Toxicological risk assessment of cyano blooms & cyanotoxins

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In cooperation with RAWAT consulting

for HYDAP

Kick-off meeting

Basic parameters used in risks evaluation of cyanotoxins:

- Biomas development trends
- Biomass density
- Cyanotoxins content
- Forecast parameters
  - Weather
  - Nutrient flow (external, internal)
  - Inoculum in sediments
  - Ecophysiology of populations

Are there any alternatives for cyanotoxins risks assessment?

- **General idea:**

CYANOBACTERIA – general parameters for cyanobacterial biomass (raw water)

- Biovolume
- Pigments composition

CYANOTOXINS (treated water)

- Sensors for general toxicity
- Sensors for selected toxins

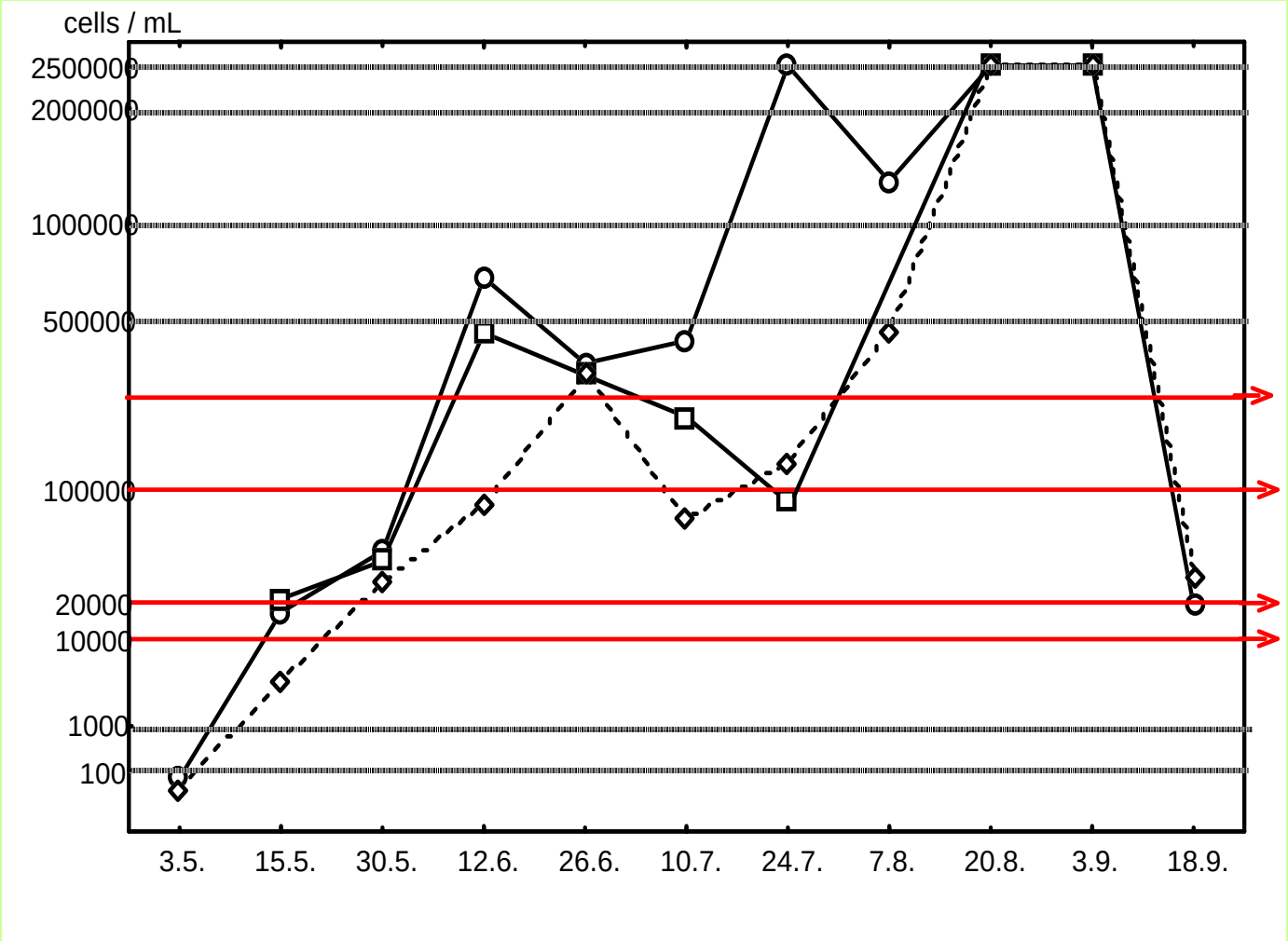
