. After centrifugation and evaporation of the methanol, the extracted solutions were cleaned using Sep-pak cartridges, and the eluted solutions were stored at −20°C for HPLC analysis (Barco et al., 2005). Duplicate lake water samples were also collected at each site from the top of the water column (0–0.5 m; surface water). The surface water samples were centrifuged (3000 × *g*, 5 min) and filtered through Whatman™ GF/C filter papers prior to storage at −20°C for the subsequent enzyme-linked immunosorbent assay (ELISA) and HPLC analysis. For PCB analysis, the sampled water and phytoplankton were passed through a solid phase extraction (SPE) column filled with 1 g XAD absorbent then dried with freeze drier. The PCBs absorbed on the SPE were further Soxhlet extracted with acetone/hexane according to the method described by Berglund et al. (2001). After a Soxhlet extraction, solvents were evaporated using a vacuum evaporator and the residuals were re-dissolved in hexane, followed by a cleanup procedure using column chromatography described by Wu et al. (1998). A mixed column was filled wet with *n*-hexane from bottom to top with 4 g silica, 10 g silica (44% conc. sulphuric acid w/w), and 2 g silica. The silica type was active and its particle size was 100–200 μm. The column was prewashed with 100 mL *n*-hexane. The elution was done with 245 mL *n*-hexane and the eluent was evaporated to a final volume of 2–3 mL for further cleanup using a second chromatography column. In the second column, 25 g alumina, basic, super active, particle size 50–200 μm, covered with 20 g sodium sulfate (anhydrous) was filled wet with *n*-hexane and prewashed with 100 mL *n*-hexane, followed by 40 mL benzene. The sample was eluted with 80 mL benzene, 200 mL *n*-hexanc/dichloromethane (98/2, V/V) and the first fraction containing PCBs were further evaporated then re-dissolved in isooctane for further gas chromatography/mass spectrum (GC/MS) quantification. Total lipid analysis was conducted using the gravimetric method.

1.4. MC determinations

For bloom samples, which contained high concentrations of MCs, the HPLC was employed to quantify the intracellular toxins. All samples containing low concentrations of MCs, such as water samples for dissolved toxins and samples for the investigation of bioaccumulation, were determined by ELISA.

HPLC analyses were performed using a Shimadzu LC-10A system with two LC-10A pumps and a ultraviolet (UV) detector. Elution conditions using a Shimadzu shim-pack (CLO-ODS6.0 150) column were 60% solution A (100% methanol) and 40% solution B (0.05 mol/L KH_2PO_4, pH 3) over 20 min at a flow rate of 1 mL/min. Column temperature was maintained at 40°C and the injection volume was 10 μL (Song et al., 2007).

The sensitivity of this method is 0.1 ng/mL. Microtiter plates were coated with monoclonalantibodies (MAB, 4.0 mg/mL) and incubated overnight at 4°C and then blocked with blocking buffer 170 mL (0.5% (W/V) gelatin in phosphate-buffered solution (PBS). They were then incubated for either 2 hr in a microplate incubator at 37°C or overnight at 4°C in 70 μL aliquots of various concentrations of MC-LR. Samples were then preincubated at 37°C for 30 min and an equal volume of biotinylated MC MAB (25 ng/mL) was added to the coated wells for 30 min. The plates were washed thoroughly, three times, with phosphate buffer saline (PBS-T) using an Immuno wash apparatus. HRP-streptavidin (Sigma) diluted 10,000 in dilution buffer (PBS containing 0.5% (W/V) gelatin and 0.05% (V/V) Tween 20) was added and incubated for 30 min at 37°C. The enzyme reaction was initiated by adding a substrate

solution (0.1 mol/L sodium acetate buffer (pH 5.0) containing 100 mg/mL of 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMBZ) and 0.005% (V/V) H_2O_2) and stopped with 1 mol/L H_2SO_4. Absorbance at 450 nm was measured with a microtiter plate reader (Song et al., 2007).

1.5. GC/MS analysis

A capillary GC/MS system equipped with DB-5 MS (60 m in length, 0.25 mm ID, 0.25 μm film) and Electron Ionization-Selected ion monitoring (EI-SIM) mode was used for identification and quantification. The temperature program was: 120°C (3 min) to 250°C (5 min) with rate of 3°C/min. The column head pressure was 31.8 psi and column flow rate was set at 1 mL/min. The injection port, interface and ion source was heated at 250, 230 and 230°C, respectively. The PCB components were identified and quantified according to the methods described Berglund et al. (2001).

