Use of cyanopigment determination as an indicator of cyanotoxins in drinking water
Wido Schmidt, Heike Petzoldt, Katrin Bornmann, Lutz Imhof and Christian Moldaenke

ABSTRACT
The indicator function of the fluorescence signals of the cyanopigments phycocyanin and phycoerythrin as early warning parameters against the microcystins in drinking water was investigated by lab- and pilot-scale studies. The early warning function of the fluorescence signals was examined with regard to the signals’ real-time character, their sensitivity and the behaviour of the cyanopigments in different treatment stages in comparison to microcystins. Fluorescence measurements confirmed the real-time character, since they can be carried out on-site without the pre-concentration of pigments. The limit of detection of phycoerythrin is determined at 0.7 μg/L and of phycocyanin at 5.3 μg/L respectively. If the pigment/microcystin ratio is known and calculated to be higher than 1, very low microcystin concentrations can be estimated by the fluorescence signals. The compared behaviour of both pigments and selected microcystins (MC-LR and MC-RR) during water treatment shows that pigments have an early warning function against microcystins in conventional treatment stages using pre-oxidation with permanganate, powdered-activated carbon and chlorination. In contrast, cyanopigments do not have an early warning function if chlorine dioxide is used as a pre-oxidant or final disinfection agent. In order to use pigment control measurements in drinking water treatment the initial pigment/toxin ratio of the raw water must be known.

Key words | cyanobacteria, cyanopigments drinking water treatment, cyanotoxins, early warning function

INTRODUCTION
The occurrence of cyanobacterial toxins in surface water bodies is recognised as a world-wide human health problem. In many countries surface water is used for drinking water production. MCL (maximum contaminant levels) for cyanotoxins in drinking water have been derived by the WHO with a provisional guideline value of 1 μg/L for daily exposure to the hepatotoxic cyanotoxin microcystin-LR (WHO 1998).

In European water bodies, the most frequently occurring cyanobacteria are the *Microcystis*, *Anabaena* and *Planktothrix* strains (e.g. Chorus & Bartram 1999; TOXIC 2005). Primary toxins produced by these organisms are different forms of microcystins.

Cyanotoxins can occur in cell-bound and in dissolved form. The irreversible release of toxins from the algal cells into the water can take place in the reservoir as well as inside the waterworks. These processes are defined as natural and induced release respectively (e.g. Chorus & Bartram 1999; Schmidt et al. 2002).

**Natural toxin release** is closely connected to the life cycle of cyanobacteria. The toxins are released during cell senescence, death and lysis. In general, the older the cells...
are, the higher the level of dissolved toxins that can be expected in water.

The induced toxin release is caused by different stress factors which have an impact on the cyanobacteria at different stages of water treatment. Mechanical stress is caused by mixing and stirring when agents such as coagulants or powdered-activated carbon are added to the water. During filtration, pressure differences and shearing forces decrease the stability of the cyanobacteria and can cause cell damage (e.g. Lawton & Robertson 1999; Drikas et al. 2001; Schmidt et al. 2002). Waterworks using oxidative treatment, e.g. pre-oxidation with permanganate, chlorine as well as chlorine dioxide or ozone, destabilize the cells by chemical oxidation (e.g. Petruševski et al. 1996; Pietsch et al. 2002).

The analysis of cyanotoxins throughout a water treatment stage is expensive and time-consuming. Nevertheless, waterworks need rapid information. A rapid analytical method for real-time process control could be the monitoring of the blue and red cyanopigments phycocyanin (PC) and phycoerythrin (PE), respectively. The determination of cyanopigments by fluorescence techniques is comparatively easy (e.g. Bryant 1982; Izydorczyk 2005; Cagnard et al. 2006). However, very little is known about the behaviour of dissolved cyanopigments during different water treatment stages and complete processes.

This article discusses the use of the two cyanopigments phycoerythrin and phycocyanin as indicators of microcystins in drinking water. The sensitivity of the pigment analysis was determined by calibration curves with standard compounds. The removal of selected microcystins, phycoerythrin and phycocyanin by activated carbon was determined and compared. Experiments for oxidation and adsorption of microcystins and both pigments were carried out in lab-scale experiments using standard analytical compounds. In order to recognize the behaviour of cyanotoxins and cyanopigments under real conditions, systematic pilot-scale studies with cyanobacteria containing water were carried out.

**MATERIAL AND METHODS**

**Raw water and cyanobacteria**

*Planktothrix rubescens* was used for all experiments involving cyanobacteria. This organism occurs seasonally in the Weida reservoir in Thuringia at biovolumes of between $0.1 < \text{BV} < 8.0 \text{mm}^3/L$ (Schmidt et al. 2002).