Cyanobacterial BIOMASS

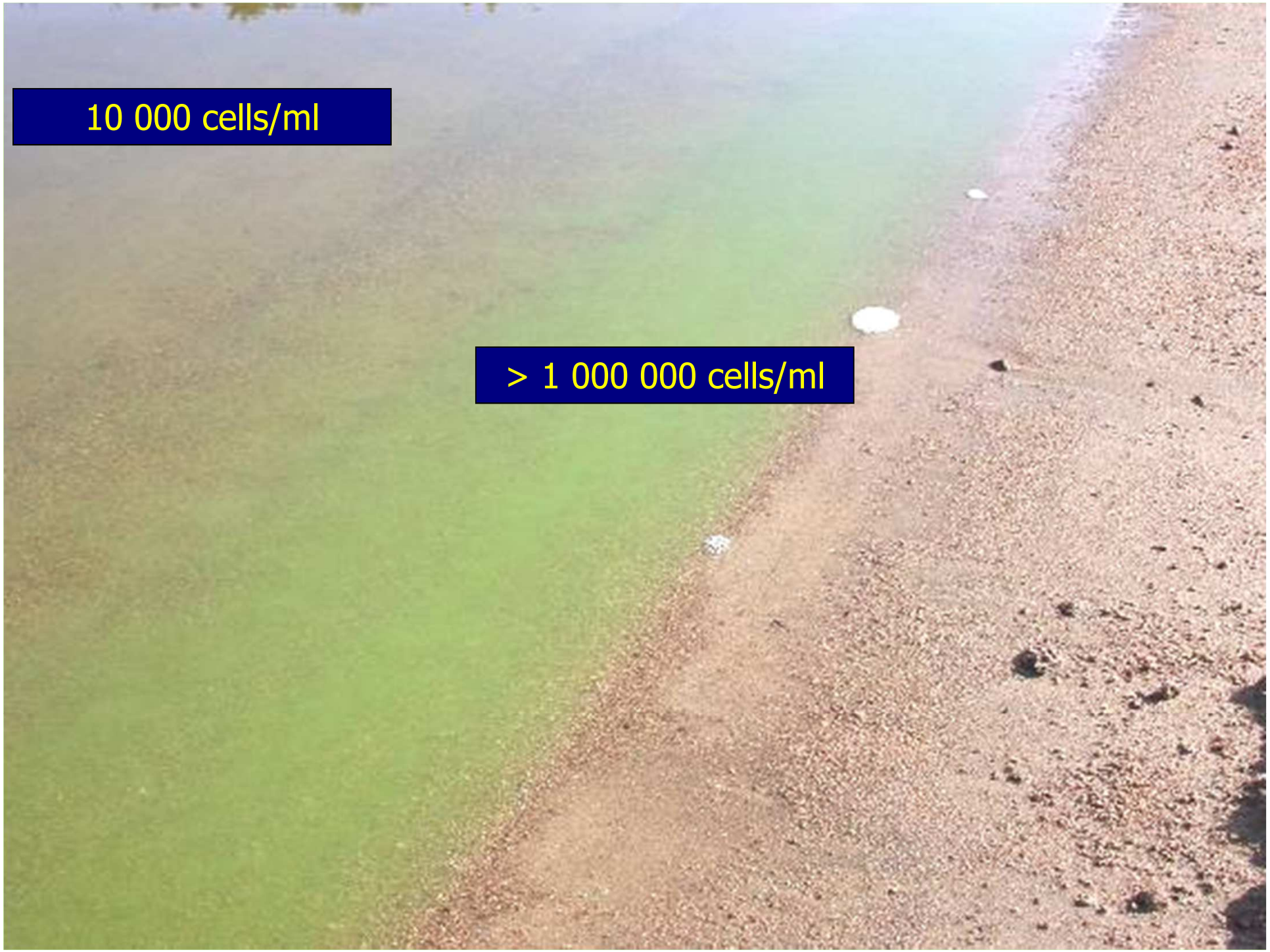
- Cyanobacterial biomass is the key monitored parameter in surface waters
  HOWEVER:
  - intercalibration study for MICROCYSTIS quantification (**cell counts**) by 37 routine laboratories give the variability unacceptable by GLP
- Chlorophyll a concentrations by ISO method and FluoroProbe fits well, but ISO method did not discriminate cyanobacteria from other phycoplankton
- Microscopic determination and biovolume give a good results, but it is time-consuming and need the experience

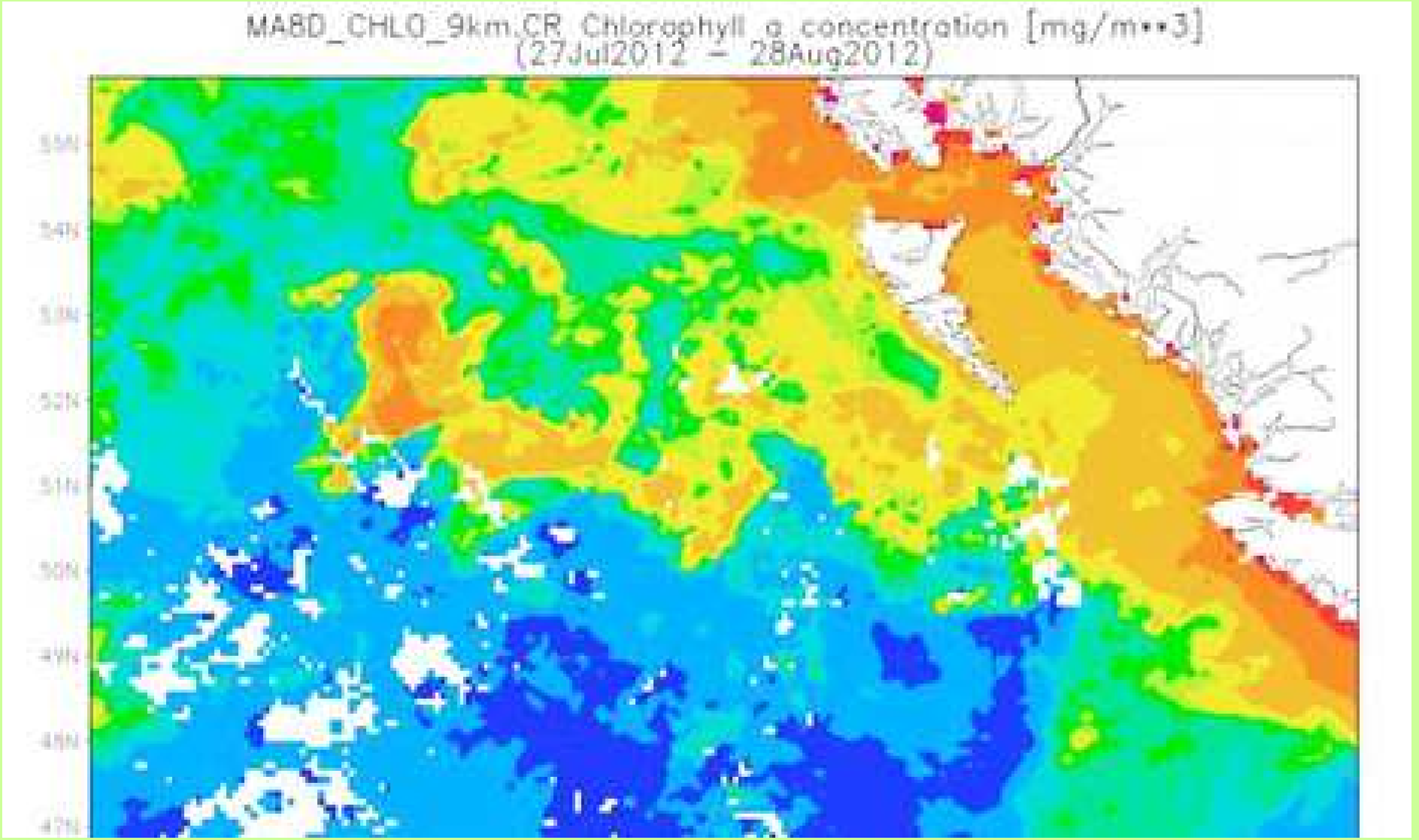
- COMBINATION OF METHODS IS NEEDED:
- Fluoro Probe and the dominant species determination seems to be a good compromisse for water management

