Development of a cell lysis method and evaluation of a field test to measure microcystins freed by cyanobacteria in surface water.

Study report and research presented to Health Canada by Christian Deblois, M. Sc., Chemist

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Centre d’expertise en analyse environnementale du Québec (CEAEQ)

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Note: The opinions expressed in this publication are those of the author and do not necessarily reflect the official views of Health Canada.
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1. INTRODUCTION

Cyanobacteria, also commonly known as blue-green algae, are aquatic organisms that proliferate at the water surface under the influence of environmental factors such as light, temperature and nutrients. The cyanobacteria class includes approximately 150 genera and 2000 species [1]. Cyanobacteria have characteristics of algae and bacteria. They differ from other algal cells in that they do not have a nucleus. They contain chlorophyll $a$, carotene, xanthophyll, phycocyanine and phycoetherin. The size of cyanobacterial cells can vary between 3 and 10µm. Cells are present in different forms, either as isolated cells, in colonies or in filaments. A significant proliferation of cyanobacteria is generally accompanied by skimming, which is easily observable during blooming. Although blooms are considered natural events, they can be significantly influenced by human activity. Scientists agree that phosphorus contributes significantly to the proliferation of cyanobacteria.

Cyanobacteria produce over 70 variants of microcystins known as cyanotoxins [2]. The most toxic of these is called Microcystin-LR (Figure 1). The different congeners of microcystin are cyclical heptapeptides comprised of a chain of amino acids that allow each congener to be identified. The general structure of microcystin (microcystin-XY) is cyclo (D-Ala-L-X-D-erythro-ß-methylisoAsp-L-Y-Adda-D-iso-Glu-N-methyldehydroAla), where X and Y represent variants of L-amino acids. Cyanobacteria may also produce other toxins.

<table>
<thead>
<tr>
<th>Microcystin-LR</th>
<th>Y</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystin-RR</td>
<td>Arginine</td>
<td>Arginine</td>
</tr>
<tr>
<td>Microcystin-YR</td>
<td>Tyrosine</td>
<td>Arginine</td>
</tr>
</tbody>
</table>

![Microcystin-LR Structure](image)

Figure 1. Microcystin-LR
Several species of cyanobacteria can synthesize cyanotoxins. The most common species include *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix*. Microcystins are considered hepatoxins because they inhibit the functioning of phosphatases 1 and 2a which are the enzymes responsible for the phosphorylation of other enzymes and proteins [3]. The approximate LD$_{50}$ toxicity of microcystin-LR varies between 15 and 150 µg/kg of body mass (b.m.) in the case of intraperitoneal injections and between 5,000 and 11,000 µg/kg b.m. orally in mice.

In recent years, the presence of cyanobacteria and the appearance of blooms have been reported in a number of countries throughout the world. In 1999, the Centre d’Expertise en Analyse Environnementale du Québec (CEAEQ) developed an analysis method to measure the amount of free and intracellular cyanotoxins that incorporates the use of liquid chromatography coupled with mass spectrometry (LC-MS/MS), in response to a need expressed by the Ministère du Développement durable, de l’Environnement et des Parcs du Québec (MDDEP) concerning the presence of cyanobacteria and its toxins in lakes and other bodies of water in Québec. Toxins are synthesized within the cells and are freed into the natural environment following cell lysis. During the growth phase, toxins are for the most part intracellular (fewer than 10 to 20% of total toxins are extracellular) [5]. The analysis methods generally used to measure cyanotoxins are liquid chromatography coupled with a UV detector, or liquid chromatography coupled with mass spectrometry. Colorimetric methods that include ELISA (Enzyme-Linked Immunosorbent Assays), by protein phosphatase-1 or -2a (PP1 or PP2a) and a PCR technique may also be used.

The MDDEP, Ontario Ministry of the Environment, Health Canada and other organizations recommend a microcystin-LR concentration of no more than 1.5 µg/l in drinking water, whereas the World Health Organization (WHO) recommends a microcystin-LR concentration of no more than 1.0 µg/l. The Institut National de la Santé Publique du Québec (INSPQ) has proposed an alert threshold of 16 µg/l for microcystin-LR and a threshold of 20,000 cyanobacteria cells per milliliter in the recreational waterways of Québec [6]. Toxicity equivalent factors (TEF) for microcystin-LR, -YR, -YM, -LA and –RR were developed by Wolf and Frank [7].

