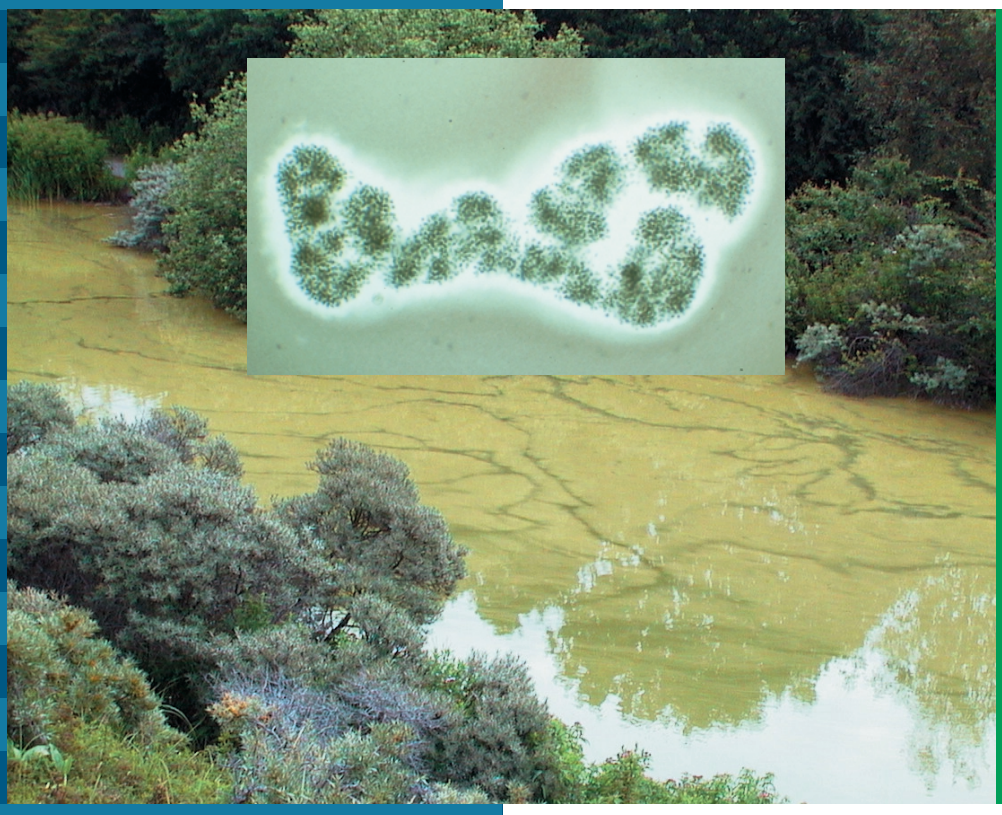
The occurrence of toxic cyanobacteria

in some Dutch surface waters used for the production of drinking water

RIWA
Rhine Water Works
The Netherlands



W. Hoogenboezem, Het Waterlaboratorium Ltd
A.J. Wagenvoort, Evides Ltd
K. Blaauboer, Het Waterlaboratorium Ltd

June 2004

Association of River Waterworks

Contents

1 Summary

A short survey of the history of cyanotoxin research is given as an introduction to the present study. The possible occurrence of cyanobacteria containing cyanotoxins in surface waters used for drinking water production in the Netherlands initiated this RIWA-study. Water samples (135) collected at various locations near drinking water utilities were fortnightly taken in the period May - September 2000. Based on the phytoplankton composition 71 of these samples were analysed for the present of microcystin, and 40 samples were analysed for the presence of anatoxin. At all studied sites intracellular microcystin was detected at least once, measured concentrations ranged from just above the detection level of 0.01 µg/l up to 6.5 µg/l. The relationship between cyanobacterial biovolume and microcystin content showed to be rather variable, although the maximum microcystin content per cell as described in the literature (0.2 pg/cell) is not exceeded in the present study, a few results seem to reach that value closely. Only in seven out of 40 selected samples a detectable amount of anatoxin has been recorded, the highest anatoxin concentration measured is 12.1 µg/l (preliminary results). Evaluation of the phytoplankton analyses showed considerable differences between several laboratories, based on sampling methods, counting and measuring methods. Some suggestions for improvement of the phytoplankton analysis method are given. The obtained results were compared with predictions made in an earlier study (Carpentier et al., 1999[b]) for most of the sampling sites. In a number of cases the actual results of measurements reach close to the theoretical predictions, while in other cases the results deviated more from the predictions. For some water utilities a new evaluation of cyanobacterial load in relation to the treatment procedure may be reconsidered.

2 Introduction

2.1 History

It has been known for many years that certain cyanobacteria, formerly known as blue green algae are able to produce toxins. One of the first papers reporting on toxic cyanobacteria dates back more than 100 years (Francis, 1878), however, only in the last two decades the notion of the importance of cyanotoxins has emerged.

2.2 Cyanotoxins

These toxins are probably the most widespread and are the worst toxins as well since some of these may have carcinogenic effects, even in very low concentrations. The primary target in mammal organs is the liver (table 1). The general mechanism of these toxins is inhibition of the protein synthesis.
Most of these toxins are soluble in water and are unable to penetrate through the lipo-membranes in cells.

Three groups of cyanotoxins have been described. The first group consists of cyclic peptides, such as microcystin (over 60 types) and nodularin, there are compounds able to induce serious liver damage.
The second group of toxins are of the alkaloid type, as produced by a relatively large group of cyanobacteria, affecting different organs in mammals such as nerves (anatoxin, saxitoxin), skin (aplysiatoxin and lyngbiatoxin-a) and the gastrointestinal tract, causing different illnesses.
The third group consists of lipopolysaccharides (LPS) which may affect any tissue exposed to the toxin; apparently LPS are produced by all groups of cyanobacteria (Dow & Swoboda, 2000).
Lipopolysaccharid (LPS) cell wall components are pyrogenic, causing fever. An outbreak of gastroenteritis in Pennsylvania is suspected to have been caused by cyanobacterial LPS (Lippy & Erb, 1976). This seems to be in contradiction with the fact that in a number of countries cyanobacteria are used as a food supplement, e.g. Spirulina spec. is cultured to be sold as dietary supplement for human consumption as health food (Whitton & Potts, 2000), Aphanizomenon is harvested from natural lakes.

Table 1. Toxins of cyanobacteria, produced by toxic strains of species belonging to these genera (after Chorus and Bartram, 1999; genera marked with an asterisk according to Komárek et al., 2001). Not necessarily all members of a genus or species produce toxins.

Toxin group	Primary target organ mammals	Cyanobacterial genera*
Cyclic peptides		
Microcystins	Liver	*Microcystis, Anabaena, Planktothrix (Oscillatoria), Nostoc, Hapalosiphon Anabaenopsis, Aphanocapsa, Romeria*, Chroococcus**
Nodularin	Liver	*Nodularia*
Alkaloids		
Anatoxin-a	Nerve synapse	*Anabaena, Planktothrix (Oscillatoria), Aphanizomenon*
Aplysiatoxins *(Oscillatoria)*	Skin	*Lyngbya, Schizothrix, Planktothrix*
Cylindrospermopsins	Liver	*Cylindrospermopsis, Aphanizomenon, Umezakia*
Lyngbyatoxin-a	Skin, gastro-intestinal tract	*Lyngbya*
Saxitoxins	Nerve axons	*Anabaena, Aphanizomenon, Lyngbya, Cylindrospermopsis*
LPS		
Lipopolysaccharides	Potential irritant; affects any exposed tissue	*All cyanobacteria*

The list of cyanobacteria species that may produce toxins is still growing. For instance Komárek et al. (2001) reanalysed material from the Brazilian disaster with haemodialysis patients. They concluded that at least seven potential toxic species were present in the reservoir sample. These species belong to the following genera: Romeria (new species), Microcystis (two species) Chroococcus, and Aphanizomenon.

The highest concentrations of toxins have been observed in scum layers formed by cyanobacteria during very dense blooms. Concentrations up to 25 mg microcystin per litre and 3.3 mg anatoxin have been reported (Chorus & Bartram, 1999). In the Netherlands however even higher concentrations were observed: 52 mg microcystin per litre in a "scum sample" from the De Gijster reservoir (Wagenvoort et al., 2000).

2.3 Cyanobacteria and drinking water production

These toxins may be of importance for drinking water companies using surface water as source. Chorus & Bartram (1999) mention several incidents with drinking water, which mainly occurred after artificial breakdown of blooms of cyanobacteria. In these cases, large numbers of decomposing cells released toxins in a relatively short period of time. In Brazil 117 patients of 136 became ill and 50 died after dialysis treatment using water containing cyanotoxins (Jochimsen et al., 1998).

Production of drinking water from surface water in the Netherlands is always carried out in treatment plants consisting of multiple barriers, to ensure proper removal of different types of micro organisms. The removal efficiency of toxic cyanobacteria or cyanotoxins is relatively unknown. To estimate the

possible risks with regard to these toxins a worst case analysis study has been done for several types of drinking water plants in the Netherlands (Carpentier et al. 1999[b]). This study was based on:

- The maximum toxin content in individual cyanobacterial cells, as reported in the literature;
- Removal efficiency of various purification steps, used in Dutch surface water treatment plants, obtained from literature;
- Actually measured phytoplankton data were used to estimate theoretical toxin loads at the abstraction points of these purification plants.

In that study it became clear that it is important in what form toxins enter the purification process, free or intracellularly. Some purification steps in drinking water purification plants may remove free toxins better, while others are better designed to remove cells instead of free toxins (Carpentier et al., 1999[b]). Estimations based on these assumptions showed a removal capacity for free toxin, usually varying 2-3 log units. Based on the highest cyanobacteria densities observed in a certain period most of the finished water may contain microcystin concentrations far below the World Health Organisation (WHO) provisional guideline for lifetime exposure (1 µg/l; Chorus & Bartram, 1999). Occasionally, however the estimated worst case concentrations are in the same (0.29-2.9 µg/l) range as the WHO guideline. These results assumed maximal toxin concentrations in each observed cell, the reported estimations are probably an overestimation of the actual toxin concentration. For this reason RIWA (Association of River Waterworks) initiated the current study on the toxic content and densities of toxic cyanobacteria at abstraction points of surface water treatment plants in the Netherlands.
The question of free or inter cellular toxins remains complicated although Chorus (pers. Comm. September, 26 2003), recently suggested that accumulations of toxic cyanobacteria on filters may pose a risk since lysis of these cells can cause unexpected high toxin concentrations.

2.4 Proposed safety levels for human consumption

Due to the lack of sufficient data based on humans and the considerable difference in sensitivity between humans and animals a reliable guideline cannot be given (Dow & Swoboda, 2000). A guideline for maximum daily intake is given in the literature (Chorus & Bartram, 1999) and the World Health Organisation (WHO) indicates that the level of microcystin-LR equivalents should be lower than 1 µg/l (lifetime consumption). Microcystin LR is one of the most toxic types of microcystin, other often less toxic microcystins are expressed as equivalents of microcystin-LR. In some countries the maximum toxin level may be slightly higher (table 2). Short periods of higher concentrations are allowed, however, without specifying the duration of shorter periods. One suggestion for a much lower safety level (0.01 µg/l) is given in a conference poster (Ueno, 1998) and in the literature (Ueno et al. 1999).

Table 2. Quality guidelines related to drinking water (after various sources: Fitzgerald, 2001):
1: proposal by Fitzgerald, 1999 cited in Fitzgerald 2001; 2: no sufficient data for guideline available;
3: proposed maximum level; 4: with dominance of cyanobacteria.

Type of toxin	WHO	Canada	Australia	Short exposure only	Remarks
Microcystin-LR	(µg/l)	1	1.5	1.3	10 1
Nodularin	(µg/l)	-	-	-	10 1
Anatoxin	(µg/l)	-	-	-	- 2
Cyl.spermopsin	(µg/l)	-	-	-	- 2
Saxitoxin	(µg/l)	-	-	3	- 3
Cyanobacteria	(cells/ml)	-	-	2000	20,000
Chlorophyll	(µg/l)	-	-	1	10 4

Well known short term effects of cyanotoxins on humans are gastro enteric problems and liver poisoning. Another short term effect is seen in swimmers and surfers, who suffer from irritation of the skin due to toxic cyanobacteria present in recreational waters. Dow & Swoboda (2000) consider that even short exposure to toxins may occasionally result in long-term injury.
In China, a higher incidence rate with liver cancer is correlated with the use of surface water containing cyanobacteria for the production of drinking water, whereas there is no increased incidence rate for people using deep groundwater as drinking water (Falconer et al. 1994).
Until now studies were mainly focused on the human exposure with microcystin-LR, since most information is restricted to this type of microcystin and because of liver damage induced and the fact that it may promote liver cancer.

2.5 Ecological consequences

Cyanotoxins can influence many different trophic levels in ecosystems: they can affect phytoplankton, plants, zooplankton and fish. The natural breakdown of already formed toxins is very important. Many studies on various aspects have been published, here only a few examples are given.
The toxicity seems to be more severe to terrestrial mammals than to the aquatic fauna, although distinct effects on aquatic invertebrates and fish larvae have been reported (Dow & Swoboda, 2000). Neurotoxins (toxic to nervous system) and hepatotoxins (liver toxins) are the best known toxins of cyanobacteria, but a relatively broad range of cytotoxins (cell toxins) have also been described for various species of cyanobacteria (Dow & Swoboda, 2000).

The ecological triggering for toxin production is still unknown, although it may depend on nitrate and phosphate concentrations, light intensities or temperature effects. A higher nitrogen concentration also stimulates higher toxin production (Heinis, 1994). Another aspect is cyanobacterial density; during bloom of these cyanobacteria more toxins may be produced. At relative low light regimes toxin production is higher.

Lampert & Sommer (1997) report that zooplankton feeding on toxic cyanobacteria may suffer from a reduced growth rate or even die. Copepods selecting individual particles are able to select non toxic cyanobacteria from toxic taxa. Cladocerans only reduce the filtering rate and eject all collected particles when a toxic particle is detected (Lampert & Sommer, 1997). The presence of toxic cyanobacteria may reduce or even stop the filtering activity of cladocerans, sometimes leading to induced changes in zooplankton composition. A number of zooplankton species avoid grazing on cyanobacteria, while some species seem to be unaffected by these toxins. In this way cyanobacteria have a structural effect on the zooplankton community. It should be kept in mind that not all zooplankters are able to forage on cyanobacteria effectively. Larger cladocerans (e.g. *Daphnia pulex*) may consume cyanobacteria but for small planktonic rotifers colonies *Microcystis* are probably too large to ingest. Concerning accumulation of microcystin, Laurén-Määttä et al. (1995) conducted an experimental study demonstrating that the majority of microcystin is metabolised and is not accumulated when toxic *Microcystis* is ingested by *Daphnia pulex* and when the *Daphnia* is ingested by a *Chaoborus* larvae (phantom midge larvae) it appeared that >90 % of the microcystin present in the prey is metabolised.

Although Eriksson et al. (1989) mention that cyanotoxins have also been found in molluscs, accumulation of these toxins seems not to influence the activity of the fresh water mussel *Anadonta cygnea*.

During cyanobacteria blooms fish mortality occurs, gill damage, but also damage of the digestive tract and liver have been observed. Pathologic changes in liver and kidney of carp (*Cyprinus carpio*) are comparable to those found in mammals, including humans. In trout (*Salmo trutta*) growth reduction has been observed due to microcystin (cf Carpentier, 1996).

Bioaccumulation is common in various aquatic invertebrates including zooplankton and fish. In the liver (hepatopancreas) of mussels the highest concentrations of microcystin have been measured. Due to bioaccumulation microcystin may influence the aquatic food web. Although it has not been demonstrated yet, sudden deaths under natural conditions may have been caused by the lack of oxygen, which often occurs when blooms of cyanobacteria break down. The role of cyanotoxins in ecosystems is not yet fully understood, but there are certainly important effects.
There is a strong indication that occasionally Water fowl is affected by cyanotoxins. October 2002 a large number of birds (4300: duck, geese, swans and cormorants) have died in the Volkerrak-Zoommeer, in the South-western part of The Netherlands, during a period of intense bloom of cyanobacteria. Several liver samples have been investigated and high concentrations of toxins have been observed (Zwart, 2003).