During the period in which the experiments were conducted *Planktothrix rubescens* from this bloom formed the red pigment phycoerythrin and the blue one phycocyanin. For laboratory experiments the cyanobacteria from the Weida reservoir was additionally cultivated in the laboratory using a basal medium (Pringsheim 1954; Rao et al. 1994).

**Analysis**

Cyanopigment calibration standards were obtained from Fluka. The standard operational procedures for pigment analysis were developed according to protocols in the European Research Project TOXIC [EC-EVK1-2001-00182 2005]. The estimations of cell-bound phycocyanin and phycoerythrin were obtained by the difference in the fluorescence measurements of unfiltered and filtered samples. The measurements were carried out with a Perkin-Elmer LS-50 fluorescence spectrometer using the exitation/emission wavelengths $560/577$ for phycoerythrin and $620/645$ for phycocyanin. The calibration curves covered the range of $1.0 < [\text{PC}] < 100 \mu g/L$ and $0.1 < [\text{PE}] < 10 \mu g/L$. The curves were made in six waters of different origin and different total organic carbon content (TOC).

Cyanotoxin standards microcystin-LR and -RR (MC-LR, MC-RR) were produced according to the protocol of the European Project TOXIC by ABO Akademi Finland. The toxin analysis was carried out by LC-MS/MS (Pietsch et al. 2001). The standard deviation of the applied method was calculated at 1.7%.

Cell-bound and dissolved toxins were separated by vacuum filtration with $0.45 \mu m$ cellulose nitrate filters. In order to avoid an artificial toxin and pigment release, the applied vacuum did not exceed 400 mbar.

**Lab-scale experiments**

**Activated carbon**

The carbon experiments were carried out using fresh products: W 35 (Norit), Hydraffin SC11 (Donau Carbon) and Adsorba N (Norit). The concentration of the dissolved
organic matter (DOC) in this water was 1.4 mg/L. The carbon doses added were 2, 4, 6, 8, 10 and 20 mg/L. The concentration of the standards phycoerythrin and phycocyanin were 10 µg/L for each and 5 µg/L MC-LR and MC-RR respectively. The residual concentrations of pigments and toxins were measured after contact times of 30 minutes and 24 h. All samples were filtered through a 0.45 µm polycarbonate filter. The pH-value was adjusted to 7.2 with sodium hydroxide. All experiments were carried out at a temperature of 18°C.

**Oxidation**

For oxidation, sodium hypochlorite solution (Riedel de Haen) and potassium permanganate (Merck) were used. Chlorine dioxide was generated in the laboratory using peroxide sulphate and sodium chlorite (DEGUSSA 1990). The chlorine doses added covered seven concentrations in the range of 1.4 to 14.1 µmol/L (see Table 3). Chlorine dioxide was added in doses of 3.0, 7.4, 14.8 and 22.4 µmol/L. Potassium permanganate was applied at six levels from 1.2 to 25.3 µmol/L (see Table 3). Ozone was adjusted to four concentrations: 4.2, 10.0, 20.1 and 31.2 µmol/L. The ozone was added from a stock solution (20 mg/L), which was produced from air by a Sorbios generator.

For oxidation experiments, model water (TOC < 0.3 mg/L) with a natural salt matrix, adjusted to pH = 7.2 was used.

The degradation of both pigments was analyzed for 60 minutes at 2 minute intervals. The cyanotoxin concentration was measured at 10 minute intervals. The contact time was adjusted by quenching the residual oxidants with thiosulphate prior to analysis.

**Pilot-scale experiments**

Pilot-scale experiments were carried out using a pilot plant with a flocculation unit, ozone generator (Sorbios), and points for dosing of permanganate and powdered-activated carbon. The filter DN 240 was filled with hydroanthracite and sand. Four sampling points were installed at several heights of the filter: 1. height I: “head of hydroanthracite”, 2. height II: “hydroanthracite/sand-line”, 3. height III: “middle of sand layer” and 4. “filter effluent”.

The operational conditions of the pilot plant are characterized in Table 1.

