Cyanobacteria and their toxins

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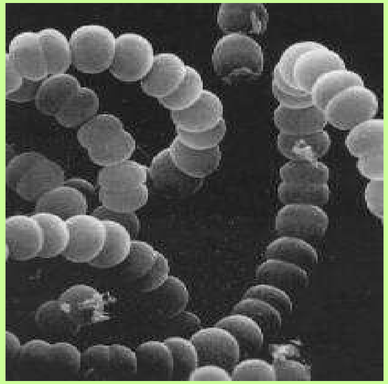
Institute of Botany
In cooperation with RAWAT consulting
for HYDAP
Kick-off meeting

Outlook of the presentation

- Cyanobacteria- **risks identification**
  - Ecological groups
  - Cell organisation
- Cyanotoxins
  - Systematics
  - Effects on human and ecosystem health
- Risks evaluation- methods for detection
  - Biomass quantification
  - Biomass movement
  - Biomass identification
- Expected activities and directions

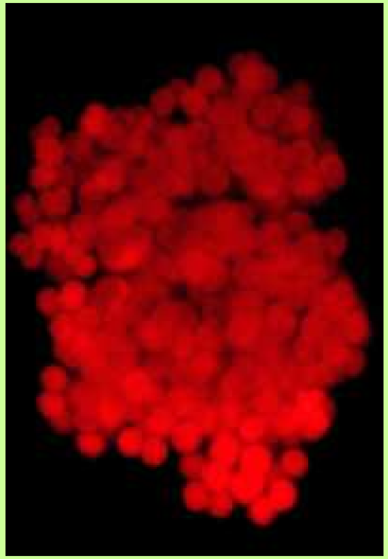
CYANOBACTERIA

• photoautotrophic prokaryotic organisms (i.e. bacteria)

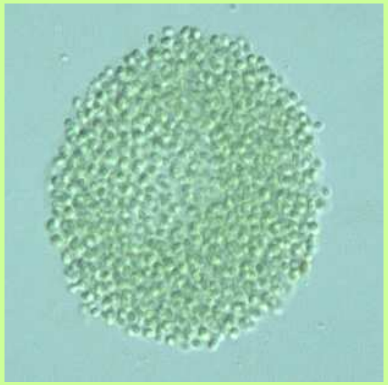


• growing in various biotops (fresh and marine water, soil, lichenes, glaciers, barks)

• most of species occur in aquatic ecosystems

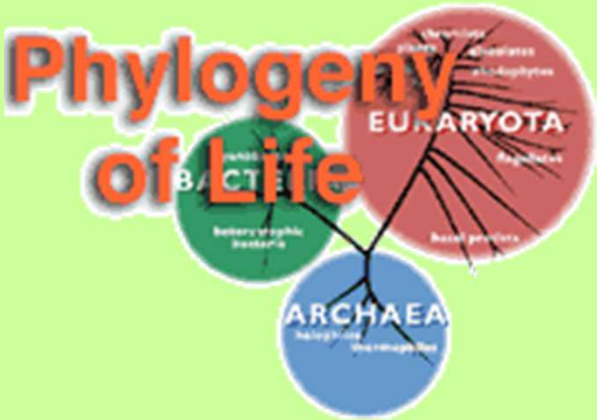




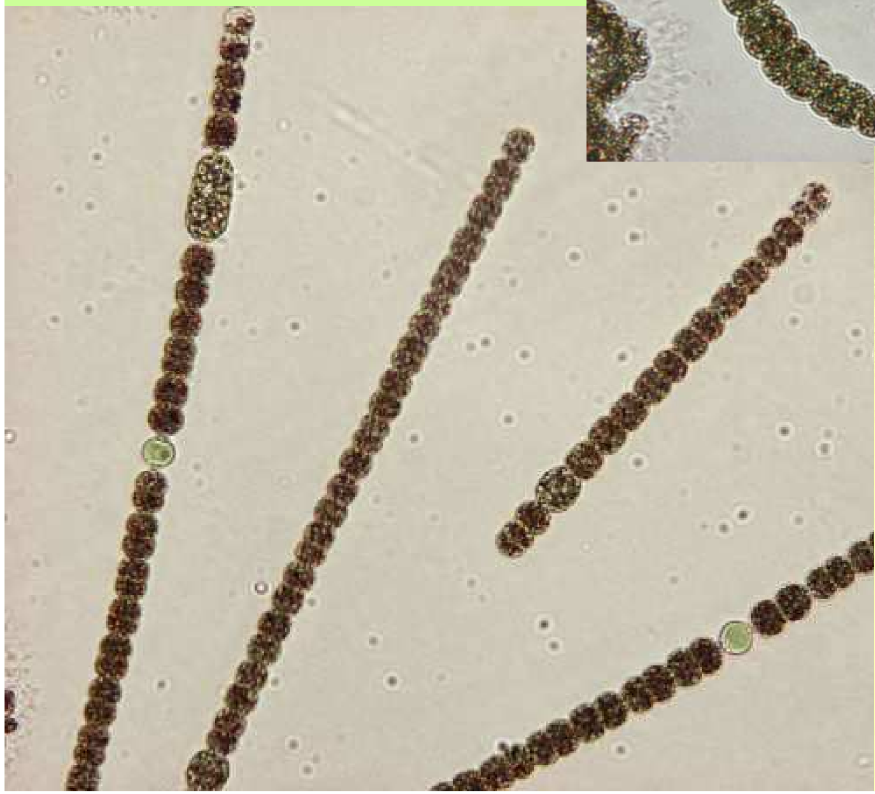
Cyanobacteria: grammnegative, photosynthetic eubacteria, 3,7mld years, responsible for the $O_{2,}$

