WHO/SDE/WSH/04.06
English only

Occurrence of Cyanobacterial Toxins (Microcystins) in Surface Waters of Rural Bangladesh - Pilot Study

Report
May 2004

Water, Sanitation and Health
Protection of the Human Environment
World Health Organization
Geneva

© World Health Organization 2004

The illustration of the cover page is extracted from Rescue Mission: Planet Earth, © Peace Child International 1994; used by permission

All rights reserved. Publications of the World Health Organization can be obtained from Marketing and Dissemination, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 2476; fax: +41 22 791 4857; email: bookorders@who.int). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to Publications, at the above address (fax: +41 22 791 4806; email: permissions@who.int).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

The World Health Organization does not warrant that the information contained in this publication is complete and correct and shall not be liable for any damages incurred as a result of its use.

Where the designation "country or area" appears in the headings of tables, it covers countries, territories, cities, or areas. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The named authors alone are responsible for the views expressed in this publication

WHO COLLABORATING CENTRE FOR RESEARCH ON DRINKING-WATER HYGIENE

at the

FEDERAL ENVIRONMENTAL AGENCY
(UMWELTBUNDESAMT)

Occurrence of cyanobacterial toxins (microcystins) in surface waters of rural Bangladesh – pilot study

Report

May 2004

Martin Welker, Ingrid Chorus and Jutta Fastner
Federal Environmental Agency
Section II 4.3 - Drinking-water Treatment
WHO Collaborating Centre for Drinking-water Hygiene
PO Box 330022, 14191 Berlin - Germany

Summary

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 source of drinking water is being 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 of 2002 for the presence of cyanobacteria and microcystins, the most frequently detected cyanobacterial toxins worldwide.

Microcystins were 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 up to >1000 µg/l, and more than half of the positive samples contained high concentrations of more than 10 µg/l. The results clearly show that concentration of microcystins well above the WHO provisional guideline value of 1 µg/l microcystin-LR can be frequently detected in Bangladesh ponds. Thus, an increasing use of surface water for human consumption would introduce a risk of replacing one health hazard by another and therefore needs to be accompanied by cyanotoxin hazard assessments.

Introduction

Drinking water in Bangladesh

The supply of clean and safe drinking water is one of the main challenges of public health care in Bangladesh. Traditionally, surface water was the main source of drinking water and was consumed without any treatment or after boiling when fuel (generally wood) was available. A rapidly increasing population density together with still insufficient sanitation frequently leads to outbreaks of water-borne infectious diseases like cholera. To avoid health hazards coupled to untreated surface water potentially contaminated with pathogens, the use of groundwater for human consumption was promoted starting about twenty years ago and some 1.3 million tube wells have been constructed since. The tube wells are mostly hand-operated and draw ground water from a depth of up to hundred meters, but more often only from a depth of a few to several tens of meters. Unfortunately the sedimentary geology of Bangladesh often contains high geogenic loads of arsenic that is soluble in dependence of the oxidation state of the element and thus of the depth of the groundwater layer that is used as reservoir.

With respect to diseases like cholera considerable progress has been made during the last two decades with respect to prevention, diagnosis, low-cost treatment, and, public education. Personal hygiene and application of basic sanitary measures are key factors for reducing water borne diseases and several education/information programs have been launched to address these issues. Amongst the alternatives to (shallow) tube wells being discussed are costly deep drilling of large wells and a return to the use of surface water. Where surface water needs to be used, low-tech water treatment options are being explored in order to significantly reduce the health hazard from surface waters. A functional local solution was the development and construction of pond sand filters (PSF) from the late 1980ies on. PSF’s principle is based on slow sand filter technology and the existing devices were built in many rural communities throughout the country. Where maintained well, PSF helped to drop incidences of cholera outbreaks considerably.

Cyanotoxins

Another potential threat from contamination of drinking water that has been recognized only recently could be the presence of toxic cyanobacteria in surface waters that are sources of drinking water. Cyanobacterial toxins (cyanotoxins) have been detected in a great number of water samples from nearly every region on earth.

Cyanotoxins can be largely classified in three major groups:

- Neurotoxic alkaloid toxins like anatoxin and saxitoxin that can be produced mainly by strains of the genera *Anabaena*, *Oscillatoria*, *Aphanizomenon*, and *Cylindrospermopsis*
- Hepatotoxic alkaloids: cylindrospermopsins that can be produced by *Cylindrospermopsis* and *Aphanizomenon* (preferentially in strains from tropical environments)

- The hepatotoxic peptides microcystins and nodularins potentially produced by strains of the genera *Microcystis*, *Planktothrix*, *Oscillatoria*, *Anabaena*, and *Nostoc*

Furthermore, toxicity not clearly attributed to known cyanotoxins can occasionally be observed in bioassays. The relevance of this to human health is poorly understood.

Among the three major groups, microcystins (Mcyst) are the most common cyanotoxins and can be expected wherever blooms of cyanobacteria occur in surface water. Their occurrence is highly likely when these blooms consist of the taxa *Microcystis*, *Anabaena*, or *Planktothrix* (see Sivonen and Jones 1999). Some of the approximately 70 structural variants of microcystin identified to date are highly toxic. These include the variants found most frequently in the taxon *Microcystis*, i.e. microcystin-LR[1] and YR.

Acute exposure to microcystin at sufficiently high concentration causes severe liver damage which is characterised by a disruption of liver cell structure (due to damage to the cytoskeleton), a loss of sinusoidal structure, increases in liver weight due to intrahepatic haemorrhage, haemodynamic shock, heart failure and finally death. The main concern with chronic exposure is promotion of tumour growth, possibly in consequence of continuous slight liver cell damage (see Kuiper-Goodman et al. 1999 for an overview).