Once technical expertise was developed, monitoring programs were introduced over the years to gather data and information on levels of cyanobacteria and concentrations of toxins in bodies of water and lakes throughout Québec. Over this period, a better understanding of the number of bodies of water affected and factors that contribute to the proliferation of blooms across Québec has emerged. The number of blooms that have occurred in recent years, however, is significant and have become so widespread that they are of a concern to the MDDEP and Ministère de la Santé et des Services Sociaux (MSSS). In 2006, the MSSS closed several Québec beaches and issued a series of no-drink warnings, particularly in southwestern Québec. Social, municipal, political and media pressure has mounted, creating an exceptional workload and requiring coordinated actions on the part of the MSSS and MDDEP.

The number of requests for analysis submitted to the CEAEQ tripled in 2006. Compared to numbers in 2005, there was a tenfold increase in requests during 2007. The CEAEQ performed over 4,000 hours of emergency analytical work in 2006 and over 12,000 hours in 2007 because so many samples of drinking water were exposed to cyanobacteria. The workload involved and the risk of having the situation repeat itself in 2008 are partly the reason why we would like to examine alternative methods, which may prove useful to MDDEP representatives in rural areas and subsequently reduce the number of analysis requests submitted to the CEAEQ.
We chose to evaluate an immuno-enzymatic (ELISA) type field kit that enables a visual assessment of the concentration of possible microcystin in water samples taken directly from bodies of water in Québec. Different types of ELISA tests are available from suppliers. We chose the QualiTube™ field kit produced by the company Envirologix™. The test requires only a few simple steps that are performed in test tubes. The test allows a visual estimation of the concentration of total microcystins in surface water where concentrations range between 0.5 and 3.0 µg/l. The colour obtained when the test is conducted is inversely proportional to the concentration of microcystins present in the water. The test must be reliable and easy to use for inexperienced testers and must require minimal training.

Envirologix™ makes two other product kits, the QuantiTube™ kit, which uses a measuring instrument and involves a visual reading, and the QuantiPlate™, which requires more specialized equipment and fixed laboratory equipment. The QuantiTube™ may prove a useful alternative, although more steps are involved and use of a spectrophotometer is required to produce measurable results.

2. CHARACTERISTICS OF THE ENVIROLOGIX™ QUALITUBE™ FIELD KIT

The advantage of the colorimetric field test, involving the use of tubes, is that non-specialized personnel can conduct tests and it requires little training. An ELISA-type test does not distinguish between the variants of microcystin and it does not measure the concentration of free toxins in water. Total levels of cyanotoxins cannot be determined because intracellular toxins have not been released. Some substances, such as natural or synthetic colouring, metals and other organic and inorganic compounds present in the area may interfere with testing because these substances may block or compete for sites where antibodies are attached in the tube. The test measures concentrations of cyanotoxins between 0.5 and 3.0 µg/l. Concentrations above 30 µg/l can be measured by diluting the sample and repeating analysis until the concentration is within the testing range. The characteristic blue colour disappears completely when a total of 3.0 µg/l or more microcystins are present.

The concentration of microcystins can also be measured using a UV spectrophotometer set at 450nm. This enables a more accurate reading than by visual means alone.

Results obtained using a QualiTube™ should be considered semi-quantitative. The test is used to estimate the absence or presence of microcystins, where the concentration is between 0.5 and 3.0 µg/l. The test cannot be used, however, to measure the amount of free microcystins in surface water. Microcystins that are contained within living algal cells are also not taken into account. This is particularly problematic since most toxins are contained within cells.

The company Envirologix™ explains on its website that the QualiTube™ is not recommended for use on drinking water or waste water.

Colorimetric tests must be validated according a precise use method because they can sometimes under- or over-estimate concentrations, depending on the characteristics and source of samples tested. If a field test can measure the total level of free and intercellular cyanotoxins, it could prove useful as a screening tool or as a means of monitoring a body of water affected by a cyanobacterial bloom. Health Canada has therefore cooperated to fund research work to assess
the reliability and suitability of the QualiTube™ kit and to develop a cell lysis method to measure total toxins in samples submitted to an ELISA type test.