1.6. Data analysis

Simple regression analyses to test the dependence of PCB concentrations and algal biomass and lipid content were performed with origin software. Mean comparisons were conducted by one-way analysis of variance (ANOVA), followed by Bonferroni tests to identify the sources of detected significance. In all cases, comparisons that showed a p value <0.05 were considered significant.

2. Results and discussion

2.1. Annual variations of algal biomass, intracellular MCs and accumulated/bioadsorbed PCBs

Cyanobacteria, composed mainly of colonial *Microcystis*, were dominant in phytoplankton samples collected from Lake Taihu and Lake Chaohu during the bloom period (May–August). For some months during cold seasons, green algae and diatom species could also be dominant. The maximum algal biomass collected occurred in July or August in Lake Taihu and Chaohu, with the maximum value of 5.9×10^8, 1.8×10^9, 7.9×10^7 and 1.7×10^9 cells/L for Taihu N_1, Taihu W_2, Taihu S_2 and Chaohu W_1, respectively. Annual variations of intracellular MCs in algae blooms from Lake Taihu and Lake Chaohu during 2009 and 2010 are shown in Fig. 1. In all four sampling sites, MC-RR and MC-LR are the major toxin species and, in most cases, the proportion of MC-RR was more than 80%. The highest MC concentrations occurred in August at 0.59 mg/g from Taihu N_1, 0.51 mg/g in October from Taihu W_2 and 0.62 mg/g in July from Taihu S_2. However, in Lake Chaohu, the highest concentration for cell-bound toxins occurred in June, with the maximum value of 0.43 mg/g from the sampling site Chaohu W_1. Compared with our previous studies in 2005 and 2007, cell-bound MCs changed dramatically in both their concentration and toxin species (Song et al., 2007; Liu et al., 2011). In 2005 and 2007, the maximum concentration of cell-bound MCs in bloom samples from all four sampling sites varied between 1.62 and 1.81 mg/g DW, which is around 3 times higher than samples taken from the same area during the same seasons. Toxin variants in the previous studies included MC-RR, MC-LR, MC-YR and Dha[7] MC-LR and MC-RR was the dominant variant from almost all bloom samples with the ratios of around 50% for most of the samples (Song et al., 2007; Liu et al., 2011). However, only MC-RR and MC-LR were recorded in our present study from the same Lake, and the ratios of MC-RR were over 80% for most of the samples analyzed. As documented in the literature, the MC concentration and the toxin variants in natural blooms may differ widely if the blooms were collected at the same sampling sites, but at different times or at the same time, but from different parts of the same lake (Song et al., 2007; Briand et al., 2009). Decreases in toxin concentration in cyanobacterial blooms were either due to the nutrient concentration or variations of toxic and non-toxic colonies (Chen et al., 2009a, 2009b, 2009c; Vezie et al., 2002). Even if the concentration of intracellular toxins decreased, there are still significant risks due to the amount of bloom biomass in the lakes, and off-flavor compounds caused by the anaerobic environments and the lysis of cyanobacterial cells, are still being released into the water (Guo, 2007). In addition, compared with the phytoplankton biomass and nutrient levels in 2005, the contamination caused by cyanobacterial blooms and excess nutrients is becoming more serious, not only in the northern part of the lakes (Taihu N_1), but throughout the whole lake, including the western part (Taihu W_2) and southern part (Taihu S_2) of the lake. An effective strategy must be implemented in these lakes, such as the mechanical collections of cyanobacterial blooms, to further avoid the potential risks associated with the release of intracellular toxins and other harmful compounds.