For most of the many structural variants of microcystins, acute toxicity trials have been conducted using the standard measure of toxicity, i.e. intraperitoneal injection (i.p.), which allows comparison of toxicity of different substances. For microcystin-LR (and also for Mcyst-YR), the i.p. LD_{50}[2] ranges from 25 to 150 µg kg^{-1} body weight in mice. This renders these two microcystins very toxic in comparison to many other substances. Among the cyanotoxins, some neurotoxins are yet more toxic, i.e. anatoxin-a(S) with an LD_{50} (i.p. mouse) of only 10 µg/kg body weight and some variants of saxitoxins with an LD_{50} of 20 µg kg^{-1}. Natural exposure pathways, however, are oral, and substantially higher amounts of a toxic substance may be required to achieve the same effect, as only a fraction of the toxin actually reaches the target organ(s) where it causes the damage. For microcystin-LR, the oral LD_{50} is 5-10 mg kg^{-1} body weight in mice. There is no evidence of hydrolysis of microcystins by peptidases in the stomach and it is apparent that a significant amount of microcystin-LR passes the intestinal barrier and is absorbed (see Kuiper-Goodman et al. 1999).

Whether or not cyanotoxins can be acutely toxic to humans depends on environmental concentrations to which people might be exposed, e.g. through oral uptake. Microcystin concentrations typically range between 1 and 100 µg/l in the open water during enhanced cyanobacterial growth, but concentrations up to 25 mg/l in cell accumulations, e.g. at shorelines, have been found and may be even higher during presence of clones with higher cellular microcystin contents. Chorus and Fastner (2001) calculated that for a averagely sensitive 10 kg toddler an acutely lethal dose could be reached by ingestion of 1-2 litres of such a cyanobacterial suspension of “pea soup” consistency which contained 25 mg/l of

---

[1] Micocystins occur in many structural variants and a nomenclature has been established, referring to amino acids in two positions of the molecule, e.g. leucine and arginine in Mcyst-LR.

[2] LD50 = lethal dose 50%; refers to the toxin dose (usually in µg per kg body mass) at which 50% of the test organisms die during a defined exposure time.

microcystin (at an LD_{50} of 5 mg per kg body weight or 50 mg for the 10 kg child). Acutely lethal intoxication, particularly of small children, through ingestion of scum material therefore cannot be dismissed as a possibility under circumstances of heavy scum formation with very high microcystin content in the cyanobacterial cells. Also, this estimate indicates that with the more toxic cyanobacterial neurotoxins, a high cellular content coupled with high cell density in the water could in extreme cases lead to uptake of an acutely lethal dose through ingestion of only 100 ml or less.

Although in most situations the biomass of toxigenic cyanobacteria under natural condition is limited to levels that cannot account for toxin concentrations in acutely lethal ranges, concern for public health through uptake of water containing cyanotoxins is substantial, particularly for microcystins and in some regions of the world also for cylindrospermopsin. While there is little indication of long-term health impairments in consequence of exposure to the cyanobacterial neurotoxins (i.e. if exposed animals or humans survive, they are likely to recover), repeated exposure to microcystins at the concentrations in which they frequently occur allows uptake of doses which can lead to illness with the potential for long-term health damage as well as eventual death from this damage. This can be concluded from animal experiments, one of which indicated that repeated exposure to a sub-acute dose may cause cumulative liver damage in a quantity which as a single exposure did not show detectable effects within 24 hours. Numerous outbreaks of human illness attributed to microcystin and/or cylindrospermopsin exposure are reported in the literature, e.g. from Brazil, Australia, Africa, England and Scandinavia (see Kuiper-Goodman et al. 1999). These observations are reinforced by a larger number of cases of animal deaths (domestic, livestock and wildlife) after drinking water with cyanobacterial scums. While documented human illness includes only exposure to microcystins and cylindrospermopsin, the animal deaths also include exposure to cyanobacterial neurotoxins.

Only one of the outbreaks attributed to exposure through drinking or recreation included human fatalities. In this case, 88 children died when a dam developed an immense cyanobacterial bloom and no other (e.g. viral or bacterial) causative agents could be identified. A further outbreak resulting in more than 70 deaths could be very clearly attributed to cyanotoxins in water used for haemodialysis.

Chronic effects of microcystins include the promotion of primary liver cancer. One of the few epidemiological studies, for example, showed that in rural areas in China the rate of liver cancer was significantly higher in communities that received their drinking water from cyanobacteria-ridden surface water compared to communities consuming well water, and the authors' interpretation that this could be related to cyanotoxin occurrence is supported by evidence from several laboratory experiments (see Fitzgerald 2001).

From the data available, WHO derived a provisional Guideline value for microcystin-LR in drinking-water of 1 µg/L. This is based on a provisional TDI (tolerable daily intake) of 40 µg per kg body weight. Assessing the available data for potential derivation of a guideline value for cylindrospermopsin is on the working programme of WHO, and a value of 1 µg/L has been proposed (Falconer et al. 2002).

Background information for cyanotoxin hazard and risk assessment

As of yet it is unclear how microcystin variants other than microcystin-LR can be included in hazard assessment, as for almost all of them, data are available only for their acute toxicity upon i.p. injection. Though microcystin-LR is one of the most frequently occurring microcystins, especially in many blooms of the taxon *Microcystis*, other microcystins occurring in a sample cannot simply be ignored. A simple worst-case approach to hazard assessment is to use the Guideline value for all microcystins (see Falconer et al. 1999). Alternatively, exposure estimates could be conducted assuming that chronic toxicity of other microcystins relates to that of microcystin-LR with the same ratio as does acute toxicity; however, to date no data support this assumption.

