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Determination of the filamentous cyanobacteria *Planktothrix rubescens* in environmental water samples using an image processing system

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Abstract

Cyanobacteria occur in surface waters worldwide. Many of these produce peptides and/or alkaloids, which can present a risk for animal and human health. Effective risk assessment and management requires continuous and precise observation and quantification of cyanobacterial cell densities. In this respect, quantification of filamentous *Planktothrix* species is problematic. The aim of this study was to develop an automated system to count filamentous *Planktothrix rubescens* using image processing. Furthermore, this study aimed to assess optimum sample volumes and filament density for measurement precision and to validate image processing measurement of *P. rubescens* for an effective risk assessment.

Three environmental samples and one cultured sample of *P. rubescens* were collected by filtration onto nitrocellulose filters. Filament lengths were determined using fluorescence microscopy combined with an image processor. Cell density could be calculated from the resulting images. Cyanobacteria could easily be discriminated from algae via their fluorescence properties. The results were found to be independent of the mode of image acquisition. The precision of total filament length determination was dependent on the total filament length on the filter, i.e. analyses of highest precision could be expected for filters containing 2000–20,000 μm filaments per mm^2. When using suitable filtration volumes, the detection limits of the described method are sufficient for an effective risk assessment. To summarise, this procedure is a fast, easy and accurate method to determine cell densities of filamentous *P. rubescens* in water samples without costly and tedious manual handling.

Keywords: Cyanobacteria; Filament; Image processing; *Planktothrix*; Cell quantification; Risk assessment

1. Introduction

Cyanobacteria occur worldwide in coastal and surface waters. Surveys in various countries have demonstrated that about 75% of samples containing cyanobacteria are toxic. Due to nutritional enrichment (eutrophication), occurrences of toxic cyanobacterial blooms in surface waters, e.g. species of the genera *Microcystis*, *Anabaena*, *Planktothrix* and *Aphanizomenon* are becoming a growing problem (Bartram et al., 1999). In addition, albeit in contrast to this situation, the intentional nutritional re-depletion of eutrophic surface waters (re-oligotrophication) resulted in regular blooms

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of *Planktothrix rubescens* in several European prealpine lakes (Mez, 1998; Ernst et al., 2001; Morabito et al., 2002; Jacquet et al., 2005).

P. rubescens is a low light adapted, filamentous cyanobacterium, made up of cells, which contain gas vesicles enabling the filaments to adjust their buoyancy in the water column in order to achieve optimal use of the ambient environment (Walsby et al., 1998). Consequently, *P. rubescens* builds blooms distributed over the whole vertical water column during winter circulation and in metalimnic layers during summer stratification. Furthermore, buoyancy disturbances can result in *P. rubescens* blooms at the lake surface (Ernst et al., 2001; Jacquet et al., 2005). *P. rubescens* blooms and layers can attain densities of up to 150,000 cells/ml (Hoeger et al., 2005).

At least 46 cyanobacterial species are able to produce neurotoxins, e.g. anatoxin-a, anatoxin-a(s) and saxitoxin, a range of dermatoxins and/or predominantly potent protein phosphatase inhibitors, such as microcystins and nodularins (Sivonen and Jones, 1999; Chorus et al., 2000). In addition to producing a range of other metabolites with unknown toxicological potential, including anabaenopeptins, microviridins and cyanopeptolins (Blom et al., 2003), species of the genera *Planktothrix* have been shown to contain the highest amounts of microcystin (<5.6 mg/g dry weight) (Fastner et al., 1999). The release of these cyanobacterial toxins may present a serious risk for wild and domestic animals as well as for human health, as recently reviewed by Dietrich and Hoeger (2005). As a result of incidents attributed to toxic cyanobacteria the World Health Organisation (WHO) and several national authorities worldwide have recommended risk assessment plans and safety levels to include cyanobacteria as a parameter, which must be monitored for water quality control (Chorus et al., 2000; Azevedo, 2001; Falconer, 2001; Codd et al., 2005).

Effective risk assessment and management requires continuous and precise observations of cyanobacterial biomass and/or cell densities (Chorus and Bartram, 1999). Quantification of *Planktothrix* species is difficult as the individual cells arranged to form a filament are hardly distinguishable. Furthermore, filament counts cannot be automatically correlated to biomass or cell densities because *Planktothrix* species exhibit large variations in filament length and filaments overlay one another and are often curved in a given sample when observed on a slide or filter, making measurement of filament length difficult and inaccurate. Quantification of cell volumes is difficult because centrifugation is laborious due to the gas vesicles incorporated in *Planktothrix* cells for buoyancy. Furthermore, quantification via determination of photopigments, e.g. chlorophyll and/or phycobilliproteins is not reliable due to regulation of pigments with various growth conditions (Feuillade, 1994) and false positive results due to pigments of eukaryotic algae and zooplankton coexisting within the same environment. Gjolme et al. (2004) demonstrated protein concentrations to best reflect cyanobacterial biomass. However, as pigment and biomass parameters, protein measurement may easily be overestimated in environmental seston samples due to the coexistence of eukaryotic algae and zooplankton within the same environment.

As many of the lakes containing *Planktothrix* species are used for recreational purposes and several even as drinking water reservoirs (Hitzfeld et al., 2000; Hoeger et al., 2005), a rapid and precise procedure for quantification of *Planktothrix* species is essential.

For cell quantification of filamentous cyanobacteria most methods of choice are based on microscopic identification and counting (Olson, 1950; Bailey-Watts and Kirka, 1981; Hoogveld and Moed, 1993). This approach has the caveat of increased demand on both manpower and skill of the personnel as well as limitations in the speed with which filament densities can be determined.

Cyanobacterial species use the biliproteins phycocyanin and allophycocyanin to harvest light for photosynthesis. Some species, including *Planktothrix* species, additionally contain the biliprotein phycoerythrin (Glazer, 1985; Anagnostidis and Komárek, 1988). When examined under blue light excitation, phycoerythrin and phycocyanin fluoresce orange and red, respectively. Therefore, cyanobacteria can be enumerated by visualising the autofluorescence of phycoerythrin and/or phycocyanin using epifluorescence microscopy (Walsby and Avery, 1996; Sieracki and Wah Wong, 1999).

Walsby and Avery (1996) designed and described a semi-automated procedure to count *Planktothrix* cell densities. This method involves the transfer of epifluorescent microscope images of filter to a computer, followed by determination of filament length via computer image analysis. This is a fast and accurate method, which measures the length of several filaments simultaneously.

The aim of our study was to develop automation in counting filamentous *P. rubescens* using arrays of filament images, i.e. to improve and expand on the method of Walsby and Avery, reducing the manual interactions required for measurement and thus reducing overall time per sample. Furthermore, this study