Degradation of microcystin by bacteria has been observed by Saitou et al. (2003). A strain of Shingomonas was isolated from a lake and was shown to be able to grow on microcystin as sole carbon source. The breakdown was relatively fast as these bacteria degraded more than 95% of the microcystin in five days. The behaviour of these bacteria with respect to microcystin is unknown, but potentially bacterial breakdown has been demonstrated in this experiment.

2.6 Detection methods for cyanotoxins

The detection methods of cyanotoxins cover bioassays as tests for mouse toxicity and tests using cell cultures or enzyme inhibition tests as protein phosphatase. Chromatographic analysis has improved considerably in the last few years especially liquid chromatography and mass spectrometry (LC-MS). Immunological test procedures have also been developed, using monoclonal or polyclonal antibodies. The first results in the early nineties with these immunological (ELISA) methods were not very sensitive but presently this method is highly sensitive for the detection of microcystins. ELISA results are provided as microcystin equivalents. Once microcystins are detected with ELISA other techniques are required to identify and quantify the toxins more precisely. Therefore the ELISA technique is considered to be very suitable as a pre-screening instrument.

This study primarily aimed to intracellular microcystin and anatoxin, since these two toxins are probably the most relevant for the Dutch situation. This assumption is based on the occurrence of cyanobacteria species producing these toxins in the Netherlands. Determination of saxitoxin has not been incorporated in this study since studies in surface waters (T. Burger-Wiersma pers. Comm. 2000) did not reveal many positive samples. The very toxic cylindrospermopsin, produced by *Cylindrospermopsis* species, for example, is known from warmer areas such as Australia, Africa and South America. Although species of *Cylindrospermopsis* have been observed in a lake near Amstelveen in the Netherlands (Anonymous, 2001), this particular species it is for the time being not expected to be a problem in Western Europe. Moreover the lack of analytical methods for the determination of Cilindrospermopsin and some other rare toxins mentioned in table 1 directed this pilot to the study of microcystin and anatoxin.

3 Materials and methods

3.1 Description of the study area

In figure 1 the investigated raw water intake locations in the Netherlands are depicted. In Appendix I some more details are given of the individual locations and some characteristics of these locations are listed.

Table 3. Institutional abbreviations, and sample point codes (second column) used throughout this report, letters in the third column refer to figure 1.

DZH		Dune water Company South-Holland Ltd
DZH-I	B1	Intake point at Afgedamde Maas
DZH-II	B1	Gravel pit at the Afgedamde Maas
DZH-III	B2	Three (a, b, c) infiltration canals in the dune area
GWA		Drinking water Company of Amsterdam
GWA-I	D	Open reservoir of recharged dune infiltration water
PWN		PWN Water Supply Company North Holland Ltd
PWN-I	C	Reservoir retaining water form Lake IJssel
WBB		Water storage company Brabantse Biesbosch Ltd
WBB-I	A	Intake from the River Meuse at Keizersveer
WBB-II	A	At the De Gijster reservoir
WBG		Drinking water company Groningen
WBG-I	E	At reservoir De Punt
WRK		Water transport company Rijn-Kennemerland Ltd
WRK-I	C	WRK intake Lake IJssel
Vitens		Drinking water company Overijssel Ltd (formerly WMO)
Vitens-I	F	Intake canal Twente
Vitens-II	F	Reservoir Weerselooseweg

Fig. 1. Sampling sites: A: WBB-I-II; B1: DZH-I-II; B2: DZH-III; C: PWN-I and WRK-I; D: GWA-I; E: WBG-I and F: Vitens-I-II (for abbreviations see table 3).

3.2 Sample collection

Samples were collected fortnightly in the period May – October 2000, using various techniques. Different sampling methods may have influenced the results; therefore the applied methods are listed in Appendix I.

3.2.1 Sample collection of deep waters

Several samples were taken with a 2 l Ruttner Sampler (Hydrobios, Kiel) at 2 m or 4 m intervals from the surface to the bottom at one or more pelagic sites. The individual samples were pooled and sub-samples were taken for determination of the chlorophyll-a content, the phytoplankton composition and the amount of microcystin and/or anatoxin, present in cyanobacteria.

3.2.2 Sample collection of rivers and shallow waters

If possible the samples from rivers were taken directly from the transport main at the abstraction site, details on the collection sites are given in Appendix I. In the other cases and the collection sites at shallow waters, samples were taken by immersing a bottle, bucket or Jerry can just beneath the surface.

3.3 Sample transport and conservation

Samples were transported at a temperature of 4°C.

3.4 Toxins of cyanobacteria

3.4.1 Microcystins (intracellular)

It is necessary to indicate that High Pressure Liquid Chromatography (HPLC) is not the most appropriate method for the determination of microcystin or anatoxin. A relative high detection level and occasionally rather large variation in duplicate determinations occur. In this determination method microcystin peaks are identified on characters exclusively specific for that compound. All different peaks meeting the

characteristics for microcystin are summed and used to calculate the total concentration of microcystin in the sample. This method is based on comparisons of microcystin-LR only and the results were not confirmed by mass spectroscopy (MS). However, since no relative easy other methods were available at that time this method has been used for our samples.

This method determines the amount of microcystin present in cyanobacteria, free toxins are not included in this measurement. These measurements are based on Lawton et al. (1994) and were carried out by AquaSense.
A quantified volume of sample water was filtered through a GF/C glass fibre filter and stored frozen at -18°C until the moment of further processing. To extract cell bound toxins the filter was thawed out and frozen several times, before actual toxin extraction started. To the filter a volume of 5 ml methanol was added after which the solution was ultra-sonicated in ice-water (0°C) for 60 minutes. The extract was air dried. The residue was eluted in 400 µl methanol and centrifuged (4000 rpm; 3245 G) for six minutes and stored in a chromatographic vial. The concentration of all types of microcystin together was determined and the concentration of microcystin-LR concentrations were measured using an HPLC-system (Shimadzu, 10A with photodiode array UV/visible detector and a Waters µBondapack C18 column (length 300 mm and internal diameter 3.9 mm), with guard column). In every series an external calibration standard of microcystin (microcystin LR) was measured. In previous studies the recovery of this method was determined at over 99% (J. Postma, AquaSense, pers. comm., January 2002). The lowest detection level of this method appeared to be 0.01 µg microcystin/l, when one litre samples were analysed.
In this survey several double and triple filters of the same sample were analysed (appendix II). The standard deviation increased with the average measured toxin concentration. The relative standard deviation was 56%. This value has the same magnitude as the quality control. The Shewart-chart of the internal standard has a standard deviation of 17%.

3.4.2 Anatoxins (intracellular)

A quantified volume of sample water was filtered through a GF/C glass fibre filter and stored frozen at -18°C until the moment of further sample processing. To the filter a volume of 5 ml methanol (hydrochloric acid (HCl), 1%) was added and 30 minutes ultra-sonicated in ice-water (0°C). The filter parts were removed by centrifugation (4000 rpm; 3245 G) during 10 minutes. The supernatant was air dried at 50°C. The dried sample was eluted in 400 µl (70% water, 30% Acetonitril, 0.5 mM SDS (sodium dodecyl sulphate) and 5 mM potasiumdihydrophosphate (KH_2PO_4)). The elute was sonicated 10 minutes and centrifuged (10 minutes 4000 rmp; 3245 G) and divided over two chromatographic vials (one backup sample). Anatoxin concentrations were measured using an isocratic HPLC-method according to Jefferies et al. (1994) and Zotou et al. (1993) using an HPLC-system (Shimadzu, 10A with photodiode array UV/visible detector and Chrompack Hypersil BDS5 C18 column (length 150 mm and internal diameter 4.69 mm)) with anatoxin-a as external standard. The detection level was 1 µg anatoxin/l, when one litre sample was analysed

3.5 Chlorophyll-a

Chlorophyll-a concentrations were determined according to the Dutch Standard Method (warm ethanol-extraction) (NNI, 1981).

3.6 Phytoplankton

Samples taken for microscopic enumeration were preserved with Lugol's iodine solution as soon as possible after sample collection.

3.6.1 General phytoplankton composition

Direct counting of preserved phytoplankton was typically carried out using a sedimentation counting chamber (2 to 100 ml) and an inverted microscope as described by Utermöhl (1958). Counting cyanobacteria involves defining the units to be counted, because the majority of planktonic cyanobacteria are presented as filamentous or colonial forms consisting of a large number of cells which are often difficult to distinguish. In appendix III the definitions of the units per genera per laboratory are listed. Cyanobacteria were identified to the genus level or species when possible.

3.6.2 Specific methods for determining cyanobacteria

The WRK- and PWN-laboratories extended the sedimentation method, by applying hydrostatic pressure to collapse the gas vesicles in order to accomplish better sedimentation of species containing these gas vesicles.

At the WBB-laboratory a special enumeration technique adapted for cyanobacteria (mainly colonies of Microcystis) was used for the reservoir samples during the season (June - November) according to Box (1981) a 500 ml sample was filtered and resuspended in 20 ml potassiumhydroxid solution (KOH, 0.1 g per l). Disintegration of colonies was achieved by alkaline hydrolysis (80°C during 30 minutes and continuous shaking, 200 rpm), followed by 60 seconds of intensive (vortex) mixing. This method usually separates cells adequately. Hydrostatic pressure was used to collapse the gas vesicles. After sedimentation the sample was quantified using an inverted microscope, at a magnification of 400 times. Cyanobacteria were identified to the genus level or species when possible.

3.6.3 Biovolume

The biovolumes were measured according to internal guidelines (appendix I).

The biovolumes of the individual measurements were log-transformed to accomplish a normal distribution. From these data the geometrical mean per genus per sample was calculated. In addition, the upper and lower 95% confidence limit level of the biovolume (µm3/n) for each genus per sample was calculated using the BACCHUS-program (Carpentier, et al. 1999[a]).

3.6.4 Calculations

The counted number per genus in each sample was used to determine the confidence level of the enumeration according to Schwoerbel (1986). These data were used to calculate the lower and upper limit at a 95% confidence level of the count per genera and these counts were transformed to density (n/ml). The densities and biovolume were multiplied resulting in a mean (mm3/l) and its upper and lower limit of the 95% confidence level of the analysis per genus per sample

4 Results

4.1 The occurrence of microcystin

At several water intake sites distributed over the Netherlands, phytoplankton samples were taken in the period May - October 2000. Phytoplankton counts were conducted on these samples. Samples containing larger numbers of cyanobacteria were selected for further investigation for microcystin and anatoxin (See Appendix IV). Since only 71 samples, containing higher cyanobacteria densities, were selected out of 135 samples the results do not represent a natural distribution. Several selection criteria have been applied. Firstly all sampling sites had to be included to obtain information on cyanobacteria collected at all Dutch water intake sites. Secondly, the number of cyanobacteria in the samples played an important role in the selection process. In 86% of the samples a detectable concentration of microcystin was observed and in 42% of the studied samples the microcystin concentration was 1 µg/l or more. In only 14% of the samples no detectable (<0.01 µg/l) concentration microcystin was obtained. The total amount of microcystin varied from non detectable <0.01 µg/l to 6.5 µg/l (Table 4).

Microcystin-LR which is considered to be one of the most toxic types of the more than 60 different types of microcystin. This special type of microcystin has been observed in samples taken from two dune infiltration canals (DZH-III, table 4).
Microcystin has been detected at all sample sites, but not in all samples investigated. The microcystin concentration varied considerable at different sites, probably due to very different types of water (reservoirs, infiltration ponds etc., see appendix I). For instance at the sampling site along the River Meuse, at the intake of the WBB reservoirs low numbers of algae and cyanobacteria were counted. At locations where water is more stagnant usually higher cyanobacteria concentrations are observed.

Table 4. Concentrations of microcystin total (µg/l), microcystin-LR, and anatoxin; n = number of samples (abbreviations see table 3).

Sampling site	Microcystin			Microcystin-LR			anatoxin		
	n	range	Mean	n	Range	Mean	n	range	Mean
WRK-I	12	1.41 - 6.51	4.12	12	<0.01	<0.01	12	<1 - 12.1	1.0
PWN-I	11	0.20 - 1.29	0.74	11	<0.01	<0.01	2	<1 - 1.07	0.56
WBB-I	5	<0.01 - 0.03	0.01	5	<0.01	<0.01	0	-	-
WBB-II	9	0.27 - 0.66	0.43	9	<0.01	<0.01	3	<1 - 2.38	0.48
Vitens-I	8	<0.01 - 6.13	1.50	8	<0.01	<0.01	8	<1 - 6.22	1.86
Vitens-II	8	<0.01 - 1.43	0.38	8	<0.01	<0.01	8	<1	<1
DZH-I	1	2.92	2.92	1	<0.01	<0.01	0	-	-
DZH-III(a)	3	2.59 - 5.21	3.5	3	0.34 - 2.37	1.67	1	<1	<1
DZH-III(b)	3	0.49 - 3.87	1.79	3	<0.01	<0.01	0	-	-
DZH-III(c)	2	1.01 - 4.36	2.69	2	1.01 - 2.23	1.62	2	<1	<1
DZH-II	1	0.64	0.64	1	<0.01	<0.01	0	-	-
GWA-I	2	<0.01 - 0.44	0.22	2	<0.01	<0.01	1	<1	<1
WBG-I	7	<0.01 - 2.50	1.07	7	<0.01	<0.01	1	2.27	2.27

4.2 The relation between cyanobacteria and the concentration of microcystin

This study focussed on cyanobacteria containing intracellular toxins microcystin or anatoxin, for each sampling site the occurrence of these algae will be described shortly before toxicity data are evaluated. The presence of microcystins dissolved in water was not determined in this study. Therefore it is important to determine whether or not there is a constant relation between the presence of cyanobacteria, measured as biovolume (mm3/l) and intracellular microcystin in the sample or not. These values are plotted in chronological order for each sample point to show possible seasonal patterns (Appendix VI). Samples from Lake IJssel (WRK-I) showed mostly higher values, in fact the highest biovolumes were observed at this site. The shallow Lake IJssel is an ideal environment for cyanobacterial growth. A seasonal pattern is visible showing the highest biovolumes in late summer. The plots for this site (appendix VI) show strongly variable values for microcystin.
The PWN-reservoir is a deep (25 m) reservoir supplied with Lake IJssel water and retains the water for about seven to eight weeks. Artificial circulation in this reservoir is generated to prevent growth of cyanobacteria and other phytoplankton species. This measure is successful since the biovolume of cyanobacteria is distinctly lower than that measured in Lake IJssel. All investigated samples contained microcystin varying from 0.20 – 1.29 µg/l (table 4). The relative position of sampling points differs considerably between WRK-I and PWN-I, the sampling point at the IJssel Lake (WRK-I) is located in the littoral zone, whereas the reservoir is sampled more or less at a point close to the centre (pelagial) of the reservoir. A distinct reduction of potential microcystin containing cyanobacteria is achieved in this reservoir. The biovolume of the counted cyanobacteria in Lake Ijssel was usually higher than in the PWN reservoir, this variation is reduced by the above mentioned artificial circulation, moreover some of the variation may be caused by differences in meteorological circumstances such as wind. Wind will have a distinct smaller effect on the smaller surface area in the reservoir compared to the large surface area of Lake IJssel. The amount of microcystin also changed much more in Lake IJssel than seen in the reservoir samples. The amount of microcystin related to the biovolume is quite different at these sampling points. Relatively low microcystin content in samples with high biovolume and occasionally high concentrations in samples with poor cyanobacteria numbers may indicate a varying microcystin content and/or may be due to a different community composition of toxic and non toxic cyanobacteria (appendix VI). The cyanobacterial composition changes form a Microcystis dominated Lake IJssel water to a more Aphanotheca and Aphanocapsa dominated water in the PWN reservoir. Due to a large variation no clear seasonal pattern is discernible in the plots for these sites.