The emerging body of knowledge on microcystin occurrence shows that concentrations in water-bodies infested with potentially toxic cyanobacteria frequently are well above 1 µg/L and rather often in the range of 100 µg/L or more (see Chorus, ed., 2001, Chapter 2 for an overview). It is therefore likely that microcystins are among the substances that occur in water in concentrations relevant to public health most frequently, and an impact on public health is likely if such water is consumed without removal of the toxic cyanobacterial cells.

With respect to water treatment technology microcystins (and other cyanotoxins) constitute a new challenge. Microcystins are cyclic peptides with considerable stability with respect to chemical and biological degradation. Thermal destruction occurs only at temperatures above 120°C, thus making microcystins insensitive to boiling, the most common low-tech water treatment (Harada 1996). Published results for biological degradation indicate that it is likely to occur with most bacterial communities (e.g. on biofilms), but lag-phases range between a few days to a few weeks (Welker et al. 2001). While this might potentially allow break-through of dissolved microcystins in treatment devices like PSF, microcystins usually occur largely within or bound to the cyanobacterial cells, and only a few per cent of the total pool are found dissolved in water, unless aging of a bloom or water treatment cause cell rupture and toxin release. The latter has been a problem when reservoirs were treated with copper sulphate. An issue currently being investigated is to which extent break-through in water treatment may also be induced through some treatment steps, e.g. if pre-oxidation damages the cells but does not oxidate the toxins, or if large amounts of cyanobacterial cell material accumulate on filters in treatment plants.

Objective of the study

The aim of the present study was to conduct a preliminary screening for the presence of toxic cyanobacteria and the magnitude of toxin concentration encountered in ponds of rural Bangladesh during a two-week sampling campaign in September 2002. On the basis of the current assessment of cyanotoxins (i.e. chronic toxicity being the major concern), the study focused on the occurrence of microcystins only. Furthermore, as sampling was conducted only once at each pond during a short time period, the data thus obtained are insufficient for assessing exposure risks through uptake with drinking-water. Rather, the objective was to assess whether and where work towards a risk assessment is likely to be needed as basis for decisions on promoting the use of surface waters as source of drinking water in substitution of arsenic contaminated tube well water.

Furthermore, for the present study the emphasis was put on the analysis of cell-bound microcystins. This was based on the general experience of cell-bound occurrence of most of the total microcystin pool in the vast majority of the samples studied from a range of settings, and is therefore adequate for a general overview of the presence and concentration of microcystins. Also, practical reasons required this focus, as the analysis of dissolved microcystins requires extended handling and processing procedures that was possible only for a small number of selected samples in the time frame and under the general conditions of the study.

Ponds in Bangladesh

The landscape of most of Bangladesh is dominated by very flat floodplains with substantial parts of the country submerged regularly during the monsoon season. The groundwater table is very high although dryness can be problematic during an extended dry season. The main natural surface waters are the rivers, the Padma and the Jamuna being the largest ones, that divide in many arms and that are connected to each other either naturally or by man-made channels. Natural standing waters are rare but artificial ponds are one of the main characteristics of rural Bangladesh.

Materials and methods

Sampling sites

Samples of pond water were taken in three regions of Bangladesh (Fig. 1). In each basic observations were also made and village members interviewed.

In and around the city of Mymensingh 46 ponds were sampled in rural communities as well as in the urban centre where ponds are also still an important water source for multiple purposes. In the Mymensingh district the arsenic concentrations in tube well water are considered to be low to moderate and surface water is used largely as a supplementary source of water, although low income families that do not have access to tube well water may also use it as drinking-water source.

In the district of Khulna 16 ponds were sampled, all of which are the water source of a PSF device. Samples were taken around the cities of Dacope and Bagherat that are located at the northern edge of the mangrove forest of the Sundarbans. As mentioned above, the groundwater in these regions has a high salinity and is unsuitable for human consumption without (unaffordable) expensive treatment. The arsenic contamination is a serious problem in Khulna district in general, but in the southern parts the use of surface water instead of tube well water is required – irrespective of arsenic concentrations – because of the levels of salinity.

The arsenic contamination of ground water is also critical in the Chandpur area, but there the salinity is low so that tube well water is widely used. Samples where taken around Chandpur and around Matlab from ponds serving as water source for PSF, and from other ponds. Some raw waters were filtered through eight layers of cotton cloth simulating the ‘saree filtration’ introduced by ICDDR,B staff in villages around Matlab.

Fig. 1: Map of Bangladesh showing the major rivers and larger cities. The sampling areas are indicated with circles.

Sampling, processing, and analysis

A total of 79 ponds in these 3 different regions of Bangladesh were visited and samples taken – a tiny fraction of the countries' myriad ponds. The sampled ponds were selected mainly for practical reasons such as their accessibility from the next road. In most cases sets of ponds in close vicinity to each other in rural settlements were visited after inquiring about the use of individual ponds by the local population.

Water samples were taken with a bucket at the site where the local people fetch water, wash dishes etc. and in many cases local people assisted in taking samples, thus providing a sample very similar to the water that is used. A sample of one litre was filled in a polyethylene bottle and stored in a cooling bag upon returning to the laboratory few hours later.

