Are Microscopic Methods for Quantification of Cyanobacteria a Good Tool for Routine Monitoring of Bathing Water?

Experience from Proficiency Testing Programs

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Introduction

Mass proliferation of Cyanobacteria in bathing waters can cause various health problems - from mild to life threatening [1]. That is why, the WHO set up limit values for Cyanobacteria in bathing waters [2]. Slightly modified WHO limit values was transposed to the Decree of the Czech Ministry of Health No. 135/2004 [3] concerning bathing water quality in the year 2004 (Table 1). Due to lack of the suitable method for Cyanobacteria quantification, a simple method based on counting cyanobacterial cells in counting chamber in up-right microscope was developed and released in 2004 as a Czech Technical Standard no. 757717 [4]. However, all microscopic methods are strongly dependent on analyst skills. It is the main reason for a relatively low reproducibility of microscopic methods comparing with e.g. chemical analyses. We use our long-term experience with organisation of proficiency testing for evaluation this Czech standard method.

Table 1. Limit values for Cyanobacteria from the Decree No. 135/2004 for bathing water quality in the Czech Republic.

Parameter	Unit	Alert level 1	Alert level 2	Alert level 3	Frequency*	Notes
Cyanobacteria	cells/mL mm^3/L	20.0000 2	100.000 10	-	2 weeks	1 1
Chlorophyll-a	µg/l	10	50	-	2 weeks	
Visual assessment				water bloom	2 weeks	2
Qualitative analysis					2 weeks	3

* When alert level 1 is exceeded, the frequency of monitoring is 5-7 days

1 Only one is needed (cells or biovolume). Analyses are done according to the Czech Technical Standard 75 7717

2 This assessment is done during sampling

3 The text information on dominant phytoplankton species with emphasis on Cyanobacteria

Methods

Czech Technical Standard no. 757717: The method is based on counting cells or measuring the length of filaments in Cyrus counting chamber (a chamber similar to haemocytometer) with upright microscope. Method for colony forming cyanobacteria is explained in Fig. 1. Quantification of filamentous cyanobacteria can be seen in Fig. 2

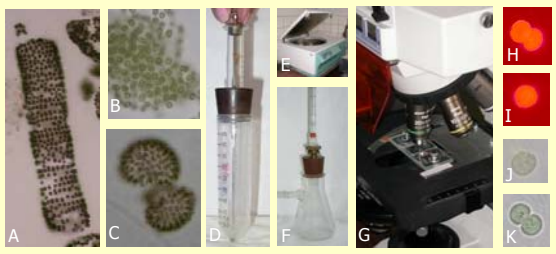


Figure 1. Colony forming cyanobacteria like *Microcystis* (1A and 1B) and *Woronichinia* (1C) have to be mechanically disintegrated (to cells or small fragments) in a plastic tube by a syringe with a thick needle before counting (1D). Centrifugation (1E) or membrane filtration (1F) can be used to concentrate sample with low numbers of cells. Cyrus counting chamber with upright microscope is used for counting cell in brighfield (1J and 1K) or with fluorescence (green excitation – 1H and 1I).

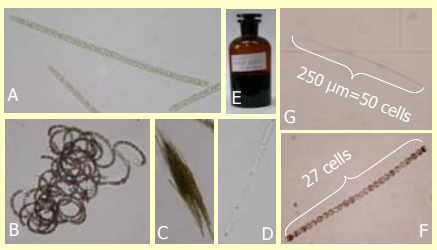


Figure 2. Filamentous cyanobacteria like *Planktothrix* (2A), *Anabaena* (2B), *Aphanizomenon* (2C), *Limnothrix* (2D) should be fixed with Lugol's solution (2E). Centrifugation (1E) or membrane filtration (1F) can be used to concentrate sample with low numbers of filaments. Cyrus counting chamber with upright microscope (1G) is used for counting or measuring. The number of cells in each filament is counted (*Anabaena*) or the length is measured by ocular micrometer (e.g. *Planktothrix, Aphanizomenon*). A conversion factor from the length of filaments to cells (5µm of length = 1 cell) was set up in the standard.

