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Dissolved microcystins in surface and ground waters in regions with high cancer incidence in the Huai River Basin of China

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HIGHLIGHTS

- The first study on microcystins pollution in groundwater in Huai River Basin.
- Quantify linear relation of microcystins between rivers and groundwater.
- Providing the evidence that MCs in groundwater from the river replenishment.
- Microcystin-RR as the most dominant toxin in groundwater.

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ABSTRACT

Microcystins (MCs) are potent hepatotoxins and have also implicated in liver tumor promotion. The present study investigates the temporal and spatial variations of MCs in different water bodies in the Huai River Basin in China. Water samples including rivers, ponds and wells were collected every quarter during December 2008 and December 2009. MCs were determined by high pressure liquid chromatography after solid phase extraction. MCs concentrations in river samples were 0.741 ± 0.623 μg L^{-1} with maximum of 1.846 μg L^{-1}. MCs in pond were 0.597 ± 0.960 μg L^{-1} with maximum of 2.298 μg L^{-1}. MCs were also detected in 51.7% of the groundwater samples, MCs in groundwater were 0.060 ± 0.085 μg L^{-1} with maximum of 0.446 μg L^{-1}. MCs concentrations in groundwater did not differ significantly among different depths or towns (Wilcoxon test, $p > 0.05$). The average MCs in groundwater in each sampling period were 0.068 μg L^{-1}, 0.118 μg L^{-1}, 0.052 μg L^{-1}, 0.059 μg L^{-1} and 0.020 μg L^{-1}. Through multi linear regression, the best fit model was built on MCs in groundwater with River B ($R^2 = 0.13$, $p < 0.05$), rather than with pond water. The results suggested that MCs contamination in groundwater originated from rivers, causing potential health risk on population who drink groundwater directly.

1. Introduction

Major rivers and freshwater lakes in China suffer from eutrophication due to industrial and agricultural wastewater emissions dramatic increasing. Cyanobacterial outbreaks caused by eutrophication have occurred in Tai Lake (Jin, 2003; Shen et al., 2003), Dianchi Lake (Zhang et al., 2003) and the Three Gorges reservoir (Li et al., 2011). These outbreaks are of national and public concerns because they adversely affect not only the water quality, but also the health of people (Li et al., 2011).

Many cyanobacteria (organisms of the phylum Cyanophyta) are known to produce cyanotoxins. These toxins are widespread exis-

Abbreviations: Chl-a, chlorophyll-a; COD, chemical oxygen demand; DO, dissolved oxygen; HPLC-DAD, high pressure liquid chromatography-diode array detection; MCE, microcystic cyanobacteria extract; MCs, microcystins; MC-LR, microcystin-LR; MC-RR, microcystin-RR; MC-YR, microcystin-YR; min, minutes; SD, secchi depth; US EPA, the United State Environmental Protection Agency; TFA, trifluoroacetic acid; TN, total nitrogen; TP, total phosphorus; TSI, trophic state indices; std, standard deviation; SMRs, standard mortality rates.

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tence in water bodies around the world and aquatic organisms (Gupta et al., 2003; Zhang et al., 2009) and led to potential effects on human (Falconer and Humpage, 2005) and wild animals (Nasri et al., 2008). Microcystins (MCs) are synthesized by a number of cyanobacterial genera, the most notable of which is the globally-distributed *Microcystis*. Over 80 structural variants have been identified from field samples or isolated strains of cyanobacteria (Kruger et al., 2009). The most commonly found MCs include microcystin-LR (MC-LR), microcystin-RR (MC-RR) and microcystin-YR (MC-YR). MCs are very potent cyanotoxins and tumor promoters (Nishiwaki-Matsushima et al., 1991). Water borne MCs led to the death of 76 patients in Brazil (Jochimsen et al., 1998). Epidemiological investigations suggest that MCs may be responsible for the high incidence of liver cancer in populations dependent upon MC-contaminated drinking water in China (Ueno et al., 1996), Serbia (Svircev et al., 2009), and Florida of the United States (Fleming et al., 2002), as well as colorectal cancer in China (Zhou et al., 2002). In addition to their widespread occurrence in eutrophic lakes and rivers, MCs may also contaminate groundwater even though no living cyanobacterial cells can be identified (Mohamed and Al Shehri, 2009), indicating that MCs can leach from other water bodies through the soil. Therefore, there is a potential risk of MCs exposure for residents who use a groundwater source for their drinking water especially in areas where the surface water is contaminated by cyanobacteria.