Dermal contact with cyanobacteria in Brno reservoir represents relatively high health risk during a substantial part of the summer season.

Concentrations of cyanobacteria in Brno reservoir 2007(cells / mL) and comparison with WHO safety "Guidance levels". Results from three localities sampled biweekly.



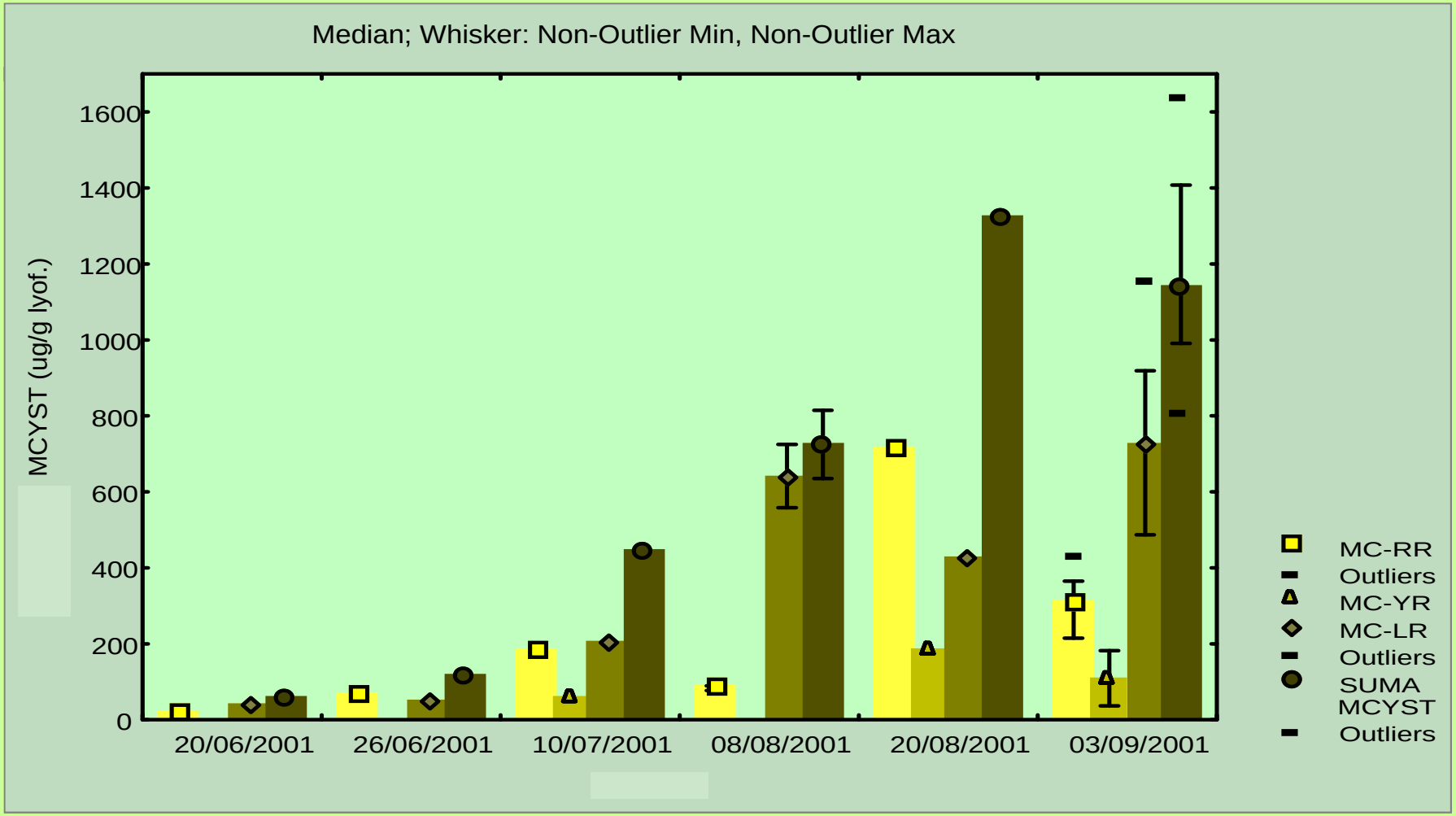

10 000 cells/ml
> 1 000 000 cells/ml

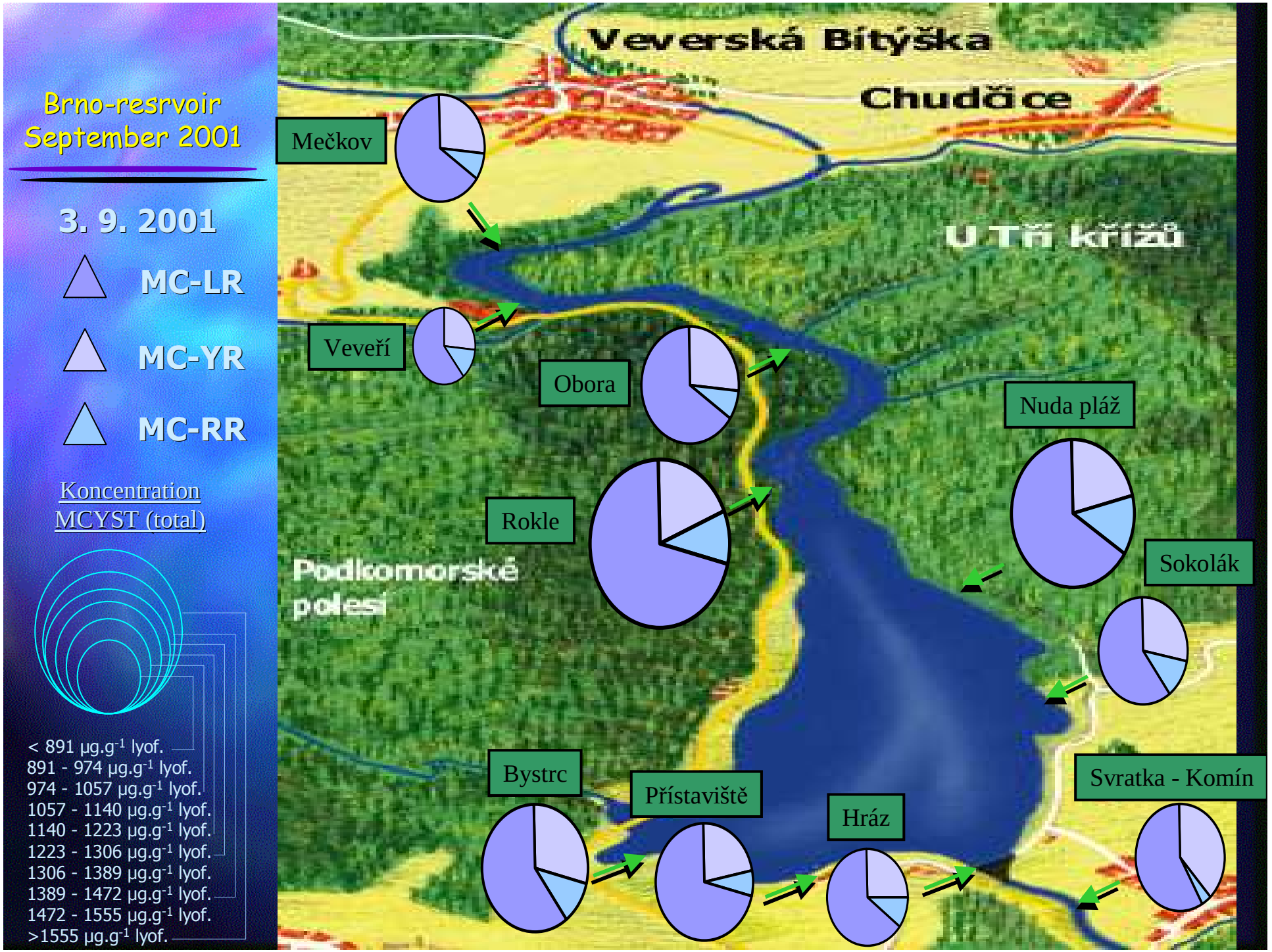