The ELISA test is not selective. If a sample containing several types of microcystins is tested, the test will indicate a value that does not reflect the actual concentration of microcystin-LR alone. Instead, it will indicate a total concentration of various forms of microcystins present in the sample. Test sensitivity for each type of microcystin variant depends on which variants are present in a sample.

The toxin that is most sensitive to the test is microcystin–LA. Microcystin-YR is the least sensitive.

Table 1 - Cross-reactivity of toxins according to the Enviroleogix™ QualiTubeTM test

<table>
<thead>
<tr>
<th>Microcystin</th>
<th>50% B/Bo¹</th>
<th>81.5 Bo DL²</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR</td>
<td>0.94</td>
<td>0.30</td>
</tr>
<tr>
<td>LA</td>
<td>0.78</td>
<td>0.43</td>
</tr>
<tr>
<td>RR</td>
<td>1.53</td>
<td>0.65</td>
</tr>
<tr>
<td>YR</td>
<td>2.53</td>
<td>0.69</td>
</tr>
<tr>
<td>Nodularin</td>
<td>1.44</td>
<td>0.53</td>
</tr>
</tbody>
</table>

¹ % B/B₀ = sample optical density
optical density of white

² DL: Detection limits

3. PROJECT OBJECTIVES

The project has two main objectives:

- Evaluating the QualiTube™ field kit and recommending its use, if applicable, within a defined framework of use.
- Development of a cell lysis method to determine total microcystin concentrations in an environment, more specifically extracellular and intracellular microcystins which can represent 80% of total microcystin levels.

Without a cell lysis method, the results obtained using an ELISA type test could significantly under-estimate levels, since recommendations are based on total cyanotoxin concentrations.

3.1 Validation of the performance of the QualiTube™ kit

- Verify how easy the test is to perform before recommending possible use.
- Verify compliance with the recommended concentration range.
- Compare results obtained using a visual reading with results obtained using a UV spectrophotometer at 450nm.
Verify results obtained using the kit, involving samples containing microcystins from cyanobacteria cultivated in the lab.

3.2 Development of a cell lysis method that can be easily used in the field to measure total microcystins.

- Evaluate different methods of cell lysis. The methods evaluated include freezing, heating, ultrasonic probe, adding a biocide (copper sulphate [CuSO4]) and centrifugation followed by filtration.
- Measure the concentration of free microcystins before and after cell lysis methods are conducted.
- Verify the effectiveness of each cell lysis method, by measuring intracellular microcystins after each cell lysis method is conducted or by using the LC-MS/MS method of intracellular microcystin analysis developed by the CEAEQ (MA. 403- Microcys 1.0)

4. RESULTS

4.1 Results of the validation of the QualiTube™ kit

The QualiTube™ field kit is relatively easy to use in controlled conditions, ideally in a commercial, public or residential building. It is harder to use, however, in a boat under windy or sunny conditions, which complicates handling and colour readings.

The kit was used according to manufacturer recommendations and operating instructions. The test is easy to perform under controlled conditions and concentrations are relatively easy to estimate. Figure 2 shows the different color levels obtained for the control, microcystin measurement standards of 0.5 and 3 µg/l and a sample containing a microcystin concentration between 0.5 and 3 µg/l of approximately 2 µg/l.

![Figure 2. Coloration obtained with the QualiTube™ test](image-url)
The sample that was used to perform the test came from several samples obtained by culturing cyanobacteria produced in the CEAEQ lab. The *Microcystis aeruginosa* strain came from the collection of the University of Toronto in Ontario bearing the identification number UTCC #299. This strain produces almost only microcystin-LR, representing the equivalent of approximately 415 µg/g of dry weight algae. The strain was cultivated in the CEAEQ laboratory under optimal growth conditions using a bg-11 culture medium, as described in the procedure published on the University of Toronto website [8].

Performance of this test sometimes requires several dilutions if the concentration of microcystins is unknown, because the test only allows for coloration at a range of concentrations between 0.5 and 3 µg/l. Several re-tests may be required if the concentration exceeds 3 µg/l. Preliminary dilutions can be conducted with demineralized water where the degree of anticipated concentration is already known.