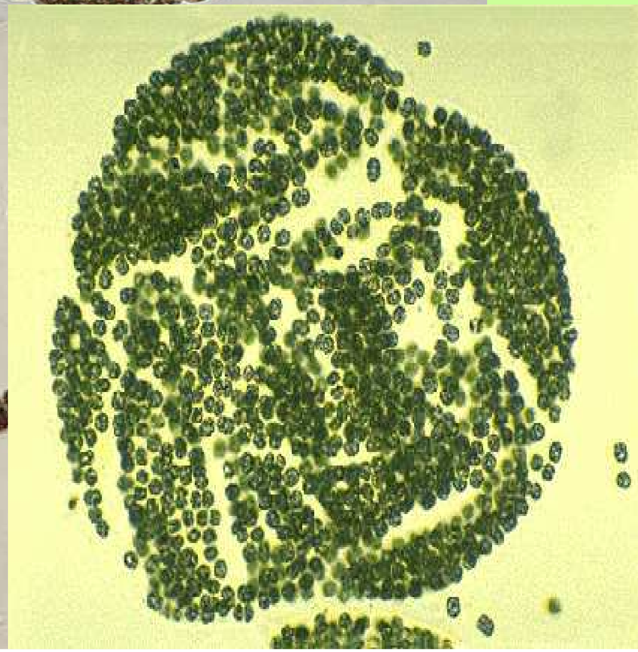
- Cell organization:
  - Unicellular
  - Colonial
  - Filamentous

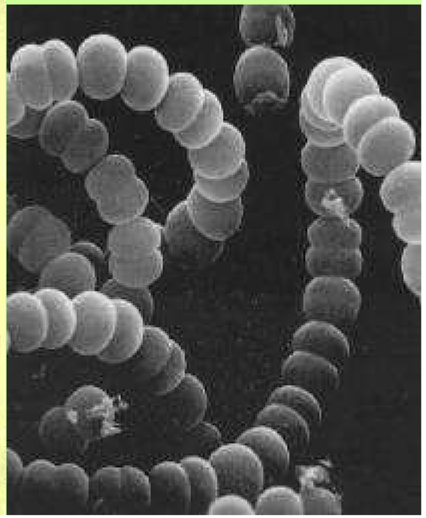


- Ecological groups:
  - Benthic
  - Soil
  - Plankton (all types up to picocyanobacteria)
  - Hot springs
  - Arctic ecosystems…
  - Dinitrogen fixation, hydrogen production, „food supplement!“

Microscopic view on cyanobacteria







Real view:
cyanobacterial water blooms
in the aquatic ecosystem

Benthic and pelagial morphotypes
of cyanobacteria
from natural samples

CYANOBACTERIA

HUMAN ACTIVITIES
(agriculture, waste waters…)