MA8D_CHLO_9km.CR Chlorophyll a concentration [mg/m**3]
(27Jul2012 - 28Aug2012)
55N
54N
53N
52N
51N
50N
49N
48N
47N

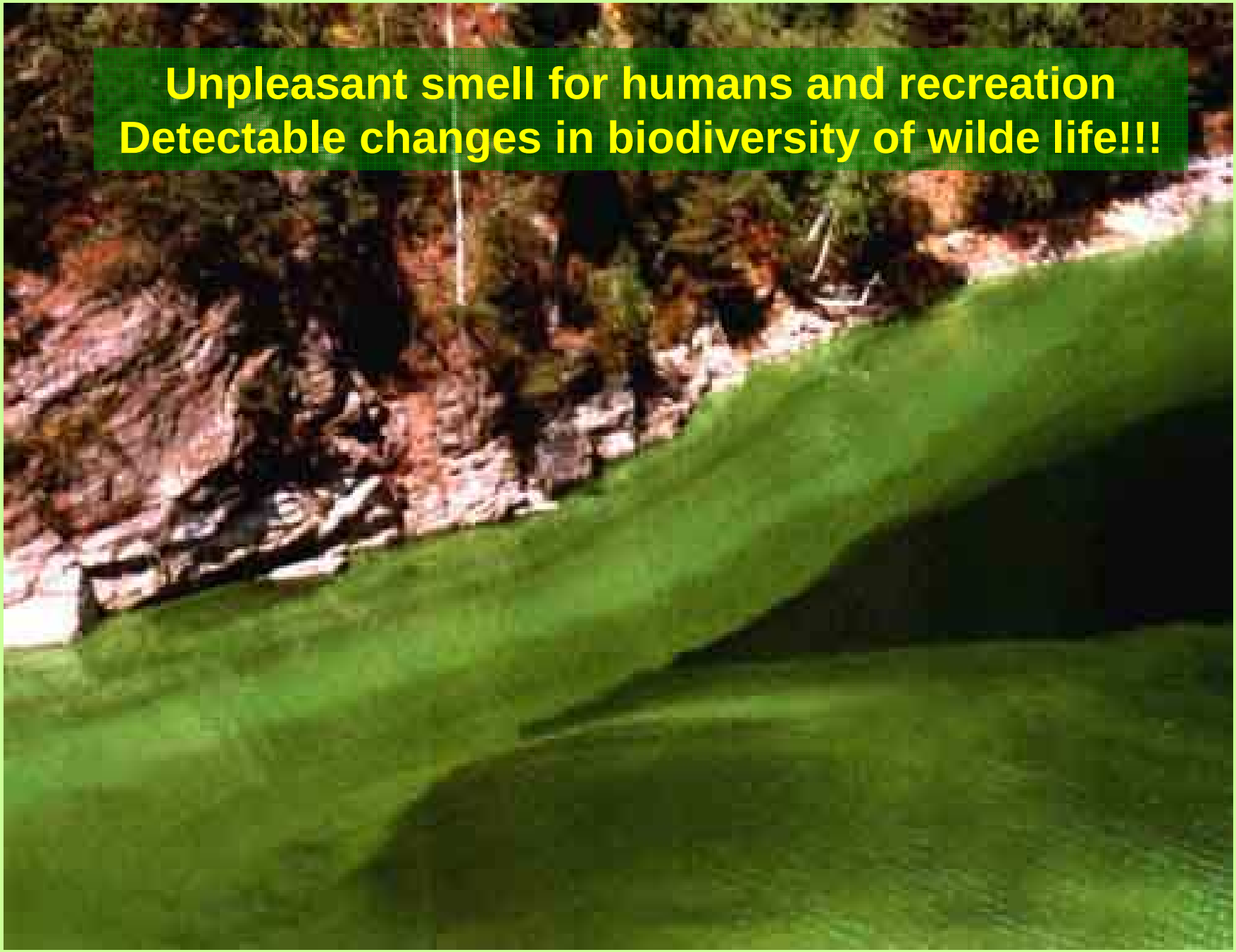
Intake of microcystins during swimming **represents highly significant risks in the Brno reservoir both for children and adults.**
Particularly, during August high HI values (up to 40 for children) were observed. HI > 1 for children were observed at 82% of localities during August 2001.