To understand the distribution and fate of PCBs in the water column, both the dissolved and particulate PCBs were investigated at three sampling sites at Lake Taihu and one sampling site at Lake Chaohu from August 2009 to July 2010. In both lakes, the concentration of dissolved PCBs varied from n.d. (under detection limit) to around 10 ng/L in all the three sampling sites. The concentration of particulate PCBs absorbed by the algal biomass varied dramatically from month to month and between sites to sites. The maximum concentration of 32.3 ng/L in Lake Taihu (Sampling site S_2) and 28.1 ng/L in Lake Chaohu (Sampling site W_1) was determined in April and March, respectively (Fig. 2). During the bloom season between May and September, the concentrations of PCBs in algae varied between 0.8 and 15 ng/L with an average concentration of 5.2 ng/L in the two lakes. For both dissolved and particulate PCBs determined from these two lakes, lower chlorinated PCBs were the major component and there was a very low concentration of higher chlorinated PCBs present in the water phase which was consistent with previous studies conducted on these lakes (Wan et al., 2011; Chen et al., 2009c).

For most of the samples from the lakes, the concentration of PCB28 and PCB52 constituted over 60% of the total PCBs throughout the year. Taking both the dissolved and particulate PCBs into consideration, 37.5% of the samples exceed the United States Environmental Protection Agency (US EPA) guideline of 14 ng/L total PCBs for surface water, while only 16.7% of the samples exceed the Chinese environmental quality standards of 20 ng/L total PCBs in surface water