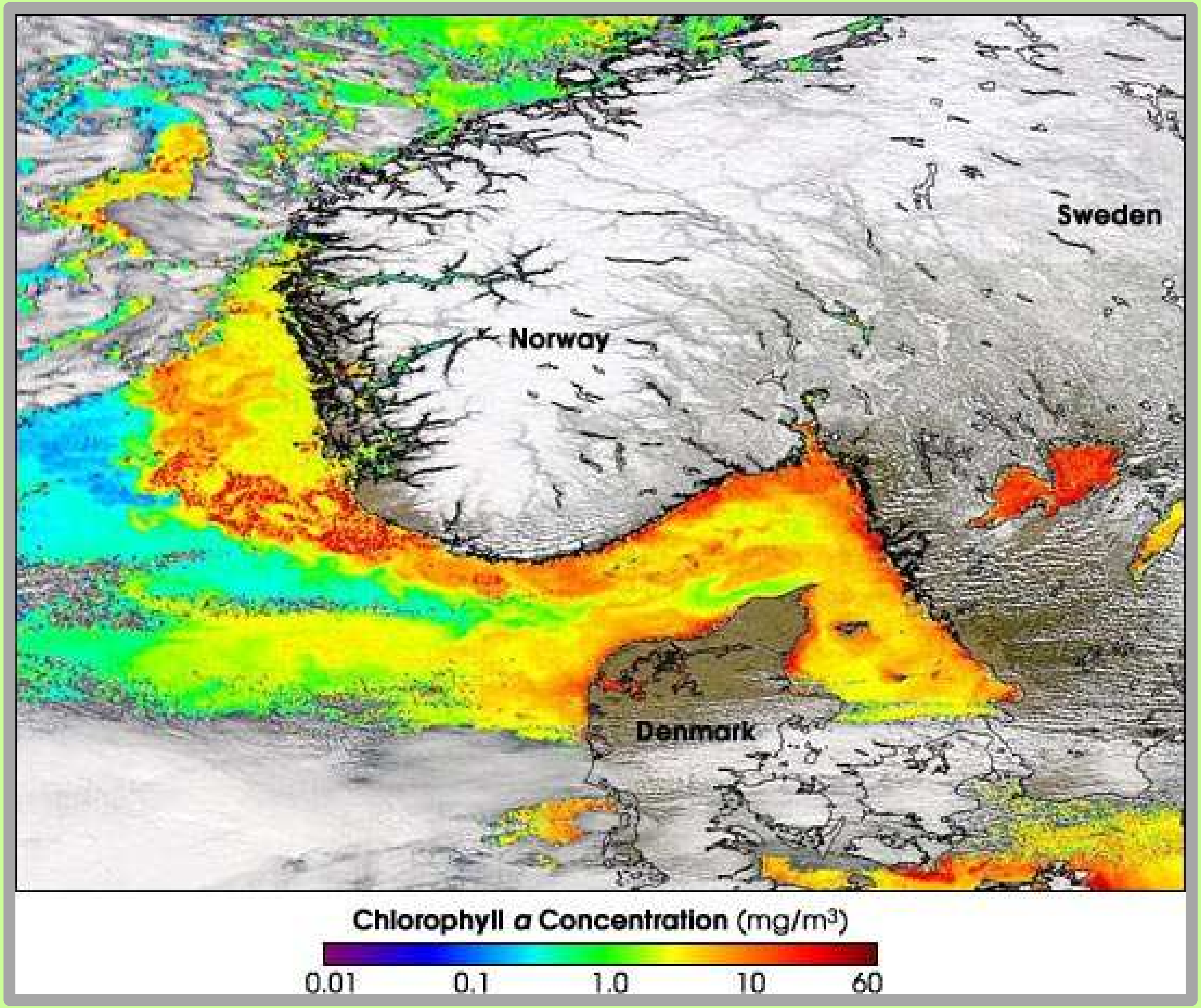
→

EUTROPHICATION
(=increased concentration of nutrients)

→

CYANOBACTERIAL MASS DEVELOPMENT



Sweden
Norway
Denmark
Chlorophyll a Concentration (mg/m³)
0.01
0.1
1.0
10
60

CYANOBACTERIA

- cyanobacterial water blooms = **serious global problem**
  - ➢affect on water chemistry (oxygen depletion, pH, pE)
  - ➢**musty odours** production
  - ➢production of **CYANOTOXINs** – toxic and biologically active compounds

- complication for water supply, recreation, fish culturing
- hazardous for other organisms

Categorization of cyanotoxins

1. **According to the chemical structure**
    - alkaloids,
    - cyclic and linear peptids
    - lipopolysaccharides

2. **According to the biological activity**
    - Detection of toxicity
    - Mechanisms of toxicity

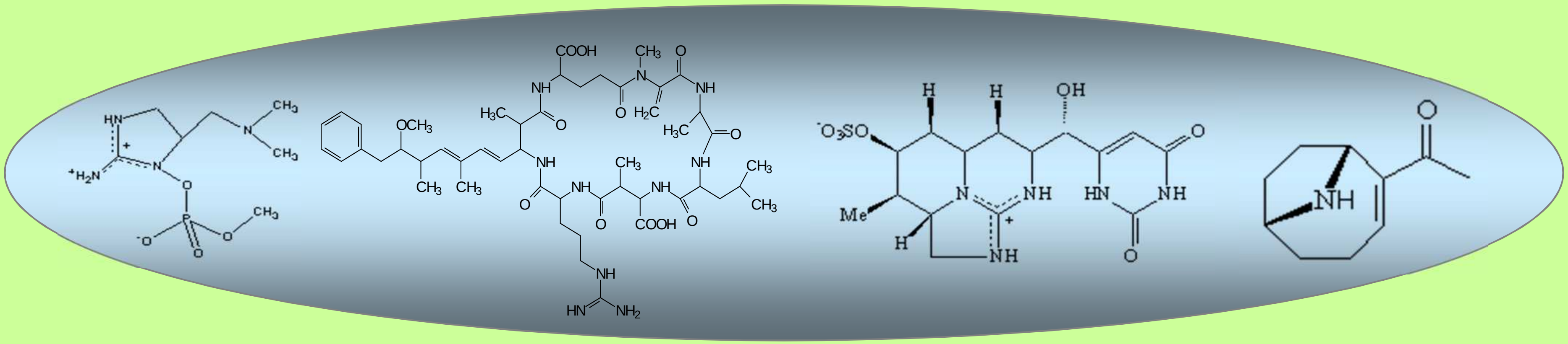
CYANOTOXINS

- become important contaminats of aquatic ecosystems
- huge group of compounds

(differ toxicologically as well as chemically)

- ➢linear and cyclic peptides a depsipeptides
- ➢low-molecular weight heterocyclic compounds
- ➢lipopolysaccharides of cell walls
- ➢etc.

➢various target organismus and modes of action
(neurotoxicity, hepatotoxicity, dermatotoxicity etc.)



Effects of selected cyanobacterial toxins

- Hepatotoxicity (83 microcystins and 145 another oligopeptides)
- Neurotoxicity (4 different mode of actions)
- Embryotoxicity, malformations
- Genotoxicity and mutagenicity, clastogenity
- Tumour promoting factors
- Immunotoxicity, immunomodulants, immunosupressors
- Phytotoxicity
- Allergens, dermatotoxins, irritants
- Cytotoxicity etc.

THE COMPARIOSON OF TOXICITY OF THE NATURAL TOXINS
(i.p. injection, acute rat test, LD50 in μg/kg)
Bacteria-cyanobacteria- animals- fungi- plants

Amatoxin	Amanita phalloides	fungus	500
Muscarin	Amanita muscaria	fungus	1100
Aphanotoxin	*Aphanizomenon flos-aquae*	cyano	10
Anatoxin -A	*Anabaena flos-aquae*	cyano	20
microcystin LR	*Microcystis aeruginosa*	cyano	43
nodularin	*Nodularia spumigena*	cyano	50
botulin	*Clostridium botulinum*	bacteria	0,00003
tetan	*Clostridium tetani*	bacteria	0,0001
kobra	*Naja naja*	snake	20
kurare	*Chondrodendron tomentosum*	plant	500
strychnin	*Strychnos nux-vomica*	plant	2 000

PLATE XII.—*Strychnos nux-vomica* (Nux Vomica). (From Jackson: *Experimental Pharmacology and Materia Medica.*)