At the Brabantse Biesbosch the concentration of cyanobacteria is very low at the abstraction point (WBB-I) in the River Meuse. In the stagnant reservoir De Gijster (WBB-II), in spite of artificial circulation, the biomass of cyanobacteria is much higher (Appendix VI). As expected for a site (WBB-I) without high cyanobacterial densities microcystin was hardly detectable in the samples (<0.01 – 0.03 µg/l). In spite of relative high cyanobacteria densities rather low microcystin concentrations (0.27 – 0.66 µg/l) were measured at De Gijster reservoir (WBB-II). In reservoir De Gijster the strongest cyanobacteria development of the three reservoirs occurs, the biomass of cyanobacteria decreases in reservoir cascades (Wagenvoort et al., 2000). In this reservoir a distinct seasonal pattern is visible, in the period with higher cyanobacteria concentrations (August and October) a limited and more or less constant amount of microcystin is seen in the samples (Appendix VI).

The shallow intake area at the Twente Kanaal (Vitens-I) and the reservoir (Vitens-II) both contain a variable cyanobacteria biomass. Seasonal variation cannot be recognised in these data. The microcystin concentration in the samples appeared also to be variable. It is remarkable that two samples (July and August) with a relatively small number of cyanobacteria contained a very high microcystin concentration (Appendix, VI). The cause of these high values cannot be explained from the present data, perhaps

inaccuracy in phytoplankton sampling or enumeration may have caused these unexpected results. The contents in samples from the reservoir (Vitens-II) are slightly lower. There are no obvious seasonal patterns visible in these graphs, although the highest microcystin values are here also recorded in July, August and September.

For DZH several sampling points were measured. The gravel pit (DZH-II), an artificial deep pit (20 - 25 m) with predominantly stagnant water, situated in De Afgedamde Maas showed a higher cyanobacterial biomass in the summer period. At this site a single measurement revealed a concentration of 3 µg microcystin per litre. At the end of the Afgedamde Maas at Brakel, the surface water abstraction point, a rather variable number of cyanobacteria were seen and a single toxin measurement conducted here showed a relative low microcystin concentration (Appendix VI). In the dune infiltration canals (DZH-III) an increasing cyanobacterial biomass and microcystin concentrations were measured (Appendix VI). The toxin concentration varied; occasionally well above 5 µg microcystin per l (table 4). These canals were the only locations where the more toxic microcystin-LR has been detected in this study (table 4 and Appendix VI). Apparently special circumstances for the development of cyanobacteria producing this type of microcystin are present in these canal systems. Although not all canals develop blooms of cyanobacteria, an investigation of the special circumstances triggering blooms in certain canals is needed. Recharged infiltrated water in the dunes of GW was sampled twice for microcystin detection. Only a single sample contained detectable amounts of microcystin (0.44 µg/l), no microcystin-LR has been identified in that sample.

Reservoir De Punt (WBG) was taken in service recently (1997). The cyanobacterial density is still relatively low but increased compared to several year ago (Carpentier et la. 1999[b]). This relatively new reservoir ecosystem is assumed to be still unstable. Once a more stable situation in reached a higher cyanobacterial biomass is expected to occur. As can be seen in appendix VI there is a rather diverse phytoplankton community. Only a single sample in the period August – October was taken and this particular sample showed a higher biovolume. Microcystin concentrations in the samples varied from <0.01 to 2.5 µg per litre (Appendix VI).

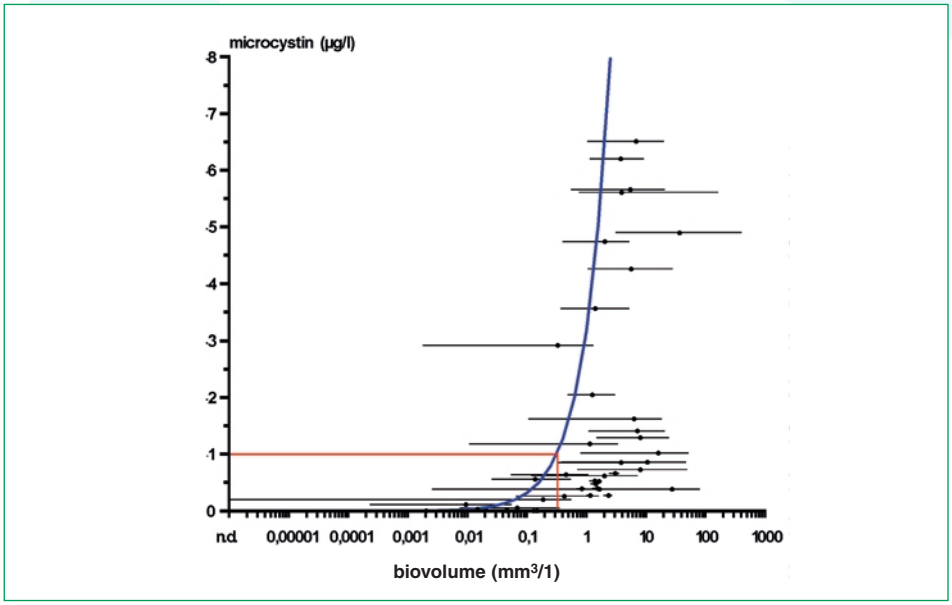
4.3 Microcystin contents and signal value

From the biovolume – microcystin plots it is concluded that no uniform pattern is visible. At some locations there is a more or less constant relation between biovolume and microcystin content, whereas at other sites a random variation seems to occur. The median ratio between microcystin (µg/l) and cyanobacterial biovolume (mm3/l) varied in the WRK reservoir from 0.1 to 2.6 and in the PWN reservoir from 0.01 – 1.1. The highest median ratio of 3.12 in Lake IJssel contrasts to the lowest (0.2) from the PWN-reservoir. It is remarkable to see that when Lake IJssel water is retained for several weeks in a deep artificially circulated reservoir the median ration drops from 3.12 to 0.01. Comparing the median ratio observed at various collection sites show quite different results, although the water may have come from the same origin. This variation indicates that storage conditions strongly influence cyanobacteria concentrations, it is not clear whether these conditions also influence the toxin production per cell or not.

In order to evaluate the degree of toxicity of the Dutch cyanobacteria, our values are compared to microcystin contents reported in literature. Average contents of 0.20 pg microcystin per cell and a maximum of 0.47 pg per cell (average diameter 3.5 µm) have been reported (Falconer et al., 1999; Wagenvoort et al. 2000). In figure 2 these data are plotted together with signal values of 1 µg per litre and 5000 cells per millilitre. Not each cyanobacterium cell is expected to contain toxin, therefore a wide range of results may occur. Based on this assumption it is expected that the results will be almost entirely right or under the curve in figure 2. Only three of the samples seem to possess more toxin than expected from

literature data. Evaluation of the collected phytoplankton data indicates that some of these analyses may have a lower reliability for instance due to the poor sampling method, sampling by immersing a bucket in the water may overestimate the actual phytoplankton density, since cells from scum layer will dominate the sample. These less reliable results were left out and the remaining data were plotted in figure 2, the cyan bars in figures in Appendix VI represent these less reliable results. All data are placed right of the model line or its confidence limits reach the model line, indicating that the Dutch situation fits to the model that individual cells probably don not contain more than 0.2 pg microcystin per cell. However, the plot shows in some samples cyanotoxin contents may reach relatively high values.

Fig. 2. Biovolume (confidence bars 95%) in relation to the observed microcystin concentration (n = 44), compared to the theoretical expected microcystin concentration when each counted cell contains 0.2 pg per cell (blue line). The red horizontal line indicates the maximum 1 µg microcystin per litre and the vertical (red) line the alarm value of 5000 cell per millilitre. Bucket samples with less reliable biovolume estimations were left out.



Within the selection of 71 samples containing high numbers of cyanobacteria only 26 (36%) contained a biovolume above the alarm value. The alarm value is based on the assumption that each cyanobacterial cell (c. 60 µm³) contains 0.2 pg microcystin; 5000 cells/ml therefore contain 1 µg microcystin per litre. Expressed in biovolume the average biovolume of 5000 cell equals 0.33 mm³/l.

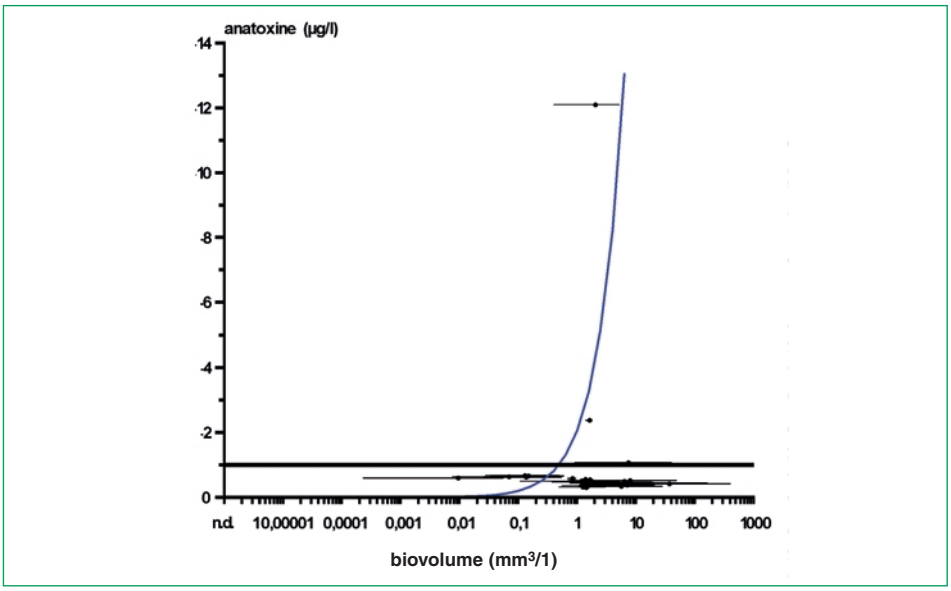
4.4 The occurrence of anatoxin

In 40 samples the numbers of *Aphanizomenon*, *Anabaena* and *Planktothrix* were high enough to expect a detectable amount of anatoxin. Only seven of these samples contained detectable concentrations of anatoxin. The concentrations in these positive samples are relatively high, since concentrations up to 12.1 µg/l (table 4) have been measured.

As the water of Lake IJssel (WRK-I) contained relatively high numbers of *Aphanizomenon* and *Anabaena*, twelve samples were selected for anatoxin measurements. Only in one of these samples (October 18) a rather high concentration (12.1 µg/l) of anatoxin was measured, indicating that phytoplankton counts do not represent a reliable estimate for anatoxin detection. The fact that one of the last samples in the series contained anatoxin initiated the question in what period the other positive samples were found. The seven positive samples were collected in June, July, September and October thus there is no indication for anatoxin production in a certain period of the year.

No samples from the River Meuse (WBB-I) were tested for anatoxin and the Gijster reservoir (WBB-II) showed only one positive sample (2.38 µg anatoxin per litre) out of three investigated samples.

Fig. 3. Biovolume (mm3/l) mass of all cyanobacteria (confidence bars 95%) in relation to intracellular anatoxin content (n = 23). Horizontal line: detection limit (1 µg/l). The theoretical blue line indicates the relationship at 4.4 mg anatoxin per gram dry weight (according to Sivonen et al., 1989); excluding less reliable surface (bucket) samples.



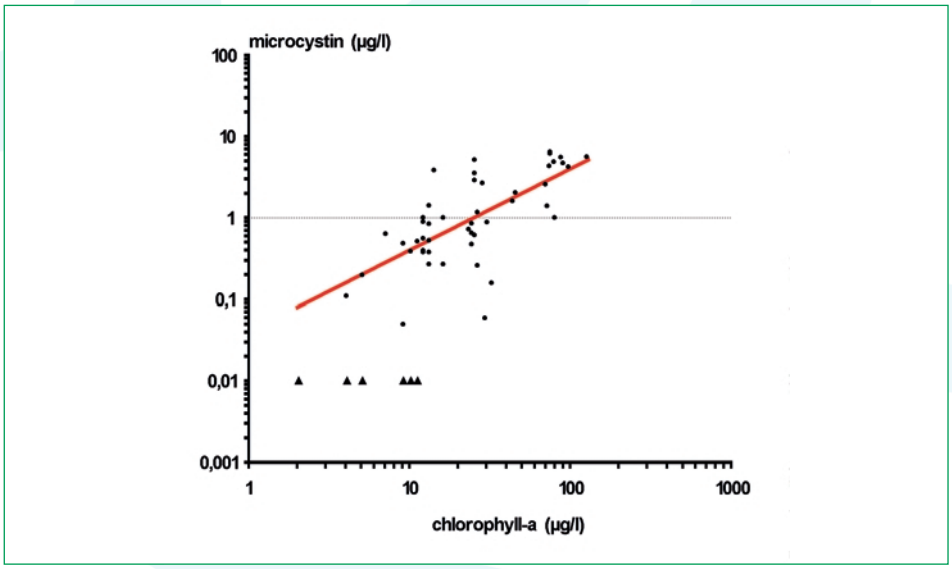
At the Vitens abstraction point (Vitens-I) three samples contained, compared to other locations, high anatoxin concentrations (3.91 – 6.22 µg/l), while five of the selected samples did not contain detectable amounts. In none of the eight selected samples of the Vitens-reservoir (Vitens-II) anatoxin was observed.

For DZH three tests were carried out in samples from infiltration canals (DZH-III), in none of these anatoxin was detected (appendix VI). The same holds for a single sample from GWA-I (table 4). In one reservoir sample at the reservoir De Punt (WBG-I) 2.27 µg anatoxin per litre was measured (Appendix VI). In order to compare the observed anatoxin concentrations with data from literature a theoretical line was constructed based on a high actual observed anatoxin concentration. The highest anatoxin concentration observed was 4.4 mg anatoxin per gram dry weight (Sivonen, 1989). In figure 3 all data based on the right sampling method and on reliable estimations methods are plotted showing our high values to be close to the model curve.

4.5 Chlorophyll-a measurements

As a measure for the total amount of phytoplankton in the samples the chlorophyll-a content was determined in all samples. This method includes all phytoplankton taxa and will be useful especially in combination with microscopic counts. Biomass estimation using chlorophyll-a contents is based on a much larger sample volume than in case of phytoplankton counts.

Fig. 4. Relation between chlorophyll-a and microcystin concentrations in all studied samples (p <0.001). Dashed line shows the 1 µg/l microcystin concentration, solid triangles indicate none detectable concentrations.



As can be seen in figure 4, this study has shown a positive correlation between the chlorophyll-a content and microcystin concentrations. It is stressed that the reliability decreases when the phytoplankton community contains higher densities of other chlorophyll-a containing species such as green algae etc. From this graph it may be deducted that chlorophyll-a concentrations of 5 µg/l may already contain detectable amounts of microcystin and oat the level of 12 tot 15 µg chlorophyll per litre concentrations of 1-4 µg microcystin may occur.

5 Discussion

5.1 Variable microcystin concentrations

At all sample sites microcystin has been measured. The concentrations, however, appeared to be rather variable. There is no clear relationship between biomass of potentially toxic cyanobacteria and the actually measured concentrations of microcystin, although almost all samples with reliable biomass estimation contain a microcystin concentration below the theoretical lines (fig. 2). From the present data it is not clear whether this variation is caused by fluctuations in numbers of toxic and non-toxic cells in the populations of the various species. Or those variations are due to fluctuations in microcystin content in the cells or to the physiological state of the cells. Variations may have originated from variation in the determination methods as well, or from matrix effects which may cause deviations in the results.