The Huai River is the sixth longest river in China and runs through the main economic areas in the middle-eastern of China. The Shaying River, one of the largest tributaries of Huai River, is suffered eutrophication and serious organic contamination (Gao et al., 2010). Some villages nearby the Shaying River have rates of liver, esophagus and gastric cancers of over 100 per 100,000 population (Wang et al., 2009). The annual average standard mortality rate of cancer in this area is 277.8/100,000, which is three to four times higher than those in the control areas (Wang et al., 2009). Residents living in this area directly use untreated groundwater as drinking water. Many ditches and ponds in the villages are contaminated by chemical fertilizers and animal and human feces, providing the proper conditions for cyanobacterial growth and the possibility for contamination by MCs in both surface and ground waters. However, little investigation is concerned MCs in surface and ground water in there.

In China, most MCs contamination data in drinking water come from urban water supply systems. Quite a few MCs monitoring data are available in rural drinking water, especially in groundwater where more than 75% of the residents in rural China draw their drinking water. Therefore, this study is important to understand the profile of MCs in rural areas and can be used as a basis for investigating the risk posed by MCs in rural drinking water sources. Here, we investigated the situations of dissolved MCs in different water bodies including river, pond, and groundwater. A relationship on MCs concentrations between rivers and groundwater was analyzed to reveal the source of MCs pollution in this area. Our results demonstrated that MCs in groundwater originated from polluted surface water.

2. Materials and methods

2.1. Study site and sample collection

Our study site is located along the Shaying River belonging to the Huai River Basin in the Province of Henan, northern China. The 1.2 million residents live along the Shaying River. Six towns, nearby the main stream and tributaries of the Shaying River including ZY, XJ, SC, XZ, KD and ZDY were selected as study areas. The towns have significantly high cancer mortality and are main residential districts with high population density. In there, groundwater severs as drinking water. Total 40 water samples sites from rivers, ponds and wells were collected every quarter between December 2008 and December 2009, sampling sites as shown in Fig. 1. Three river sites designated as River A, River B and River C. River A and River B are the upstream and midstream of the main river. And River C is the tributary of the main river. Ponds scattered around the residential areas are open water storage places, which is not connected to the rivers, mainly formed by rainwater and domestic sewage (i.e. main sewage discharge sites). One representative pond was selected based on its large size, high resident population and persistence of the water body. Thirty-six wells covering shallow (<40 m) and deep wells (40–400 m) scattering in six towns were selected as sampling sites for groundwater. During the whole sampling period, the total number of groundwater samples for MC analysis was 180 and for river and pond samples was 20 (Table S1). The surface water was collected by descending glass bottles to the depth of 0.5 m and groundwater was collected into High Density Polyethylene water samplers (Nalogene, U.S.A., 5L, 1100) from the hand pumps. Before sampling, the water was allowed to run for 10 min to minimize contamination from water in the line. Water samples were kept at 4 °C less than 7 d before laboratory analysis.

2.2. Environmental temperature and physical–chemical analysis of water

The environmental temperature was gathered from historical database of local meteorological bureau. And the temperature changes were shown in Fig. 2. The secchi depth (SD) for surface water was determined with secchi disk.

All regular items for water quality analysis were referred to the methods of the United States Environmental Protection (US EPA) recommended. Total phosphorus (TP) (US EPA Method 365.4), total nitrogen (TN) (US EPA Method 351.4), chemical oxygen demand (COD) (US EPA Method 410.4), and chlorophyll-a (Chl-a) (US EPA Method 445.0) were determined according to the standard EPA methods (US EPA, 1983).

2.3. Phytoplankton analysis

Algal cell density was determined and algal cells were identified to the species level. One liter of water was fixed with 10 mL Lugol's iodine solution (Eaton and Moss, 1966). After sedimentation for 48 h, algal cells were counted and identified as detailed by Zhang et al. (2006). Briefly, a total of 50 mL of the sediment liquid was collected and a 100 μL aliquot was dropped onto an algal cell counting plate (Institute of Hydrobiology, Chinese Academy of Sciences). Algal cells were analyzed using an inverted microscope (Leica Inc.).

2.4. Analysis of MCs in water

MCs in water were analyzed via solid phase extraction followed by high pressure liquid chromatography-diode array detection (HPLC-DAD), as described in Lawton and Edwards (2001). To remove suspended solids, 5 L of water was passed through a GF/C membrane (Whatman, 1.2 μM). The filtrate was then passed through a cartridge, containing 5 g ODS (Octadecylsilyl Sunchrom) preconditioned with 50 mL of methanol and 50 mL of ultrapure water (Milli-Q, Millipore Inc.). The cartridge was then rinsed with 50 mL of ultrapure water, followed by 50 mL of 20% methanol. MCs absorbed on the cartridge were eluted with 50 mL of 80% methanol with 0.01% trifluoroacetic acid (TFA). The eluate was evaporated to approximately 5 mL at 56 °C and was suspended in 10 mL of 20% methanol. The suspension was passed through a sec-