The results of the QualiTube™ test are determined on the basis visual reading and by comparing standard colour solutions with the colours of samples collected. Concentrations can also be evaluated using a UV spectrophotometer set to 450nm. Concentrations obtained using a visual reading were compared to concentrations obtained using a UV spectrophotometer.

## 4.2 Results of the cell lysis methods evaluated

### 4.2.1 Evaluation of the QualiTube™ test

The different cell lysis methods were evaluated and were used to compare results from the two types of readings. The results obtained are shown in Table 2. In the case of each of the trials conducted, the visual reading was compared to the spectrophotometric reading.

Table 2 - Comparison of results to determine the concentration of free toxins in water using the QualiTube™ and a UV spectrophotometer set at 450nm

<table>
<thead>
<tr>
<th>Sample characteristics</th>
<th>Total toxins QualiTube™ µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Visual reading</td>
</tr>
<tr>
<td><strong>R1 J7</strong> 2 x 10^6 cell/ml</td>
<td></td>
</tr>
<tr>
<td>Without treatment</td>
<td>&gt; 0.5 &lt; 3</td>
</tr>
<tr>
<td>Frozen</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>Frozen + CuSO₄ 0.001 %</td>
<td>&gt; 3</td>
</tr>
<tr>
<td>Frozen + CuSO₄ 0.001 % 1 hour wait time before freezing</td>
<td>&gt; 3</td>
</tr>
<tr>
<td><strong>R2 J5</strong> 4 x 10^6 cell/ml</td>
<td></td>
</tr>
<tr>
<td>Without treatment</td>
<td>&gt; 0.5 &lt; 3</td>
</tr>
<tr>
<td>Frozen (4x)</td>
<td>&gt; 0.5 &lt; 3</td>
</tr>
<tr>
<td>Frozen (4x) + CuSO₄ at 0.016 % + ultrasound bath (4 x 10 min)</td>
<td>≈3</td>
</tr>
</tbody>
</table>
Table 3 – Comparative study of the QualiTube™ and LC-MS/MS

<table>
<thead>
<tr>
<th>Toxin</th>
<th>QualiTube™ µg/l (n=4)</th>
<th>LC-MS/MS µg/l (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcystin-LR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7 J8</td>
<td>6.3</td>
<td>6.6</td>
</tr>
<tr>
<td>CV%</td>
<td>16.3</td>
<td>15.3</td>
</tr>
<tr>
<td>Microcystin-LR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R7 J8</td>
<td>3.5</td>
<td>3.9</td>
</tr>
<tr>
<td>CV%</td>
<td>24.8</td>
<td>7.3</td>
</tr>
</tbody>
</table>

Results based on visual readings and a spectrophotometric reading are consistent, so long as the range of concentrations a QualiTube™ is able to measure are respected, because the positive results obtained were evaluated and sorted according to concentration intervals. In the field, a spectrophotometric reading is therefore unnecessary for an approximate estimate of the concentration of toxins in a sample, although a spectrophotometric reading does produce more accurate results.

Results using the QualiTube™ were compared to results using an LC-MS/MS to measure free cyanotoxins at two levels of concentration in a cyanobacteria sample cultivated in the laboratory. The results are shown in Table 3.
Average concentrations to measure microcystin-LR (n=4) using the QualiTube™ test were similar to LC-MS/MS results. The relative coefficient of variation is higher in the case of the QualiTube™ test compared to the LC-MS/MS for both concentrations evaluated in the number of samples n=4.

4.2.2 Evaluation of cell lysis methods

The cell lysis methods that were evaluated included ultrasound baths, heating, freezing, addition of hydroquinone and copper sulphate, as well as methanol/water and acetonitrile/water extraction, followed by centrifugation and filtration.

The toxin results for each cell lysis technique evaluated are shown in Table 2. The cell lysis techniques were conducted on cyanobacterial cells cultivated in the laboratory at two different phases of maturity. Depending on the stage of cell maturity, the concentration of free toxins in the water varied significantly. As Table 2 shows, concentrations of free toxins varied according to the growth phase of cells, growth conditions and the restarting of cultures.