5.2 Intra- and extracellular toxins

An aspect not covered in this study is that toxins can also be dissolved in the water, especially after breakdown of a cyanobacterial bloom. The toxin detection method used here did not include extracellular toxins since they are washed out and lost during the filtration phase and the preparation of the sample. Studies carried out in several cell cultures demonstrated that only in young slowly growing cultures all formed toxins remain intracellular. In slowly growing older cultures 20 – 30 % of the toxins is extracellular. In old cultures with decaying cells up to 70% of the toxin leaks out of the cells (Chorus & Bartram, 1999). Thus, measurements based on a filtration step focussing on intracellular toxins will, under certain circumstances, underestimate the actual total toxin content of the sample.

5.3 Determination of numbers and biomass

Komárek (1999) suggests that especially the smaller cyanobacteria species may have an important contribution to the amount of toxin in samples. The counting procedure may also have an important influence on the results, especially in samples containing colonies of toxic cyanobacteria. Such colonies consist of a large number of individual cells but have a relatively small chance of being observed in the microscopical survey, because only a small sample volume is examined. Densities of very large Chroococcal colonies are usually low; a number of 100 colonies per litre is a considered to be a high density, considering that much less than a millilitre is counted. There is a very small chance of encountering a colony. Once a colony is observed the estimation of the number of constituent cells is very difficult and rather inaccurate. Since the morphology and cell numbers in different colonies is quite variable and, additionally, it is not possible to estimate the number of cells in the third dimension of the microscopical image. Consequently, the actual number of cells may be strongly underestimated when no colonies are encountered in the survey. On the other hand, when a colony is observed the cyanobacterial density will probably be overestimated. Such factors may have influenced the present data. At the WBB–laboratory, a procedure of disintegration of colonies in a large volume has been applied, consequently the cells of the colonies are distributed more randomly in the counting chamber resulting in a much more accurate counting of the more uniform units (single cells instead of amorph colonies), as can be seen in the much smaller confidence limits in Appendix VI.

Evaluation of the present counting data resulted in a distinction between more and less reliable phytoplankton enumeration. Moreover, the sampling methods appeared to be different at various locations.

For instance the use of a bucket for sampling has a disadvantage that floating cells or colonies or even cells in scum layers may become over-represented in the samples, with probably a large variation between duplicate samples. It is better is to sample below the water surface with special devices such as Ruttner of Friedinger samplers. It is obvious that sampling methods have influenced our results. This is illustrated (Appendix II) by the fact that in particular "bucket samples" had to be excluded due to less reliable results. Especially a number of low biomass and high toxin concentration samples appeared to be unreliable. Identification of cyanobacteria needs more attention. Until now drinking water laboratories identified cyanobacteria to genus level. More frequent analyses and detailed identifications may help to discover the actual species causing the toxin problems in raw waters. The present study has shown that a large number of cyanobacteria not necessarily mean high amounts of toxin. Especially anatoxin was not determined in all samples counting high numbers of Anabaena, Planktothrix or Aphanizomenon cells.

5.4 Biomass estimation using chlorophyll

Inaccurate counting occurs when only a small portion of the samples is investigated, as illustrated by the results of the phytoplankton counts in several laboratories. However, short confidence limits measured by the WBB laboratory show that special care for the phytoplankton analyses can improve the results considerably. One of the aspects is disintegration of colonies providing a more homogeneous distribution of phytoplankton cells. Biomass determinations with chlorophyll analysis are based on much larger sample volumes, up to 500 ml. A disadvantage is that occasionally large portions of the chlorophyll-a content can originate from groups of non-toxic algae, such as green algae (Chlorophyceae). However, in cyanobacteria dominated samples chlorophyll-a is probably a better alternative for biomass estimation. New techniques such as fluorescent pigment analysis or phytoplankton pigment analysis using HPLC-techniques (Ietswaart et al., 2000) may give better estimates for cyanobacterial biomass than chlorophyll-a determination, since these techniques can discriminate between various groups of phytoplankton. The best approach is probably a procedure using improved microscopical counting techniques to obtain taxonomic information on the community structure followed by an analysis of the biomass using chlorophyll or one of the other additional techniques.

5.5 Implications for drinking water production

Carpentier et al. (1999[b]) estimated the theoretical raw water contents of cyanotoxins in the Netherlands in a worst case risk analysis based on counted numbers of cyanobacteria and the assumption that each cell contains the maximum known toxin concentration (table 5). From this it is expected that actual measurement will be lower than the worst case estimates, which is the case for the microcystin samples of DZH-III, PWN-I and WRK-I. However, in a number of cases the range of microcystin concentrations is comparable (WBB-II, GW-I) to the worst case situation and in some cases the measured microcystin concentration is higher than in the worst case (WBG), water samples from De Punt contained hardly any Microcystis cells. For anatoxin only two locations were mentioned in the worst case analysis, at the WRK the concentration is slightly higher than the lowest worst case estimation. A single studied sample from the reservoir De Punt (WBG-I) contained 2.27 µg anatoxin per litre, which is larger than the worst case estimate (table 5).

Table 5. Comparison between worst case estimations of toxin concentrations (Carpentier et al., 1999[b]) and measured concentrations in selected samples in the year 2000. Selection of the samples was based on high numbers of potentially toxin producing cyanobacteria. For some companies all samples were lumped together to compose a single range (all sites); a dash indicates that no worst case estimate was available.

Microcystin	**Anatoxin**			
	µg/l	µg/l	mg/l	µg/l
	Worst case	Measured	Worst case	Measured
DZH	9.2 - 261	0.49 - 5.21	-	< 1
GW	0.14 - 0.52	< 0.01 - 0.44	-	< 1
PWN	4.7 - 61.2	0.2 - 1.29	-	< 1 - 1.07
WBB	0.08 - 0.61	< 0.01 - 0.66	-	< 1 - 2.38
WBG	0.3	< 0.01 - 2.5	0.2 - 1.0	2.27
Vitens	-	< 0.01 - 6.13	-	< 1 - 6.22
WRK-INY	10 - 137	< 1.41 - 6.51	7 - 87	< 1 - 12.1

The results of some of the present measurements were unexpectedly higher than predicted in the worst case analysis (Carpentier et al., 1999[b]). This is a remarkable result, since the impression was that the development of cyanobacteria in 2000 did not lead to high biomass, probably due to a rather poor summer period in that year. When worst case values are already exceeded in a period with relatively low biomass a new evaluation of this analysis is appropriate. A new worst case analysis for the sites of Vitens and reservoir De Punt (WBG) may be considered. It is therefore advised to continue the measurements to estimate the normal development of potentially toxic cyanobacteria in the various raw waters, monitoring using more detailed identification and quantification methods e.g. measurements using the Optical Plankton Analyser (OPA), using refined and standardised techniques. Toxin measurements are monitored over the cyanobacterial growing period, so that not only "presumed" worst case samples are measured.

Although removal of these toxins during water treatment has been described in several studies (see for references: Carpentier et al., 1999[b]; Chorus et al., 2001) many aspects of removal are still unknown. In particular quantitative information on the removal of toxins in different processes is required. Data on the removal during micro straining, rapid sand filtration, coagulation, slow sand filtration etc. is described. But the removal during bank filtration (Chorus et al. 2001) or artificial recharge of infiltrated surface water much less information seems to be available. Cell bound or dissolved toxin load require different purifications principles. For the evaluation of possible threats for the drinking water production in some purification plants a special analysis is needed to make sure that toxin removal is indeed adequate. While in other situations in which the toxin load is much lower, there is apparently no need for further analysis.

6 Conclusions and recommendations

- Microcystin containing cyanobacteria are widely distributed in the Netherlands, and were found at all sampling points in this study. The (intracellular) microcystin concentration varied from <0.01 – 6.51 µg per litre.
- Anatoxin containing cyanobacteria seem to occur at a slightly lower level in the studied samples but occasionally relatively high anatoxin concentrations were measured (<1 – 12.1 µg/l).
- The ratio between the biomass of cyanobacteria and the toxin concentration is variable. Factors influencing this variation were not analysed. This observation indicates that estimation of the toxin risk based on phytoplankton counts is relatively unreliable. Based on a relatively small number of samples it seems that samples containing less than 5000 cells per ml will not exceed the WHO-guideline (for intracellular toxins additional regulations.
- The present study shows that phytoplankton estimations, based on counting small sample volumes containing cyanobacterial colonies occasionally may give unreliable results.
- For more detailed information on the occurrence of toxic cyanobacteria in water used for the production of drinking water it is necessary to optimise the sampling and enumeration methods. Since counting methods usually are based on small portions of the sample, they are prone to errors. The methods applied by WBB where colonies are disintegrated and biovolumes are measured significant better results can be obtained. When cyanobacteria dominate in a sample the chlorophyll-a content may be a fairly good alternative for biomass estimation. In the near future, modern fluorescent techniques for determination of certain pigments may appear even a better method for biomass estimations or the use of the optical plankton analyser as long as larger sample volumes are used. Especially cyanobacteria are vulnerable for counting errors given the fact that these organisms possess gas vesicles enabling them to form floating scum layers. These layers influence the sampling procedure strongly.
- It is stressed that microscopical identification and counting remains an important part of the procedure, in order to identify possible toxic cyanobacteria.
- In the present study a selection of samples was investigated, this selection was based on the number of potentially toxic cyanobacteria present in the sample. Since it became clear that their numbers or biomass is not the most reliable method to estimate possible toxin risk it is recommended to study toxin contents in all samples, at least containing some potentially toxic cyanobacteria during the growing season May - October.
- Investigations to removal efficiency of cyanobacteria and their toxins in purification plants are necessary, for both intracellular and free toxins.

Acknowledgements

Lab technicians of the various laboratories are kindly acknowledged for their cooperation in counting the samples, identifying the cyanobacteria and preparing the data for this report: drs. Henk Ketelaars (WBB), ing. Eric Penders (Het Waterlaboratorium) ir. Hans Smeenk (WLB), drs. Petra Scholte (WLB) were kind enough to read the manuscript critically and provided us with valuable suggestions. Some colleagues participated only shortly in this project: drs. Ruud te Welscher, drs. Tineke Burger-Wiersma and Ernst de Bokx and are acknowledged for their help.

8

Literature

Anonymous, 2001. Tropisch blauwwier in Nederland aangetroffen. H2O 32: 12-13.

Box, J.D., 1981. Enumeration of cell concentrations in suspensions of colonial freshwater microalgae, with particular reference to Microcystis aeruginosa. Br. Phycol. J. 16: 153-164.

Carmichael, W.W. & I.R. Falconer, 1993. Diseases related to freshwater Blue-green algal toxins, and control measures. In: Falconer, I.R. (ed). Algal toxins in seafood and drinking water. Academic press London pp 189-209.

Carpentier, C.J. 1996. Cyanobacterie toxine: gevolgen voor de volksgezondheid en ecologische relevantie. Afstudeerscriptie Universiteit Utrecht. pp 1-30 (in Dutch).

Carpentier, C.J., H.A.M. Ketelaars & A.J. Wagenvoort. 1999[a]. Rapid and versatile routine measurements of plankton biovolumes with BACHUS. J. Plankton Res. 21: 1877 – 1889.

Carpentier, C.J., W. Hoogenboezem & H.A.M. Ketelaars, 1999[b]. Cyanobacterie toxines en drinkwater bereid uit oppervlaktewater, een worst case benadering. Report PWN, Haarlem and WBB Werkendam. pp. 1-64 (in Dutch).

Chorus, I. & J. Bartram. 1999. Toxic cyanobacteria in water, a guide to their public health consequences, monitoring and management. E. and F.N. Spon. London and New York. pp. i -xv; 1- 416

Dow, C.S. & U.K. Swoboda 2000. Cyanotoxins. In: B.A. Whitton and M. Potts (eds). The ecology of cyanobacteria their diversity in time and space. I - xviii, 1 - 669. Kluwer Dordrecht.

Eriksson, J.E., J.A.O. Meriluoto & T. Lintholm, 1989. Accumulation of a peptide toxin from cyanobacterium Oscillatoria agardhii, in freshwater mussel Anadonta cynea. Hydrobiologia 183: 211-216.

Falconer, I.R., M.D. Burch, D.A. Steffensen, M. Choice & O.R. Coverdale. 1994. Toxicity of blue green algae (cyanobacterium) Microcystis aeruginosa in drinking water to growing pigs as an animal model for injury and risk assessment. Environmental Toxicology and Water Quality 9: 131 – 139.

Falconer, I.R , J. Bartram, I. Chorus, T. Kuiper-Goodman, H. Utkillen, nM. Bruch & G.A. Godd. 1999. Save levels and save practices. In : Chorus, I & J. Bartram (eds.) Toxic cyanobacteria in water. A guide to their public health consequences, monitoring and management. E. &F.N. Spon, London 156 – 178.

Fitzgerald, D.J., 2001. Cyanotoxins and human health- overview. In: Chorus, I., (ed.). Cyanotoxins, occurrence causes consequences. pp i – xviii; 1-357.

Francis, 1878. Poisonous Australian Lake. Nature 18: 11-12.

Heinis, F. , 1994. Toxinen in cyanobacteriën: de situatie in Nederland. AquaSense Rapportnr. 94.052. Riza, Lelystad (in Dutch).

Ietswaart, Th., T. Burger Wiersma, H.A.M. Ketelaars, L. Gille, C. de Vries & B. Zegers. 2000. Estimating phytoplankton biomass by means of microscopical counts and HPLC pigment analysis: a comparison. Association of River Waterworks-RIWA 1-51.

Jefferies, T.M., G. Brammer, A. Zotou, P.A. Brough & T. Gallagher, T., 1994. Determination of anatoxin-a, homo-anatoxin and propyl-anatoxin in cyanobacterial extracts by HPLC, GC-mass spectrometry and capillary electrophoresis. In: Codd G.A. et al (eds), Detection methods for cyanobacterial toxins, Royal Society of Chemistry, pp. 34-39 .

Jochimsen, E.M., W. W. Carmichael, J.A. Denise, M. Cardo, S.T. Cookson, C.E.M. Holmes, M.B. Antunes, D.A. de Melo Filho, T.M. Lyra, V.S.T. Barreto, S.M.F.O. Azevedo, W.R. & Jarvis. 1998. Liver failure and death after exposure to microcystins at a haemodialysis centre in Brazil. The New England Journal of Medicine Vol. 338(13): 873-878.

Karner D.A., J.H. Standridge, G.W. Harrington & R.P. Barnum. 2001. Microcystin Algal toxins in source and finished water. Journal AWWA. pp 72 – 81.

Komárek, J. 1999. Übersicht der Planktischen Blaualgen (Cyanobakterien) im Einzugsgebiet der Elbe. Internationale Kommission zum Schutz der Elbe, Mezinárodní Komise pro Ochranu Labe. pp.1 – 54.

Komárek, J., S.M.O. Azevodo, P. Domingos, J. Komárková & M. Tich_. 2001 Background of the Caryaru tragedy; a case taxonomic study of toxic cyanobacteria. Arch. Hydrobiol. Suppl. 140: 9-29.

Lampert, W.,& U. Sommer. 1997. Limnoecology: the ecology of lakes and streams. Oxford University press. New York – Oxford, pp. i – xiv: 1 – 382.

Laurén-Määttä, C., J. Hietala, M. Reinikainen & M. Walls. 1995. Do Microcystis aeruginosa toxins accumulate in the food web; a laboratory study. Hydrobiologia 304: 23-27.

Lawton, L.A., C. Edwards & G.A. Codd. 1994. Extraction and High-performance liquid chromatographic method for the determination of microcystins in raw and treated waters. Analyst 119. 1525 – 1530.

NNI. 1981. Spectrofotometrische bepaling van het gehalte aan chlorofyl-a. NEN 6520, NNI, Delft. (in Dutch).

Saitou, T., N. Sugiura, T. Tayama, Y. Inamori and M. Matsumara. 2003. Degradation characteristics of microcystins by isolated bacteria from Lake Kasumigaura. Journal of watersupply: research and technology Aqua 52(1): 13 - 18.