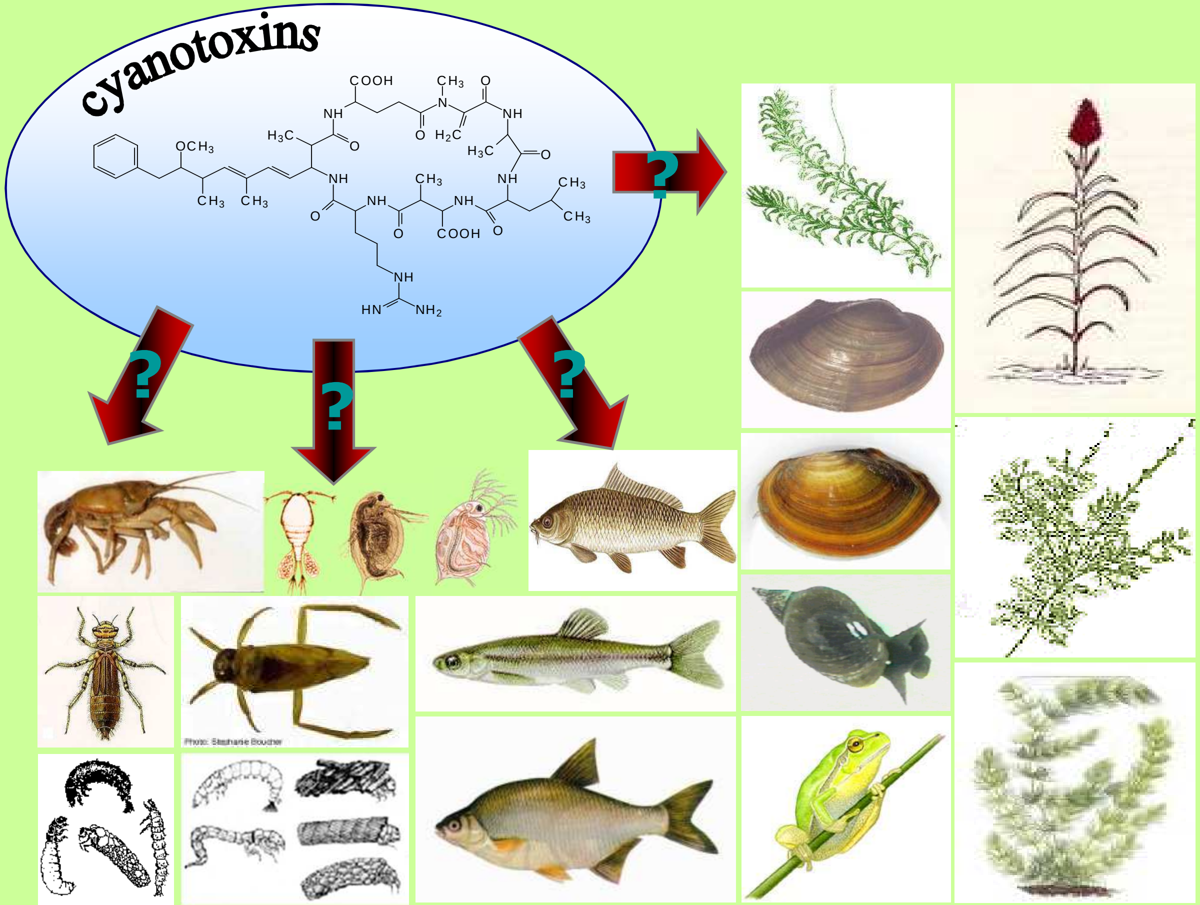
Genus	Toxins produced
Anabaena	Anatoxins, Microcystins, Saxitoxins, LPS's
Anabaenopsis	Microcystins, LPS's
Anacystis	LPS's
Aphanizomenon	Saxitoxins, Cylindrospermopsins, LPS's
Cylindrospermopsis	Cylindrospermopsins, Saxitoxins, LPS's
Hapalosiphon	Microcystins, LPS's
Lyngbia	Aplysiatoxins, Lyngbiatoxin-a, LPS's
Microcystis	Microcystins, LPS's
Nodularia	Nodularin, LPS's
Nostoc	Microcystins, LPS's
Phormidium (*Oscillatoria*)	Anatoxin, LPS's
Planktothrix (*Oscillatoria*)	Anatoxins, Aplysiatoxins, Microcystins, Saxitoxins, LPS's
Schizothrix	Aplysiatoxins, LPS's
Trichodesmium	yet to be identified
Umezakia	Cylindrospermopsin, LPS's