Sample processing and preservation was performed in the following laboratories: Fisheries Department of Bangladesh Agricultural University (BAU) in Mymensingh district, Department on Public Health Engineering (DPHE) local laboratory in Khulna, and department of environmental microbiology of the Centre for Health and Population Research (ICDDR,B), Dhaka for the samples taken in Chandpur district.

For analysis of cell-bound microcystins a subsample of the well mixed water sample was filtered over a membrane filter (cellulose nitrate 0.45µm, Sartorius). Folded filters were placed in a drying oven at a temperature of maximally 50°C. With some water samples only some 10 mL could be filtered before the filter was blocked. After about 10h (over night) in the drying oven the filters were checked for dryness and when dry were stored cool (when possible at 4°C) in an airtight box with dry silica gel. From each water sample two subsamples for toxin analysis were prepared following this protocol and transported to Germany. The sample processing and preparation up to this point was partly performed by students of BAU, and after an introduction this was done reliably and with accuracy. Selected samples for the analysis of dissolved microcystins were concentrated with solid phase cartridges. A volume of 500 to 1000 mL was drawn through a preconditioned SPE-cartridge for each sample and the retained organic fraction was eluted with pure methanol.

In parallel to the toxin samples phytoplankton samples for biomass determination were taken. 12 mL of raw water were filled in polycarbonate centrifuge tubes and fixed with Lugol’s iodine solution and stored cool and dark for later microscopic counting. A detailed analysis of the plankton communities was beyond the scope of our study, so quantitative counting was performed only for the cyanobacteria while cell density of other microalgae was estimated semi-quantitatively.

The extraction and analysis of the samples was performed at the Umweltbundesamt (Federal Environmental Agency) in Berlin, Germany, as described in Fastner *et al.* (1998).

The sampling and processing method proved suitable for local conditions and can be applied independent of the availability of electrical power everywhere. The only critical point is the drying procedure that requires a drying oven under the given climatic conditions. Dry samples can be stored cool and dark for a time of weeks to months without decay of the toxin. The extraction method was tested before and has been shown to be efficient to allow detection and quantification of microcystins well below the critical value of 1 µg/L.

Results

Description of ponds and their use

Most ponds are in a size range of less than 30 m in diameter and depths of about 1 to 1.5 m, as reported by the local people for the ponds we sampled. Each settlement of some dozen households has several artificial ponds in its close vicinity. Neighbouring ponds can differ substantially in water depth, degree of shading by trees, accessibility, food-web structure, and nutrient supply, to name the factors most important for controlling cyanobacterial development. As a result, ponds situated next to each other can show highly differing phytoplankton communities. This hampers any generalizing approaches to evaluation of the occurrence of cyanotoxins in these waters.

Pond water is directly used intensively and extensively for human consumption, personal hygiene, washing of clothes and dishes, bathing of cattle, and aquaculture. An individual pond can serve for several purposes at once. A common picture is stone or concrete steps at one edge allowing easy access to the water, and these sites are the main sites for water usage and abstraction.

Water for human consumption is abstracted from ponds in all regions visited during the sampling campaign, with differing intensity depending on alternative sources of drinking water. The following information on water use was compiled from oral accounts by local people, employees from the Department of Public Health Engineering (DPHE), accompanying scientists from the Bangladesh Agricultural University (BAU) and the Centre for Health and Population Research (ICDDR,B), and personal observation. This provides only a preliminary indication of water use, and for estimating exposure it would need to be assessed to which extent pond water is actually ingested either through drinking or through use in cooking and mouth washing.

In the Mymensingh district water from tube wells is generally available in most villages without the need of long transportation (less than 1 km) and the arsenic contamination of groundwater in that region is considered low. Nonetheless pond water is used for cooking, personal hygiene, and ritual bathing including mouth washing. In slum areas of and around Mymensingh pond and river (Jamuna) water are likely the main sources of drinking water. In some villages we encountered tube wells that were only partly functional or dysfunctional and in such cases the need for drinking water likely is to be covered with surface water.

In Southern Khulna district (around Bagherhat and Dacope) the ground water is unsuitable for human consumption due to high salinity rather than due to arsenic contamination that may be of importance in the Northern parts of the district. Therefore drinking water supply relies fully on surface waters treated by pond sand filter devices (PSF). For each individual PSF one or more persons living nearby were responsible and in conversations showed pride in the good maintenance of the devices and the quality of the water. At some PSF people reported that water from this device is used because of its good taste even by people that have to travel considerably to gain access. The ponds from which water was abstracted to be treated by PSF generally were also well maintained and protected by a fence. These fences are efficient in denying access of the omnipresent domestic animals. Together with an obvious ban of human wastes in the immediate vicinity they appear effective in reducing the immediate nutrient input to the ponds. At the time of our visit, the ponds were comparatively clear and without surface blooms of cyanobacteria.

In Chandpur district the groundwater is contaminated with arsenic to a degree indicating its unsuitability for human consumption without treatment to remove arsenic. Similar to Khulna district a number of PSF can be found in villages, but here, most of them proved to be out of function. In some PSF essential parts were missing such as connecting tubing, or the filter chamber was empty or used for other purposes and the water was directed from the pond to the tap by a short-cut.

Occurrence of cyanobacteria

The large-scale climatic conditions in Bangladesh can be considered as favourable for cyanobacterial growth in all ecological niches that can be occupied by cyanobacteria, although for the present study only those cyanobacteria growing in surface waters were of interest.

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 either Euglenophyceae, Chlorophyceae, 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 6425 mm^3/l, but also occurred 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 µm) filaments.