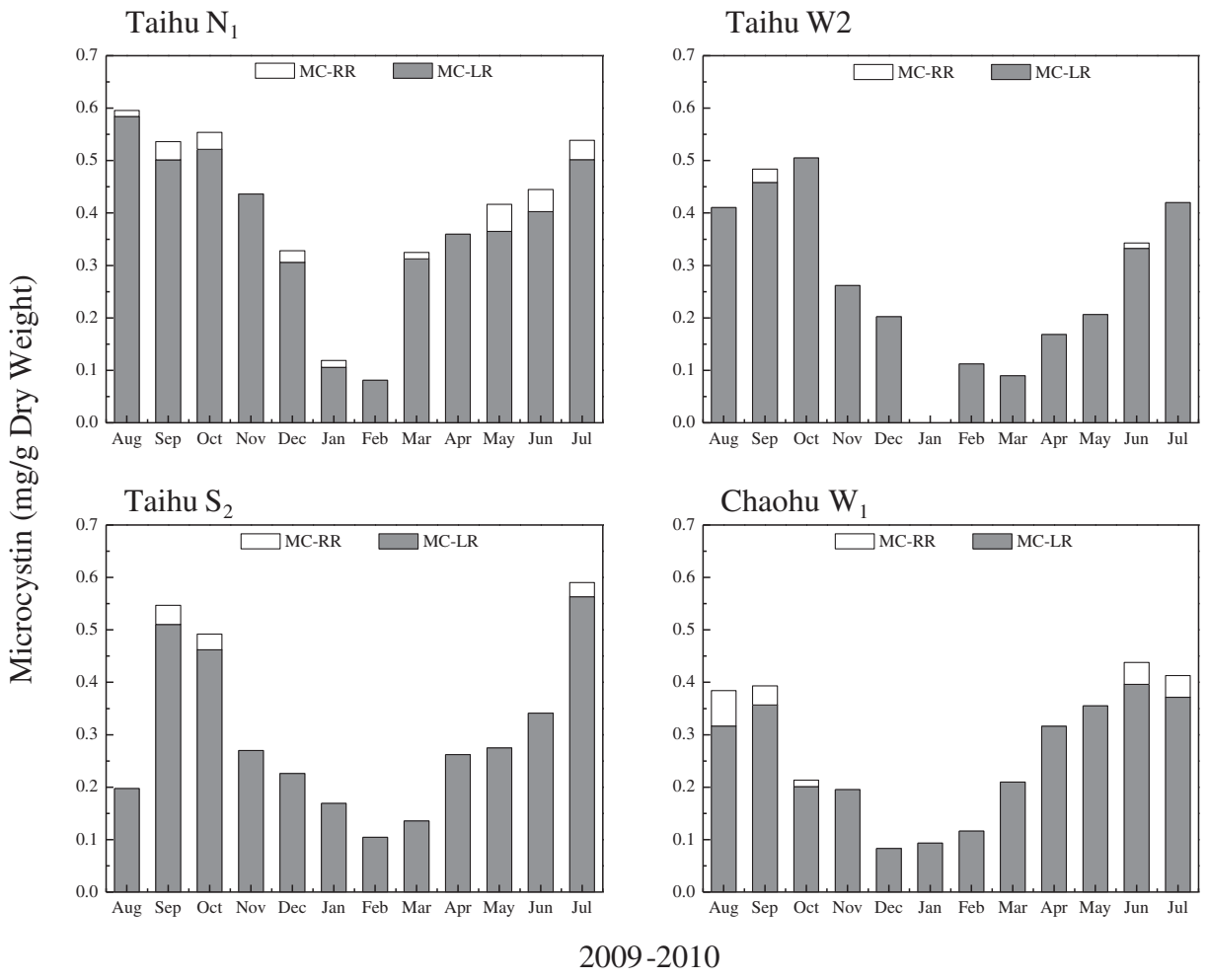

Fig. 1 – Concentrations of microcystins in the naturally occurring algal blooms in Lake Taihu and Lake Chaohu, from August 2009 to July 2010. Detection limit of high performance liquid chromatography method in the present study is 0.05 μg/mL. Data are expressed as the mean value of two independent experiments with duplicate determinations.

(US EPA 2002; GB 3838–2002). This indicates that there is a substantial health risk in these lakes due to the toxic, carcinogenic and mutagenic potential of PCBs and other contamination in the aquatic ecosystem (Song et al., 2007).

2.2. Routes of transfer and fate of PCBs via phytoplankton in eutrophic lakes

In past decades, a number of studies were conducted addressing the accumulation of PCBs and other POPs in phytoplankton in both marine and freshwaters (Berglund et al., 2001; Fitzgerald and Steuer, 2006; Fytianos et al., 1997; Halling-Sorensen et al., 2000; Gewurtz et al., 2009; Roessink et al., 2010). However, detailed routes and mechanisms underlining the bioaccumulation and eventual fate of PCBs *via* phytoplankton are not yet well understood. The present study was designed to understand these mechanisms by analyzing the relation between accumulated PCB content and total phytoplankton biomass, biomass of individual species of alga and total lipid content. Results from Fig. 3a clearly indicated that the PCB content in algal samples from both Lake Taihu and Lake Chaohu has no significant liner correlation with total algal biomass (R^2 = 0.02, P = 0.3). However, there was a quite significant positive correlation between the accumulated PCB content of the alga and the biomass of green alga and diatoms (R^2 = 0.27, P = 0.00018, Fig. 3b) or total lipid content in alga (R^2 = 0.54, P < 0.0001, Fig. 3c) in the investigated lakes. In several early studies, the bioaccumulation of POPs in phytoplankton was considered to be correlated with the trophic status of lakes and biomass intensity (Berglund et al., 2001; Taylor et al., 1991; Halling-Sorensen et al., 2000). The negative relationship between lake trophic status and bioaccumulated hydrophobic POPs was usually attributed to the "biomass dilution theory", in which, biodilution may occur under rapid growth conditions when the growth rate of phytoplankton exceeds the partition rate of POPs and thereby preventing equilibrium (Berglund et al., 2001; Taylor et al., 1991). Other studies, however, reported positive correlations between lake trophic status and bioaccmulated POPs (Gunnarsson et al., 2000). Subsequent studies also indicated lake trophic status could significantly influence the production of algal lipids and

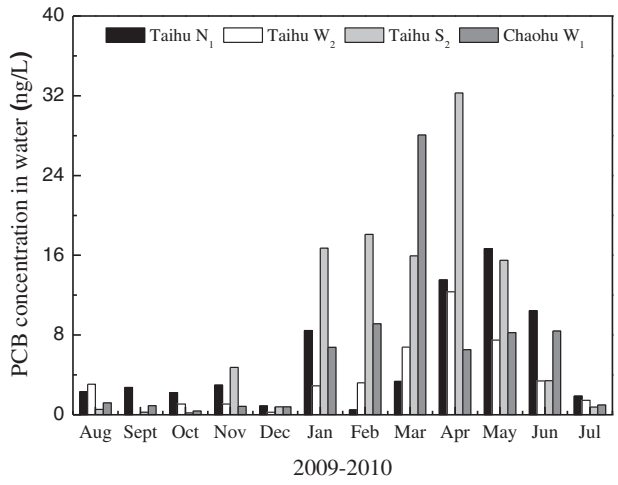

Fig. 2 – Total concentration of polychlorinated biphenyls (PCBs) absorbed/bioaccumulated in the naturally occurring algal blooms in Lake Taihu and Lake Chaohu, from August 2009 to July 2010. Detection limit of gas chromatography–mass spectrometer method in the present study is 0.1 ng/mL. Data are expressed as the mean value of two independent experiments with duplicate determinations.