CYANOTOXINS

• intensively studied through **human health aspects**

• **ecological and ecotoxicological attributes not fully understood**

• about some cyanotoxins only reduced information exist



HEPATOTOXIC MICROCYSTINS

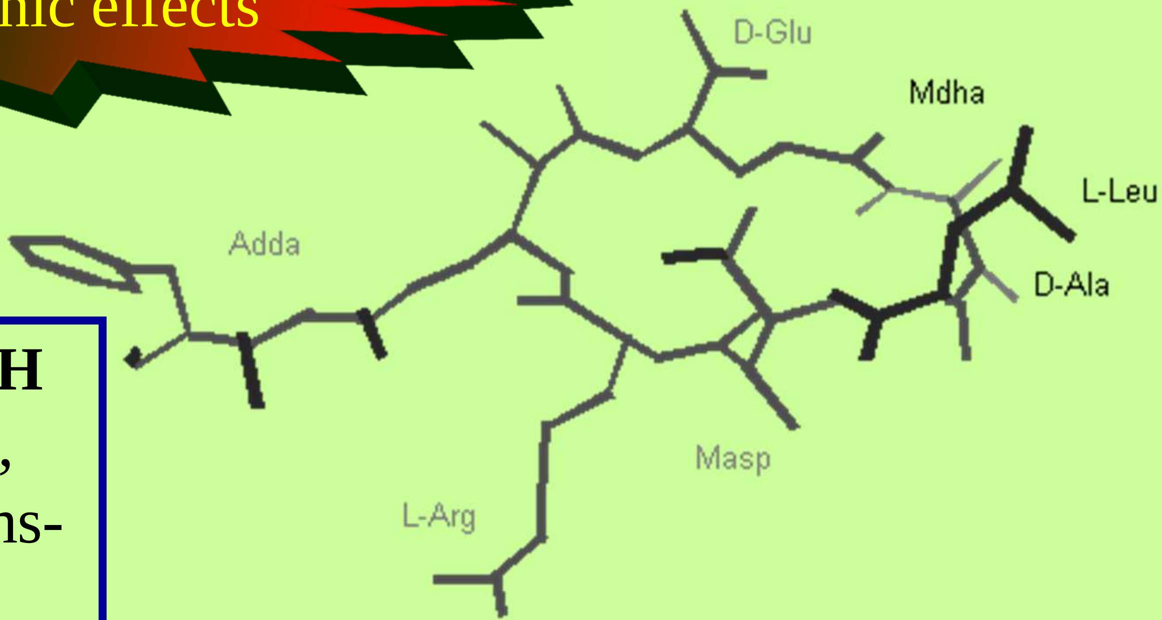
• the most studied cyanotoxins, commonly detected in biomass

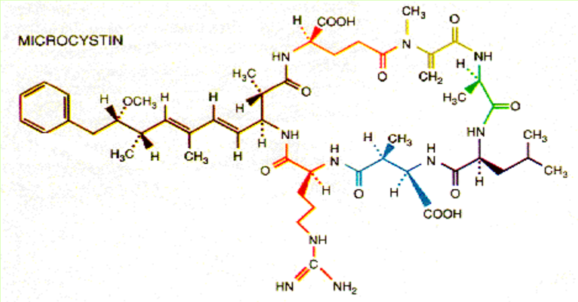
• cyclic heptapeptids, more than 60 structure variants

• produced by *Microcystis*, *Planktothrix*, *Oscillatoria*, *Nostoc*, *Anabaena*...

Acute toxic for vertebrates, chronic hepatokarcinogenic effects

EXTENSIVE RESEARCH
of human-health aspects,
effect on aquatic ecosystems-
gaps of knowledges

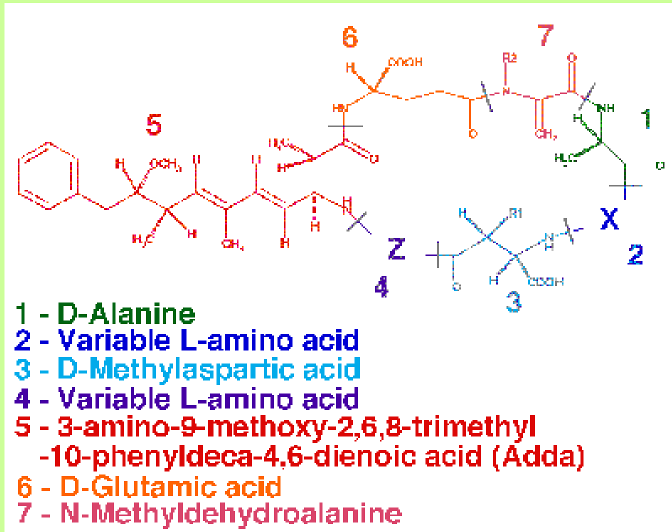




MICROCYSTINS

- The microcystins are a group of cyclic heptapeptide (7 amino acids) hepatotoxins (liver toxins) produced by a number of cyanobacterial genera
- MODE OF ACTION: PP2A INHIBITION
- It was found that extremely low concentrations

of microcystin-LR could strongly inhibit protein phosphatases 1 and 2A from both plants and mammals, thereby causing hyperphosphorylation of the cell and a massive disruption of a number of important cellular mechanisms.



MICROCYSTINS MODE OF ACTION

Microcystins = inhibitors of proteinphosphatases, e.g. the key enzyme of signalisation and regulation in eucaryotic cells