The results of trials R1 J7 and R2 J5 show that freezing released approximately 2 to 3 times more toxins than the initial amount of free toxins. In the case of R2 J7, however, freezing did not result in any significant increase of free toxins. The copper sulphate and ultrasound bath show levels of free toxins that are almost identical to those obtained by freezing. We also evaluated cell lysis by boiling samples. Results were similar to those of freezing.

Results obtained through immunoassays using different cell lysis methods, showed that the level of free toxins increased by a factor of 2 to 3. Based on these results, we believe these methods of cell lysis have an efficiency ratio in the range of 10 to 60%. In literature, Pietsch [9] reports that the content of intracellular toxins represents over 90% of total toxins in cells that are less than 10 days old. We also noted that the average intracellular content generally represents 80% of total cyanotoxins when the method developed by the CEAEQ is used on cells at different phases of maturity.

Table 4 shows the results of trials to determine the concentration of intracellular and extracellular toxins using LC-MS/MS. These methods did not yield satisfactory results except for one of the freezing trials using R2 J7 cells. The results of all the freezing trials were not reproducible. They also varied significantly from one trial to another. Cell maturity, number of cells and the volume of a sample were factors that significantly influenced results.

Based on the experimental conditions present and results obtained, these methods cannot be recommended to determine the total concentration of toxins in an environment. During some trials we observed a decline in the number of free or extracellular toxins compared to the initial concentration of toxins present. A decline in the concentration of free toxins is likely attributable to substances that are freed during lysis or to an unidentified chemical degradation process.
Table 4 – Results obtained by LC-MS/MS for the different cell lysis methods evaluated.

<table>
<thead>
<tr>
<th>Methods of cell lysis</th>
<th>Total intra and extracellular toxins µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2 J7 1.03 x 10^7 cell/ml</td>
<td></td>
</tr>
<tr>
<td>Reference method sample</td>
<td>55.3</td>
</tr>
<tr>
<td>Sample frozen one night</td>
<td>61.9</td>
</tr>
<tr>
<td>Frozen sample + 0.032 % CuSO₄ 2 hour wait time</td>
<td>39.9</td>
</tr>
<tr>
<td>R9 J6 4.23x10^7 cell/ml</td>
<td></td>
</tr>
<tr>
<td>Reference method sample</td>
<td>85.7</td>
</tr>
<tr>
<td>Sample frozen one night + hydroquinone</td>
<td>16.4</td>
</tr>
<tr>
<td>Frozen sample 3x + hydroquinone</td>
<td>28.7</td>
</tr>
<tr>
<td>Ultrasound sample + hydroquinone</td>
<td>6.6</td>
</tr>
<tr>
<td>Hot sand 100°C sample + hydroquinone</td>
<td>16.4</td>
</tr>
</tbody>
</table>

Figure 3 shows the effects of ultrasound treatment on the condition and number of cyanobacterial cells after treatment. The number of cyanobacteria declined significantly after treatment, as shown in the right half of the photo. The presence of cellular debris is also detectable.

We also evaluated an extraction–centrifugation method as a possible alternative, which appeared to be more effective than the conventional methods evaluated above. The method was modeled after the extraction procedure described by Song [10]. The following centrifugation tubes were used: Ultrafree-CL LH, 0.45µm, sold by Millipore™. Samples were extracted using a methanol/water (80/20) mixture. The mixture was then centrifuged to recover the extraction solvent. A second extraction was performed adding the same extraction mix. The filtrate was recovered and the methanol evaporated over an argon jet at 22°C. If the extract contained more
than 15% methanol, the real concentration of toxins was under-evaluated. The filtrate was then
directly analyzed using the QualiTube™.

An acetonitrile/water mixture was also assessed to determine its extraction effectiveness. The
methanol/water mix proved more effective for the extraction of intracellular cyanotoxins.

Table 5 shows the results obtained for cell lysis through centrifugation, using a methanol/water
and acetonitrile/water mixture. This cell lysis method was verified using cyanobacteria samples
at different phases of maturity and was compared to the results of the intracellular and
extracellular method used by the CEAEQ.

A test was also conducted on a sample of surface water from a lake containing a mixture of
microcystin-LR, -RR and -YR.