Water temperatures in ponds rarely drops below 20°C and then only for a short period of time allowing persistence of water blooms without a distinct ‘winter-break’ typical for temperate surface waters.

Nonetheless, temporal dynamics need to be considered when interpreting our analytical data. Although the major part of the country of Bangladesh has a more or less uniform landscape, seasonal dynamics obviously are not synchronized. Local authorities (BAU, ICDDR,B) reported that blooms of cyanobacteria occur during or shortly after the monsoon season in Mymensingh district while in the Chandpur district the blooming season starts only in October and lasts throughout the winter. For the moment, reliable data on plankton dynamics are rare. In regions critical with respect to current or future use of ponds as drinking-water source, further studies are necessary for a more complete assessment of potential exposure risks. These should follow monthly or fortnightly sampling regimes. For this purpose, microscopic counting techniques should be applied for identification of species and for quantifying cyanobacterial cell density.

Occurrence of microcystins

The results of HPLC analyses are compiled in Table 1 and in Fig. 2. Microcystins could be detected in 39 out of 79 pond samples, most of them with macroscopically visible blooms of cyanobacteria but also in some in which cyanobacteria were only sub-dominant. High concentrations of microcystins were linked to blooms of *Microcystis* in many ponds. The genera *Planktothrix* and *Anabaena*, known as toxin producers in temperate lakes, seem to be less important toxin producers in Bangladesh, either due to lower biomasses or due to reduced or absent toxigenity of the predominating clones. With the available data this can not be assessed and in the course of the seasonal phytoplankton succession higher biomasses as well as toxigenic clones of *Planktothrix* and *Anabaena* could well occur.

From the 39 positive samples 14 showed only trace concentration, below one µg/L, i.e. below the WHO provisional Guideline value for microcystin-LR. In the remaining 25 pond samples concentrations between 1 and more than 1000 µg/L could be detected. All ponds with high toxin concentrations were located in the Mymensingh district with the exception of ponds 75 and 76 in the Chandpur district. The most abundant structural variants in all samples were microcystins-RR, YR, and LR that are also the major ones in most samples from *Microcystis* dominated water bodies in temperate and subtropical latitudes. Nine other variants with a minor share of the total microcystin concentration could be detected in the samples. At maximum they contributed up to 30% of the total microcystin concentration. With respect to the relative contribution of particular structural variants some samples can be grouped due to their high similarity. Examples are ponds 01, 13,14, and 15 with microcystin-LR contributing around 60% to total microcystins and only about 10% being microcystin-RR. Most likely in all of these ponds the same species or clone is responsible for the toxin production – in the ponds 13 to 15 this is very likely since these ponds were situated next to each other.

Microcystins were not only detected with the highest frequency in ponds of Mymensingh district (two thirds of the samples were positive for microcystins), but were also detected with the highest concentrations: one third showed concentrations above 10µg/L with a maximum concentration of 1390 µg/L. In the Chandpur samples microcystins were detected in 8 out of 17 samples and only in two ponds concentrations exceeded 1 µg/L. In the Khulna district only in one pond (out of 16) trace amounts (<< 1 µg/L) were detected. This pattern most likely reflects seasonal patterns that differ in the particular regions according to the accounts of Dr. Khan, BAU and Dr. Islam, ICDDR,B. In Mymensingh peak abundances of cyanobacteria generally are reported during the summer month from July to September whereas in the Chandpur area these are expected later in the year, mainly in October and November. For the Khulna district no data or oral accounts have been available on the seasonality of cyanobacteria. The sampled ponds, however, were possibly generally less prone to cyanobacterial due to the protective measures of PSF ponds mentioned above.