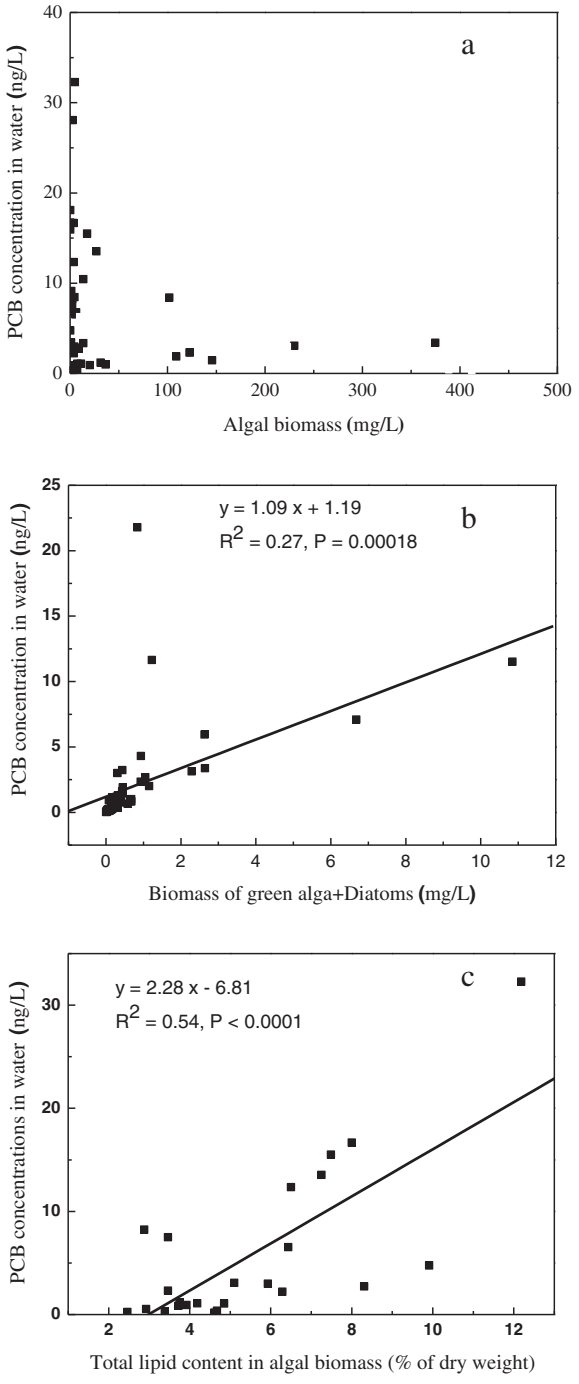

Fig. 3 – Liner regression analysis relating to the algal biomass (a), biomass of green alga + diatoms (b) and total algal lipids to the total PCB concentrations (c) in Lake Taihu and Lake Chaohu.

further influence the uptake of PCBs (Berglund et al., 2001; Halling-Sorensen et al., 2000).

In a laboratory study, bioconcentration factors for several hydrophobic organic compounds including PCBs increased up to nine times as the total algal lipid content of green algae *Selenastrum carpricornutum* increased from 17% to 44% of the total dry weight as a consequence of nitrogen starvation (Halling-Sorensen et al., 2000). However, in highly eutrophic lakes in China, nitrogen or phosphate starvation usually occurred during the summer bloom season. This is based on our monitoring results of nitrogen and phosphate concentrations especially in their dissolved state. It is difficult to explain the relative lower PCB accumulations in algal biomass during summer bloom season using this theory. Interestingly, a significant positive correlation was observed between PCB accumulation and biomass of green algae and diatoms throughout the year at all sampling sites investigated. Further analysis indicated that PCB accumulation could be explained by the equilibrium partitioning theory if the total lipid content and the biomass dilution concept were taken into consideration in Lake Taihu and Lake Chaohu (Fitzgerald and Steuer, 2006).