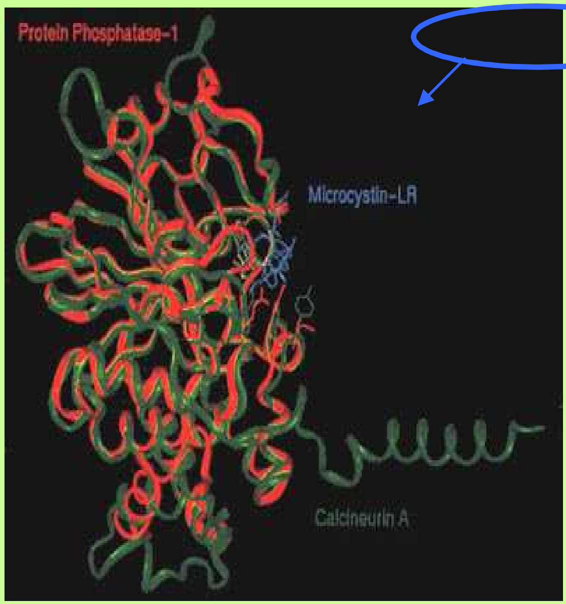
IN VITRO STUDIES

⇒microcystins inhibite PP in plant, invertebrate, fungi and vertebrate cells

⇒activity of key metabolic enzymes

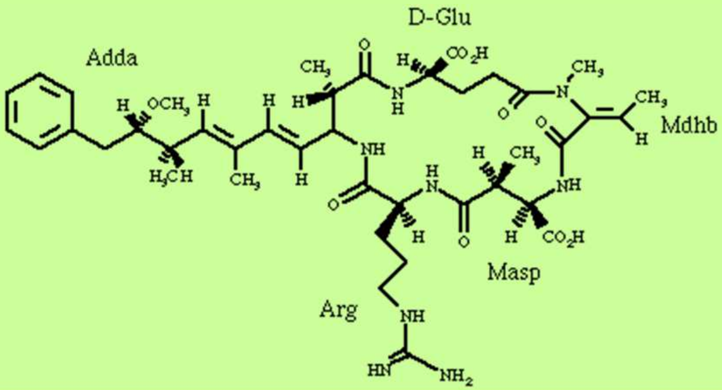
⇒signal pathways of gene expression

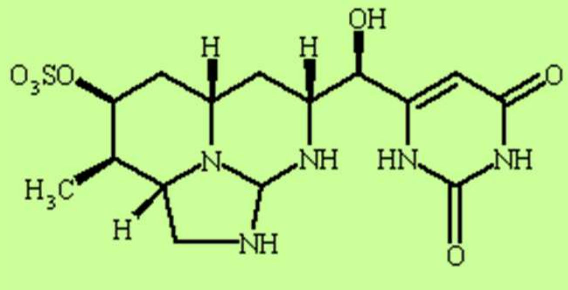
⇒**potencial for modification of the growth and reproduction of wholle populations**



Other hepatotoxins:

- Nodularin (cyclic peptide like microcystins)
- Cylinrospermopsin (Although originally described from *Cylindrospermopsis raciborskii*, cylindrospermopsin can be found also in *Aphanizomenon ovalisporum* and *Umezakia natans*. The toxin is a cyclic alkaloid and, like microcystins, primarily affects the liver, although causes considerable damage to other major organs – nefrotoxin, cardiatoxin, mutagen, TPF…)





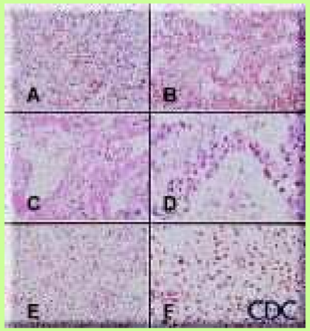
CYLINDROSPERMOPSIN

Ecological consequences of hepatotoxins

- Stabile molecules
- Tend to bioacumulation in the food web!!!
- Documented increased concentrations from zooplankton to fish
- HEALTH RISKS

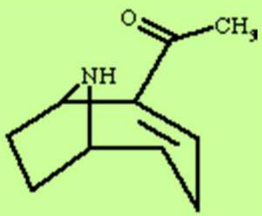
For humans and

ecosystem

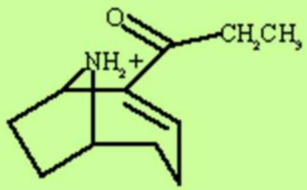


Neurotoxins

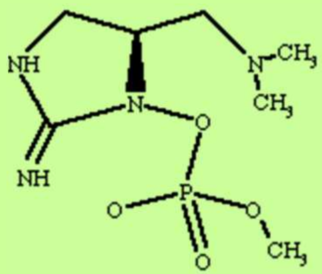
- The anatoxins are a **group of neurotoxic alkaloids** produced by a number of cyanobacterial genera including *Anabaena*, *Oscillatoria* and *Aphanizomenon*. The toxicity of these compounds (LD50)varies from 20 µg kg-1 (by weight, I.P. mouse) for anatoxin-a(S) to 200-250 µg kg-1 for anatoxin-a and homoanatoxin-a, making them **<u>more toxic than many microcystins.</u>**



ANATOXIN-A



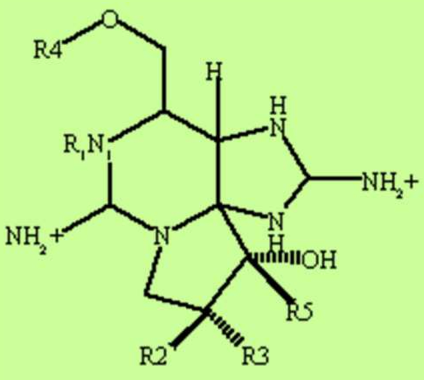
HOMOANATOXIN-A



ANATOXIN-A(S)

SAXITOXINS-another neurotoxins

- Like anatoxins, the saxitoxins (STX) are **neurotoxic alkaloids** which are also known as PSP's (paralytic shelfish poisons) due to their occurance and association with seafood.
- They **block sodium channels in nerve cells,** thus casuing their neurotoxic effects.
- **There are a number of STX variants** generally divided into groups based on their structure or organism of origin

