Microcystins (cyanobacterial toxins) in surface waters of rural Bangladesh: Pilot study

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ABSTRACT

In Bangladesh the exposure of millions of inhabitants to water from (shallow) tube wells contaminated with high geogenic loads of arsenic is a major concern. As an alternative to the costly drilling of deep wells, the return to the use of surface water as a source of drinking water is considered. In addition to the well-known hazards of water borne infectious diseases associated with the use of surface water, recently the potential public health implications of toxic cyanobacteria have been recognized. As a first step towards a risk assessment for cyanotoxins in Bangladesh surface waters, seston samples of 79 ponds were analysed in late summer 2002 for the presence of cyanobacteria and microcystins (MCYST), the most frequently detected cyanobacterial toxins worldwide.

Microcystins could be detected in 39 ponds, mostly together with varying abundance of potentially microcystin-producing genera such as *Microcystis*, *Planktothrix* and *Anabaena*. Total microcystin concentrations ranged between <0.1 and $>1{,}000\,\mu g\,l^{-1}$, and more than half of the positive samples contained high concentrations of more than $10\,\mu g\,l^{-1}$. The results clearly show that concentrations of microcystins well above the provisional WHO guideline value of $1\,\mu g\,l^{-1}$ MCYST-LR can be frequently detected in Bangladesh ponds. Thus, an increasing use of surface water for human consumption introduces a risk of replacing one health hazard by another and therefore needs to be accompanied by cyanotoxin hazard assessments.

Key words | Bangladesh, cyanobacteria, microcystin, pond, surface water, toxins

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INTRODUCTION

In Bangladesh surface water has traditionally been the main source of drinking water, but an increasing population density and a lack of adequate sanitation has led to severe microbial contamination of surface water causing disease and mortality, notoriously by infections with *Vibrio cholerae*. In the 1970 s the use of groundwater for human consumption was propagated to overcome this problem and in the following decade tube wells, small-diameter cased wells fitted with a cast iron suction hand pump, were installed all over the country (Briscoe 1978). Although the exact number of tube wells is uncertain, estimates are in the region of 10 million, of which some 1.3 million were constructed as public supplies (Smith *et al.* 2000). However, in 1993, high levels of arsenic were found in drinking water from tube wells. The presence of arsenic appears to be linked in particular to the age of the aquifer from which water is abstracted; Holocene alluvial aquifers are principally affected (Ahmed 2002).

Immense efforts to analyse water samples from hundreds of thousands of wells showed that critical arsenic concentrations had a very patchy distribution and suggested

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that water from shallow tube wells is unsuitable for human consumption in large areas. Water supply is – and will be – extremely decentralized in Bangladesh prohibiting the application of central water treatment procedures that could be applied in more developed regions. There are a number of alternatives to (shallow) tube wells including the costly drilling of deep wells, a return to the use of surface water, utilisation of rainwater and arsenic removal. The draft national policy on arsenic mitigation states a preference for the use of surface water over groundwater, although this does not necessarily take into account the potential risks of using surface water.

Different from pathogens such as *V. cholerae*, toxic cyanobacteria have been recognized only recently as a potential health hazard in surface waters used as a drinking water source. The type of toxin most frequently found in freshwater are microcystins (MCYST), hepatotoxic cyclic peptides produced by different genera of cyanobacteria such as *Microcystis*, *Nostoc*, *Anabaena* and *Planktothrix/Oscillatoria*, which frequently occur in dense water blooms in nutrient rich water bodies. In all potentially toxigenic taxa both microcystin-producing and non-microcystin-producing species/genotypes exist (Sivonen & Jones 1999). Microcystins have been detected in a great number of water samples from nearly every region on Earth and thus can be expected wherever blooms of cyanobacteria occur in surface waters (summarized, e.g. in Sivonen & Jones 1999). These toxins are usually found largely bound within the cells, whereas extracellular concentrations typically are low unless sudden and massive cell death leads to their lysis and toxin release (Welker *et al.* 2001).

In high doses, microcystins lead to acute liver failure due to the disruption of hepatocyte cytoskeletal components. Lethal doses (LD_{50}) of microcystins vary between their structural variants (some 90 have been described so far) and range from 100 to 600 $\mu g\ kg^{-1}$ bodyweight when applied by intraperitoneal injection, but are a factor of 10 – 100 higher by oral uptake (summarized by e.g. Dawson 1998; Sivonen & Jones 1999). Acute toxicity is therefore expected only under circumstances of heavy surface bloom formation and a direct uptake of water from such surface blooms, particularly when cells accumulate along shorelines by up to a factor of 1,000 more compared to the open water (Fastner *et al.* 1999a; Welker *et al.* 2003). Otherwise, the biomass of potentially toxigenic cyanobacteria in a well-mixed water body is generally limited to values that cannot lead to sufficiently high microcystin concentrations to cause acute toxicity after accidental uptake or consumption of water from respective sources. However, concentrations are frequently found in ranges that are chronically hazardous, including the promotion of tumours. A (provisional) WHO guideline (1998) has been developed with regard to chronic effects and proposes a maximally tolerable concentration of 1 μgl^{-1} microcystin-LR equivalents (microcystin-LR being the most toxic and one of the most abundant structural variants) for long-term exposure. Concentrations above this value can be encountered frequently in cyanobacteria-dominated water bodies (Sivonen & Jones 1999).

One of the few epidemiological studies available so far showed that in rural areas in China the rate of primary liver cancer was significantly higher in communities that received their drinking water from surface water infested with toxic cyanobacteria compared with communities consuming well water (Yu 1995; Kuiper-Goodman *et al.* 1999). However, in addition to toxic cyanobacteria the endemic rate of hepatitis B during the study period was high in China. Outbreaks of cyanotoxin-related diseases associated with the distribution of contaminated drinking water have been reported from several countries, particularly from Australia and Brazil (Falconer *et al.* 1983; Jochimsen *et al.* 1998; Kuiper-Goodman *et al.* 1999).