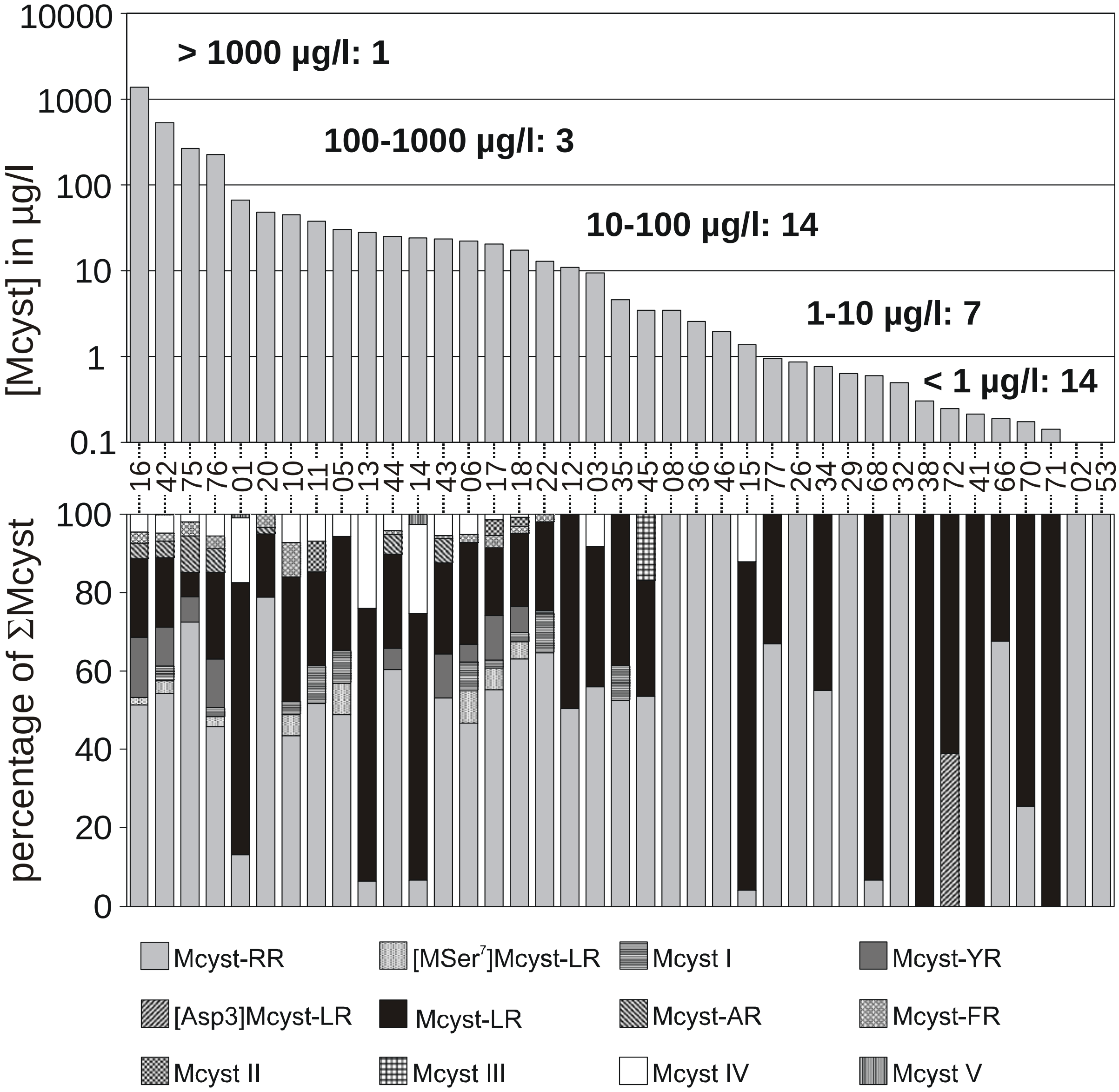

Fig. 2: Summary of microcystin occurrence in pond-water samples of Bangladesh. The upper panel shows the results for the samples in the order of concentration as calculated from duplicate samples. The lower panel shows the relative contribution of individual structural variants to the total concentration 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.

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. Quanitative phytoplankton data for potentially microcystin-producing genera (*Microcystis, Planktothrix*, *Anabaena*) given as biovolume and semi-quantitative phytoplankton data for other cyanobacteria (NDTF: various **n**ot **d**etermined (very) **t**hin **f**ilaments, most probably Oscillatoriales) and algae (B: Bacillariophyceae. Ch: Chlorophyceae, Eu: Euglenophyceae, Cr: Cryptophyceae). Semiquantitative categories: +: rare; ++: common; +++: abundant. Mcyst concentrations are given as total MCYST concentrations in µg/L. n.d.: not detectable.

Pond	Location		Cyanobacterial biovolumes (mm^3/l) and taxa				Other algae	Mcyst
#	division	Upazila/town	*Microcystis*	*Planktothrix*	*Anabaena*	other		µg/L
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	Mymansingh	12.6			*Pseudanabaena* sp.		48
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	Isshorganji	3.8			NDTF	B, Ch, Eu	n.d.
26	Dhaka	Isshorganji	7.5	14.7	109.7	NDTF	Ch	0,87
27	Dhaka	Isshorganji	+			NDTF	Ch	n.d.
28	Dhaka	Isshorganji	+			NDTF		n.d.
29	Dhaka	Isshorganji	5.3		25.6	NDTF		0,63
30	Dhaka	Isshorganji	+			Merismopedia *sp., NDTF*	Ch	n.d.
31	Dhaka	Isshorganji	+			NDTF	Ch	n.d.
32	Dhaka	Isshorganji	2.6			NDTF	Ch	0,49
33	Dhaka	Isshorganji	5.5	14.5	1.2			n.d.
34	Dhaka	Isshorganji	6.8			*Merismopedia* sp., NDTF	Ch	0,76
35	Dhaka	Isshorganji	9.0	3.0	28.7	*Oscillatoria* sp., NDTF		4.6
36	Dhaka	Isshorganji	18.8	4.6	35.7	*Oscillatoria* sp., NDTF		2.6
37	Dhaka	Isshorganji		7.8	+	*Oscillatoria* sp., NDTF, *Pseudanabaena* sp.	B, Ch	n.d.
38	Dhaka	Isshorganji	2.2	235.6		NDTF, *Aphanizomenon* sp.		0.30
39	Dhaka	Mym., BAU	2346		+		Eu	n.d.
40	Dhaka	Mym., Bhailar	129.3	+	6.1	NDTF	B, Eu	n.d.
41	Dhaka	Mym., Bhailar	+			*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
44	Dhaka	Muktagacha	119.0	+	+	NDTF		25
45	Dhaka	Muktagacha	231.5			*Merismopedia* sp., NDTF		3.5
46	Dhaka	Muktagacha	*sample*	*lost*				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
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.

Conclusions

Preliminary assessment of the public health relevance of the results

The results indicate that toxic cyanobacteria are quite common in Bangladesh ponds. Moreover, they show that concentrations of microcystins can reach very high concentrations. Depending on the water usage habits of the local population the uptake of microcystins occurring in concentrations of > 10 µg/L for successive days is likely to lead to hazardous exposure.