date	water conc. of MCYST ($\mu g.l^{-1}$)	children (25 kg, intake of 80 ml water.h^{-1})				adults (70 kg, intake 50 ml.h^{-1})			
		regular swim. (2,6 h.d^{-1})		weekend swim. (5 h.d^{-1})		regular (2,6 h.d^{-1})		weekend (5 h.d^{-1})	
		intake MCYST ($\mu g.kg^{-1}$ b.w./d)	HI	intake MCYST ($\mu g.kg^{-1}$ b.w./d)	HI	intake MCYST ($\mu g.kg^{-1}$ b.w./d)	HI	intake MCYST ($\mu g.kg^{-1}$ b.w./d)	HI
26.6.	1,46	0,0122	0,30	0,0234	0,58	0,0027	0,07	0,0052	0,13
10.7.	5,86	0,0488	1,22	0,0938	2,35	0,0109	0,27	0,0209	0,52
7.8.	48,19	0,4010	**10,02**	0,7711	**19,28**	0,0895	**2,24**	0,1721	**4,30**
20.8.	116,18	0,9666	**24,17**	1,8589	**46,47**	0,2158	**5,39**	0,4149	**10,37**
3.9.	99,76	0,8300	**20,75**	1,5961	**39,90**	0,1853	**4,63**	0,3563	**8,91**

Fig. 1: Variations of three dominating microcystin variants in the cyanobacterial water blooms collected from Brno reservoir during the 2001 season. Median values (+/- maximum/minimum).




Brno-resrvoir
September 2001
3. 9. 2001
MC-LR
MC-YR
MC-RR
Koncentration
MCYST (total)
< 891 µg.g-1 lyof.
891 - 974 µg.g-1 lyof.
974 - 1057 µg.g-1 lyof.
1057 - 1140 µg.g-1 lyof.
1140 - 1223 µg.g-1 lyof.
1223 - 1306 µg.g-1 lyof.
1306 - 1389 µg.g-1 lyof.
1389 - 1472 µg.g-1 lyof.
1472 - 1555 µg.g-1 lyof.
>1555 µg.g-1 lyof.
Veverská Bítýška
Chudčice
U Tří křížů
Podkomorské polesí
Mečkov
Veveří
Obora
Rokle
Nuda pláž
Sokolák
Bystrc
Přístaviště
Hráz
Svratka - Komín


Unpleasant smell for humans and recreation
Detectable changes in biodiversity of wilde life!!!

Trends in the risk evaluation

- Toxicity identification require special expertise and it is expensive
- All cyanobacteria produce toxins
- The most practical way is to quantify cyanobacterial biomass (pigments or cells by microscope, or special device like Fluoro Probe or CCD kinetic fluorescence immaging

Methodological approaches- prediction

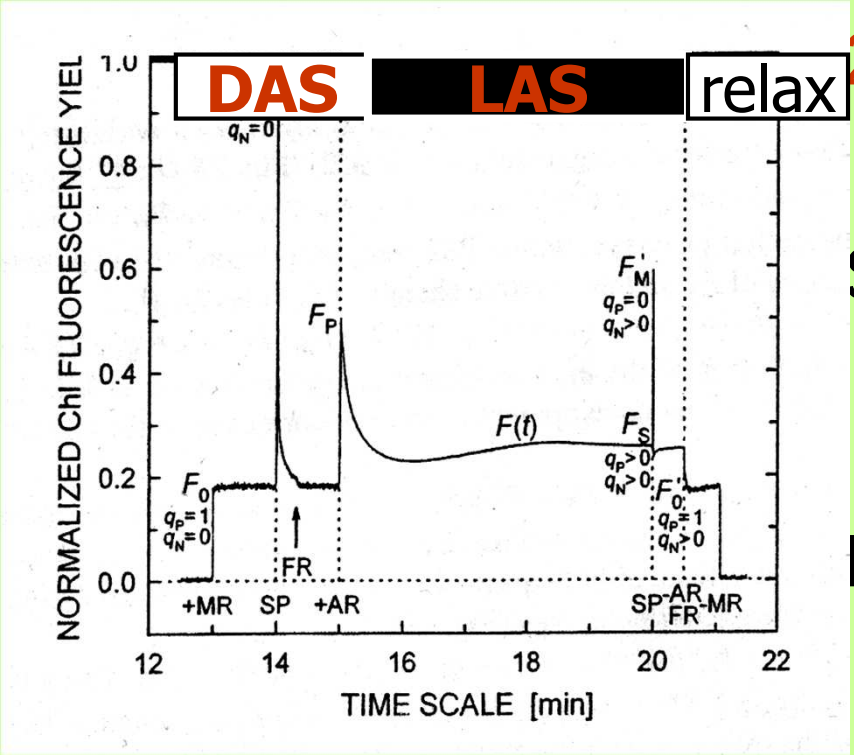
- Fluorescence microscopy and probes
- Laboratory cultivation
- CCD camera with FLIA was proved as powerful tool for the study of annual cycle of colony formation and ecophysiology of *Microcystis*
- meticulous care and long time under microscope was spend to build up base for
    - next comparative study of seasons
        - other localities
        - European countries
        - other species