Schwoerbel, J., 1986. Methoden der Hydrobiologie Susswasserbiologie. 4. Auflage Gustav Fischer Verlag Stuttgart. pp i - xi; 1-368.

Sivonen, K., K. Himberg, R.Luukainen, S.I. Niemelä, G.A. Poon, G.A. Codd. 1989.Preliminary characterization of neurotoxic Cyanobacteria blooms and strains from Finland. Toxicity assesment 4, 339 - 352.

Ueno, Y. 1998. Acute , chronic toxicities and hepatocarcinogenity of microcystin-LR. 4th international conference on toxic cyanobacteria p. 129.

Ueno, Y., Y. Makita, S. Nagata, T. Tsutsumi, F. Yoshida, S. Tamura, M. Seikijima, F. Tashiro, T. Harada and T. Yoshida. 1999. No chronic oral toxicity of a low dose of microcystin-LR, a cyanobacterial hepatotoxin, female BALB/c mice. Wily and Sons Inc., pp. 45 – 55.

Utermöhl, H., 1958. Zur Vollkomnung der quantitativen Phytoplankton-Methodik. Mitt. Int. Ver. Limnol. 9: 1-39.

Wagenvoort, A.J., S. Novemsia-Danuhyarsu, A. van der Made-Engelberts, C.P.R. Pikaar & R. Trompetter. 2000. Produktie van microcystine door natuurlijke Microcystis populaties in de Biesbosch spaarbekkens. rapport aw/aba 2000-35115 N.V. Waterwinningsbedrijf Brabantse Biesbosch. pp 1-47 (in Dutch)

Whitton, B.A. & M. Potts. 2000. The ecology of cyanobacteria, their diversity in time and space. i-xviii, 1-669. Kluwer Dordrecht.

Zotou A., Jefferies T.M., Brough P.A. & Galagher T. 1993. Liquid chromatography with electrospray ion-trap mass spectrometry for the determination of anatoxins in cyanobacteria and drinking water. Analyst: 118: 753.

Zwart, G. 2003 Cyanobacteriën nieuwsbrief, t.b.v. waterbeheerders en deelnemers van het cyanobacteriën werk overleg. Nieuwsbrief 2; p.p1 – 6 (Dutch newsletter on cyanobacteria).

Appendix I

Characteristics of the sampling sites, including the number of stations per sites and the types of phytoplankton and chlorophyll a analysis conducted.

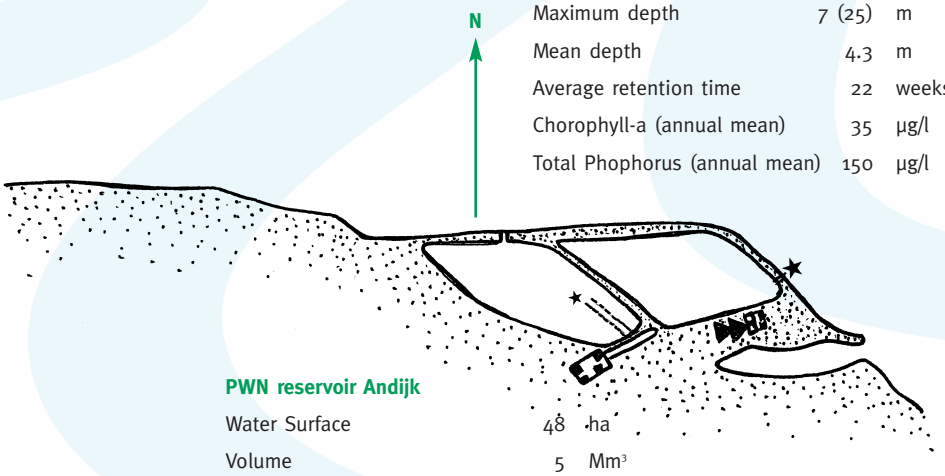
Method	WRK-I	PWN-I	WBB-I	WBB-II	GWA-I	WBG-I
Sample collection						
Type of water	lake	reservoir	river	reservoir	recharged	reservoir
Collection	directly out of a pipe (inlet)	over column at one station	directly out of a pipe (inlet)	over column at three station	reservoir immersion of a bottle	immersion of a bucket (10 l)
Number of stations	1	1	1	3	1	1
Number of subsamples	-	4 (2 l Ruttner)	1	15 (2 l Ruttner)	1	1
Intervals (m)		2	-	4	-	-
Chlorophyll-a	+	+	+	+	-	-
Phytoplankton						
General composition	+	+	+	+	+ (in 1 of 2 cases)	+
Specific determination of Cyanobacteria	-	+	+	+	-	-
Measurement of Biovolume	+	+	+	+	-	+
Criteria	max. 10 per genera	max. 10 per genera	max. 30 per genera or 95% BHI Ln transformed values ≤ 0,4 (BACCHUS)	max. 30 per genera or 95%BHI BHI Ln transformed values ≤ 0,4 (BACCHUS)	-	-

Method	DHZ-I	DHZ-II	DHZ-III	Vitens-I	Vitens-II
Sample collection					
Type of water	gravel pit at Afgedamde Maas	intake at Afgedamde Maas	infiltration ponds at Meijendel and Monster	canal	Reservoir
Collection	over column at one station	over column at one station	immersion of a bottle or jerry-can	immersion of a bottle	directly out of a pipe
Number of stations	1	1	1	1	1
Number of subsamples	15 (2 l Ruttner)	3 (2 l Ruttner)	1	1	1
Intervals (m)	2	2	-	-	-
Chlorophyll-a	+	+	+	+	+
Phytoplankton					
General composition	+	+	+	+	+
Specific determination of Cyanobacteria	-	-	-	c	-
Measurement of Biovolume	+	+	+	+	+
Criteria	max. 30 per genera or 95%BHI ≤ 40% (BACCHUS)	max. 30 per genera or 95%BHI ≤ 40% (BACCHUS)	max. 30 per genera or 95%BHI ≤ 40% (BACCHUS)	-	-

Schemes including some characteristics of water bodies, such as water surface, maximum depth, mean depth, volume, retention time and chlorophyll-a (annual mean) and total phosphorus (annual mean) studied here. Asterisk indicates a sample location, dotted lines represent subterranean water mains; shaded area is land.

WRK Intake Lake IJssel

Water Surface	1130	ha
Maximum depth	7 (25)	m
Mean depth	4.3	m
Average retention time	22	weeks
Chorophyll-a (annual mean)	35	µg/l
Total Phophorus (annual mean)	150	µg/l

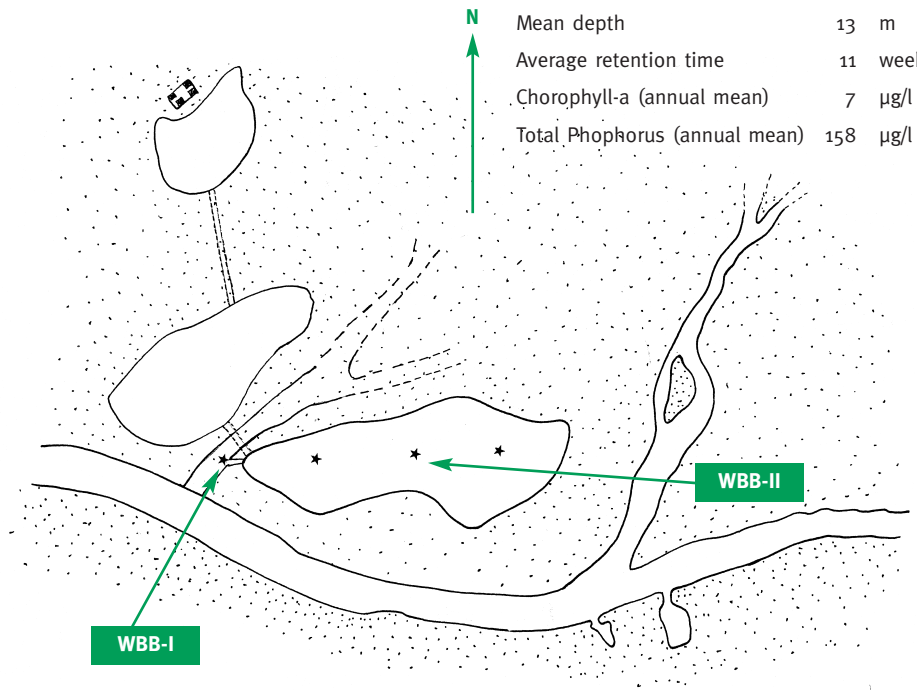


PWN reservoir Andijk

Water Surface	48	ha
Volume	5	Mm^3
Maximum depth	21	m
Mean depth	12	m
Average retention time	7.5	weeks
Chorophyll-a (annual mean)	-	µg/l
Total Phophorus (annual mean)	-	µg/l

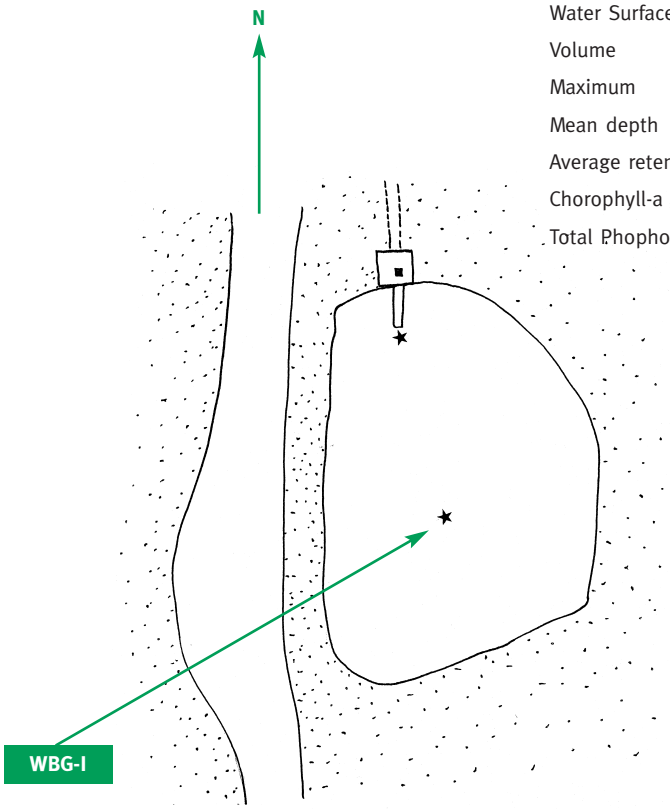
WBB reservoir De Gijster

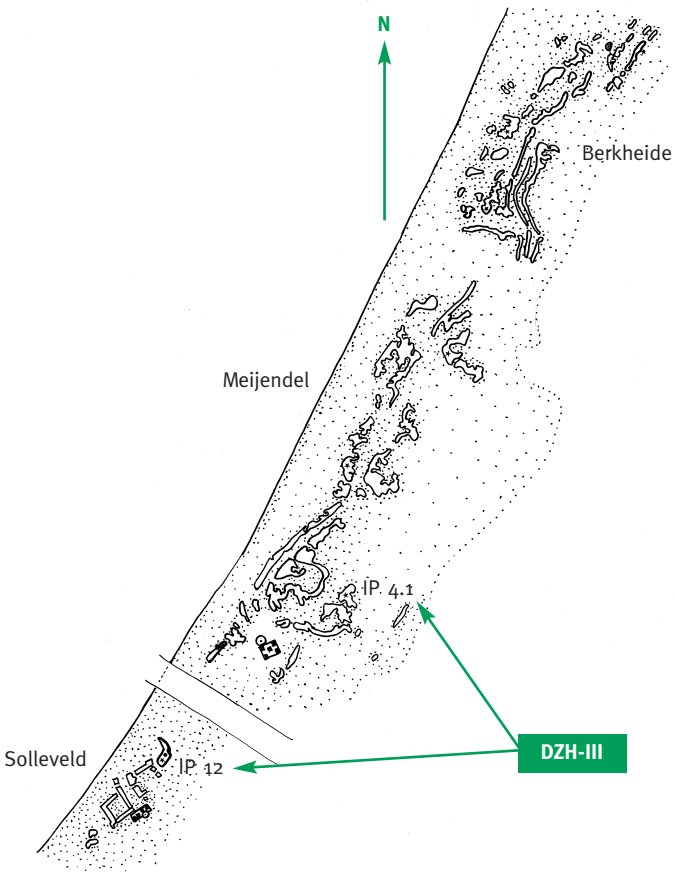
Water Surface	350	ha
Volume	40	Mm^3
Maximum depth	27	m
Mean depth	13	m
Average retention time	11	weeks
Chorophyll-a (annual mean)	7	µg/l
Total Phophorus (annual mean)	158	µg/l