**Table 1 | Experimental conditions for pilot water treatment plant (PWT)**

<table>
<thead>
<tr>
<th>Treatment stage</th>
<th>Chemical</th>
<th>Dose, g/m²</th>
<th>Filter size, cm</th>
<th>Retention time in the PWT, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flocculation</td>
<td>Aluminium sulphate</td>
<td>2.6–3.0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Praestol 2540 TR</td>
<td>0.1</td>
<td></td>
<td></td>
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<tr>
<td>Filtration</td>
<td></td>
<td></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Pre-ozonation</td>
<td>Ozone</td>
<td>0.8–1.0</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Flocculation</td>
<td>Aluminium sulphate</td>
<td>2.6–3.0</td>
<td>8</td>
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</tr>
<tr>
<td></td>
<td>Praestol 2540 TR</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td></td>
<td></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Permanganate</td>
<td>Potassium permanganate</td>
<td>0.67</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td></td>
<td></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Flocculation</td>
<td>Aluminium sulphate</td>
<td>2.6–3.0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Praestol 2540 TR (polyacrylamide based)</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td>Powdered-activated carbon</td>
<td>10</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Filtration</td>
<td></td>
<td></td>
<td>64</td>
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</tr>
</tbody>
</table>

Filter DN 240; size: 4400 × 240 cm; Filter material: hydroanthracite: 900 mm; diameter (DM) = 1.40–2.25 mm; Sand: 1,000 mm; DM = 0.71–1.25 mm; Gravel: 100 cm; DM = 2.00–3.15 mm.
The dosage of permanganate, ozone and powdered-activated carbon before the flocculation was adjusted on the basis of pre-experiments with the same source water. All pilot-scale experiments were carried out as long-term experiments over the entire filter run periods, which focused on the turbidity breakthrough, observed after time spans ranging between 16 and 32 h. During each filter period three measurements of cyanopigments and cyanotoxins were carried out. For discussion, the arithmetical average of all single measurements from four filter periods was calculated.

All runs were controlled by continuous measurements of pH-values, turbidity, and filter pressures.

RESULTS

Analysis of cyanopigments

In Figure 1 the measured fluorescence intensities for phycerythrin and phycocyanin are correlated with the concentration of the standards. The fluorescence intensities measured in the range between 0.05 to 25 units correlates linearly with the concentration. The correlation coefficients were calculated to $R^2 = 0.9965$ for PC and $R^2 = 0.9994$ for PE. The corresponding limits of determination using the Perkin Elmer LS-50 software SQS 3.3 were calculated to LDPE: $0.7 \mu g/L$ and LDPC: $5.3 \mu g/L$. The red PE showed approximately 25 times more intensive fluorescence than the blue PC. Nevertheless, the determination of both compounds with simple fluorescence measurements without pre-concentration in the $\mu g/L$ range was possible. Tests for pigment determination were made in six waters of different origin by dosing the same pigment concentrations. The average standard deviations were calculated at 12% for PE and 16% for PC respectively.

During the experimental period, the native *Planktothrix rubescens* in the Waida Reservoir was characterized by an average total content of $8 \mu g/L$ phycerythrin, $53 \mu g/L$ phycocyanin and $2.5 \mu g/l$ microcystin (sum of MC-LR and MC-RR). The ratio of pigment to toxin level was calculated at 5 (PE/MC) and at 21 (PC/MC) respectively (Table 2). The cultivated *Plankthotrix rubescens* also formed both pigments. As in the native organisms, more phycocyanin was formed in the cells. The average toxin production of the cultivated algae cells was higher than in the native organisms of the Waida reservoir (see Table 2).

Laboratory-scale experiments

Oxidation

The most recent studies have shown that the reaction of oxidants with MC-LR is second order (e.g. *Onstad et al.* 2007; *Rodriguez et al.* 2007). In such a case, the pseudo-first-order plot $\ln [CP]/[CP]_0$ versus the reaction time $t$ delivers with the slope of the curve the pseudo-first-order reaction constant $k_1$ (pseudo). The second-order reaction constants are obtained by plotting the $k_1$ (pseudo) values versus the concentrations of oxidants added to the water.

The rate constants for the reaction of permanganate, ozone, chlorine and chlorine dioxide with phycerythrin and phycocyanin were measured in a batch system under comparable conditions of a constant water matrix, pH-value and temperature. Pseudo-first-order conditions, that is $c$ (ozone, permanganate, chlorine, chlorine dioxide) $\gg c$ (PC, PE) were adjusted. The calculation is explained using permanganate as an example.

Figure 2 shows the time-dependent degradation of phycerythrin in the model water as an example. In Figure 3 the linear correlation of $k_1$ (pseudo) via the concentration of permanganate is shown.