Principle

- Light-induced PSII fluorescence kinetic
- Shows photosystem associated electron transport chain processes
- Fluorescence yield is connected to chlorophyll and redox state of chinons as well as to their binding sites disposition in photosystem

=> Detection of effects at base level of energy metabolism of autotrophic cell

Chlorophyll fluorescence kinetic record



1. Dark Adapted State
2. Light Adapted State

Several basic terms are measured: F_0 F_m F_p F_s $F_{m'}$ $F_{0'}$

Fast and slow kinetic could be used

Purpose of evaluation

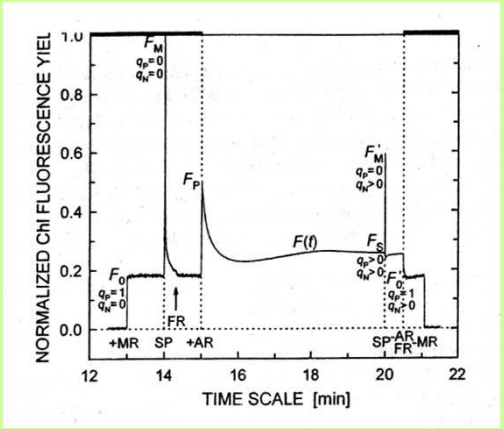
Fluorescence parameters + fluorimeters

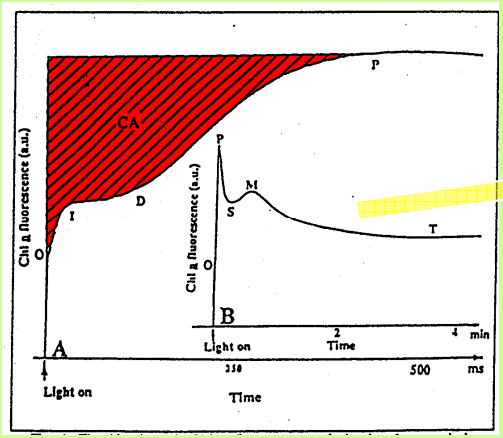
- specific mode of action
- studies of physiology or processes in photosynthesis

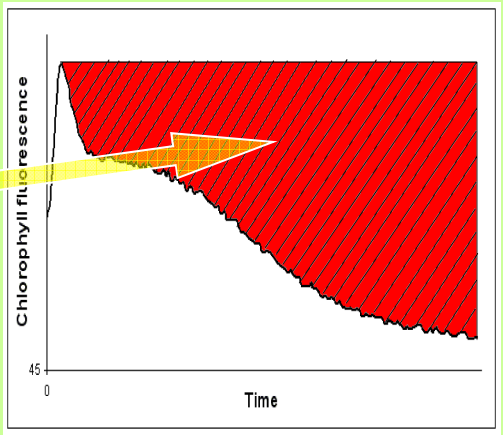
Complementary area + fluorescence imaging

- Screening bioassays
- Fluorescence imaging of heterogeneous and micro scale objects

Evaluation of the processes







1] by the terms and fluorescence parameters of the slow kinetics

2] by calculating definite integral residual (complementary area) from fast and slow kinetic records of fluorescence

Benefits of evaluation by slow complementary area

- Integrates several modes of action (ETS, proton pump, processes on membranes, ATP synthesis, Calvin cycle)
- Simple setup, simple interpretation
- Increase sensitivity for CCD fluorescence imaging systems
- Give the chance to observe heterogeneous object with low chlorophyll content
- Shows small variability in replicates

Instruments

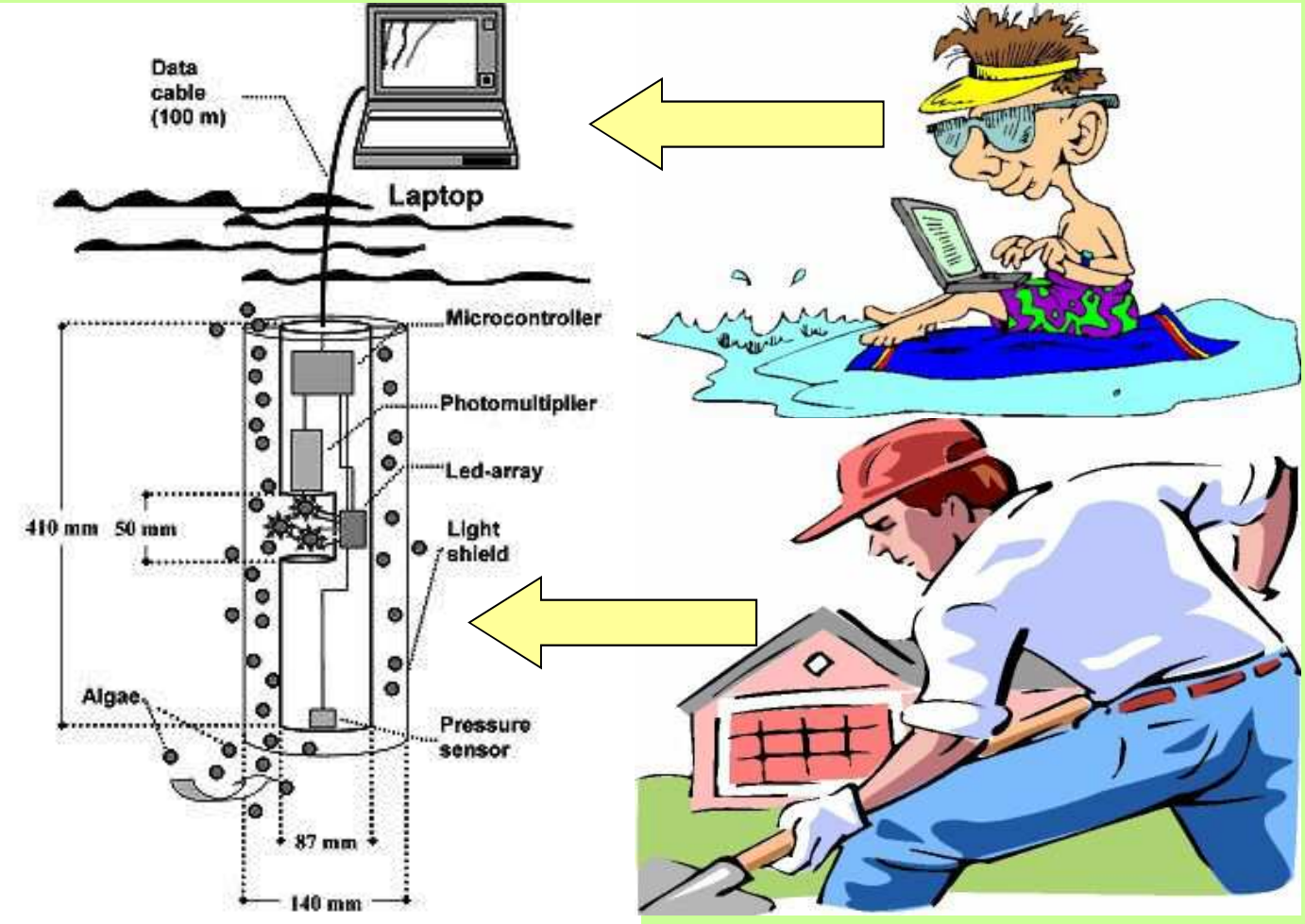
- Our instrumentation is based on fluorescence imaging systems (PSI, Brno, Czech Republic) with CCD camera detection

