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Application of plow-tillage as an innovative technique for eliminating overwintering cyanobacteria in eutrophic lake sediments☆

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ABSTRACT

Surface sediment in eutrophic lakes is both a destination and a habitat for overwintering cyanobacteria. The resuspension and recovery of viable, overwintering cyanobacteria from the surface sediment during warm spring weather is usually the primary stage of cyanobacterial blooms (CBs) in shallow eutrophic lakes. Therefore, the elimination of overwintering cyanobacteria in sediment is vital to control CBs. In the present study, sediment plow-tillage (PT) was introduced as an innovative technique for eliminating overwintering cyanobacteria in sediments from Lake Chaohu. Four depths of PT (2, 5, 10, and 15 cm) were tested during the 42-day experiment. The results showed that rapid cell death during the first 0–7 d after PT was accompanied by high oxygen uptake rates. The viable cells in deeper sediment died more quickly and at a higher rate after PT. A PT depth of >10 cm effectively eliminated viable cyanobacteria (with a removal rate of 82.8%) from the sediment and prevented their resuspension. The activity of the viable cyanobacteria also decreased quickly as cyanobacteria were eliminated. It appears that the dark, anoxic environment of the deeper sediment after PT was responsible for the elimination of viable cells. Although high release rates of nitrogen and phosphorus were found to accompany the dying and decomposition of cyanobacteria during days 0–7 of the experiment, greater depth of PT was found to decrease nutrient concentrations in the overlying water. In conclusion, we recommend sediment PT as a new technique for eliminating overwintering algae in sediments. However, the release of nutrients from the sediment and the *in situ* control of CBs in lakes after PT should be further studied.

1. Introduction

Cyanobacterial blooms (CBs) have attracted considerable attention from both academic and governmental organizations in recent years because of their various adverse effects on public health, tourism, fisheries, and ecosystems (Paerl et al., 2011a; Yang et al., 2008). Climate change and nutrient overload play important roles in freshwater eutrophication and CBs (Heisler et al., 2008; Sondergaard et al., 2001). In the last 30 years, global surface temperature has increased by about 0.2 °C per decade (Hansen et al., 2006), resulting in a major overall increase (0.55 °C) during that time. This increase influences iron, sulfur, and phosphorus cycling in aquatic ecosystems, intensifying water eutrophication and eventually promoting the formation of CBs (Bullock et al., 2013; Jiang et al., 2008; Robador et al., 2009). In developing countries like China, an overload of nutrients like nitrogen (N) and phosphorus (P) in waters and sediments is usually the primary cause of CBs in freshwater lakes. Numerous large, shallow lakes such as Lake Taihu and Lake Chaohu have suffered serious CBs from late spring to autumn since the 1990s (Duan et al., 2009; Jiang et al., 2014). The drinking water crisis that occurred in Wuxi City in 2007 actually originated from a severe CB in the drinking water source, Lake Taihu (Qin et al., 2010). Therefore, the elimination of CBs is now an urgent environmental concern for the government and people living near these waters.

Numerous methods of eliminating or suppressing CBs have been attempted or studied, including flocculation (Pierce et al., 2004), coagulation–flotation (Gao et al., 2010), oxidation (Chen and Yeh, 2005), and ultrasonic irradiation (Rajasekhar et al., 2012). However, all of these techniques can only be used after a CB outbreak, and inevitably cause massive algae biomass deposition on the surface

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sediment and increased pollution loading in the sediment (Chen et al., 2014). Moreover, considering their huge cost, most of these techniques are limited in practical application. Therefore, increasing attention has been given to physiological knowledge about cyanobacteria to prevent CBs from occurring in the first place. In the four-stage hypothesis of cyanobacterial bloom formation proposed by Kong and Gao (2005), overwintering and recruitment were identified as the bottleneck stages for algal development. Numerous other studies (Fallon and Brock, 1981; Julesz and Miezin, 1980) have also revealed that overwintering cyanobacteria in the surface sediment are the major source of CBs the following year. Therefore, elimination of overwintering cyanobacteria in the sediment may effectively eliminate the source of CBs.

Some chemical and biological algicides, such as H_2O_2 and rice straw, have been used to eliminate overwintering cyanobacteria (Jia et al., 2014). However, the application of algicides may have unknown environmental impacts on the aquatic ecosystem. In previous studies, plow-tillage (PT) was implemented for ecological reconstruction of soil and sediment (Gu et al., 2012). It provides a relatively cost-effective and non-invasive technique for sediment remediation by relocating polluted surface sediment to a greater depth (Ferland et al., 2014). In the present study, we introduced PT for the first time, to our knowledge, as a method of eliminating overwintering cyanobacteria in sediment. Different depths of PT were simulated in the laboratory to bury viable cyanobacteria in deeper layers of sediment. Our objective was to determine whether viable, overwintering cyanobacteria could be eliminated by PT and to provide a new *in situ* technique for eliminating CBs in hypereutrophic lakes in China.