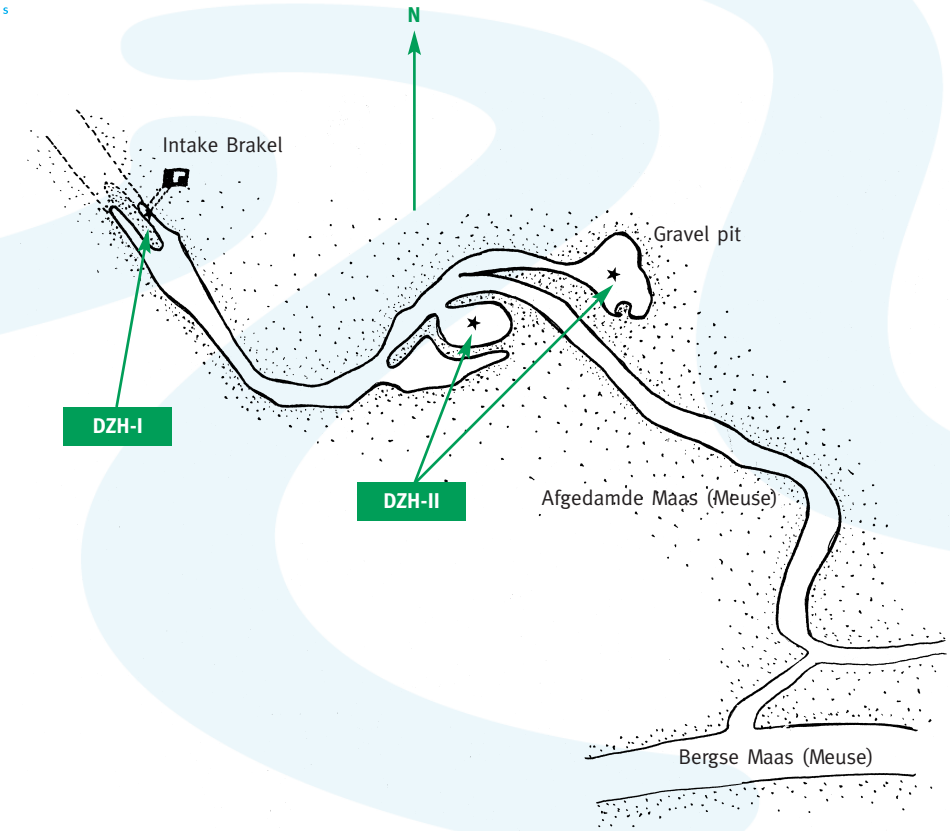


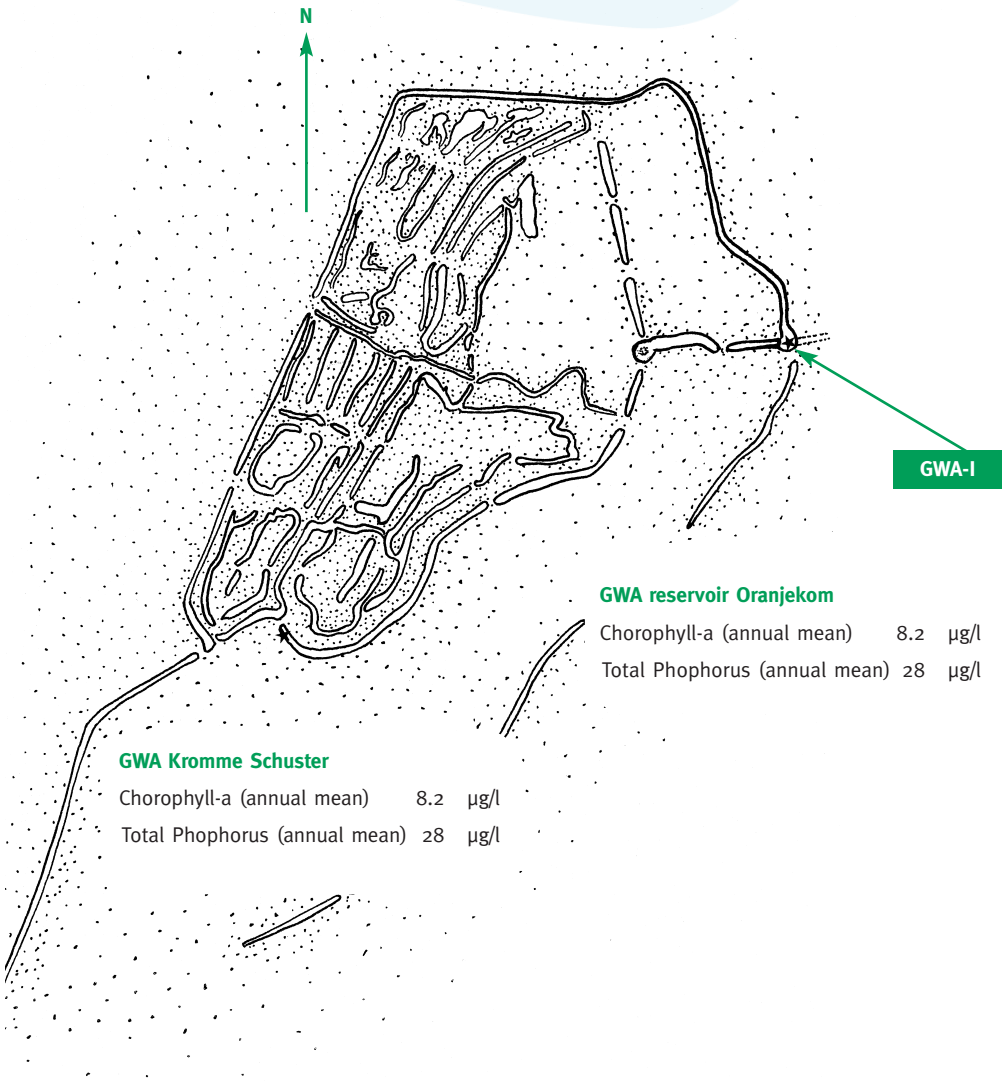
WBG reservoir De Punt

Water Surface	14	ha
Volume	1.2	Mm^3
Maximum	depth	22 m
Mean depth	10	m
Average retention time	10	weeks
Chorophyll-a (annual mean)	8.2	µg/l
Total Phophorus (annual mean)	28	µg/l







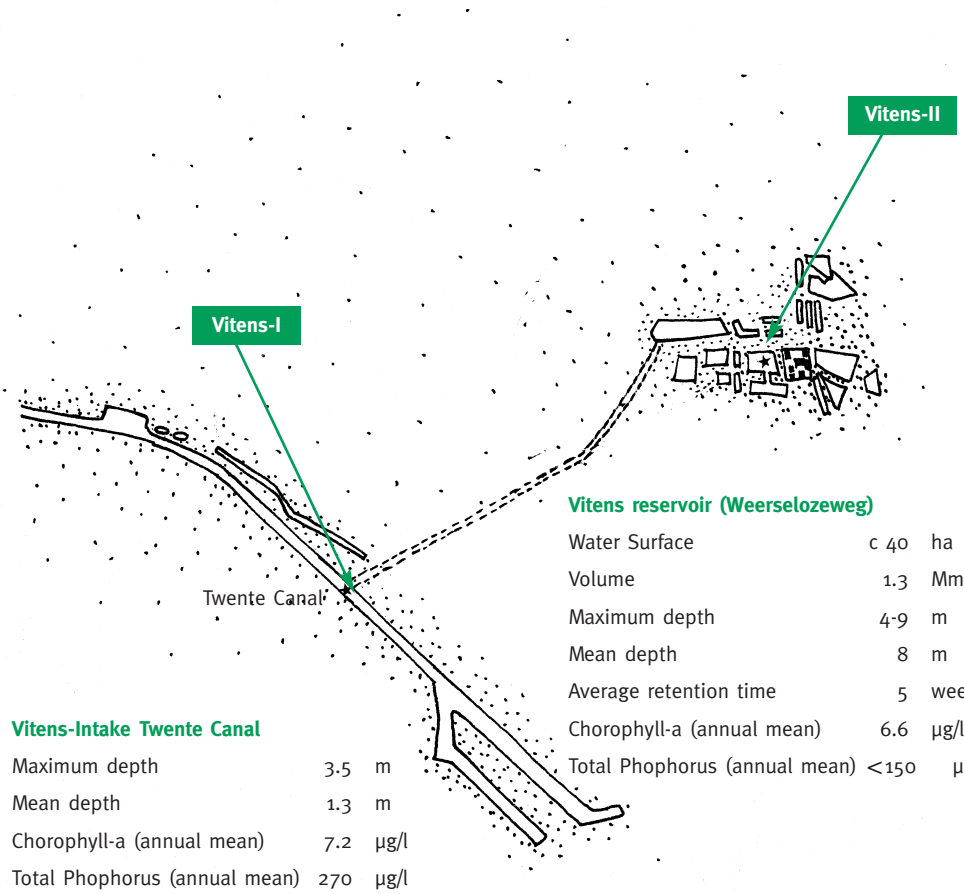


GWA reservoir Oranjekom

Chorophyll-a (annual mean)	8.2	µg/l
Total Phophorus (annual mean)	28	µg/l

GWA Kromme Schuster

Chorophyll-a (annual mean)	8.2	µg/l
Total Phophorus (annual mean)	28	µg/l



Vitens reservoir (Weerselozeweg)

Water Surface	c 40	ha
Volume	1.3	Mm^3
Maximum depth	4-9	m
Mean depth	8	m
Average retention time	5	weeks
Chorophyll-a (annual mean)	6.6	µg/l
Total Phophorus (annual mean)	<150	µg/l

Vitens-Intake Twente Canal

Maximum depth	3.5	m
Mean depth	1.3	m
Chorophyll-a (annual mean)	7.2	µg/l
Total Phophorus (annual mean)	270	µg/l

Appendix II

Results of double and triple microcystin measurements to estimate variation in determinations (analysis by AquaSense).

Sampling site	Sampling date	Total microcystin (µg/l) 1	2	3	reported	s.d.	s.d. related to avg (%)
WRK-I	31-Jul-00	1.79	1.02	1.41	1.41	0.39	27.4
PWN-I	12-Jul-00	1.01	1.57		1.29	0.4	30.7
PWN-I	26-Jul-00	0.55	1.44	1.06	1.02	0.45	43.9
PWN-I	18-Oct-00	1.44	0.91		1.18	0.37	31.9
GWA-I	11-Oct-00	2.94	0.005	0.005	<0.01	1.69	172
DZH-III	18-Jul-00	0.62	1.25		1.02	0.45	43.9
Vitens-I	15-Aug-00	4.82	7.43		6.13	1.85	30.1
Vitens-I	12-Sep-00	0.57	0.6		0.59	0.02	3.6
Vitens-II	1-Aug-00	0.09	0.005		0.05	0.06	120.2
Average (%)							**56.1**

Appendix III

Observed cyanobacteria taxa, arranged per sampling site, including type of colony, trichome or single cell. Biovolume range is indicated by smallest and largest biovolume.

		Biovolume (µm3/unit)		
sampling site	**taxon**	**unit**	**smallest**	**largest***
WRK-I	*Anabaena*	trichome (100µm)	347	
	Aphanizomenon	trichome (100µm)	345	1333
	Aphanocapsa	colony	301	2047
	Chroococcus	colony	54	7238
	Cyanobacteria nn	cell	7	11
	Cyanobacteria nn	colony	181	7980
	Cyanobacteria nn	trichome (100µm)	266	
	Cyanodictyon	colony	216	7238
	Microcystis	cell	13	54
	Microcystis	colony	905	26486
	Pseudanabaena	cell	8	22
	Radiocystis	colony	2483	57906
	Snowella	colony	905	3706
PWN-I	*Anabaena*	trichome (100µm)	1436	5798
	Aphanizomenon	Trichome (100µm)	442	4418
	Aphanizomenon flos aquae	Trichome (100µm)	478	1580
	Aphanocapsa	colony	65	3949
	Aphanothece	colony	221	523599
	Chroococcus	colony	2721	8180
	Cyanobacteria nn	trichome (100µm)	649	
	Merismopedia	colony	29302	
	Microcystis	cell	8	128
	Microcystis	colony	1186	127832
	Phormidium	trichome (100µm)	160	
	Plankthothrix	trichome (100µm)	479	3313
	Pseudanabaena	cell	52	86
	Woronichinia	colony	4189	17974
WBB-I	*Anabaena*	cell	48	57
	Aphanizomenon	trichome (200µm)	3123	3291
	Cyanobacteria nn	cell	80	120
	Microcystis	cell	60	66
	Oscillatoria	trichome (200µm)	2000	3000
	Pseudanabaena	cell	38	48

		Biovolume (µm3/unit)		
sampling site	**taxon**	**unit**	**smallest**	**largest***
WBB-II	*Anabaena*	cell	48	57
	Aphanizomenon	trichome (200µm)	3123	3291
	Cyanobacteria nn	cell	100	
	Microcystis	cell	60	66
	Oscillatoria	trichome (200µm)	2500	
	Pseudanabaena	cell	38	48
WBG-I	*Anabaena*	trichome (100µm)	1102	2045
	Aphanizomenon	trichome (100µm)	1102	1651
	Aphanocapsa	colony	500	500
	Chroococcales nn	cell	46	46
	Chroococcales nn	colony	1102	1102
	Merismopedia	colony	100	100
	Microcystis	cell	63	
	Microcystis	colony	6880	
	Oscillatoria	trichome (100µm)	60	
	Pseudanabaena	cell	4	
DZH-I	*Aphanizomenon*	trichome (100µm)	1033	3304
	Microcystis	cell	16	98
	Microcystis	colony	7841	49082
	Pseudanabaena	trichome (100µm)	344	521
DZH-II	*Aphanizomenon*	trichome (100µm)	1649	3919
	Chroococcus	colony	239	
	Microcystis	cell	40	60
	Microcystis	colony	19985	30229
	Oscillatoria	trichome (100µm)	1262	
	Pseudanabaena	trichome (100µm)	412	484
DZH-III	*Anabaena*	trichome (100µm)	398	2276
	Aphanizomenon	trichome (100µm)	1789	3919
	Microcystis	cell	14	70
	Microcystis	colony	6883	34801
	Oscillatoria	trichome (100µm)	4025	11724
	Pseudanabaena	trichome (100µm)	232	730
Vitens-I	*Anabaena*	trichome (100µm)	594	2045
	Aphanizomenon	trichome (100µm)	734	1242
	Chroococcales nn	cell	46	62
	Chroococcales nn	colony	2	1102
	Merismopedia	colony	50	125
	Microcystis	cell	26	56
	Microcystis	colony	6880	27500
	Pseudanabaena	cell	4	8
	Pseudanabaena	trichome	240	
	Snowella	colony	446	446
	Woronichinia	colony	500	500

		Biovolume (µm3/unit)		
sampling site	**taxon**	**unit**	**smallest**	**largest***
Vitens-II	*Anabaena*	trichome (100µm)	1790	2045
	Aphanizomenon	trichome (100µm)	734	1108
	Aphanocapsa	colony	500	
	Chroococcales nn	cell	55	55
	Chroococcales nn	colony	500	1102
	Merismopedia	colony	50	80
	Microcystis	cell	53	53
	Microcystis	colony	68	80
	Oscillatoria	cell	60	60
	Pseudanabaena	trichome	4	302
	Snowella	colony	446	446
	Woronichinia	colony	500	

** in case only the smallest biovolume is displayed, means only one measurement is carried out.*

RIWA
Rhine Water Works
The Netherlands

Appendix IV

Appendix IV

Cyanobacterial biovolumes (mm^3 per l) as observed at various sampling sites, listed per taxon.

sampling site	sampling date	*Anabaena*	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	Chroococcales nn	*Chroococcus*	Cyanobacteria nn	*Cyanodictyon*	*Merismopedia*	*Microcystis*	*Oscillatoria*	*Phormidium*	*Planktothrix*	*Pseudanabaena*	*Radiocystis*	*Snowella*	*Woronichinia*
WRK-I	17-May-00							0.423	0.152							5.629		
	31-May-00						0.011	1.001	0.221		0.003							
	28-Jun-00		13.152	4.776			1.409	1.684	0.504		0.100				0.005		0.360	
	12-Jul-00		1.476	5.699			4.222	0.361	1.411		0.136				0.159	0.483		
	31-Jul-00		19.955				0.126	3.319	1.407		0.384				0.154			
	09-Aug-00		0.813					0.088	2.947		0.362				0.122		0.528	
	23-Aug-00		1.358				0.147	1.015			34.686				0.896			
	06-Sep-00		0.929						0.764		2.954				0.051			
	20-Sep-00								1.655		5.053				0.040			
	04-Oct-00	0.270					1.958		0.350		0.338				0.005			
	18-Oct-00			0.234				0.973	0.976						0.013			
PWN-I	18-May-00		1.620	0.009	0.034													
	31-May-00	6.958		4.421							0.000							
	14-Jun-00			24.484	0.011		2.454				0.328							
	28-Jun-00		0.096	14.083	0.184					11.721	3.659				0.062			
	12-Jul-00		3.160	0.298	2.761		1.633				1.038			0.331	0.194			
	26-Jul-00			9.151							13.474				0.258			
	09-Aug-00	1.149		2.358							0.788				0.156			1.257
	23-Aug-00			6.742							5.684			0.449	0.089			
	06-Sep-00		2.787	1.962							1.213			0.144				4.909
	20-Sep-00		0.243	0.219	26.180						0.006		0.008					1.227
	04-Oct-00			0.872				0.130			0.071							
	18-Oct-00			0.136	0.011						0.025			0.147				2.696

Cyanobacterial biovolumes (mm^3 per l) as observed at various sampling sites, listed per taxon.

sampling site	sampling date	*Anabaena*	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	Chroococcales nn	*Chroococcus*	Cyanobacteria nn	*Cyanodictyon*	*Merismopedia*	*Microcystis*	*Oscillatoria*	*Phormidium*	*Planktothrix*	*Pseudanabaena*	*Radiocystis*	*Snowella*	*Woronichinia*
WBB-I	13-Jun-00	0.000	0.000					0.000			0.000	0.000			0.000			
	19-Jun-00	0.000	0.000					0.000			0.000	0.000			0.000			
	26-Jun-00	0.000	0.000					0.003			0.000	0.000			0.000			
	03-Jul-00	0.000	0.000					0.034			0.000	0.000			0.000			
	10-Jul-00	0.000	0.115					0.000			0.000	0.000			0.000			
	17-Jul-00	0.001	0.000					0.000			0.001	0.000			0.006			
	24-Jul-00	0.000	0.000					0.000			0.000	0.000			0.008			
	31-Jul-00	0.000	0.375					0.025			0.001	0.000			0.000			
	07-Aug-00	0.000	0.062					0.000			0.000	0.000			0.022			
	14-Aug-00	0.000	0.150					0.000			0.001	0.000			0.004			
	21-Aug-00	0.000	0.000					0.001			0.000	0.000			0.010			
	04-Sep-00	0.000	0.000					0.024			0.000	0.000			0.014			
	11-Sep-00	0.000	0.281					0.034			0.000	0.000			0.005			
	18-Sep-00	0.000	0.000					0.023			0.002	0.000			0.000			
	25-Sep-00	0.000	0.088					0.074			0.000	0.000			0.006			
	02-Oct-00	0.000	0.112					0.006			0.000	0.000			0.003			
	09-Oct-00	0.000	0.075					0.009			0.000	0.000			0.000			
	16-Oct-00	0.000	0.655					0.006			0.000	0.016			0.001			
	23-Oct-00	0.000	0.225					0.000			0.000	0.000			0.000			
	31-Oct-00	0.000	0.000					0.000			0.000	0.000			0.000			
	06-Nov-00	0.000	0.187					0.061			0.000	0.032			0.000			
	13-Nov-00	0.000	0.019					0.000			0.000	0.000			0.000			
	20-Nov-00	0.000	0.000					0.000			0.000	0.000			0.000			
	27-Nov-00	0.002						0.007			0.000	0.032			0.000			