Proficiency testing (PT) programs: We have organized PTs for the determination and quantification of Cyanobacteria since the year 2000. From the year 2004 each participant of PT obtains two different samples of surface water with cyanobacteria. The first contains colony forming species (various *Microcystis*) and the second filamentous species (usually *Planktothrix agardhii*). The range of cyanobacterial cells per ml is dozens of thousands or more often hundreds of thousands.

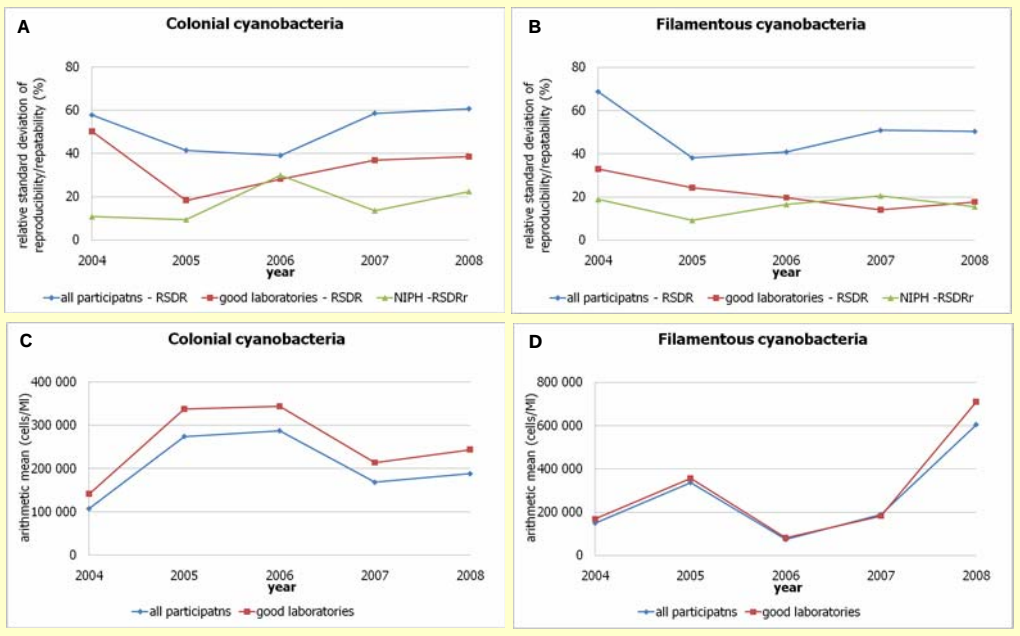
Our PTs are open to everyone, who wants to participate. Some of the participants had serious problems with the method. That is the reason, why we selected nine „good laboratories" from all participants and use their results for reproducibility evaluation of the method. The main criterion for classification as a "good" laboratory was a skilled analyst (according to our personal experience). Of course, this criterion is very subjective, but the issue of analyst abilities is the keystone of all microscopic analyses. We calculated the arithmetic mean, median and the relative standard deviation of reproducibility (RSD_R) from the results of all participants and from nine "good" laboratories and the arithmetic mean and the relative standard deviation of repeatability (RSD_r) from the results of our laboratory (data for homogeneity testing).

Results

We analyzed data from five PTs (2004 – 2008; Table 2). The number of participants varied between 16 (2008) and 40 (2005). The participants were mainly from public health service laboratories, but laboratories of river basin authorities and laboratories of drinking water producers took part in our PTs too. RSD_R of all participants for filamentous cyanobacteria (mean 50%, max 69%, min 38%; Fig. 3A) is nearly the same as for colony forming species (mean 52%, max 61%, min 39%). Of course, RSD_R of "good" laboratories is much better (filamentous - mean 22%, max 33%, min 14%; colonial - mean 37%, max 50%, min 18%; Fig. 3B) and it is almost the same as RSD_r from the results of National Institute of Public Health.