Microcystins (and other cyanotoxins) constitute a new challenge to water treatment technology (Hrudey *et al.* 1999). Microcystins are quite stable with respect to chemical and biological degradation. Thermal destruction occurs only at temperatures above 120°C thus making microcystins insensitive to boiling, the treatment most people use to render water safe for drinking. Biological degradation is believed to occur with most bacterial consortia (e.g. biofilms), but after a lag-phase ranging between days and weeks after release of the toxin from the cells (Welker *et al.* 2001). As microcystins largely occur cell-bound, they are effectively removed by filtration of intact cells with filter maintenance regimes that avoid cell rupture and lysis on the filters, which otherwise can cause microcystin release and breakthrough.

In Bangladesh, the main natural surface waters are rivers – the Padma (Ganges) and Jamuna (Brahmaputhra) being

the largest ones – which are divided into many arms and connected to each other either naturally or by man-made channels. Natural standing waters are rare, but man-made ponds are one of the main characteristics of rural Bangladesh. In Bangladesh, most of the houses are constructed on elevated ground for protection against flooding during the rainy season. The elevated ground is created by excavating the neighbouring area and building a compact earthen platform. The resulting depressions of the excavations fill with water during the rainy season forming ponds. Therefore, every house or group of houses is located near a pond (Islam *et al.* 2000). These ponds play an important role in the everyday life of the population around them. Pond water is directly used intensively and extensively for personal hygiene, washing of clothes and dishes, bathing of cattle and aquaculture. To a highly varying degree pond water is also used in rural areas for cooking rice and by the poor as drinking water. An individual pond can serve several purposes simultaneously. Due to the nutrient rich soils (mainly alluvial sediments) and high population densities in conjunction with rudimentary sanitation facilities the ponds can be generally considered as eutrophic. In many of these intensively used eutrophic ponds cyanobacterial blooms are common, and microcystins have been detected occasionally in pond water from several regions, mainly associated with high abundance of *Microcystis* sp. (Khan *et al.* 2001; Ahmed *et al.* 2002; Affan *et al.* 2002).

The aim of the present work was a pilot study assessing cyanobacterial abundance and microcystin concentrations in pond waters of rural Bangladesh. This study is limited, since the number of ponds in Bangladesh is overwhelming and since it could not follow seasonal changes in individual ponds. While the resulting dataset is insufficient as a basis for risk assessment, it does elucidate the scale of the potential health hazard associated with cyanotoxin occurrence in Bangladesh.

METHODS

Sampling sites

A total of 79 ponds in different regions of Bangladesh were sampled in September 2002. These were selected mainly by practical criteria such as their accessibility from the next road. In most cases we sampled sets of ponds in close proximity to each other in rural settlements after enquiring about the respective use of individual ponds by the local population. Most ponds were in a size range of less than 20 m in diameter and had a depth of about 1 to 1.5 m as reported by local people.

Samples were taken in three regions of Bangladesh (Figure 1, Table 1). In and around the city of Mymensingh 46 ponds were sampled in rural communities as well as in the urban centre where ponds are still an important water source for multiple purposes. In the Mymensingh district surface water is used only to supplement tube well water or by the lowest income families that do not have access to tube well water.

In the Khulna division 16 ponds were sampled all of which are the raw water source of pond sand filtration (PSF) devices. Samples were taken around the cities of Dacope and Bagerhat, which are located at the northern edge of the mangrove forest of the Sundarbans. The groundwater in these regions has a high salinity, making it unsuitable for human consumption without (unaffordably) expensive treatment. Thus, the use of surface water instead of tube well water is common irrespective of high arsenic concentrations in the groundwater at particular sites.

The arsenic contamination of groundwater is critical in the Chandpur area, but there the salinity is low so that tube well water is still widely used. Samples were taken near Chandpur and Matlab from ponds serving as a water source for PSF as well as from other ponds.

Sampling and sample processing

Water samples were taken with a bucket at the site where the local people take water for domestic use, wash dishes, etc. and in many cases local people assisted in taking water, thus guaranteeing a sample very similar to the water that is commonly used. A sample of 1 litre was collected in a polyethylene bottle and stored in a cooling bag upon returning to the laboratory.

For analysis of cell-bound microcystins, a subsample (10–200 ml) of the well-mixed water sample was filtered over a membrane filter (cellulose nitrate 0.45 μm, Schleicher and Schuell, Germany). The filters were then folded, put in a 2 ml reaction tube and placed in a drying oven at a maximum temperature of 50 °C. After about 10 h (over-

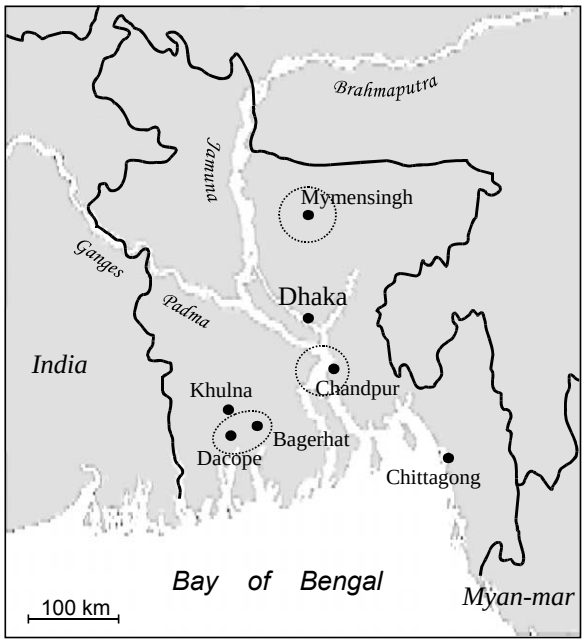

Figure 1 | Map of Bangladesh showing the major rivers and larger cities. The respective sampling areas are indicated with circles.

night) in the drying oven the filters were checked for dryness and dry filters were stored cool (when possible at 4 °C) in an airtight box with dry silica gel. From each water sample duplicate subsamples for toxin analysis were prepared and transported to Germany.