RIWA
Rhine Water Works
The Netherlands

Cyanobacterial biovolumes (mm^3 per l) as observed at various sampling sites, listed per taxon.

sampling site	sampling date	*Anabaena*	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	*Chroococcales nn*	*Chroococcus*	*Cyanobacteria nn*	*Cyanodictyon*	*Merismopedia*	*Microcystis*	*Oscillatoria*	*Phormidium*	*Planktothrix*	*Pseudanabaena*	*Radiocystis*	*Snowella*	*Woronichinia*
WBB-II	10-Jul-00	0.063	0.544								0.115				0.001			
	17-Jul-00	0.000	0.255					0.000			0.620	0.000			0.007			
	24-Jul-00		0.255								0.620				0.007			
	31-Jul-00	0.027	0.229								0.427				0.002			
	07-Aug-00	0.224	2.446								1.933				0.037			
	14-Aug-00	0.445	5.860								3.146				0.207			
	21-Aug-00	0.189	4.077								9.846				0.099			
	28-Aug-00	0.186	16.816								12.580				0.138			
	04-Sep-00	0.021	2.930								10.087							
	11-Sep-00	0.066	4.077								17.254				0.050			
	18-Sep-00	0.018	2.803								12.320							
	25-Sep-00	0.003	1.783								12.832							
	02-Oct-00	0.063	3.949								8.961							
	09-Oct-00		0.892								6.581				0.029			
	16-Oct-00	0.005	0.382								9.903				0.046			
	23-Oct-00	0.023	0.127								6.611				0.017			
	06-Nov-00	0.015									1.368							
	13-Nov-00										0.196							
	24-Nov-00	0.000	0.000								0.090				0.000			

Cyanobacterial biovolumes (mm^3 per l) as observed at various sampling sites, listed per taxon.

sampling site	sampling date	*Anabaena*	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	*Chroococcales nn*	*Chroococcus*	*Cyanobacteria nn*	*Cyanodictyon*	*Merismopedia*	*Microcystis*	*Oscillatoria*	*Phormidium*	*Planktothrix*	*Pseudanabaena*	*Radiocystis*	*Snowella*	*Woronichinia*
DZH-I	29-May-00		0.021															
	05-Jun-00		0.135															
	19-Jun-00		0.516															
	03-Jul-00		0.111															
	17-Jul-00		0.831								0.034							
	31-Jul-00		0.466								1.976							
	14-Aug-00		0.043								0.175				0.171			
	28-Aug-00		0.812								0.048				0.006			
	25-Sep-00		0.134								0.099				0.004			
	23-Oct-00										0.004							

RIWA
Rhine Water Works
The Netherlands

Cyanobacterial biovolumes (mm^3 per l) as observed at various sampling sites, listed per taxon.

sampling site	sampling date	*Anabaena*	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	*Chroococcales nn*	*Chroococcus*	*Cyanobacteria nn*	*Cyanodictyon*	*Merismopedia*	*Microcystis*	*Oscillatoria*	*Phormidium*	*Planktothrix*	*Pseudanabaena*	*Radiocystis*	*Snowella*	*Woronichinia*
DZH-II	19-Jun-00		0.354															
	03-Jul-00										0.025							
	17-Jul-00		0.087								0.001							
	31-Jul-00		0.135								0.236							
	14-Aug-00		1.617				0.003				2.091							
	28-Aug-00		2.152								0.014				0.000			
	25-Sep-00		1.905								0.215				0.001			
	23-Oct-00											0.022						
DZH-III	30-May-00										0.005				0.003			
	06-Jun-00	0.058																
	20-Jun-00	0.841									0.057	0.165			0.009			
	04-Jul-00	1.111									0.423				0.009			
	18-Jul-00										0.243				0.028			
	01-Aug-00	0.258	0.126								1.685	0.236						
	15-Aug-00		0.096								10.978				0.008			
	29-Aug-00	0.029									14.612				0.086			
	12-Sep-00										22.849				0.893			
	26-Sep-00		392.390															
	17-Oct-00		23.694								0.008				0.090			

Cyanobacterial biovolumes (mm^3 per l) as observed at various sampling sites, listed per taxon.

sampling site	sampling date	*Anabaena*	*Aphanizomenon*	*Aphanocapsa*	*Aphanothece*	*Chroococcales nn*	*Chroococcus*	*Cyanobacteria nn*	*Cyanodictyon*	*Merismopedia*	*Microcystis*	*Oscillatoria*	*Phormidium*	*Planktothrix*	*Pseudanabaena*	*Radiocystis*	*Snowella*	*Woronichinia*
Vitens-I	14-Jun-00	0.009	0.500			0.050				0.003	1.118						0.007	
	13-Jul-00		0.159			0.091				0.006	0.084							
	17-Jul-00		0.459			0.096				0.022	0.254							
	02-Aug-00		0.040			0.052				0.009	0.125							
	17-Aug-00		0.397			0.823				0.023	0.229				0.044		0.002	
	06-Sep-00		0.448			0.015				0.000	11.664				0.053		0.006	
	14-Sep-00	0.051	0.834			0.063				0.012	5.690				0.016		0.015	0.013
	09-Oct-00	0.084	1.006			0.372				0.000	0.538				0.004		0.065	0.020
Vitens-II	14-Jun-00		0.085			0.004				0.000		0.002						
	13-Jul-00	0.878	0.174			0.017				0.006		0.000					0.003	
	02-Aug-00	0.276				0.059				0.023	0.054							
	17-Aug-00	0.065	0.089			0.167				0.071	0.028				0.004		0.157	
	14-Sep-00		0.297	0.087		0.010				0.034	0.164				0.000		0.137	0.010

Appendix V

All sample dates listed for each sampling site, the amount of microcystin, microcystin-LR anatoxin and Chlorophyll a is given.

		toxin (µg/l)			
sampling site	sampling date	microcystin	Microcystin_LR	anatoxin	chlorophyll a (µg/l)
WRK-I	17-May-00	1.62		< 1	43
	31-May-00	2.05		< 1	45
	28-Jun-00	4.26		< 1	96
	12-Jul-00	5.66		< 1	125
	31-Jul-00	1.41		< 1	71
	09-Aug-00	6.2		< 1	74
	23-Aug-00	4.9		< 1	78
	06-Sep-00	5.61		< 1	86
	20-Sep-00	6.51		< 1	74
	04-Oct-00	3.56		< 1	25
	18-Oct-00	4.74		12.1	89
PWN-I	18-May-00	0.2			5
	31-May-00				9
	14-Jun-00			1.07	21
	28-Jun-00	0.86			24
	12-Jul-00	1.29			
	26-Jul-00	1.02			16
	09-Aug-00	0.62			25
	23-Aug-00	0.73		< 1	23
	06-Sep-00	0.85			13
	20-Sep-00	0.38			13
	04-Oct-00	0.26			26
	18-Oct-00	1.18			26
WBB-I	13-Jun-00				8
	19-Jun-00				16
	26-Jun-00				4
	03-Jul-00				6
	10-Jul-00				4
	17-Jul-00				3
	24-Jul-00				8
	31-Jul-00				5
	07-Aug-00				7
	14-Aug-00				6
	21-Aug-00	0.01			5
	04-Sep-00	0.03			6
	11-Sep-00	0.01			4
	18-Sep-00				4

sampling site	sampling date	toxin (µg/l) microcystin	Microcystin_LR	anatoxin	chlorophyll a (µg/l)
WBB-I	25-Sep-00	<0.01			5
	02-Oct-00				3
	09-Oct-00				3
	16-Oct-00	<0.01			2
	23-Oct-00				2
	31-Oct-00				
	06-Nov-00				
	13-Nov-00				
	20-Nov-00				
	27-Nov-00				
WBB-II	10-Jul-00				2
	17-Jul-00				4
	24-Jul-00				15
	31-Jul-00				11
	07-Aug-00				16
	14-Aug-00				8
	21-Aug-00	0.4			12
	28-Aug-00	0.66			24
	04-Sep-00	0.48		< 1	24
	11-Sep-00	0.27			16
	18-Sep-00	0.38		< 1	12
	25-Sep-00	0.52		2.38	11
	02-Oct-00	0.53		< 1	13
	09-Oct-00	0.39		< 1	10
	16-Oct-00	0.27			13
	23-Oct-00				6
	06-Nov-00				
	13-Nov-00				
	24-Nov-00				
GWA-I	20-Sep-00	0.44		< 1	
	11-Oct-00	<0.01	0		
WBG-I	16-May-00	2.5			
	07-Jun-00	1.35			
	14-Jun-00	2.17		2.27	
	26-Jun-00	<0.01			
	10-Jul-00	0.72			
	26-Jul-00	0.45			
	05-Sep-00	0.27			
DZH-I	29-May-00				
	05-Jun-00				
	19-Jun-00				
	03-Jul-00				
	17-Jul-00				
	31-Jul-00	2.92			25
	14-Aug-00				

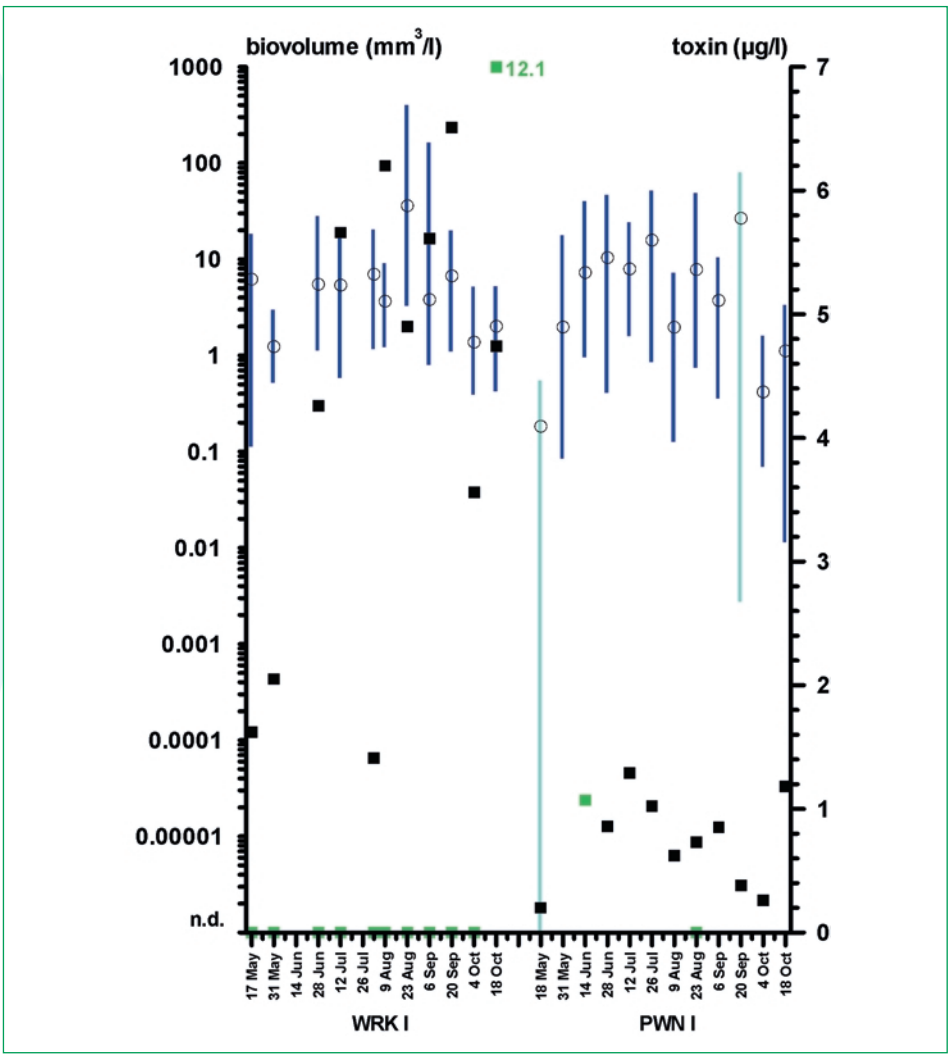
		toxin (µg/l)			
sampling site	sampling date	microcystin	Microcystin_LR	anatoxin	chlorophyll a (µg/l)
DZH-I	28-Aug-00				
	11-Sep-00				
	25-Sep-00				
	23-Oct-00				
DZH-II	25-Apr-00				
	08-May-00				
	22-May-00				
	05-Jun-00				
	19-Jun-00				
	03-Jul-00				
	17-Jul-00				
	31-Jul-00				
	14-Aug-00	0.64			7
	28-Aug-00				
	11-Sep-00				
	25-Sep-00				
	23-Oct-00				
DZH-III	30-May-00				
	06-Jun-00				
	20-Jun-00				
	04-Jul-00	0.49			9
	18-Jul-00	1.02			12
	01-Aug-00	3.87			14
	15-Aug-00	2.59	0.34	< 1	69
	29-Aug-00	2.71	2.37		28
	12-Sep-00	5.21	2.31		25
	26-Sep-00	1.01	1.01	< 1	79
	17-Oct-00	4.36	2.23	< 1	73
Vitens-I	14-Jun-00	0.89		3.91	30
	13-Jul-00	0.06		4.78	29
	17-Jul-00	0.16		6.22	32
	02-Aug-00	2.85		< 1	
	17-Aug-00	6.13		< 1	
	06-Sep-00	1.31		< 1	
	14-Sep-00	0.59		< 1	
	09-Oct-00	0		< 1	11
Vitens-II	14-Jun-00	0.11		< 1	4
	13-Jul-00	0.56		< 1	12
	02-Aug-00	0.05		< 1	9
	17-Aug-00	0		< 1	10
	06-Sep-00	1.43		< 1	13
	14-Sep-00	0		< 1	9
	26-Sep-00	0.9		< 1	12

Appendix VI

Cyanobacterial biovolume (mm^3/l), plotted per date for each sampling site, bar indicates confidence interval, open circles represent mean value and solid squares microcystin concentration (right axis) the amount of intracellular microcystin.