Table 5 – Comparison of cell lysis results obtained by centrifugation using the QualiTube™ and
LC-MS/MS reference method

<table>
<thead>
<tr>
<th>Sample characteristic</th>
<th>Total intracellular and extracellular toxins</th>
<th>QualiTube™ (µg/l)</th>
<th>LC-MS/MS* (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R11 J7</td>
<td>Reference method</td>
<td>–</td>
<td>85</td>
</tr>
<tr>
<td>5.4 x 10⁶ cell/ml</td>
<td>Centrifuged MeOH (80/20)</td>
<td>–</td>
<td>126</td>
</tr>
<tr>
<td></td>
<td>Centrifuged ACN</td>
<td>34</td>
<td>–</td>
</tr>
<tr>
<td>R12 J*</td>
<td>Without treatment</td>
<td>11</td>
<td>–</td>
</tr>
<tr>
<td>12.4 x 10⁶ cell/ml</td>
<td>Centrifuged ACN/MeOH (75/25)</td>
<td>16</td>
<td>–</td>
</tr>
<tr>
<td>R13 J6</td>
<td>R13 J6 Reference method</td>
<td>–</td>
<td>85</td>
</tr>
<tr>
<td>8.0 x 10⁶ cell/ml</td>
<td>R13 J6 Centrifuged MeOH (80/20)</td>
<td>91</td>
<td>82</td>
</tr>
<tr>
<td>R10 J28</td>
<td>R10 J28 Reference method</td>
<td>–</td>
<td>64</td>
</tr>
<tr>
<td>34.0 x 10⁶ cell/ml</td>
<td>R10 J28 Centrifuged MeOH (80/20)</td>
<td>62</td>
<td>54</td>
</tr>
<tr>
<td>Surface water</td>
<td>Reference method</td>
<td>–</td>
<td>18.5</td>
</tr>
<tr>
<td>400,000 cell/ml</td>
<td>Centrifuged MeOH (80/20)</td>
<td>19.4</td>
<td>–</td>
</tr>
</tbody>
</table>

* The reference method is the method used in the CEAEQ laboratory to measure intracellular and extracellular
cyanotoxins.

– Not determined.

The methanol/water mixture proved more effective for extraction of intracellular microcystins.

By using extraction by centrifugation with methanol, the concentration of total toxins was
relatively similar to the concentration obtained using the reference method.
5. CONCLUSION

Of all the cell lysis methods that were evaluated (freezing, heating, ultrasound, biocides and centrifugation), the centrifugation method using a methanol/water extraction yielded the best results to determine total cyanotoxins (intracellular and extracellular). In the case of surface water, the results of this method are equivalent to the CEAEQ method (MA. 403 – Microsys 1.0).

The Envirologix™ QualiTube™ test is a qualitative test that produces an approximate measurement of total cyanotoxin concentrations, subsequent to cell lysis, for the following range of concentrations: < 0.5 µg/l, between 0.5 and 3.0 µg/l or > 3 µg/l. The test cannot directly determine concentrations above 3.0 µg/l. Successive dilutions must be performed to bring concentrations to within the testing range. Results are more accurate when used in combination with a portable ultraviolet spectrophotometer.

The QualiTube™ test is 25 to 100 times less sensitive than conventional methods of liquid chromatography coupled with a mass spectrometer - the method currently in use at the CEAEQ laboratory. The test does not distinguish between variants of cyanotoxins that are present in a sample. The test does not single out variants and therefore cannot be used to evaluate concentrations of cyanotoxins as they compare to a microcystin-LR-based standard or recommendation.

The QualiTube™ test does not directly measure intracellular microcystins. To generate this type of information, the test must be preceded by an efficient cell lysis method to determine the total level of microcystins. The test can be used, however, to measure and examine toxins that cannot be analyzed or detected using conventional methods, since all measurement standards for microcystins are not available.

In summary, over a period of two hours, using the cell lysis method developed by the CEAEQ, followed by measurement using a QualiTube™, it is possible to determine an approximate concentration of microcystins in the environment. The test is a tool that can be used to monitor a body of water affected by cyanobacteria, where a previous characterization has been performed and where the concentration of toxins is above 0.5 µg/l.
REFERENCES