In Table 3, the second-order constants obtained for PC and PE are compared with those of MC-LR [*Onstad et al.* 2007].
Chlorine effectively decomposes both types of molecules, the cyanotoxin MC-LR as well as the pigments PE and PC. The constants determined show nearly the same degradation rates. Permanganate, often used as a pre-oxidant, also decomposes microcystins and pigments with comparable level of rate constants. Differences were measured for chlorine dioxide application. The degradation of MC-LR is at between 2 and 3 orders of magnitude slower in comparison to phycoerythrin and especially phycocyanin. The applied ozone doses degraded both pigments very rapidly and completely. In these experiments no explicit rate constant could be obtained.

### Adsorption on activated carbon

One of the most efficient measures for microcystin removal from water is the application of powdered-activated carbon (Lambert et al. 1996; Donati et al. 1997; Cook & Newcomb 2000). A decisive question is that of the adsorbability of the dissolved cyanopigments phycocyanin and phycoerythrin. Microcystins are peptide-like molecules with a mol mass of about 1000 a.u. In contrast, the mol masses of both cyanopigments are approximately 100,000 a.u.. In such a case significant differences in adsorption behaviour can be expected.

In **Figures 4 and 5**, the reduction of microcystin concentrations MC-LR and MC-RR are shown in comparison to both pigments after contact times of 30 minutes and 24 h.
The reduction of microcystins depends on the dose of carbon. After 30 minutes contact time, 40 to 80% of toxin was removed by all carbons tested. After 24 h the removal was nearly complete. In comparison, a significant reduction of the concentration of pigment macromolecules after 30 minutes could not be achieved. Only in the case of the highest doses of carbon (20 mg/L) and on equilibrium contact time of 24 h was the removal efficiency higher than 90%. These results indicate that, in contrast to microcystins, dissolved cyanopigments such as phycocyanin and phycoerythrin cannot be removed with activated carbon in water treatment processes with adequate efficiency.

Pilot-scale studies

The removal efficiencies of microcystins and pigments were measured in four treatment stages evaluating conventional flocculation and filtration, ozone, permanganate and powdered-activated carbon, prior to filtration. The experiments were carried out consecutively. The bars in Figure 6 indicate the level of total cell-bound toxins (MC-LR and MC-RR). In comparison, the lines show the concentrations of dissolved toxins. The algae cells were efficiently removed in all treatment stages so that the concentration of cell-bound toxins was lower than 0.1 μg/L in the effluent of the filter. On the other hand, during all treatment trains a toxin release was measured.

The highest level of dissolved toxins was measured at filter height II, the hydroanthracite/sand-line. This fact indicates an accumulation of algal cells in the filter which is linked to an increase in toxin release. In such a case, the oxidation capacity of residual permanganate and ozone added prior to filtration did not guarantee the destruction of the released toxins. Nevertheless, if powdered-activated carbon was applied, the concentration of dissolved microcystins could be kept at the lowest level at all sampling points downstream of the point of carbon addition.

The main effect of the carbon was reached in the filter bed indicated by the low level of dissolved toxins determined at height II. Obviously, powdered carbon entered the filter up to the hydroanthracite/sand-line.

The concentrations of phycoerythrin and phycocyanin throughout the treatment are given in Figure 7. The course of the cell-bound pigments (bars) indicates the removal of algal cells. These results are comparable to that of cell-bound microcystines in Figure 6. The concentrations of the two dissolved pigments phycoerythrin and phycocyanin increases like the dissolved toxins in all treatment stages examined. The highest levels indicated by

![Figure 3](image-url) Plot of the pseudo-first-order rate constant versus the initial concentration of permanganate.

![Figure 4](image-url) Removal rates of Microcystins-RR and -LR by different types of activated carbon in comparison with the cyanopigments phycoerythrin and phycocyanin; contact time 30 minutes, pH = 7.2; DOC = 1.2, T = 18°C.

![Figure 5](image-url) Removal rates of Microcystin-RR and -LR by different types of activated carbon in comparison with the cyanopigments phycoerythrin and phycocyanin; contact time 24 hours, pH = 7.2; DOC = 1.2 mg/L, T = 18°C.
fluorescence intensities were measured at filter height II, the anthracite/sand-line. This was the height with the highest content of dissolved toxins. A significant difference between microcystin and pigment removal was measured in treatment stage IV (powdered-activated carbon dosage). The carbon reduces the toxins MC-LR as well as MC-RR.

Nevertheless, the removal of dissolved phycoerythrin and phycocyanin was considerably lower in comparison, because the adsorbability of both protein-like macromolecules on activated carbon is low.

In Figure 8, the ratio between both pigments and the sum of all toxins analysed is shown throughout different
treatment stages. It is remarkable that both ratios increase from approximately 3 to 8 for PE/MC and 20 to 50 for PC/MC in the average throughout the treatment stages with the exception of the carbon stage. After carbon dosage, both ratios increase significantly to 20 for PE/MC and 170 for PC/MC. The reason is the better adsorbability of microcystins on carbon.