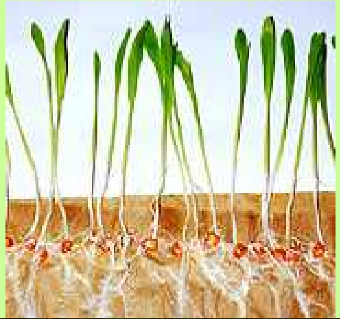


SAXITOXIN - GENERAL STRUCTURE

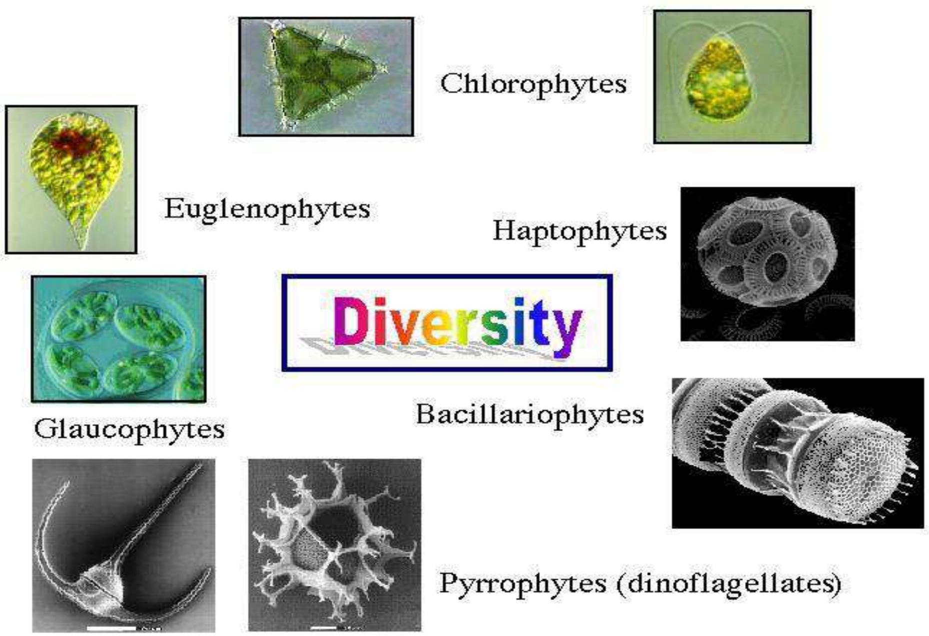
Ecological effects of neurotoxins

- Unstable molecules (UV, temperature)
- No biocumulation
- **Extremly toxic –**
- **3-5min after ingestion= mortality** (even rat, dogs, catle..)
- Frequently in benthic cyanobacteria, no only water blooms)

Phytotoxicity



- Toxicity to:
  - Phytoplankton
  - Aquatic mactophyte
  - Terestrial plants



IN VIVO STUDIES WITH TERESTRIAL PLANTS

- model species (*Sinapis alba*, *Lepidium sativum*, *Phaseolum vulgaris*, *Solanum tuberosum*)
- **active intake** of microcystins – through epidermis of roots and leaves
- growth inhibition
- inhibition of photosyntesis
- changes of biochemical markers of stres indication :

  activity of detoxication enzymes (GST, GSHPx),
  activity ssDNases, etc...

!!! Ecologically relevant concentrations of microcystins !!!

IN VIVO STUDIES WITH AQUATIC PLANTS

• experiments with *Elodea*, *Ceratophyllum*, *Myriophyllum*, *Vesicularia*, *Phragmites*, *Lemna*, *Spirodella*

•**active intake** of microcystins proved

• detoxiction via glutathion S-transferase

• inhibition of photosyntesis

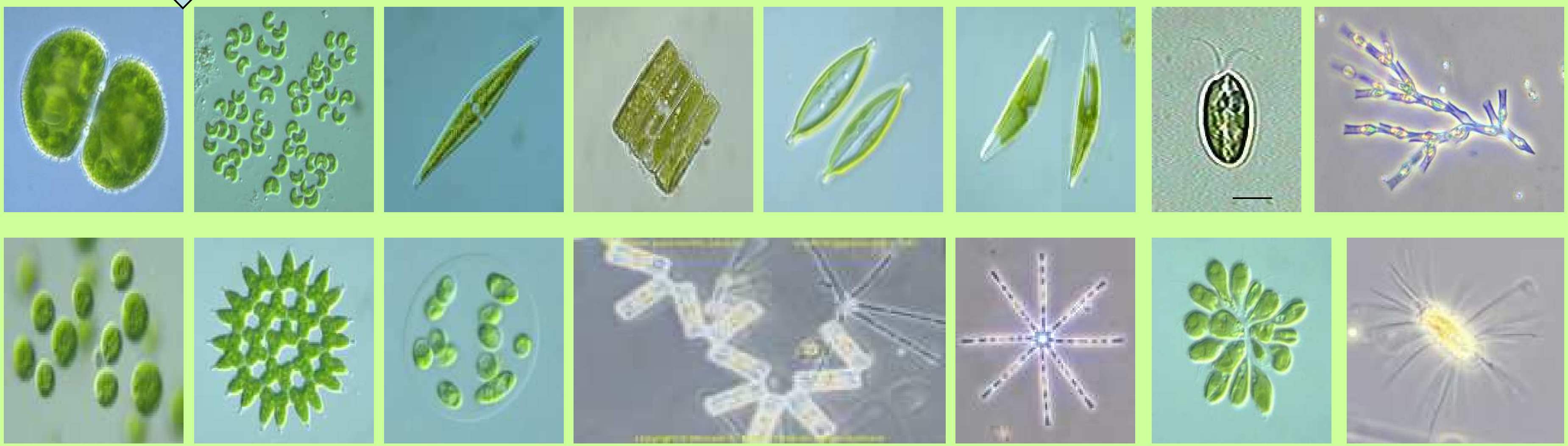
• changes of biochemical markers of stress indication :