Discrepancy between results of "good" laboratories and all participants was bigger for colony forming species. Within the five PTs, the arithmetic mean of all participants in particular PT posed 76-84% of arithmetic mean of "good" laboratories for colony forming species (Fig. 3C) and 85 – 104% for filamentous species (Fig. 3D).

Figure 3. RSD_R and RSD_r and the arithmetic mean for the results from the PTs organised by the National Institute of Public Health (NIPH) for Cyanobacteria quantification in 2004 – 2008.



Conclusions

The method for filamentous cyanobacteria seems to be sufficient for routine monitoring of bathing water, if it is done by skilled analyst. The method for colonial species is worse. Underestimation (by about 20%) of the results is more probable and the uncertainty is greater.

Significance of the work

Our work can serve (1) as a basis for the revision of the Czech technical standard method, (2) as an information on the validity of the results from monitoring of bathing water containing cyanobacteria in the Czech Republic and (3) as an information on the precision of microscopic quantification methods at all.

Table 2. Summary of the results from the PTs organised by the National Institute of Public Health for Cyanobacteria quantification in 2004 – 2008.

Year	All participants					„Good" laboratories"					National Institute of Public Health			
	N	median cells/mL	mean cells/mL	σ cells/mL	RSD_R %	N	median cells/mL	mean cells/mL	σ cells/mL	RSD_R %	N_r	mean cells/mL	σ cells/mL	RSD_r %
Colonial Cyanobacteria														
2008	16	167291	188 641	114 581	**60,7**	7	261105	244 177	93 901	**38,5**	10	176 400	39 596	**22,4**
2007	23	164000	169 010	99 037	**58,6**	9	207740	214 133	78 941	**36,9**	10	282 540	38 509	**13,6**
2006	29	311400	287 999	112 213	**39,0**	8	351750	344 382	97 172	**28,2**	14	191 464	57 250	**29,9**
2005	40	270118	274 773	113 695	**41,4**	8	342528	337 906	61 677	**18,3**	10	379 798	35 752	**9,4**
2004	39	104830	107 942	62 349	**57,8**	8	153875	141 337	70 950	**50,2**	12	219 997	23 836	**10,8**
Filamentous Cyanobacteria														
2008	16	645400	605 299	304 184	**50,3**	7	711720	710 246	126 006	**17,7**	12	687 847	106 845	**15,5**
2007	23	188000	189 023	95 962	**50,8**	9	192437	182 241	25 451	**14,0**	13	195 409	40 155	**20,5**
2006	29	73304	73 415	29 963	**40,8**	8	74512	80 690	15 908	**19,7**	12	77 166	12 754	**16,5**
2005	40	331815	336 493	128 095	**38,1**	8	346684	355 480	86 234	**24,3**	11	209 969	19 398	**9,2**
2004	39	150000	149 840	102 932	**68,7**	8	172810	168 473	55 594	**33,0**	10	152 780	28 655	**18,8**

N – number of participants; N_r – number of results; σ - ; RSD_R - relative standard deviation of reproducibility; RSD_r - relative standard deviation of repeatability

References

[1] Stewart I, Webb PM, Schluter PJ, Shaw GR. Recreational and occupational field exposure to freshwater cyanobacteria – a review of anecdotal and case reports, epidemiological studies and the challenges for epidemiologic assessment. Environmental Health: A Global Access Science Source 2006, 5:6 doi:10.1186/1476-069X-5-6.

[2] WHO. Guidelines for safe recreational water environments. Volume 1: Coastal and Fresh Waters. WHO; 2003.

[3] Decree of the Ministry of Health of the Czech Republic No. 135/2004 from 17 March 2004 specifying hygienic requirements for natural bathing waters, swimming-pools, and hygienic limits of sand in children sandpits (in Czech).

[4] Czech Technical Standard no. 757717 Water quality – Determination of planktonic cyanobacteria, 2004, revision in 2008 (in Czech).

Acknowledgement

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