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Hyperspectral remote sensing of cyanobacterial pigments as indicators of the iron nutritional status of cyanobacteria-dominant algal blooms in eutrophic lakes

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ABSTRACT

Iron can stimulate cyanobacterial growth. Determining iron availability to cyanobacteria is therefore essential for timely warnings of bloom development. The objectives of this study were to determine the key spectral parameters indicating cellular iron status in cyanobacteria and to establish reliable equations for estimating iron nutrition in cyanobacterial cells. Cells, pigments, cellular iron, and spectra of cyanobacteria were measured monthly at 17 sites in Meiliang Bay of Taihu Lake during the summer period of cyanobacterial blooms from 2010 to 2013. Pronounced spatial and temporal variability of cellular iron of cyanobacteria was observed. The previously developed structure-insensitive pigment index (*SIPI*) and plant senescence reflectance index (*PSRI*) and the newly proposed chlorophyll *a*/phycocyanin index ($R_{Chl/PC}$) exhibited strong relationships with cyanobacterial cellular iron content. The relationships between the cellular iron concentration and *SIPI*, *PSRI* and $R_{Chl/PC}$ could be expressed as linear, quadratic and cubic functions, respectively. The equations derived herein were tested using independent data from 2008 to 2009, obtained from 31 sites within Taihu Lake. For the three models that included *SIPI*, *PSRI* and $R_{Chl/PC}$ as predictors, the coefficients of determination (R^2) between the measured and estimated cellular iron concentration were 0.549, 0.584 and 0.909, and the mean relative errors (*RE*) were 17.1%, 18.1% and 8.0%, respectively. The overall results indicated that use of the three key hyperspectral parameters, *SIPI*, *PSRI* and $R_{Chl/PC}$, could be used for non-destructive and real-time monitoring of the iron nutritional status of cyanobacteria-dominant algal blooms in eutrophic lakes.

1. Introduction

Cyanobacteria are photosynthetic microorganisms commonly found in diverse aquatic environments, including fresh, brackish and marine waters (Catherine et al., 2013; de Oliveira et al., 2015; Hunter et al., 2010). They are particularly well adapted for growth in nutrient-enriched and slow-flowing lakes during summer and autumn in temperate latitudes. An excessive growth of cyanobacteria produces agglomerates known as algal blooms, which are a troubling indicator of eutrophication and pose a serious threat to freshwater ecosystems (de Oliveira et al., 2015; Natalia et al., 2013). Cyanobacterial populations can also pose significant risks to animal and human health because several of the constituent microorganisms can produce cyanotoxins. To date, among approximately 150 described genera of cyanobacteria, 40 have been found to produce toxic compounds (Borges et al., 2015). Potent toxins have been linked to numerous incidences of ill health in humans, including fatal poisonings (Borges et al., 2015; Cheung et al., 2013).

It has long been known that the availability of the macronutrients nitrogen and phosphorus strongly influence algal blooms (Do Nascimento et al., 2015; Ma et al., 2015; Paerl et al., 2011). Since the early 1990s, evidence has accumulated to suggest that algal growth can also be influenced by the micronutrient iron (Dillon and Evans, 2001; Kosakowska et al., 2007; Martin, 1990; Nagai et al., 2007; Wang et al., 2014). Cyanobacteria, as prokaryotes, have higher cellular iron requirements than eukaryotic algae, and increased iron concentrations are found to selectively stimulate cyanobacterial growth at the expense of competing chlorophytes (Imai et al., 1999). Iron has also been shown to be involved in cyanotoxin production and the musty odor of cyanobacteria (Dillon and Evans, 2001; Lukač and Aegerter, 1993; Lyck et al., 1996; Utkilen and Gjølme, 1995).

Monitoring the availability of iron to cyanobacteria is necessary so that timely warnings of bloom development can be provided

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to safeguard human and animal health. Traditional in situ nutrient element detection methods consist of field sample collection and laboratory analysis; such methods can be a time-consuming, labor-intensive and highly skilled activity. Because cyanobacterial blooms may change rapidly in space and time (Hunter et al., 2010; Randolph et al., 2008), there is a clear need for real-time monitoring to better understand the iron nutritional status of cyanobacterial blooms. Remote sensing may provide a tool for such monitoring. Remote sensing has been used successfully to monitor other aquatic variables, such as chlorophyll *a* (Li et al., 2015; Tebbs et al., 2013), phycocyanin (Li et al., 2015; Randolph et al., 2008) and cell populations (Hunter et al., 2009, 2010). However, most of the studies involving cyanobacterial remote sensing have focused primarily on optically active variables (Kutser et al., 2008; Matthews et al., 2010; Shi et al., 2015). Because iron is a non-optically active element in phytoplankton, estimating the concentration of cellular iron can be challenging.

Fortunately, studies have increasingly focused on the remote-sensing assessment of non-optically active compounds in water. The studied compounds include total nitrogen (He et al., 2008), total phosphorus (Gao et al., 2015; Wu et al., 2010), temperature (Alcântara et al., 2010), chemical oxygen demand (Wang et al., 2004) and biochemical oxygen demand (Wang et al., 2004). More recently, researchers (Hunter et al., 2009, 2010) have succeeded in quantifying microcystins in eutrophic lakes using hyperspectral remote sensing of cyanobacterial pigments as indicators.