Physiochemical sorption of POPs in live phytoplankton in either freshwater or marine water was influenced by many factors such as the characteristics of sorbing phases (lipid content, surface area, etc.) and the hydrophobicity of the POPs themselves, usually expressed by the octanol–water partition coefficient (K_{ow}) (Fitzgerald and Steuer, 2006). The process of POPs sorption to alga could be divided into two steps: (1) passive uptake of POPs from the water phase onto the surface of algal cells and (2) bioaccumulation of POPs in algal cells through equilibrium partitioning between intracellular lipids and the membrane. Therefore, for the first step, the cell or colony size of phytoplankton will dramatically influence the uptake of POPs from the water phase onto the surface of algal cells. Single-cell green alga and diatoms have more surface area than the colonial cyanobacteria such as *Microcystis* (Chen et al., 2009a, 2009b, 2009c), providing more binding points for POPs on the cell surface. For the second step, the total lipid content is the key parameter which could

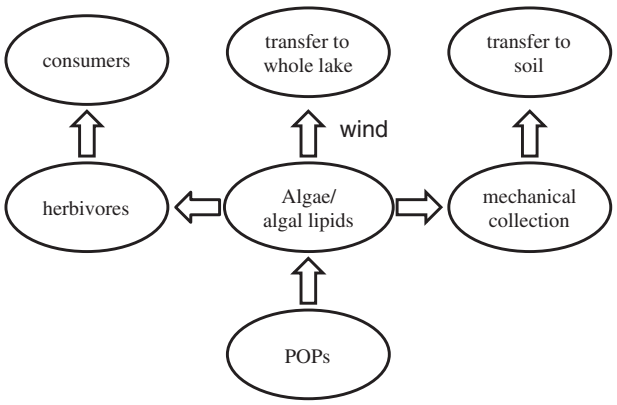

Fig. 4 – Conceptual routes of transfer and fates of PCBs *via* phytoplanktons in eutrophicated freshwaters.

influence the bioaccumulation of PCBs and other POPs in algal cells. Under certain conditions, diatoms and green alga could produce 30%–70% total lipid (dry weight), while cyanobacteria usually could only produce around 5% total lipid and produced mainly in the form of membrane lipids (Hu et al., 2008). The significant correlation between PCB content and biomass of green alga and diatoms or total lipid content could be explained based on the above theory. The lower content of PCBs in algal biomass during the summer was not only influenced by the total lipid content, but also by the lack of equilibrium partitioning to lipids, which is rarely reached in rapid proliferation of cyanobacterial blooms during warm seasons. Although PCBs tend to accumulate in phytoplankton with high-oil content, the bioadsorption/bioaccumulation of PCBs in huge amounts of cyanobacterial bloom biomass represents a significant amount of particulate PCBs in the freshwater column (Fig. 2).

In recent years, the fate of POPs in aquatic systems *via* phytoplankton and macrophytes has received increased interest, because of their bioaccumulation and biomagnification potential in food chains (Berglund et al., 2001; Gerofke et al., 2005; Roessink et al., 2010). PCBs can be highly absorbable and accumulate in phytoplankton in Chinese eutrophic freshwaters. There are mainly three possible routes for the transfer of PCBs and other POPs *via* phytoplankton (See also Fig. 4). Since high-oil algal biomass growing in these lakes during the spring and winter is a high-quality food source for zooplankton, PCBs may accumulate first in zooplankton and then further transfer to other aquatic organisms with higher trophic levels in the food chain (Berglund et al., 2001; Roessink et al., 2010). The second possible route for the transfer and fate of PCBs is due to the temporal and spatial distributions and migrations of cyanobacterial blooms. Based on the results from this study, PCBs and other POPs might be transferred and migrated from the highly contaminated area to other bays or to the whole lake through the migration of floating mat-like algal blooms under the help of wind waves. This process may accelerate the pollutant diffusion in some freshwaters with point source pollution characters. In many Chinese freshwater locations, mechanical collection of cynobacterial bloom biomass has been implemented as an effective emergency measure to control cyanobacterial blooms and to minimize hazards. The route is, when the non-point source pollution of PCBs in freshwater is due to atmospheric fallout, then the biomass could be concentrated and removed by mechanical collection. In this way, POPs contamination status could be gradually improved with current practices of lake management. Currently, very little information is available on the fate of POPs through the second and third routes. More intensive studies regarding these routes should be conducted, not only for the understanding of the fate of contaminants, but for more efficient management strategies regarding these persistent environmental contaminations.