**DISCUSSION**

The effective use of cyanopigments as indicator of microcystins in raw water and during the water treatment process is subjected to the following limitations.

1. **Real-time character of measurements:** Pigment measurements can be carried out by simple on-site measurements with mobile techniques without pre-concentration. In order to obtain information about the dissolved pigment content, the sample has to be filtered on-site. The pigment analysis takes minutes in comparison to microcystin ELISA kits, which takes hours, or chromatographic toxin analysis, which takes one day minimum.

2. **Analytical sensitivity:** The calibration curves covered the range of 1.0 < [PC] < 100 μg/L and 0.1 < [PE] < 10 μg/L. The limits of determination were calculated at LD₉⁰: 0.7 μg/L and LD₀: 5.3 μg/L. But, the sensitivity of the method can be changed by water matrix effects, indicated by the higher standard deviation of pigment measurements in comparison to microcystins. Therefore the limit of pigment detection has to be estimated for each (type of) water under consideration. For the water used in these experiments the sensitivity of the analytical method seems to be adequate enough to indicate toxins in the low μg/L range, because in all cases the ratio of pigments to microcystins was clearly higher than 1 (compare Table 2). Nevertheless, the need for systematic determination of the pigment/toxin ratio in raw waters containing cyanobacteria is urgent and could form the basis for the real-time indication of cyanotoxins in drinking water.

3. **Behaviour of indicator compounds during water treatment:** The measurement of cell-bound cyanopigments by fluorescence during water treatment stages indicates on average the removal of cyanobacteria cells and the course of the cell-bound toxin level.

The prediction of dissolved microcystins by fluorescence measurements is much more difficult. An early warning function, e.g. against induced toxin release can be defined only if the oxidation rate and (or) adsorbability of pigments and toxins is known. If both types of compounds can be removed with comparable efficiency, the pigments could have an early warning function. In cases in which
toxins but not pigments are removed by oxidants and (or) carbon very efficiently, the occurrence of cyanopigments in treated water does not automatically mean the occurrence of toxins. The pigments indicate cyanobacteria-originated organic matter only. On the other hand, if pigments are more efficiently removed than toxins, they cannot be seen as an indicator.

In Table 4, the indicator potential of cyanopigments for cyanotoxins is classified for various stages of the treatment process. Both cyanopigments can be efficient indicators of microcystins and cyanobacteria-originated organic matter when chlorine and permanganate are used. In the case of ozone, the degradation of pigments seems to take place at nearly the same rate as for microcystin degradation. When the dose of ozone is not high enough to oxidize toxins and pigments, the early warning function of pigments is given. But, if chlorine dioxide is applied, the fluorescence measurements cannot indicate the presence of microcystins since MC-LR will be degraded much more slowly.

The indicator function of the fluorescence in the case of powdered-activated carbon is reduced because of significant differences of the adsorbability of cyanopigments and pigments on carbon. Nevertheless, cyanopigments remaining in solution after carbon addition indicate cyanobacteria-originated organic matter in the water.

The pilot-scale experiments with native algae showed how the release and degradation process of toxins and pigments takes place under conditions of practise.

The general course of release of microcystins and both cyanopigments is comparable during all treatment stages examined.

In the filter effluent, toxins and pigments could be measured in the case of conventional filtration and if ozone and permanganate were applied. In all cases the presence of microcystins was indicated by pigment fluorescence. The addition of powdered-activated carbon guaranteed complete microcystin elimination whereas traces of PE and PC were found in the filter effluent.

CONCLUSIONS

Fluorescence measurements of cyanobacterial pigments during water treatment indicated the occurrence of cyanobacterial cells and cell bound toxins. The indication of dissolved toxins by fluorescence measurements depends on the behaviour of toxins and pigments in water treatment processes.

The impact of ozone, chlorine and permanganate on the degradation of dissolved pigments and microcystins was comparable. The indicator function of pigments can be confirmed. In the case of chlorine dioxide application, the indicator function of both pigments was less significant, because the pigments were oxidized much more rapidly by chlorine dioxide in comparison to microcystins.

If powdered-activated carbon was applied, the microcystins were more effectively removed than pigments.

The behaviour of pigments and microcystins during the filtration steps, especially its accumulation in the filter bed was comparable.

The results show that in many cases pigment fluorescence can be used as an indicator of microcystin occurrence in drinking water. The application of fluorescence measurements in raw water and different stages of water treatment can help to reduce time-consuming and expensive toxin measurements.

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