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Estimating nodularin content of cyanobacterial blooms from abundance of *Nodularia spumigena* and its characteristic pigments—a case study from the Baltic entrance area

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Abstract

The presence of toxin-producing cyanobacteria is of concern for the management of recreational waters. The abundance of toxic cyanobacteria and environmental concentrations of their toxins may fluctuate substantially within a short time emphasising the need for rapid methods to estimate toxin concentrations in the water. During late June-early September 2002 the abundance, biomass and characteristic pigments of *Nodularia spumigena* Mertens and the hepatotoxin nodularin produced by *N. spumigena* were analysed in water samples collected from the Baltic entrance area. Significant relationships were found between cell-bound concentrations of nodularin and the abundance and biomass of *N. spumigena* with a relationship of approximately 1 pg nodularin per *Nodularia*-cell. It is suggested that simple counts of *Nodularia* under the microscope may be used as a rapid on-site technique to estimate potential nodularin concentrations in recreational waters. Comparison with data from Australia shows that cell-bound concentration of nodularin per *Nodularia*-cell differs between geographically distant areas and therefore such relationships should be established for individual areas. The carotenoids echinenone, canthaxanthin and a *cis*-canthaxanthin-like caroteniod, identified from a laboratory culture of *N. spumigena* isolated from the Baltic Sea, were also significantly correlated with concentrations of cell-bound nodularin. However, *Aphanizomenon* and *Anabaena*, two genera commonly co-occurring with *Nodularia*, also contain these pigments and thus the significant correlations obtained presumably originate from *Nodularia* being the dominant cyanobacterium in all samples collected in 2002.

Keywords: *Nodularia spumigena*; Cyanobacteria; Nodularin; Hepatotoxin

1. Introduction

The cyanobacterium *Nodularia spumigena* Mertens forms recurring massive blooms in the Baltic Sea (Karhu, 1997). In addition, *N. spumigena* regularly blooms in estuaries and brackish lakes in Australia, New Zealand and inland USA (Hammer, 1981; Horne and Galat, 1985; Carmichael et al., 1988; Codd et al., 1994; Jones et al., 1994), and it has been reported from coastal lakes along the North Sea coast (Nehring, 1993) and South Africa (Harding et al., 1995).

In recreational waters blooms of cyanobacteria are an esthetic nuisance and may cause skin irritation, allergic reactions and flu-like symptoms in humans upon exposure (Pilotto et al., 1997). In addition, mass abundance of *N. spumigena* poses a potential human health hazard through the production of the toxin nodularin, a cyclic pentapeptide chemically and toxicologically similar to the heptapeptide microcystins commonly

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found in freshwater cyanobacteria (Sivonen and Jones, 1999). Like microcystins, nodularin inhibits protein phosphatases 1 and 2A and acts as a tumour promoter (Yoshizawa et al., 1990). In addition, carcinogenic effects have been attributed to nodularin (Ohta et al., 1994). The intraperitoneal LD_{50} in mice is approximately 50 $\mu g\,kg^{-1}$ (Eriksson et al., 1988) which renders the toxicity of nodularin comparable to the most potent microcystins (Sivonen and Jones, 1999).

Mortality of domestic animals after consumption of water with high abundance of *N. spumigena* has been reported from Australia (Francis, 1878), South Africa (Harding et al., 1995) and the Baltic Sea and German North Sea coast (see Nehring, 1993).

WHO (1998) has adopted a provisional guideline value of 1.0 μg microcystin-LR l^{-1} for drinking water. Due to its similarity to microcystins this value may be useful for nodularin where *N. spumigena* occurs in near-fresh water areas used for drinking water (e.g. Lake Alexandrina, Australia). Similarly a series of guidelines for safe practice in managing bathing waters described for areas with microcystin-producing cyanobacteria (Falconer et al., 1999) may be applicable to areas with dominance of nodularin-producing *Nodularia*. The guidelines relate to cyanobacterial abundance with the first level set at 20,000 cells ml^{-1} or 10 μg chlorophyll *a* l^{-1}. At or below this level the primary concern about human health impairment arises from irritative or allergenic cyanobacterial compounds while concentrations of toxins like microcystins are expected to be below or close to the provisional guideline for drinking water and thus unlikely to pose a risk through accidental consumption.

The second level of 100,000 cyanobacterial cells ml^{-1} or 50 μg chlorophyll *a* l^{-1} describes moderate probability of adverse health effects. In addition to the increased probability of irritative symptoms, this level represents potential health impairment through ingestion of cell-bound cyanotoxins like microcystins when blooms are dominated by genera like *Microcystis* or *Planktothrix*. In these blooms microcystin concentrations of 20 $\mu g\,l^{-1}$ are likely and such cell densities should trigger health authorities to issue on-site warnings (Falconer et al., 1999).

The third level represents conditions with surface scum and high risk of adverse health effects. In German recommendations this level corresponds to >150 μg chlorophyll *a* l^{-1} or >100 μg microcystins l^{-1} (Chorus and Fastner, 2001). Immediate action to prevent contact with scums, e.g. through prohibition of swimming etc., is recommended at this level.

Methods for analysis of cyanobacterial toxin concentrations require substantial time for analysis and in most cases results will not be available until after at least a day. Thus, there is a need for rapid and simple on-site methods to analyse toxin concentrations in the water or procedures that will allow reasonable estimates of the toxin concentrations likely to be present.

Phytoplankton is commonly quantified using chlorophyll *a* as an indirect measure of total biomass or by enumeration of individual species/genera under the microscope with subsequent conversion of abundance to biomass. In addition, chemical analysis of phytoplankton pigments may be used for the characterisation of phytoplankton communities from pigment signatures of natural samples (see Jeffrey et al., 1997). From knowledge on the pigment composition of different phytoplankton groups the contribution to total chlorophyll *a* from the different groups can be assigned using linear regression, multiple simultaneous equations or matrix factorisation (Jeffrey et al., 1999). In marine areas the carotenoid zeaxanthin is generally defined as the characteristic pigment of cyanobacteria (Jeffrey et al., 1997) and the presence of zeaxanthin in sediment cores has been used as an indication for the presence of cyanobacteria in the Baltic Sea during the last 7000–7500 years (Bianchi et al., 2000).

Most monitoring programmes include measurements of chlorophyll *a* concentrations, microscopy of phytoplankton abundance and/or characterisation of phytoplankton through pigment analysis. The aim of the present work was to examine the relationship between occurrence of *N. spumigena*, expressed by cell counts or characteristic pigments, and the cell-bound concentration of nodularin in the phytoplankton to evaluate the usefulness of these measurements for estimation of the cell-bound nodularin concentration in water.

2. Material and methods

2.1. Sampling area and procedures

Water samples were collected from the Sound and Køge Bay in the Baltic entrance area (Fig. 1). Køge