2.3. Simultaneous removal of cyanobacterial toxins and PCBs by mechanical collection of bloom biomass from eutrophic freshwaters

Some POPs contaminants can persist over 50 years in an aquatic environment, and the half life of PCBs in the environment is over 20 years (Harner et al., 1995). Once an aquatic environment is contaminated by PCBs, it will be very difficult for the environment to recover. At present, the only effective method to treat PCB contamination in sediment is sediment dredging, which can be very expensive and can only be implemented at the point-source contaminated area (Renner, 2001). So far, there is no inexpensive way to remove PCBs from non-point source contaminated waters. According to the findings, related to the bioadsorption/bioaccumulation of PCBs *via* phytoplankton in water columns, mechanical collections of toxic cyanobacteria and then transferring it

Table 2 – Simultaneously elimination of microcystins and PCBs *via* mechanical collection of bloom biomass and treated with three-level system.

Parameter	Inlet cannel	Sedimentation ponds			Outlet cannel
		1st level	2nd level	3rd level	
Algal cell intensity ($\times 10^7$ cells/L)	1296.4	78124.8	988.5	322.5	2.2
∑PCBs	271.1 (ng/g) in dry algae	7.8 (ng/g) in sediment	5.6 (ng/g) in sediment	0.9 (ng/g) in sediment	n.d. in water
Cell-bound MCs (mg/g DW)	0.5	0.2	0.1	0.003	n.d.
Dissolved MCs (μg/L) in water	22.6	84.5	2.3	0.6	0.1
MCs (μg/g DW) in sediment	217.5	178.4	235.0	113.4	14.7

PCBs: polychlorinated biphenyls; n.d.: under detection limit; MCs: microcystins.

to soil for further treatment, possibly removes toxins and PCBs and other hydrophobic POPs simultaneously. To assess this possibility and further evaluate the fate and removal efficiency of both the toxins and PCBs in a three-level treatment system near Lake Taihu, algal cell intensities, PCB concentrations, cell-bound MCs, dissolved MCs as well as the MC concentration in the sediment were systematically investigated throughout the treatment processes. Results indicated from Table 2 clearly showed that the three-level system is very efficient in removing cyanobacterial cells, toxins and PCBs. After discharging the collected blooms into the soil-based treatment system, over 99% of cyanobacterial cells, toxins and PCBs were restrained by soil filtration and almost no toxins or PCBs could be detected in the outlet channel to Lake Taihu. In this way, PCBs and toxic cyanobacterial metabolites could be effectively removed from the lakes. Assuming the moisture content in mechanically collected biomass is 99% and daily harvesting amount is 1000 tons of fresh biomass from May to October, around 0.5 kg of PCBs and 900 kg of MCs, 144 tons of nitrogen and 9 tons of phosphate will be removed from Lake Taihu simultaneously. Furthermore, the PCB adsorption capabilities of sediments in three-level treatment system will be increased due to the increasing amount of organic carbons in the sediment caused by lysis and sedimentation of cyanobacterial blooms (Skoglund, and Swackhamer, 1999).

3. Conclusions

Results from the present study strengthen the evidence for the fate of POPs *via* the bioaccumulations and bioadsorptions in phytoplankton. Significant positive correlations between PCB content and total lipid content in phytoplankton indicates this adsorption mechanism could be explained by the partitioning theory. In addition, PCB adsorption by phytoplankton could also be influenced by phytoplankton species and high-oil content single-cell alga tended to have stronger absorption abilities and could capture more hydrophobic contaminates from the water column. Based on these findings, a novel conceptual route of transfer and fate of PCBs *via* phytoplankton was proposed for eutrophic freshwater. Mechanical collection of bloom biomass in eutrophic freshwaters could effectively eliminate PCBs, intracellular toxins simultaneously, with estimated annual eliminations of 0.5 kg of PCBs and 900 kg of MCs, 144 tons of nitrogen and 9 tons of phosphate in Lake Taihu. This study will lead to a better understanding of the role of phytoplankton in the migration and transfer of contamination, and the route and fate of POPs *via* phytoplankton in eutrophic freshwater lakes. These findings and proposed concepts in this study will provide also new information and knowledge in managing POPs contaminations in highly eutrophic freshwater Lakes in China.

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