A positive result is, however, that in many ponds no microcystins could be detected, i.e. concentrations were well below 1 µg/L, and this was also observed in localities where concentrations were high in neighbouring ponds. Thus, ponds without critical microcystin concentrations are likely to be available to the population – probably throughout Bangladesh (even though the climatic conditions and nutrient supplies of respective water bodies seem to be very similar). This offers options for avoiding exposure by educating the population to identify and avoid the critical situations.

This preliminary study shows that further work for cyanotoxin risk assessment is important to protect public health in Bangladesh where surface water is used as drinking-water source.

Recommendations

Public awareness

A major problem with cyanobacterial toxins in developing as well as in industrialized countries often is a lack of awareness by people potentially exposed to cyanotoxins. Regarding exposure through recreational use of contaminated waters, simple avoidance measures could minimize the risk of exposure considerably. Since some toxigenic cyanobacteria tend to accumulate at the surface and especially at near-shore sites the avoidance of 'blooms' after visual inspection is the simplest measure of risk reduction. Efforts to inform the public on recognising blooms are ongoing in many countries. In analogy, a direct simple way to substantially reduce cyanotoxin exposure would be the avoidance of water consumption from ponds with cyanobacterial blooms. This, however, requires some training to recognize cyanobacterial blooms without the aid of laboratory equipment.

Avoidance of high concentrations of microcystins could be attained with some basic advice and training – though the task would still be a major effort with regard to population number and settlement structures. Possibly, this may be combined with other campaigns of education/information on sanitation and drinking water.

Pond Sand Filtration (PSF)

During our 2-week stay we did not encounter a functional PSF that received water from a pond carrying a cyanobacterial bloom and therefore no data on the efficiency of microcystin removal could be collected. There is, however, little doubt about the improvement of water quality also with respect to microcystins by this treatment where it effectively removes particles. This can be assessed already visually through improved clarity of the water. The only critical point could be the accumulation of cyanobacterial cells in the top layer of the sand filter, which might lead to occasional 'flash' release of cyanotoxins when these cells die and lyse. However, results emerging from currently ongoing research on cell lysis and microcystin release in water treatment indicate that even if this happens, sand filtration is likely to substantially reduce microcystin concentrations. This is largely due to bacterial biofilms on the sand filters which effectively degrade microcystins, particularly at high temperatures. Although incidences of break-through are reported in the literature, there is no doubt that concentrations are nonetheless substantially reduced.

Therefore, PSF treated water certainly is an improvement to non-filtered or sari-filtered water and an increased application of well-functioning PSF in critical regions is recommended.

Pond care

Should the use of surface waters for drinking water supply increase in the future some recommendations for the selection and care of source-ponds should be compiled and provided. This can already be developed on the basis of general knowledge and includes, for example, the reduction of nutrient input, e.g. by a ban of latrines and defecation near the pond, a ban of washing clothes, dishes, persons, cattle, etc. in the pond used explicitly as drinking-water source. The good condition of the ponds encountered with a fence can be used as illustrative example. In this context it may be important to assess whether most rural communities have easy access to more than one pond, so that allocating functions to ponds would be an option. More complex measures such as biomanipulation by introduction of predatory fish, or by designing size and depth of ponds, require further studies as outlined below.

Saree filtration

The efficiency of the 'saree-filtration' with respect to cyanotoxin removal could not be tested to a satisfying extent during our stay. Two trials indicated that saree filtration is an improvement to non-filtered water by a reduction of phytoplankton biomass and toxin concentration to about 20-50% (data not shown). A further propagation of this lowest-tech water treatment may therefore merit support with respect to cyanobacterial toxins, though further experiments should be conducted to confirm this.

Future studies

The data presented here should be taken as a basis for more detailed subsequent studies at selected sites. Further, in view of the results the present report also aims to highlight exposure to toxic cyanobacteria as a risk to consider when developing strategies for safe drinking-water.

1. The concentration of microcystins and other cyanotoxins depends mainly on the presence of toxigenic species/clones, and this can be subject to large fluctuations on a seasonal scale. Our analyses only reflect a point measurement in a dynamic process. For some selected sites, **seasonal monitoring** is recommended in order to understand the seasonality of cyanobacteria and toxin concentrations and to allow some basic estimates when peak concentrations are to be expected in different regions of Bangladesh. Toxin concentration, and cyanobacterial identity and biomass need to be surveyed as a basis for improved hazard assessment. Furthermore, data from temperate settings have shown patterns of toxin occurrence in relation to species dominance, from which microcystin production can be expected with high likelihood for a number of species. It is uncertain to what extent this experience can be applied to tropical settings such as Bangladesh. For *Microcystis* spp., the results reported here indicate, that also in Bangladesh the occurrence of microcystin is highly likely when this genus is present. For other taxa, such relationships would need to be established.

For such a more profound study, support for introduction of techniques in Bangladesh would be desirable.

2. Since the efficiency of **PSF** for microcystin removal could not be tested, a study should be designed to assess the treatment performance of PSF for microcystin affected ponds. For these investigations also, an important first step is to monitor whether in protected ponds cyanobacterial blooms and cyanotoxins occur at all by monitoring the seasonal phytoplankton dynamics. This would best be done by cell counts or chlorophyll/pigment analyses. If this is not possible, macroscopic observation of the ponds by the responsible local inhabitants could be an alternative option, following training for recognition of blooms together with occasional

sampling of ponds when blooms are reported by these persons. If cyanobacterial blooms occur, the efficiency of PSF can only be tested by analysing pre-filter and post-filter samples.