2. Materials and methods

2.1. Study site and sampling

Both the sediment and cyanobacterial samples were obtained from a CB-prone area of Lake Chaohu (31°41′36.91″N, 117°.23′45.32″E). Lake Chaohu, which covers an area of 760 km^2 and has a mean depth of 3.06 m, is the fifth-largest shallow freshwater lake in China, and is one of the most severely eutrophic lakes in China. Serious algal blooms (mainly *Microcystis* and *Anabaena*) have occurred annually during late spring and autumn with increasing frequency and intensity in recent decades (Yang et al., 2006). Here, 144 sediment cores were collected using a gravity core sampler (Rigo Co. Ltd., Ø110 mm × L500 mm, Japan). The cyanobacterial sample was collected by sieving lake surface water through a 50-μm plankton net and then preserved at 4 °C. Both the sediment and cyanobacterial samples were transported to the laboratory within 4 h. The sediment columns were transferred to the laboratory without disturbance to ensure that the sediment-water interface was maintained as it was in the lake. All samples were treated within 24 h.

2.2. Experimental design

Initially, the cyanobacterial sample was diluted with lake water, which had been previously filtered through a medium-speed qualitative filter to remove algae and other particulate matter. The cyanobacteria were then incubated at 6 ± 0.2 °C (average water temperature of the lake during winter) in dark for 5 d of adaptation. Subsequently, the sinking cyanobacteria were collected by sieving with a 20-μm plankton net for use in the experiment.

Different depths of plow-tillage were implemented by mixing the filtered cyanobacteria with various thicknesses of surface sediments. Four depths of PT were used: 2 cm (PT2), 5 cm (PT5), 10 cm (PT10), and 15 cm (PT). Approximately 10 g of filtered cyanobacteria were homogenized with the plowed surface sediment of each treatment. The undisturbed sediment was set as the blank treatment (Blank). In addition, 10 g of filtered cyanobacteria were added to the undisturbed surface sediment, and this was set as the control treatment (CK). Subsequently, filtered lake water was gently injected to avoid disturbance of the interface. Thus, six treatments were implemented: Blank, CK, PT2, PT5, PT10, and PT15. Each treatment had 24 duplicated columns. All of the sediment columns were incubated at 6 ± 0.2 °C in black plastic barrels filled with filtered lake water in a dark environment for 42 d. In our preliminary experiments, we found that more than 99% of viable cyanobacteria could be eliminated with PT by day 42 (data not shown). Therefore, the experiment duration was set to 42 days. Sample collection and determination of cyanobacteria in the sediment were carried out on days 0 (beginning), 1.5, 4, 7, 11, 17, 28, and 42 of the experiment. At each sampling period, three triplicate columns for each treatment were used for testing.

2.3. Chemical analyses

To obtain cyanobacterial colonies from the sediment, a certain volume (about 5–20 g, according to the cyanobacterial content of the sediment) of the sediment solution was taken after the solution was thoroughly mixed by shaking. It was then filtered with 50-μm mesh sieves to isolate the colonies from the sediment (Latour and Giraudet, 2004). Cyanobacterial colonies isolated from the sediment were dispersed into single cells using an ultrasonicator (JY88, Scientz, China) for cell enumeration. The sonications were conducted at 25 kHz with cyclic operation intervals of 5 s. Cell concentrations of the solution were manipulated to the range of 10^3–10^8 cells mL^{-1} using a flow cytometer-based protocol for quantitative analysis of cyanobacteria in lake sediments to guarantee analytical precision by dilution or centrifugation. Then, total cyanobacterial counts were measured by flow cytometry (CytoBuoy b.v., Nieuwerbrug, The Netherlands). The instrument was equipped with a bare solid-state laser (488 nm, 20 mW) and five detectors for forward scatter (FWS), side scatter (SWS), red fluorescence (>655 nm, FLR), orange fluorescence (585–655 nm, FLO), and yellow fluorescence (518–548 nm, FLY). Data recording was triggered by the FWS signal and controlled by a peristaltic pump, with a flow rate ranging from 120 to 600 μL min^{-1}. The injection time for each sample varied from 3 to 10 min, depending on particle concentration. An external sheath fluid system was employed to reduce sediment recycling, with a flow rate of 80 mL min^{-1} for the distilled water. The tip of the sampling tube was enlarged by a 1500-μm nozzle to facilitate the sampling of large colonies. The performance of the instrument was tested with fluorescent beads (Micron, Polysciences Inc., USA). CytoClus software (CytoClus 3, CytoBuoy b.v., The Netherlands) was used for data analysis. The cyanobacteria cells (and colonies) were selected by considering the amplitude and shape of the different signals (FWS, SWS, FLR, FLO, and FLY). The specific relative removal rate (SRRR, μ, %) of viable cyanobacteria cells was calculated according to the following equation (Xu et al., 2010):

$$m = (X_1 - X_2)/X_1 * 100\%$$

where X_1 is the concentration of viable cells at the previous incubation stage (T_1) and X_2 is the concentration of viable cells at the next incubation stage (T_2).

Fluorescein diacetate (FDA) and propidium iodide (PI) staining methods were used with a CytoSense flow cytometer to investigate the activity of cyanobacterial cells in the sediments (Franklin et al., 2001). Standard PI solution (Sigma, P-4170) was diluted to

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