Aliquots of all water samples were prepared for the determination of phytoplankton biomass. Polycarbonate centrifuge tubes were filled with 12 ml of raw water and fixed with Lugol's iodine solution and stored in a cool, dark location.

Phytoplankton determination and quantification

Determination of dominant phytoplankton taxa was performed with a light microscope immediately on fresh samples (Bourelly 1970; Anagnostidis & Komárek 1988; Komárek & Anagnostidis 1989; Komárek & Anagnostidis 1999). Cyanobacterial biomass was determined from fixed samples by cell-counts and estimation of cell volumes according to Utermöhl (1958). *Microcystis* and *Anabaena* species were identified to species level, but quantified by genera. The abundances of cyanobacterial picoplankton and very thin filaments ($< 1\ \mu m$) of cyanobacteria as well as other phytoplankton taxa were determined semi-quantitatively.

Microcystin analysis

The dried filters were extracted thrice by sonication with 1.5 ml 70% methanol. Combined supernatants were completely dried in an evaporator and stored dry at −20°C until analysed by high-performance liquid chromatography (HPLC). Dry samples were resolved in 300 µl of 50% methanol, centrifuged and the clear supernatant was injected (200 µl). The HPLC system consisted of a Waters 600S controller, a 616 pump, a 717plus autosampler, and a 996 photodiode array detector (PDA). A gradient of water and acetonitrile (with 0.05% TFA) according to Lawton *et al.* (1994) was applied to a RP-C18 column (Lichrospher, Merck) at a flow rate of 1 ml min^{-1}. Chromatograms at a wavelength of 238 nm were derived from PDA data and microcystins were quantified by calibrating the system with microcystins -LR, -RR and -YR. Other microcystins were identified by their UV-spectra and quantified as MCYST-LR equivalents. Several fractions with microcystins were collected and analysed further by MALDI-TOF mass spectrometry as described in detail previously (Fastner *et al.* 1999b; Welker *et al.* 2002).

The extraction method has been tested previously and has been shown to be efficient to allow detection and quantification of microcystins well below the critical value of 1 µg l^{-1}.

RESULTS

Phytoplankton abundance

The large-scale climatic conditions in Bangladesh can be considered as favourable for cyanobacterial growth in all ecological niches. High temperatures, high irradiation intensities, and high precipitation and humidity make cyanobacteria thrive in epiphytic, epilithic and epipsammic microhabitats as well as in aquatic ecosystems.

A pronounced regional difference regarding the abundance of cyanobacteria could be observed for the ponds sampled in September 2002 (Table 1). Cyanobacteria were most frequent in the Mymensingh area, somewhat less frequent in the Chandpur and Matlab area, and were only rarely found in ponds sampled in the Khulna area. In ponds with no or low cyanobacterial abundance, the phytoplankton was dominated by Euglenophyceae, Chlorophyceae,

Table 1 | Compilation of information on sampled ponds in rural Bangladesh. Geographic information is related to the nearest larger town. Detailed information on exact locality names was not available. Quantitative phytoplankton data for potentially microcystin-producing genera (*Microcystis*, *Planktothrix*, *Anabaena*) given as biovolume, and semi-quantitative phytoplankton data for other cyanobacteria and algae. Microcystin concentrations are given as total MCYST concentrations in $\mu g\ l^{-1}$

Pond location	Cyanobacterial taxa and biovolumes ($mm^3\ l^{-1}$)						Other algae	MCYST
No.	Division	Upazila/town	Microcystis	Planktothrix	Anabaena	Other taxa	++/+++	$\mu g\ l^{-1}$
01	Dhaka	Mym., Maskanda	13.9	6.8				68
02	Dhaka	Mym., Maskanda	+		20.8		B, Ch	<0.1
03	Dhaka	Mymensingh City	8.2	+	+		B	9.4
04	Dhaka	Mymensingh City	1.8			NDTF	Ch	n.d.
05	Dhaka	Fulbaria	267.2	8.2		NDTF		31
06	Dhaka	Fulbaria	94.3	3.6	+	NDTF		22
07	Dhaka	Fulbaria	3.2			*Merismopedia* sp., NDTF		n.d.
08	Dhaka	Fulbaria	27.7	50.9				3.4
09	Dhaka	Fulbaria		24.5			Ch	n.d.
10	Dhaka	Fulbaria	868.2	+				45
11	Dhaka	Fulbaria	23.4	8.5	+	*Pseudanabaena* sp.		38
12	Dhaka	Fulbaria	41.1					11
13	Dhaka	Fulbaria	1.3				Ch, Eu	28
14	Dhaka	Fulbaria	13.1	11.3				24
15	Dhaka	Mym., Shankipara	4.5			*Oscillatoria* sp.		1.4
16	Dhaka	Mym., Shankipara	1322	12.8		NDTF		1390
17	Dhaka	Mym., Shankipara	33.7	60.2				20
18	Dhaka	Mym., Shankipara	54.0	36.7				17
19	Dhaka	Mym., Shankipara	1.1			*Merismopedia* sp.		n.d.
20	Dhaka	Mymensingh	12.6			*Pseudanabaena* sp.		48

Table 1 | (*continued*)