3. The feasibility of effective **pond protection** should be investigated further since by protecting ponds from excess nutrient input the cyanotoxin problem could be reduced at its origin. This would best be addressed by a study comparing PSF-ponds and nearby non-protected ponds in the Khulna district. If the observation during our visit holds true for extended time periods, i.e. that in the ponds protected for PSF cyanobacterial blooms occur less frequently than in other non-protected ponds, then the system of pond protection should be investigated in more depth by interviewing the local communities about the rules and measures that were establish for protection.

3. Further measures to avoid cyanobacterial blooms could be the **biomanipulation** of the food web by introduction of selected fish species into ponds. In temperate lakes, for example, the introduction of piscivorous fish species has in some cases been successful in reducing phytoplankton biomass. This works by changing the food chain: Piscivorous fish feed on smaller planktivorous fish which in turn feed on zooplankton such as *Daphnia*, which in turn feed on phytoplankton. A reduction of planktivorous fish by introducing their predators thus means more *Daphnia*, and this in turn may mean less planktonic algae and cyanobacteria. However, cyanobacteria are rather poor food for *Daphnia*. This is one reason why the impact of biomanipulation specifically to reduce cyanobacteria is uncertain, though some successful examples exist.

Experiments to study the influence of fish stock on the phytoplankton composition were conducted in experimental ponds at BAU, Mymensingh, during our stay. It is recommended to encourage further research in this area at BAU, where facilities and expertise for tropical aquaculture are available. However, this approach should not be given high priority as success is uncertain. Also, the urgent need of nutritional protein for the local population through maximising fish production would need to be balanced against protecting ponds as drinking-water source, although this might be solved by allocating functions to ponds provided that enough ponds are available in a given locality.

4. **Saree filtration** as lowest-cost technology has been studied and introduced to rural populations by ICDDR,B in the study site of Matlab: For lowest income families water treatment by saree filtration could be the only minimal water treatment. Therefore, it is recommended to test the efficiency of removal of cyanobacterial cells under different conditions, i.e. with different cyanobacterial species (among those that potentially produce cyanotoxins) as these can differ substantially in their retention efficiency by filtration.

Acknowledgement

We are very grateful to Dr. Saleha Khan, Dr. Md. Mahfuzul Haque and the students from the Bangladesh Agricultural University (BAU), to the staff of the Department on Public Health Engineering (DPHE) local laboratory in Khulna, and to Dr. Sirajul Islam and Dr. Nurul Huda Khan from the Centre for Health and Population Research (ICDDR,B) for their engaged aid with sampling and sample processing. We sincerely thank the staff from the WHO Environmental Health Unit in Dhaka for their constant and friendly support during our stay in Bangladesh. In Berlin, we particularly thank Katrina Laskus for extensive support with counting cyanobacteria.

WHO wishes to express its appreciation to those who contributed to the preparation and finalization of this pilot study, in particular to Ingrid Chorus, Jutta Fastner and Martin Welker, Federal Environmental Agency, Berlin, Germany.

Literature

Chorus, I. & Fastner, J. (2001). Recreational exposure to cyanotoxins. Chorus, I. (ed.): Cyanotoxins - occurrence, causes, consequences. 190-199.

Chorus, I. (ed.) (2001). Cyanotoxins - occurrence, causes, consequences. Berlin, Springer.

Falconer, I. R. (1999). An overview of problems caused by toxic blue-green algae (Cyanobacteria) in drinking and recreational water. *Environ Toxicol* 14, 5-12.

Fastner, J., Flieger, I. & Neumann, U. (1998). Optimised extraction of microcystins from field samples - A comparison of different solvents and procedures. *Wat Res* 32, 3177-3181.

Harada, K.-I. (1996). Chemistry and detection of microcystins. In: Watanabe, M. F., Harada, K.-I., Carmichael, W. W. & Fujiki, H. (ed.): Toxic *Microcystis*. CRC, Boca Raton, USA. 1, 103-148.

Kuiper-Goodman, T., Falconer, I. R. & Fitzgerald, D. J. (1999). Human health aspects. Chorus, I. & Bartram, J. (ed.): Toxic cyanobacteria in water. E & FN Spoon, London. 113-153 .

Sivonen, K. & Jones, G. J. (1999). Cyanobacterial toxins. Chorus, I. & Bartram, J. (ed.): Toxic cyanobacteria in water. E & FN Spoon, London. 41-111.

Welker, M., Steinberg, C. & Jones, G. J. (2001). Release and persistance of microcystins in natural waters. Chorus, I. (ed.): Cyanotoxins - occurrence, effects, and ecological consequences. Springer, Berlin. 85-103.

Other relevant publications from WHO

Guidelines for Drinking-water Quality, 3rd edition, Volume 1: Recommendations

Arsenic, Drinking-water and Health Risks Substitution in Arsenic Mitigation: a Discussion Paper

Toxic Cyanobacteria in Water

The Arsenic Monograph (in preparation)

Report on evaluating household impact of arsenic

Towards an assessment of the socioeconomic impact of arsenic poisoning in Bangladesh

Access to the World Health Organization (WHO) web site: www.who.int

Access to Water, Sanitation and Health at WHO: www.who.int/water_sanitation_health/

Access to current and previous World Water Days web site: www.worldwaterday.org

Access to WHO fact sheets: www.who.int/inf-fs/en/index_n.html

Access to information on safe health care waste management: www.healthcarewaste.org

Access to information on environmental sanitation and health: www.sanicon.net

Access to drinking water supply surveillance and monitoring in developing and transitional countries: www.lboro.ac.uk/watermark

The text of most of the Guidelines and information on their updating are available on the Internet: http://www.who.int/water_sanitation_health/GDWQ/index.html