■ = anatoxin, ■ = microcystin and ■ = microcystin-LR

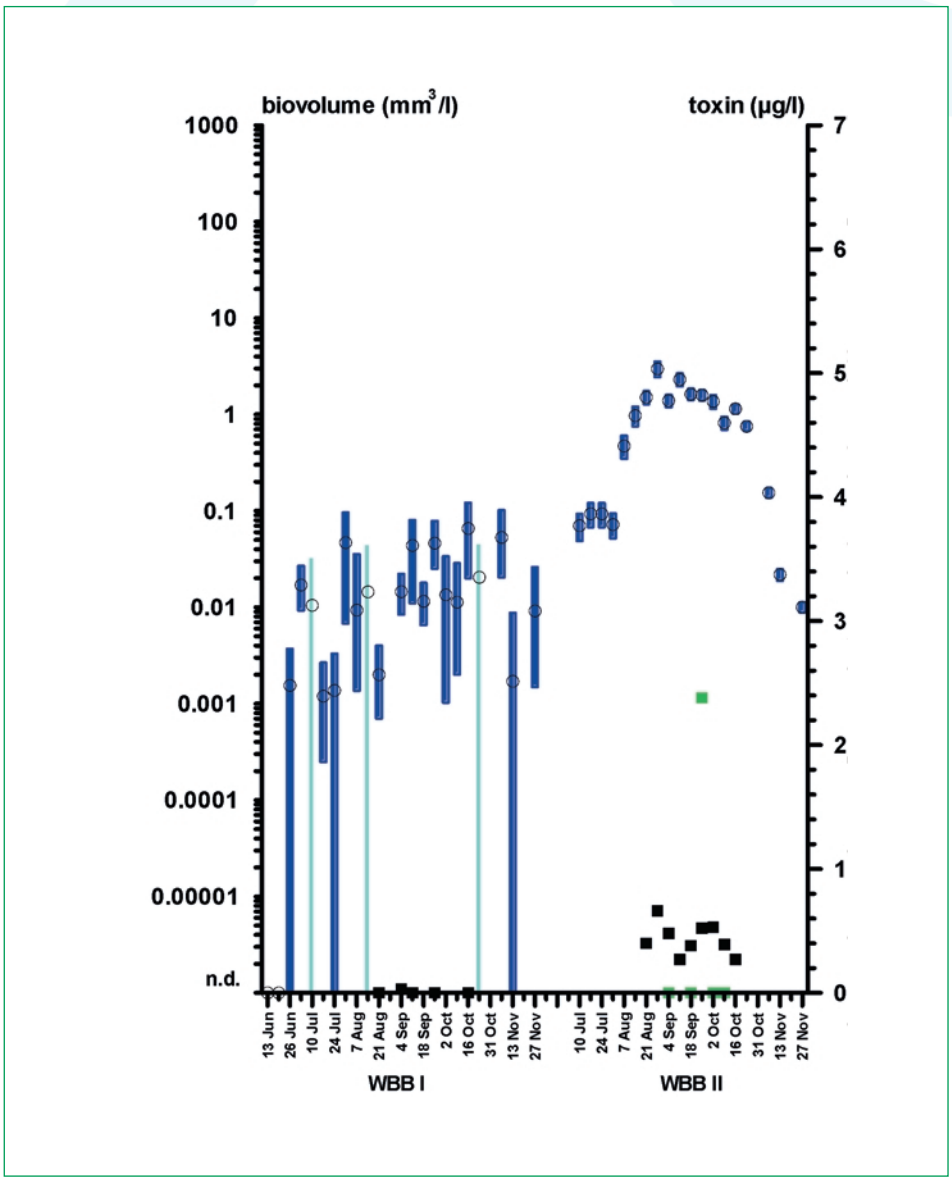
■ = less reliable results (more than 3 log units)



Cyanobacterial biovolume (mm^3/l), plotted per date for each sampling site, bar indicates confidence interval, open circles represent mean value and solid squares microcystin concentration (right axis) the amount of intracellular microcystin.

■ = anatoxin, ■ = microcystin and ■ = microcystin-LR

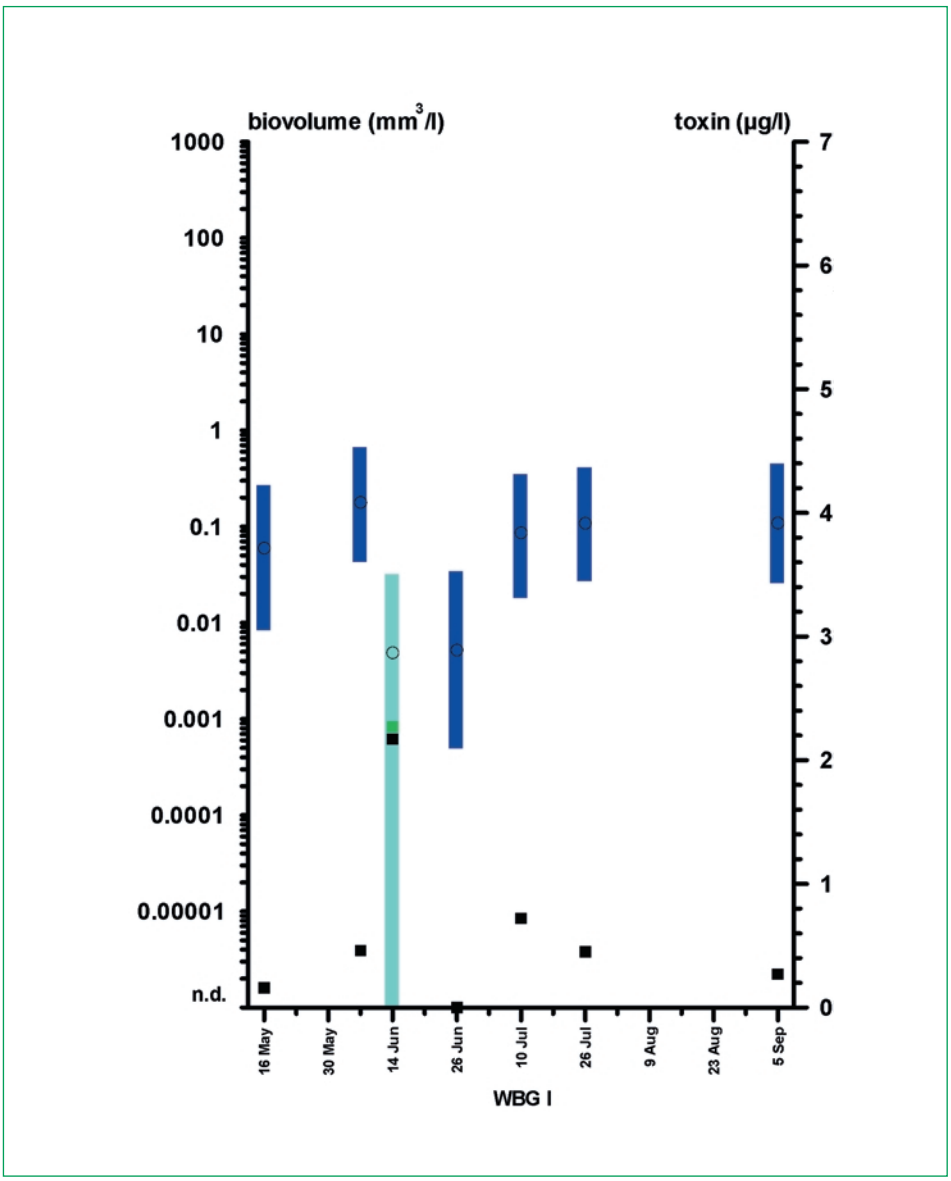
▬ = less reliable results (more than 3 log units)



Cyanobacterial biovolume (mm^3/l), plotted per date for each sampling site, bar indicates confidence interval, open circles represent mean value and solid squares microcystin concentration (right axis) the amount of intracellular microcystin.

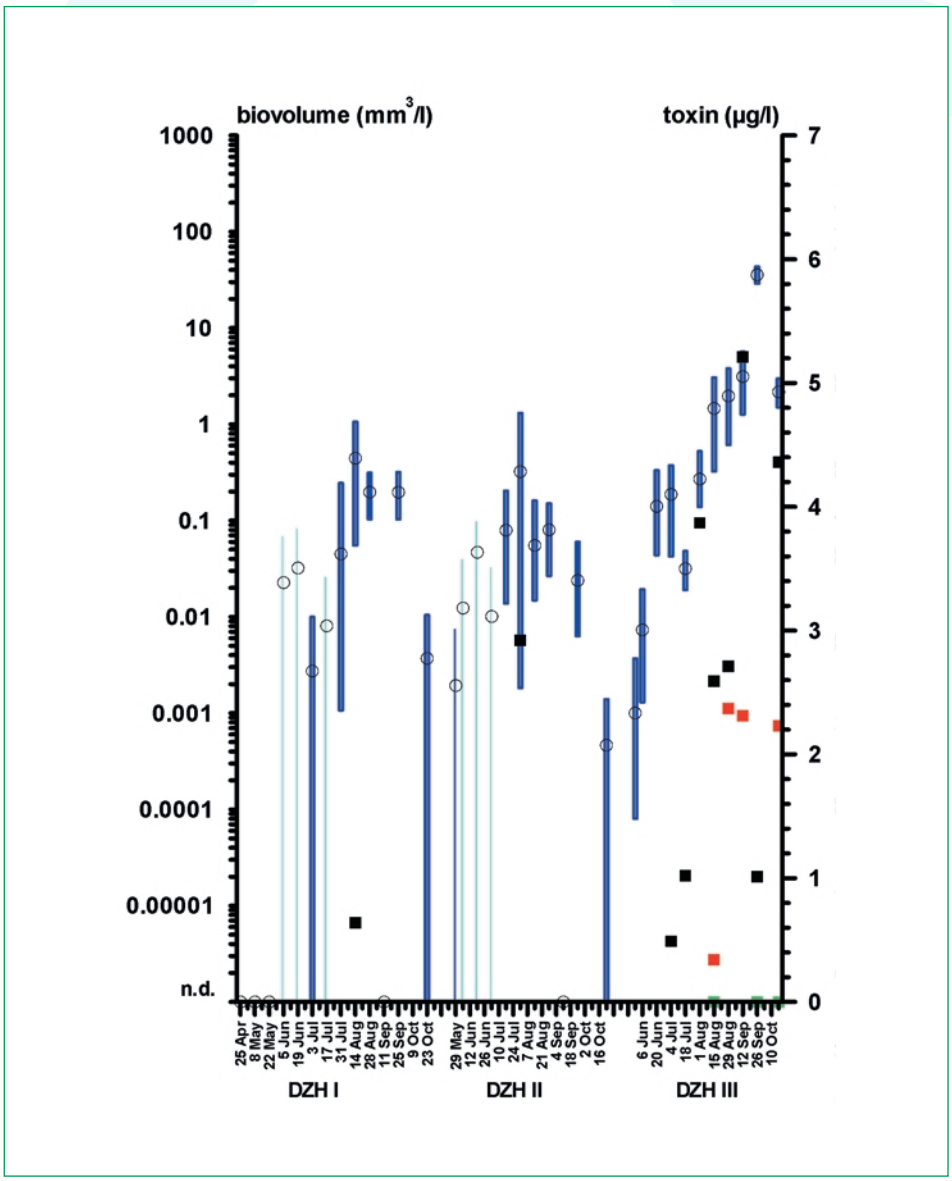
■ = anatoxin, ■ = microcystin and ■ = microcystin-LR

▬ = less reliable results (more than 3 log units)



Cyanobacterial biovolume (mm^3/l), plotted per date for each sampling site, bar indicates confidence interval, open circles represent mean value and solid squares microcystin concentration (right axis) the amount of intracellular microcystin.

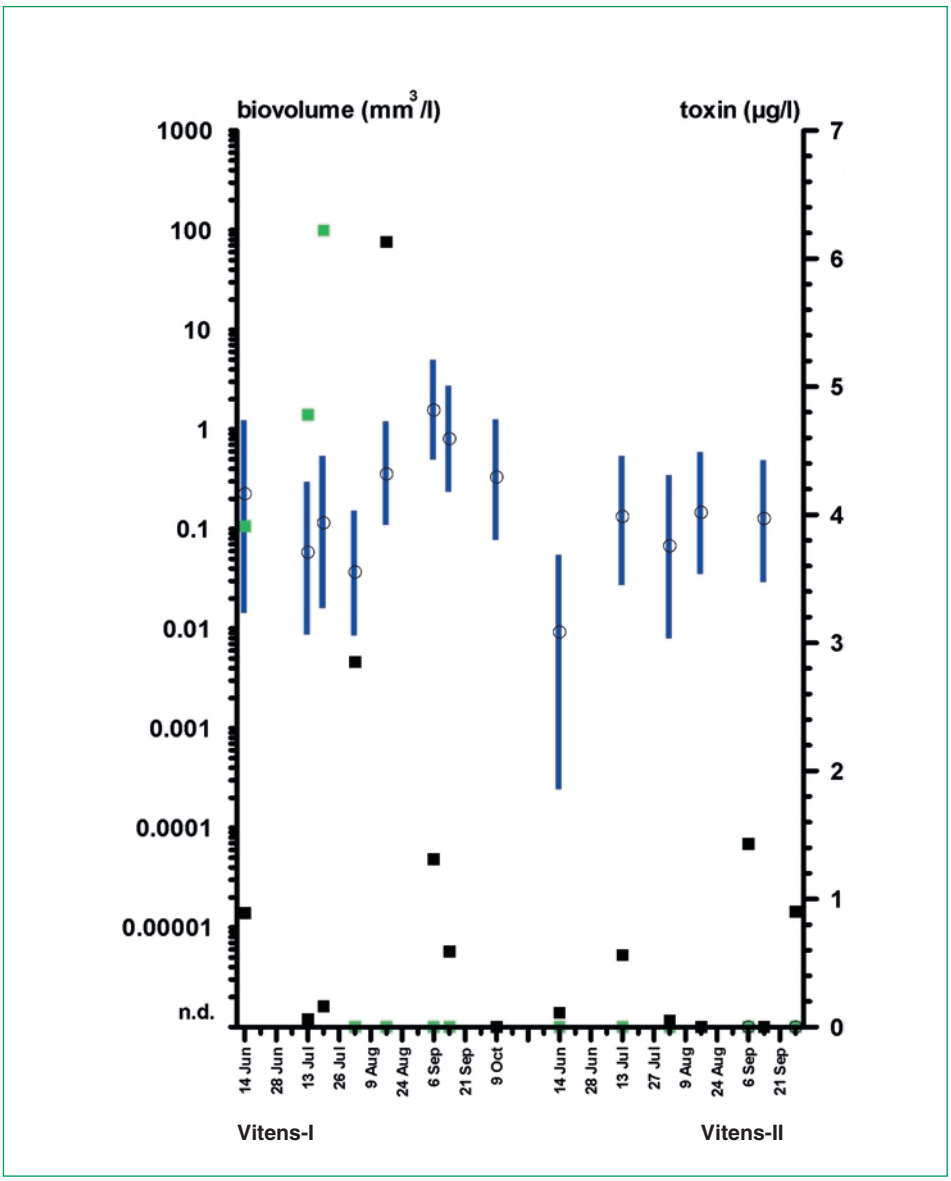
■ = anatoxin, ■ = microcystin and ■ = microcystin-LR
▬ = less reliable results (more than 3 log units)



Cyanobacterial biovolume (mm^3/l), plotted per date for each sampling site, bar indicates confidence interval, open circles represent mean value and solid squares microcystin concentration (right axis) the amount of intracellular microcystin.

■ = anatoxin, ■ = microcystin and ■ = microcystin-LR

▬ = less reliable results (more than 3 log units)



RIWA
Rhine Water Works
The Netherlands

Colofon

Authors:

W. Hoogenboezem, Het Waterlaboratorium Ltd

K. Blaauboer, Het Waterlaboratorium Ltd

A.J. Wagenvoort, Evides (formerly WBB)

RIWA-Project group

G.L. Bakker (Vitens, Overijssel; formerly Drinking Water Company Overijssel)
Ing. K. Blaauboer (Het Waterlaboratorium; formerly WRK; secretary)
M. Boer (Het Waterlaboratorium; formerly PWN)
Dr. W. Hoogenboezem (Het Waterlaboratorium, formerly PWN; chairman)
Dr. Ir. E.J.T.M. Leenen (RIVM)
Dr. J. Postma (AquaSense)
Ir. A.I.A. Soppe (Waterlaboratorium Noord)
A. Visser (Het Waterlaboratorium; formerly DZH)
Drs. A.J. Wagenvoort (Evides, formerley WBB)

Publisher: Vereniging van Rivierwaterbedrijven (RIWA)

Design: Meyson Communicatie, Amsterdam

Print: ATP Digitale Media, Zwanenburg

ISBN: 90-6683-108-1

June 2004