Pond location	Cyanobacterial taxa and biovolumes ($mm^3\ l^{-1}$)						Other algae	MCYST
No.	Division	Upazila/town	Microcystis	Planktothrix	Anabaena	Other taxa	++/+++	$\mu g\ l^{-1}$
21	Dhaka	Mym., BAU	6425					n.d.
22	Dhaka	Mym., BAU	9.2		2.1	c.f. *Microcystis*		13
23	Dhaka	Mym., Nowmohal	6.3	897.0	+	*Oscillatoria* sp.		n.d.
24	Dhaka	Mym., Nowmohal	1.4			*Merismopedia* sp., NDTF		n.d.
25	Dhaka	Ishwargonj	3.8			NDTF	B, Ch, Eu	n.d.
26	Dhaka	Ishwargonj	7.5	14.7	109.7	NDTF	Ch	0,87
27	Dhaka	Ishwargonj	+			NDTF	Ch	n.d.
28	Dhaka	Ishwargonj	+			NDTF		n.d.
29	Dhaka	IIshwargonj	5.3		25.6	NDTF		0,63
30	Dhaka	Ishwargonj	+			*Merismopedia* sp., NDTF	Ch	n.d.
31	Dhaka	Ishwargonj	+			NDTF	Ch	n.d.
32	Dhaka	Ishwargonj	2.6			NDTF	Ch	0,49
33	Dhaka	Ishwargonj	5.5	14.5	1.2			n.d.
34	Dhaka	Ishwargonj	6.8			*Merismopedia* sp., NDTF	Ch	0,76
35	Dhaka	Ishwargonj	9.0	3.0	28.7	*Oscillatoria* sp., NDTF		4.6
36	Dhaka	Ishwargonj	18.8	4.6	35.7	*Oscillatoria* sp., NDTF		2.6
37	Dhaka	Ishwargonj		7.8	+	*Oscillatoria* sp., NDTF, *Pseudanabaena* sp.	B, Ch	n.d.
38	Dhaka	Ishwargonj	2.2	235.6		NDTF, *Aphanizomenon* sp.		0.30
39	Dhaka	Mym., BAU	2346		+		Eu	n.d.
40	Dhaka	Mym., Bailar	129.3	+	6.1	NDTF	B, Eu	n.d.
41	Dhaka	Mym., Bailar	+			*Anabaenopsis* sp. *Merismopedia* sp., NDTF	B, Ch	0.21
42	Dhaka	Muktagacha	216.1	+	15.5	*Pseudanabaena* sp.		535
43	Dhaka	Muktagacha	51.2	+	+	NDTF		23

Table 1 | (*continued*)

Pond location	Cyanobacterial taxa and biovolumes ($mm^3\ l^{-1}$)						Other algae	MCYST
No.	Division	Upazila/town	Microcystis	Planktothrix	Anabaena	Other taxa	++/+++	$\mu g\ l^{-1}$
44	Dhaka	Muktagacha	119.0	+	+	NDTF		25
45	Dhaka	Muktagacha	231.5			*Merismopedia* sp., NDTF		3.5
46	Dhaka	Muktagacha	*No data*					1.9
50	Khulna	Dacope	0.5					n.d.
51	Khulna	Dacope						n.d.
52	Khulna	Dacope	+					n.d.
53	Khulna	Dacope	+				Eu, Cr	<0.1
54	Khulna	Dacope	+				Eu, Cr	n.d.
55	Khulna	Dacope, Barikhali	+					n.d.
56	Khulna	Bagherat, Fultala	+				Eu	n.d.
57	Khulna	Bagherat, Fultala	+				Eu	n.d.
58	Khulna	Bagherat, Fultala	+					n.d.
59	Khulna	Bagherat, Kazapara	+					n.d.
60	Khulna	Bagherat, Kazapara	+				B	n.d.
61	Khulna	Bagherat, Khachna	+					n.d.
62	Khulna	Bagherat, Khachna	+					n.d.
63	Khulna	Bagherat, Kochoa	+					n.d.
64	Khulna	Bagh., Gimta Khali	+					n.d.
65	Khulna		+					n.d.
66	Chittagong	Chandpur	+			NDTF	Ch, Eu	0.19
67	Chittagong	Chandpur	+				Eu	n.d.
68	Chittagong	Chandpur	2.3	0.7	2.9	*Merismopedia* sp., NDTF	B, Ch, Eu	0.60
69	Chittagong	Chandpur, Bagadi	0.5			NDTF	B, Ch, Eu	n.d.
70	Chittagong	Chandpur, Pakhidia	1.2		+	*Merismopedia* sp.		0.17

Table 1 | (*continued*)

Pond location	Cyanobacterial taxa and biovolumes ($mm^3\ l^{-1}$)						Other algae	MCYST
No.	Division	Upazila/town	Microcystis	Planktothrix	Anabaena	Other taxa	++/+++	$\mu g\ l^{-1}$
71	Chittagong	Chandpur				*Oscillatoria* sp.	Ch	0.14
72	Chittagong	Chandpur	+			*Merismopedia* sp.	Eu	0.25
73	Chittagong	Chandpur	+				Ch, Eu, Cr	n.d.
74	Chittagong	Chandpur	+			NDTF	Cr	n.d.
75	Chittagong	Chandpur	510.6					268
76	Chittagong	Chandpur	147.6			*Pseudanabaena* sp.		226
77	Chittagong	Matlab	0.6		2.3		B, Ch	0.95
78	Chittagong	Matlab	0.4			NDTF	Ch	n.d.
79	Chittagong	Matlab	11.1			*Anabaenopsis* sp., *Merismopedia* sp.	Ch	n.d.
80	Chittagong	Matlab	+					n.d.
81	Chittagong	Matlab, Gazipur				NDTF	Ch, Eu, Cr	n.d.
82	Chittagong	Matlab, ICDDR	1.4	22.1		*Oscillatoria* sp.	Eu, Cr	n.d.

NDTF: various not determined (very) thin filaments, most probably Oscillatoriales
B: Bacillariophyceae. Ch: Chlorophyceae, Eu: Euglenophyceae, Cr: Cryptophyceae
Semiquantitative categories: +: rare; ++: common; +++: abundant. n.d.: not detectable.

Bacillariophyceae or Cryptophyceae, or by mixtures of these phyla (Table 1). Occasionally Euglenophyceae formed stable surface films of a reddish colour in some ponds. The lowest abundance of phytoplankton was observed for ponds in the Khulna area, in some of which only very low numbers of planktonic algae could be found.