As an essential element in photosynthetic tissues (Guerinot and Yi, 1994), iron promotes the structural integrity of photosynthetic reaction centers (Msilini et al., 2011; Pereira et al., 2013; Yadavalli et al., 2012) and can be involved in chlorophyll *a*, phycocyanin and carotenoid pigment biosynthesis (Geider and La Roche, 1994; van Leeuwe and Stefels, 1998). Here, we hypothesize that the concentration of cellular iron might be expressed by cyanobacterial pigments (concentrations and/or ratios) according to the following relationship:

$$\text{Concentration of cellular iron} \propto \text{f}\ (\text{chlorophyll } a,\ \text{phycocyanin},\ \text{carotenoids}) \quad (1)$$

Chlorophyll *a*, phycocyanin and carotenoids have direct optical properties, and previous studies showed that the pigments can be expressed through spectral indices according to the following expression (Giardino et al., 2007; Hunter et al., 2008; Kutser et al., 2008; Li et al., 2015, 2012; Shi et al., 2015; Tebbs et al., 2013):

$$\text{f}\ (\text{chlorophyll } a,\ \text{phycocyanin},\ \text{carotenoids}) \propto \text{Spectral indices} \quad (2)$$

Following from these two equations, the concentration of cellular iron can be expressed as follows:

$$\text{Concentration of cellular iron} \propto \text{Spectral indices} \quad (3)$$

The objectives of the present study were (1) to examine the relationships between pigments and cellular iron concentrations to determine the extent to which pigments provide useful indicators of iron nutritional status in cyanobacteria, (2) to determine the key spectral parameters indicating cellular iron status in cyanobacteria and (3) to establish reliable regression equations for estimating iron nutrition in cyanobacterial cells. The anticipated results would lay a technical foundation for the non-destructive and real-time monitoring of the iron nutritional status of cyanobacteria-dominant algal blooms in eutrophic lakes, using hyperspectral remote sensing.

2. Materials and methods

2.1. Study area and experimental design

Taihu Lake, the third largest freshwater lake in China, is located in the middle and lower reaches of the Yangtze Delta, one of the most developed areas in China. The lake has a surface area of 2338 km^2 and mean depth of 1.9 m (Wang et al., 2013) and is an important freshwater resource for approximately 10 million inhabitants in nearby cities (Zhu et al., 2014). With the rapid urbanization of the region, Taihu Lake has shown evidence of eutrophication and summer cyanobacterial blooms since the 1980s (Chen et al., 2003; Jiang et al., 2015). In 2007, the accumulation of toxic cyanobacterial blooms in Taihu Lake left approximately 2 million people without drinking water for at least a week (Guo, 2008; Qin et al., 2010).

This study was conducted using samples taken monthly from Taihu Lake during the summer cyanobacterial bloom period (May–Aug.) from 2008 to 2013. Two experiments were undertaken; an overview of the environmental samples used in each experiment is given in Table 1.

The first experiment (Exp. 1), with 31 sample stations (sites 1–31) throughout Taihu Lake, was undertaken monthly from May to Aug. of 2008–2009. The second experiment (Exp. 2), with a total of 17 sample stations (site M_1–M_{17}) in Meiliang Bay, was conducted from May through Aug. during 2010–2013 (Fig. 1); Meiliang Bay is one of the most highly eutrophic bays in Taihu Lake.

At each station, the spectra of the algal blooms were measured, and water samples were collected using Niskin bottles (KC-Denmarks A/S, Silkeborg, Denmark). These water samples were immediately preserved at 4 °C and brought back to the laboratory for the analysis of the cyanobacterial cell densities, pigments and cellular iron.

2.2. Reflectance measurements

Hyperspectral reflectance measurements were taken from a boat using a Unispec spectroradiometer (Unispec, PP Systems, Haverhill, MA, USA) with a sampling interval of 3.3 nm and a spectral resolution of 0.3 nm. The spectroradiometer was calibrated using a white Spectralon panel before collecting the algal spectra. An optical fiber of the spectroradiometer was mounted on a telescopic pole that was extended over the side of the vessel at 0.5 m above the water surface. Spectra were acquired at a viewing zenith angle of $\theta = 40^\circ$ upwards from the vertical, to avoid instrument self-shading and boat shadow, and an azimuth angle of $\Phi = 135^\circ$, to minimize sun glint effects. The entire field sampling campaign was made over solar zenith angles $\theta_o = 40$–60° under completely clear skies. The experiments were undertaken during flat water conditions (wind speed $U < 2\ m\ s^{-1}$) when a large number of cyanobacteria assembled on the surface of the water. The contribution of bottom reflectance wasn't considered in this study because all simple sites were covered by floating algae during the reflectance measurements. Ten individual reflectance spectra were collected and averaged for each station within the lake. Noise at both ends of the spectrum limited the useful data range to between 400 and 900 nm.

2.3. Determination of pigments

The concentrations of chlorophyll *a* and carotenoids were determined by the spectrophotometric method (Xing et al., 2007). A 5 mL cell suspension sample was clarified by centrifugation (8000*g*, 10 min), and the supernatant was resuspended in 95% ethanol. The resuspended cells were then extracted by manual grinding, and the extract was held overnight in the dark. Subsequently, the sample was clarified again by centrifugation (8000*g*, 10 min). The