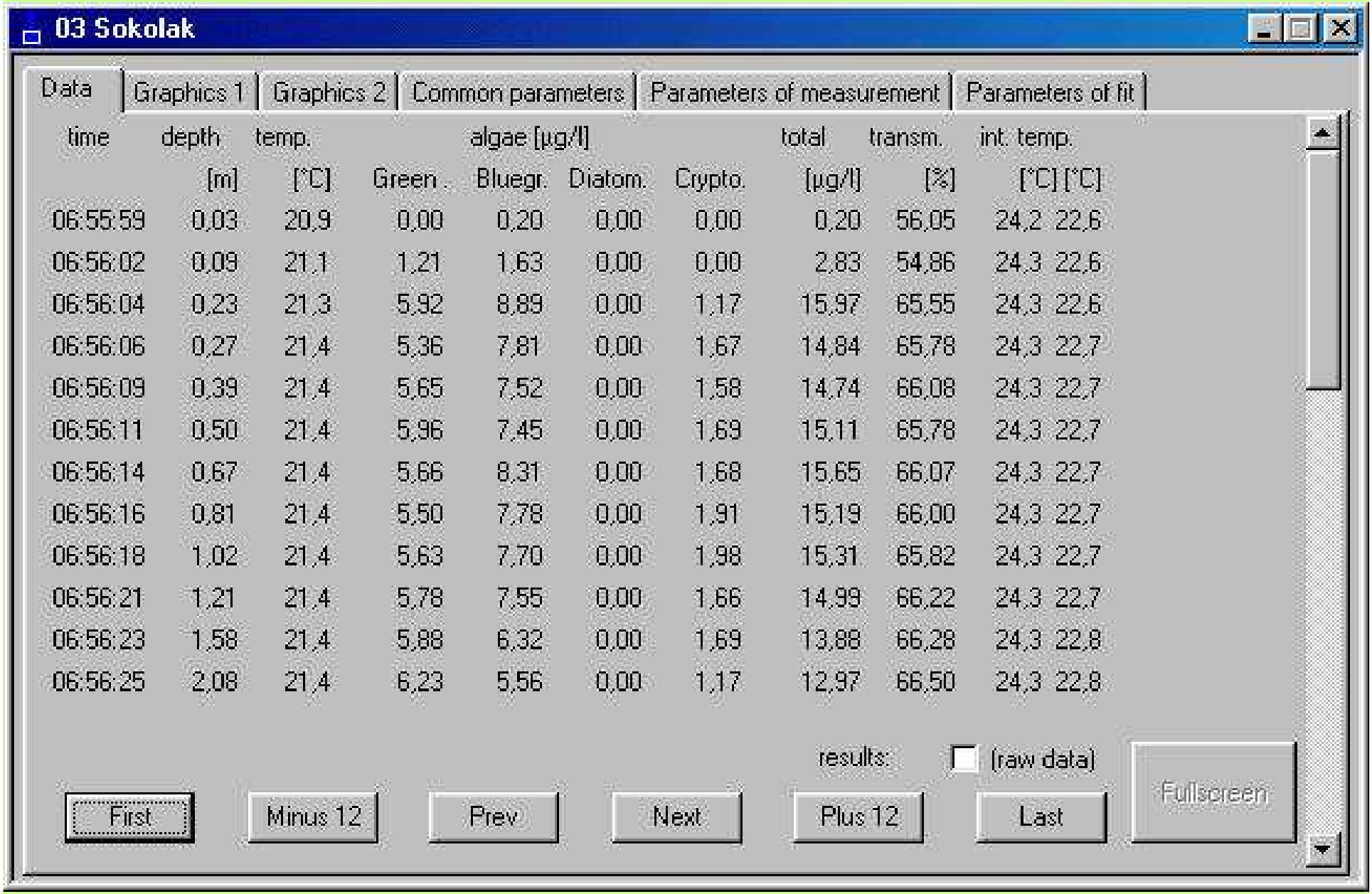
Photosynthetic pigments

group	*pigments*	*Excit. (nm)*	*Emis. (nm)*
Chlorophyta	Chlorophyll-b	480	685
Chromophyta Dinophyta	Chlorophyll-c Karotenoidy	460 525	685
Cyanobacteria	phycocyanin	610	650-685
Cryptophyta	phycoerythrin Chlorophyll-c	550 460	685