The cyanobacterial populations frequently included potentially microcystin-producing taxa such as *Microcystis*, *Planktothrix* and *Anabaena* in varying abundances (Table 1). *Microcystis* was dominant in many ponds with maximal biovolumes of up to 6,425 $mm^3\ l^{-1}$, but also occurred as co-dominant with *Planktothrix* and/or *Anabaena*. Exclusive dominance of either *Planktothrix* or *Anabaena* was rarely observed. Other abundant cyanobacterial taxa in the ponds were *Oscillatoria* sp., *Merismopedia* sp., *Pseudanabaena* sp., *Anabaenopsis* sp., and not further identified thin ($< 1\ \mu m$) filaments.

Occurrence of microcystins

The results of HPLC analyses of seston samples are compiled in Table 1 and in Figure 2. Microcystins could be detected in 39 out of 79 pond samples, most of them with obvious blooms of cyanobacteria, i.e. presence of surface scums, but also in some in which cyanobacteria were only sub-dominant (Table 1). Ponds in the Mymensingh (30 out of 46) and Chandpur/Matlab (8 out of 17) districts frequently contained microcystins, while in only one out of 16 ponds in the Khulna area trace amounts ($\ll 1\ \mu g\ l^{-1}$) were detected.

From the 39 positive samples 14 showed only trace concentrations below $1\ \mu g\ l^{-1}$, i.e. below the WHO

guideline value for microcystins. In the remaining 25 pond samples containing microcystins, concentrations were above 1 μg l^{-1} and ranged up to more than 1,000 μg l^{-1}. All ponds with high toxin concentrations were located in the Mymensingh district with the exception of ponds no. 75 and 76 in Chandpur district.

High concentrations of microcystins were coincident with blooms of *Microcystis* in many ponds, though not all *Microcystis* blooms (e.g. ponds 21 and 39) contained detectable amounts of microcystins. Most samples with high abundance of *Planktothrix* and/or *Anabaena*, and also some samples with co-occurrence of several potentially toxigenic taxa showed no or only low concentrations of microcystins suggesting that *Planktothrix* and *Anabaena* are less important microcystin producers in Bangladesh.

The structural variants that dominated in all samples were microcystins RR, YR and LR (Figure 2). Nine other variants with a minor share could be detected in the samples making up a combined share of some 30% of total microcystins at maximum. With respect to the relative contribution of particular structural variants some samples can be grouped owing to their high similarity. In ponds 01, 13, 14 and 15, for example, microcystin-LR contributed approximately 60% to the total microcystin concentration, and microcystin-RR contributed only around 10%. Most likely in all these ponds the same species or genotype is responsible for the toxin production; in ponds 13 to 15 this is very likely since these ponds were located next to each other.

DISCUSSION

Choice of detection method

The occurrence of cyanobacteria and cyanotoxins is increasingly addressed in many drinking-water monitoring programmes, and experience with the practicality of an array of chemical and biochemical techniques for their detection is increasingly available. However, implementing monitoring schemes in settings such as tropical developing countries faces specific challenges. One target of the present pilot study was to assess the possibilities for implementing an effective monitoring programme under these circumstances in cooperation with local authorities and scientists.

Analysis of seston samples by HPLC following a ubiquitously applicable sample preparation protocol by filtration was given preference to other techniques such as immunochemical tests (e.g. enzyme-linked immunosorbent assay, ELISA). ELISA analyses, though generally simple in handling, require an uninterrupted cooling chain of the kit that cannot be guaranteed in Bangladesh because of frequent power cuts and often (unexpectedly) long transportation times. The toxins themselves, especially microcystins, have proved to be very stable when dried, allowing transportation and storage for a period of some months without significant degradation. They can subsequently be analysed in a laboratory equipped with HPLC. Though ELISA is more sensitive than HPLC, the results of the HPLC analyses clearly showed that their sensitivity was sufficient: the lowest detected concentration was more than one magnitude lower than the provisional WHO guideline value for microcystin-LR. A further advantage of HPLC is that it differentiates microcystin variants, which is relevant because of their differences in toxicity.

Frequency of occurrence and concentrations of microcystins

The frequency of microcystin occurrence and concentrations in Bangladesh ponds agrees well with other findings from both temperate and subtropical water bodies. Worldwide, most surveys have detected microcystins in more than 50% of the water bodies investigated with concentrations usually ranging between <1 up to 1,000 μg l^{-1}, and the likelihood of microcystin occurrence increases with the abundance of potentially toxigenic taxa (Sivonen & Jones 1999). Microcystin concentrations in more than half of the ponds tested positive for microcystins in the present survey exceeded, in some cases substantially, the provisional WHO guideline value of 1 μg l^{-1} (microcystin-LR).

For assessing the regional differences found in both the abundance of cyanobacteria and microcystin concentrations, seasonality as well as the trophic status and the ecology of the ponds have to be considered. In eutrophicated ponds, the concentration of microcystins and other cyanotoxins depends mainly on the presence of potentially toxigenic species/genotypes and their biomass, which may fluctuate on a seasonal scale. Blooms of cyanobacteria

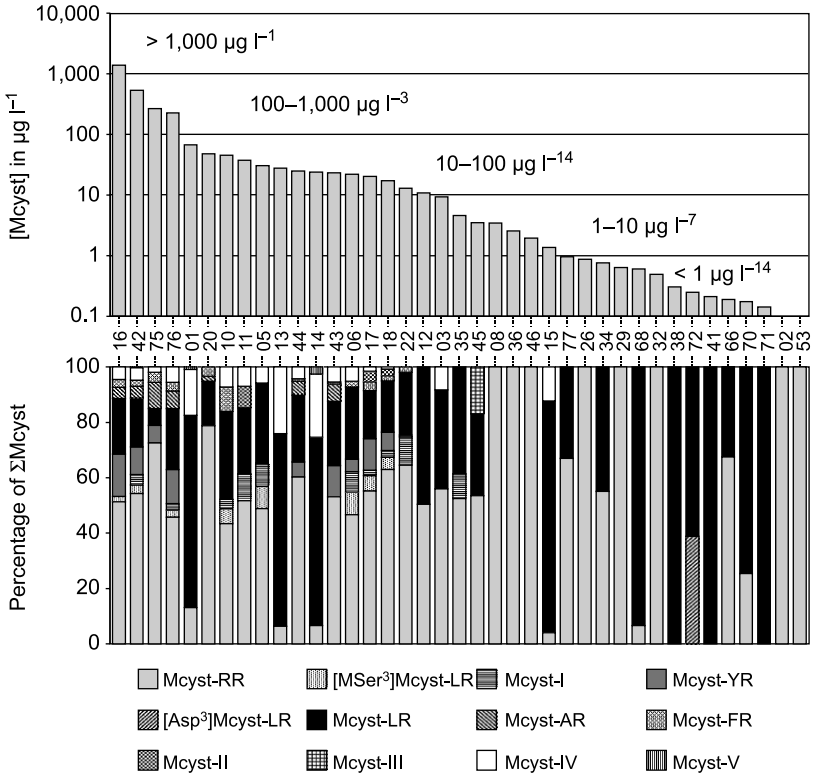

Figure 2 | Summary of microcystin occurrence in pond-water samples of Bangladesh. The upper panel shows the results for the samples in the order of concentration as calculated from duplicate samples. The lower panel shows the relative contribution of individual structural variants to the total concentration of microcystins. The numbers between the panels refer to the pond number as listed in Table 1. The structure of microcystins I to V could not be determined unambiguously.

occur during or shortly after the monsoon season in Mymensingh district while in the Chandpur district the blooming season starts mostly in October and lasts through the winter (Saleha Khan, Sirajul Islam, Nurul Huda Khan, unpublished data). Nevertheless, water temperatures in ponds rarely drop below 20°C, thus allowing persistent cyanobacterial growth without a distinct winter break that is typical for temperate surface waters. Reliable data on plankton dynamics are, however, scarce and thus for some selected sites an extended monitoring should be carried out to understand the seasonality of toxin concentrations and to allow some basic estimates of when peak concentrations are to be expected in different regions of Bangladesh.

In many ponds no or only low numbers of cyanobacteria could be found and microcystin concentrations were well below 1 µg l^{-1}. This applies especially to the ponds investigated in the Khulna area, all of which are used as source water for subsequent PSF and thus are well maintained and protected by a fence. These fences efficiently prevent access by the omnipresent domestic animals and also exclude human wastes from the immediate vicinity, as reported by the local people. This appears to reduce the direct nutrient input to the respective ponds considerably and could be the reason why densities of cyanobacteria and algae were comparatively low, at least during the sampling campaign in September 2002. Further studies including the seasonal phytoplankton dynamics should investigate whether or not in the protected ponds cyanobacterial blooms and cyanotoxins occur at all.

Ponds without critical microcystin concentrations could also be found in regions where concentrations were high in neighbouring ponds. Neighbouring ponds sometimes differed substantially in important factors such as water depth, degree of shading by trees, food-web structure (e.g. different fish stocks) and nutrient supply (e.g. supplementary fish feeds, organic waste due to access by ducks, cattle, etc.). As a result, ponds situated next to each other showed highly differing phytoplankton communities. This precludes any generalizing approaches to evaluation of the occurrence of cyanotoxins even on a limited regional scale.

Microcystin occurrence in relation to dominant cyanobacterial taxa

Microcystis could be clearly identified as the main microcystin-producing cyanobacterial genus in Bangladesh ponds. Concentrations were high in some almost monospecific blooms of this taxon, and the structural microcystin variants that dominated in all samples were MCYST-RR, -YR and -LR (Figure 2), the same major variants found in most samples from *Microcystis*-dominated water bodies in temperate and subtropical latitudes (e.g. Henriksen 1996; Park *et al.* 1998; Fastner *et al.* 1999b). While MCYST-RR is less toxic, the toxicity of MCYST-YR is equal to that of MCYST-LR (Sivonen & Jones 1999). Previous studies in Bangladesh also determined MCYST-RR, -YR, -LR and -LA as major microcystins in blooms of *M. aeruginosa*, but similar to our findings not all *Microcystis* blooms were found to contain microcystins (Affan *et al.* 2002; Ahmed *et al.* 2002). Indeed, in the ponds with the highest *Microcystis* biovolumes, ponds 21 and 39, no microcystins could be detected. This is probably due to differences in the predominating species/genotypes between the water bodies: while some may be dominated by non- (less)toxic genotypes, others may be dominated by toxic ones. Toxin contents of *Microcystis* strains isolated from a single water body have been shown to vary over several orders of magnitude (Rohrlack *et al.* 2001).

The genera *Planktothrix* and *Anabaena*, known as microcystin producers in temperate lakes, seem to be less important microcystin producers in the ponds investigated in the present study. Demethylated variants of MCYST-RR, -HtyR and -LR, frequently observed with several *Planktothrix* species in temperate lakes (Fastner *et al.* 1999b), could not be detected in any of the samples. From the available data it cannot be excluded that the *Planktothrix* species present in Bangladesh produce the same microcystin variants as *Microcystis*. Furthermore, the *Planktothrix* species present in the ponds could not be identified, as the taxonomy of tropical cyanobacteria is poorly developed and respective modern identification keys are not available. Further potentially microcystin producing cyanobacterial taxa in Bangladesh might be identified with isolated strains. Also, in the course of seasonal phytoplankton succession (which was not assessed in this preliminary survey), microcystin-producing species/genotypes of *Planktothrix* and *Anabaena* could well occur in Bangladesh.