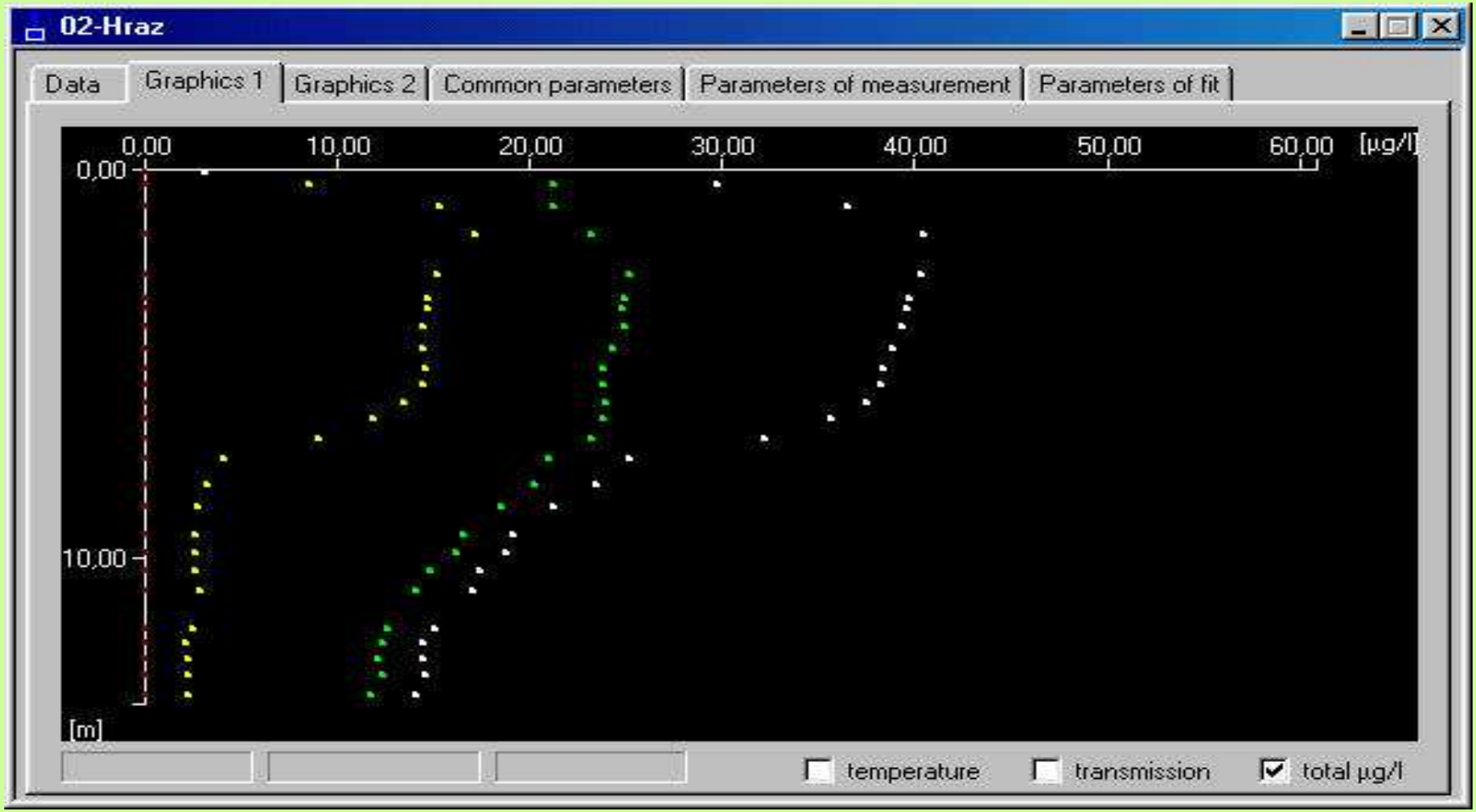
Fluoro Probe

FluoroProbe




03 Sokolak
Data
Graphics 1
Graphics 2
Common parameters
Parameters of measurement
Parameters of fit
time
depth
temp.
algae [µg/l]
total
transm.
int. temp.
[m]
[°C]
Green .
Bluegr.
Diatom.
Crypto.
[µg/l]
[%]
[°C] [°C]
06:55:59 0,03 20,9 0,00 0,20 0,00 0,00 0,20 56,05 24,2 22,6
06:56:02 0,09 21,1 1,21 1,63 0,00 0,00 2,83 54,86 24,3 22,6
06:56:04 0,23 21,3 5,92 8,89 0,00 1,17 15,97 65,55 24,3 22,6
06:56:06 0,27 21,4 5,36 7,81 0,00 1,67 14,84 65,78 24,3 22,7
06:56:09 0,39 21,4 5,65 7,52 0,00 1,58 14,74 66,08 24,3 22,7
06:56:11 0,50 21,4 5,96 7,45 0,00 1,69 15,11 65,78 24,3 22,7
06:56:14 0,67 21,4 5,66 8,31 0,00 1,68 15,65 66,07 24,3 22,7
06:56:16 0,81 21,4 5,50 7,78 0,00 1,91 15,19 66,00 24,3 22,7
06:56:18 1,02 21,4 5,63 7,70 0,00 1,98 15,31 65,82 24,3 22,7
06:56:21 1,21 21,4 5,78 7,55 0,00 1,66 14,99 66,22 24,3 22,7
06:56:23 1,58 21,4 5,88 6,32 0,00 1,69 13,88 66,28 24,3 22,8
06:56:25 2,08 21,4 6,23 5,56 0,00 1,17 12,97 66,50 24,3 22,8
results:
(raw data)
First
Minus 12
Prev
Next
Plus 12
Last
Fullscreen

FluoroProbe - graph




Cyanotoxins and
cyanobacterial blooms
managament represents a great
potential for the remote sensing
application!!!!!
THANK YOU

Expected results

- Joint effort for validation and calibration of data measured by in-lake and aircraft spectra
- Evaluation of RS tools potential for risks assessment of cyanobacterial blooms (dynamic of biomass movement)
- Cross discussion - RS tools potential for water quality assessment (soil erosion, nutrients pollution, use of new devices, spectra combination etc.).