Cyanotoxin hazard assessment

As discussed above, many of the samples analysed in this preliminary survey contained the most toxic microcystin variants LR and YR, and concentrations substantially above the provisional WHO guideline value of 1 $\mu g\,l^{-1}$ were found quite frequently. For microcystins, hazardous exposure occurs through oral uptake and thus depends on the local populations' practices of water use. However, this is a major uncertainty in estimating current exposure – it is unknown to what extent the local population uses pond water for drinking and food preparation. If water is used for drinking, concentrations found in this survey are likely to lead to exposure at a dose that may impair health. As discussed by Chorus & Fastner (2001), a child of 10 kg body weight will already be exposed to the TDI (tolerable daily intake) proposed by WHO through consumption of 4 ml of water containing 100 $\mu g\,l^{-1}$ of microcystin-LR (or the equally toxic YR), or 40 ml of water containing 10 $\mu g\ l^{-1}$. At concentrations of 1,000 $\mu g\ l^{-1}$, consumption of 0.5–1.0 l per day would expose a 10 kg child to concentrations that caused liver damage in animal experiments run over several weeks.

For assessing health hazards due to cyanobacteria, the current understanding is that microcystins are the most important cyanotoxins on the basis of their frequency of occurrence in concentrations above the provisional WHO guideline level of 1 $\mu g\ l^{-1}$. However, cyanobacteria can produce other toxic substances, and various bioassays demonstrate bioactivity not accounted for by their known metabolites (e.g. Oberemm *et al.* 1999).

Cyanotoxin risk management

Consolidating current scientific evidence indicates that there is no way to influence whether or not a prevailing cyanobacterial population will produce microcystins: cells possessing the gene encoding for microcystin production also contain the toxins in fairly stable concentrations. Management measures must therefore address controlling concentrations of cyanobacterial biomass. This has the advantage of encompassing all of their known toxins together with other, poorly understood bioactive metabolites. In this context,

microcystin concentrations may be used as a point of reference for setting health-based targets for cyanobacterial cell densities in water used for drinking. As mentioned above, the preliminary results of the survey indicate a considerable effect of some simple measures of protecting ponds such as fencing and shading on the total phytoplankton biomass as well as on cyanobacterial biomass. First actions could make use of recent educational campaigns on hygiene and sanitation in rural Bangladesh by underlining the necessity of the selection of ponds for drinking water supply that have then to be protected by the available respective measures. Such campaigns might also include demonstrations of how cyanobacterial proliferation can be identified by pond users from the greenish discolouration and high turbidity of the water and/or surface scums. In this context, local populations should be informed that boiling water is not effective to remove microcystins.

Where no pond water with low cyanobacterial cell density is available, locally feasible approaches to removing cells need to be explored. These may be quite effective particularly for removing microcystins, as these occur mainly cell-bound and scarcely dissolved in water except for cases of massive and synchronous cell lysis. One lowest cost technology approach, the saree-filtration, which applies a folded cotton cloth (in general a piece of the traditional dress of Bengali women), has been shown to reduce infections of cholera by removing germ-carrying vectors (Colwell *et al.* 2003). Preliminary experiments to estimate phytoplankton and microcystins reduction through saree filtration showed a reduction of biomass and toxin concentration to about 20 to 50% in two water samples treated, respectively. Other approaches to remove cells from pond water, such as the PSF schemes already conducted in the Khulna division, merit assessment of their efficacy for reliable removal of cyanobacteria and cyanotoxins.

CONCLUSIONS

1. This survey shows toxic cyanobacteria to occur quite commonly in surface waters of Bangladesh. A generalization on 'safe' and 'critical' waters is hampered by the patchiness of occurrence of cyanobacteria on very small scales.
2. Microcystins can reach very high concentrations. From respective waters even the use for cooking can lead to cyanotoxin uptake in concentration ranges likely to be hazardous for health. Depending on the water usage by the local population, people will be exposed to concentrations well above the provisional WHO guideline value for microcystin-LR.
3. A first step to avoid excessive cyanotoxin uptake could be the dissemination of knowledge: the simplest measure in many cases could be the use of water from nearby ponds with less dense or absent cyanobacterial blooms. The survey results suggest that pond protection measures as well as water filtration methods already practised in some settings merit further assessment of their reliability and efficacy for removing cell-bound cyanotoxins.

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REFERENCES

Affan, A., Jewel, A. S., Ueno, Y., Haque, M. M., & Khan, S. 2002 *Microcystis aeruginosa* growth stages and the occurrence of microcystins in natural and drinking waters of Bangladesh. *Abstract, Xth International Conference on Harmful Algae*, 21–25 October 2002, *St Peter Beach, FL*, p. 7.

Ahmed, K. M. 2002 Geological and hydrogeological controls on the occurrence and distribution of arsenic in Bangladesh groundwater. In *Arsenic Contamination: Bangladesh Perspective* (ed. M. F. Ahmed), ITN Bangladesh, Dhaka, Bangladesh, pp. 134–162.

Ahmed, S. M., Dahlmann, J. & Luckas, B. 2002 Toxic cyanobacteria blooms and the occurrence of microcystin from freshwater eutrophic ponds in Bangladesh. *Abstract, Xth International Conference on Harmful Algae*, 21–25 October 2002, *St Pete Beach, FL*, p. 8.

Anagnostidis, K. & Komárek, J. 1988 Modern approach to the classification system of cyanophytes: 3 - Oscillatoriales. *Arch. Hydrobiol. Suppl.* **80**, 327–472.

Bourelly, P. 1970 *Les algues dèau douce - Tome III: Algues bleues et rouges*. Editions N. Boubee & C., Paris, France.

Briscoe, J. 1978 Domestic water use in a village in Bangladesh. I. A methodology and a preliminary analysis at use patterns during the 'cholera season'. *Prog. Wat.* **11**, 131–141.

Chorus, I. & Fastner, J. 2001 Recreational exposure to cyanotoxins. In *Cyanotoxins - Occurrence, Causes, Consequences* (ed. I. Chorus), Springer Verlag, Berlin, pp. 190–199.

Colwell, R. R., Huq, A., Islam, M. S., Aziz, K. M. A., Yunus, M., Khan, M. H., Mahmud, A., Sack, R. B., Chakraborty, J., Sack, D. A. & Russek-Cohen, E. 2003 Reduction of cholera in Bangladeshi villages by simple filtration. *Proc. Natl Acad. Sci. USA* **100**, 1051–1055.

Dawson, R. M. 1998 The toxicology of microcystins. *Toxicon* **36**, 953–962.

Falconer, I. R., Beresford, A. M. & Runnegar, M. T. C. 1983 Evidence of liver damage by toxin from a bloom of the blue-green alga. *Microcystis aeruginosa*. *Med. J. Aust.* **1**, 511–514.

Fastner, J., Neumann, U., Wirsing, B., Weckesser, J., Wiedner, C., Nixdorf, B. & Chorus, I. 1999a Microcystins (hepatotoxic heptapeptides) in German fresh waterbodies. *Environ. Tox.* **14**, 13–22.

Fastner, J., Erhard, M., Carmichael, W. W., Sun, F., Rinehart, K. L., Rönicke, H. & Chorus, I. 1999b Characterization and diversity of microcystins in natural blooms and strains of the genera *Microcystis* and *Planktothrix* from German freshwaters. *Arch. Hydrobiol.* **145**, 147–163.

Henriksen, P. 1996 Microcystin profiles and contents in Danish populations of cyanobacteria/blue-green algae as determined by HPLC. *Phycologia* **35**, 102–110.

Hrudey, S. E., Burch, M., Drikas, M. & Gregory, R. 1999 Remedial measures. In *Toxic Cyanobacteria in Water* (ed. I. Chorus & J. Bartram), E & FN Spoon, London, pp. 276–312. www.who.int/docstore/water_sanitation_health/toxicyanobact/ch17.html

Islam, M. S., Begum, A., Khan, S. I., Sadique, M. A., Khan, M. N. H., Albert, M. J., Yunus, M., Huq, A. & Colwell, R. R. 2000 Microbiology of pond ecosystems in rural Bangladesh: Its public health implications. *Int. J. Environ. Stud.* **58**, 33–46.

Jochimsen, E. M., Carmichael, W. W., An, J., Cardo, D. M., Cookson, S. T., Holmes, C. E. M., Antunes, M. B., de, C., Filho, D. A., de, M., Lyra, T. M., Barreto, V. S. T., Azevedo, S. M. F. O. & Jarvis, W. R. 1998 Liver failure and death after exposure to microcystins at a dialysis center in Brazil. *New Engl. J. Med.* **339**, 873–878.

Khan, S., Affan, A., Haque, M., Imokawa, M. & Ueno, Y. 2001 Determination of microcystins in natural and drinking water of Bangladesh by ELISA. In: *Book of Abstracts, Fifth International Conference on Toxic Cyanobacteria, 16–20 July 2001, Noosa, Australia*.

Komárek, J. & Anagnostidis, K. 1989 Modern approach to the classification system of cyanophytes: 4 - Nostocales. *Arch. Hydrobiol. Suppl.* **82**, 247–345.

Komárek, J. & Anagnostidis, K. 1999 Cyanoprokaryota 1. Teil: Chroococcales. In *Süβwasserflora von Mitteleuropa* (ed. H. Ettl, G. Gärtner, H. Heynig & D. Mollenhauer), Band 19/1. Gustav Fischer Verlag, Stuttgart, pp. 113–153.

Kuiper-Goodman, T., Falconer, I. R. & Fitzgerald, J. 1999 Human health aspects. *Toxic Cyanobacteria in Water* (ed. I. Chorus & J. Bartram). E & FN Spon, London, www.who.int/docstore/water_sanitation_health/toxicyanobact/ch12.html

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

Oberemm, A., Becker, J., Codd, G. A. & Steinberg, C. E. W. 1999 Effects of cyanobacterial toxins and aqueous crude extracts of cyanobacteria on the development of fish and amphibians. *Environ. Toxicol.* **14**, 77–88.

Park, H. D., Iwami, C., Watanabe, M. F., Harada, K.-I., Okino, T. & Hayashi, H. 1998 Temporal variabilities of the concentrations of intra- and extracellular microcystin and toxic *Microcystis* species in a hypertrophic lake, Lake Suwa, Japan (1991–1994). *Environ. Toxicol. Wat. Qual.* **13**, 61–72.

Rohrlack, T., Henning, M. & Kohl, J.-G. 2001 Isolation and characterization of colony-forming *Microcystis aeruginosa* strains. In *Cyanotoxins: Occurrence, Causes, Consequences* (ed. I. Chorus), Springer Verlag, Berlin, pp. 152–158.

Sivonen, K. & Jones, G. J. 1999 Cyanobacterial toxins. In *Toxic Cyanobacteria in Water* (ed. I. Chorus & J. Bartram), pp. 41–111. E & FN Spon, London, www.who.int/docstore/water_sanitation_health/toxicyanobact/ch05.html.

Smith, A. H., Lingas, E. O. & Rahman, M. 2000 Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull. Wld Health Organ* **78**, 1093–1103.

Utermöhl, H. 1958 Zur Vervollkommnung der quantitativen Phytoplanktonmethodik. *Mitt. Int. Ver. Limnol.* **9**, 1–38.

Welker, M., Steinberg, C. & Jones, G. J. 2001 Release and persistence of microcystins in natural waters. In *Cyanotoxins: Occurrence, Causes, Consequences* (ed. I. Chorus), Springer Verlag, Berlin, pp. 85–103.

Welker, M., Fastner, J., Erhard, M. & von Döhren, H. 2002 Application of MALDI-TOF MS in cyanotoxin research. *Environ. Toxicol.* **17**, 367–374.

Welker, M., von Döhren, H., Täuscher, H., Steinberg, C. E. W. & Erhard, M. 2003 Toxic *Microcystis* in shallow lake Müggelsee (Germany): Dynamics, distribution, diversity. *Arch. Hydrobiol.* **157**, 227–248.

WHO 1998 *Guidelines for Drinking-water Quality. Addendum to Volume 2, Health criteria and other supporting information*, 2nd edition. World Health Organization, Geneva.

Yu, S.-Z. 1995 Primary prevention of hepatocellular carcinoma. *J. Gastroenterol. Hepatol* **10**, 674–682.

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