pigments composition activity of GST

EFFECTS ON WHOLLE ECOSYSTEM – SERIOUS <u>REDUCTION OF MACROPHYTE VEGETATION AND ENERGY FLOW</u> IN THE ECOSYSTEM

EFFECTS OF MICROCYSTINS ON PHYTOPLANKTON

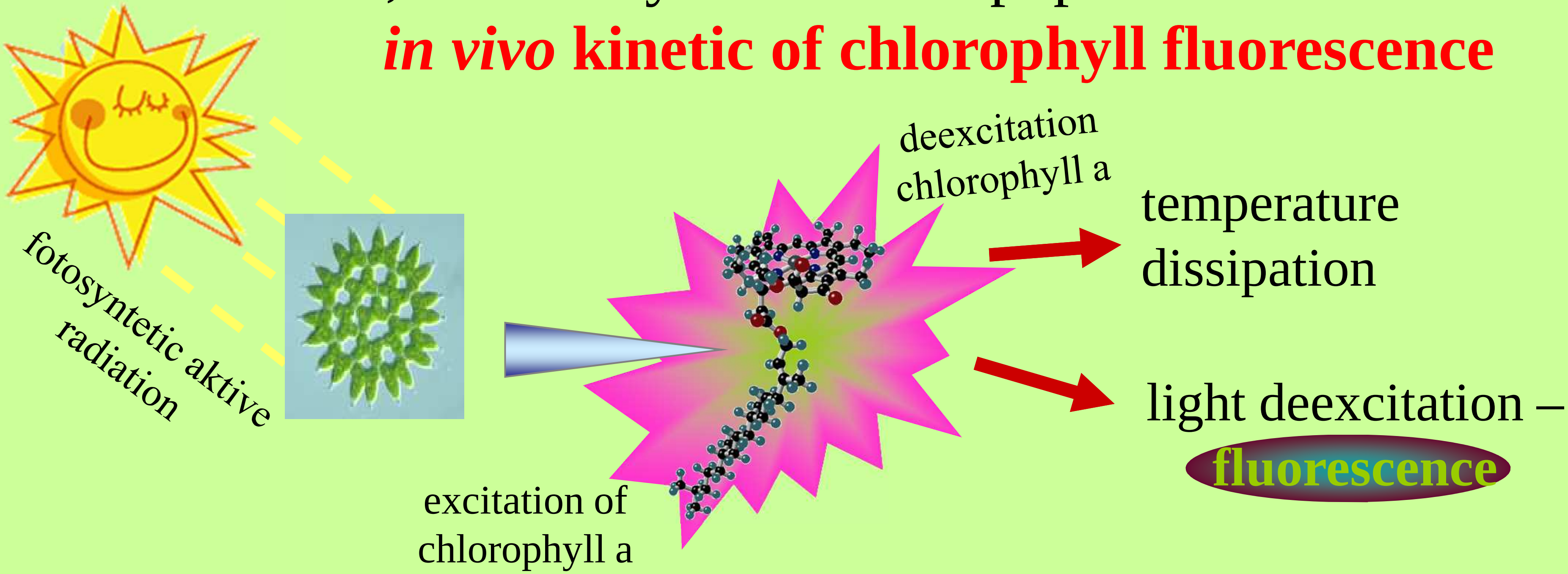
- Effects detected in environmentally relevant concentrations
- Difference within phytoplankton species in sensitivity to microcystins
  - diatoms, heterocontes, cryptophyces, dinophlagelates, green algae and cyanobacteria



EFFECTS OF MICROCYSTINS ON PHYTOPLANKTON:EXPERIMENTAL DESIGN

- Endpoints –**growth and cell division** of pure or wild strains, laboratory and natural populations

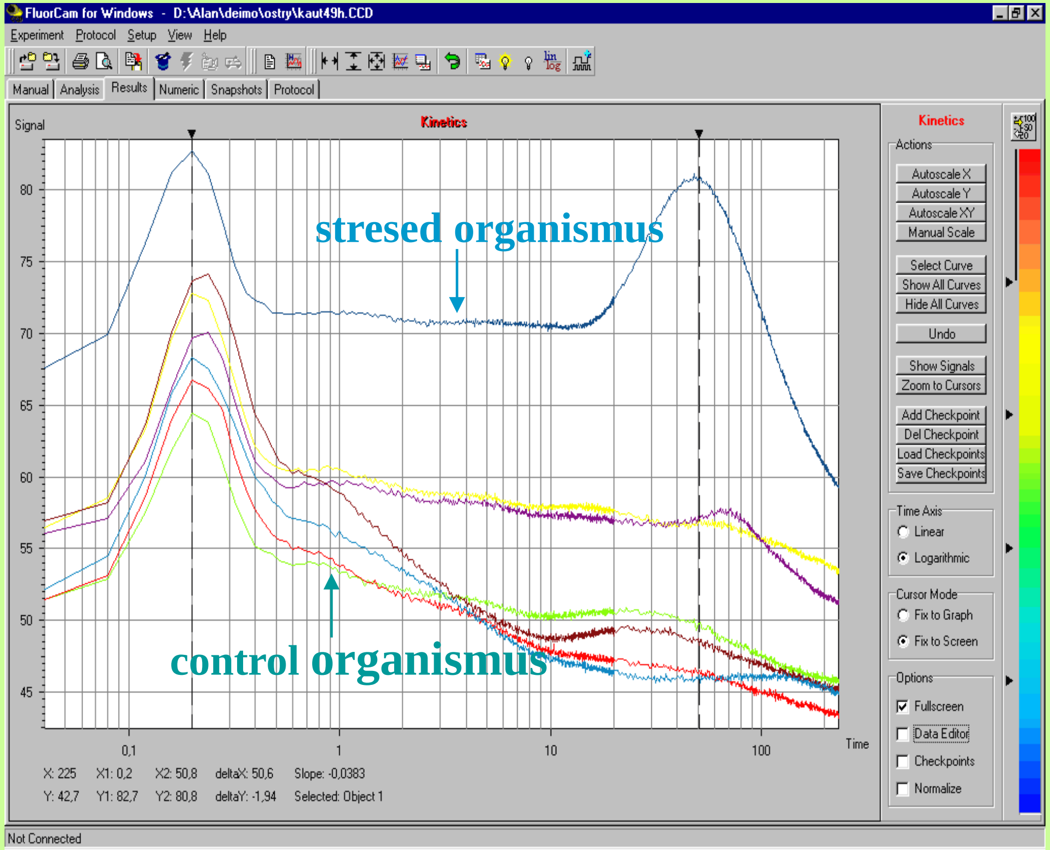
***in vivo* kinetic of chlorophyll fluorescence**



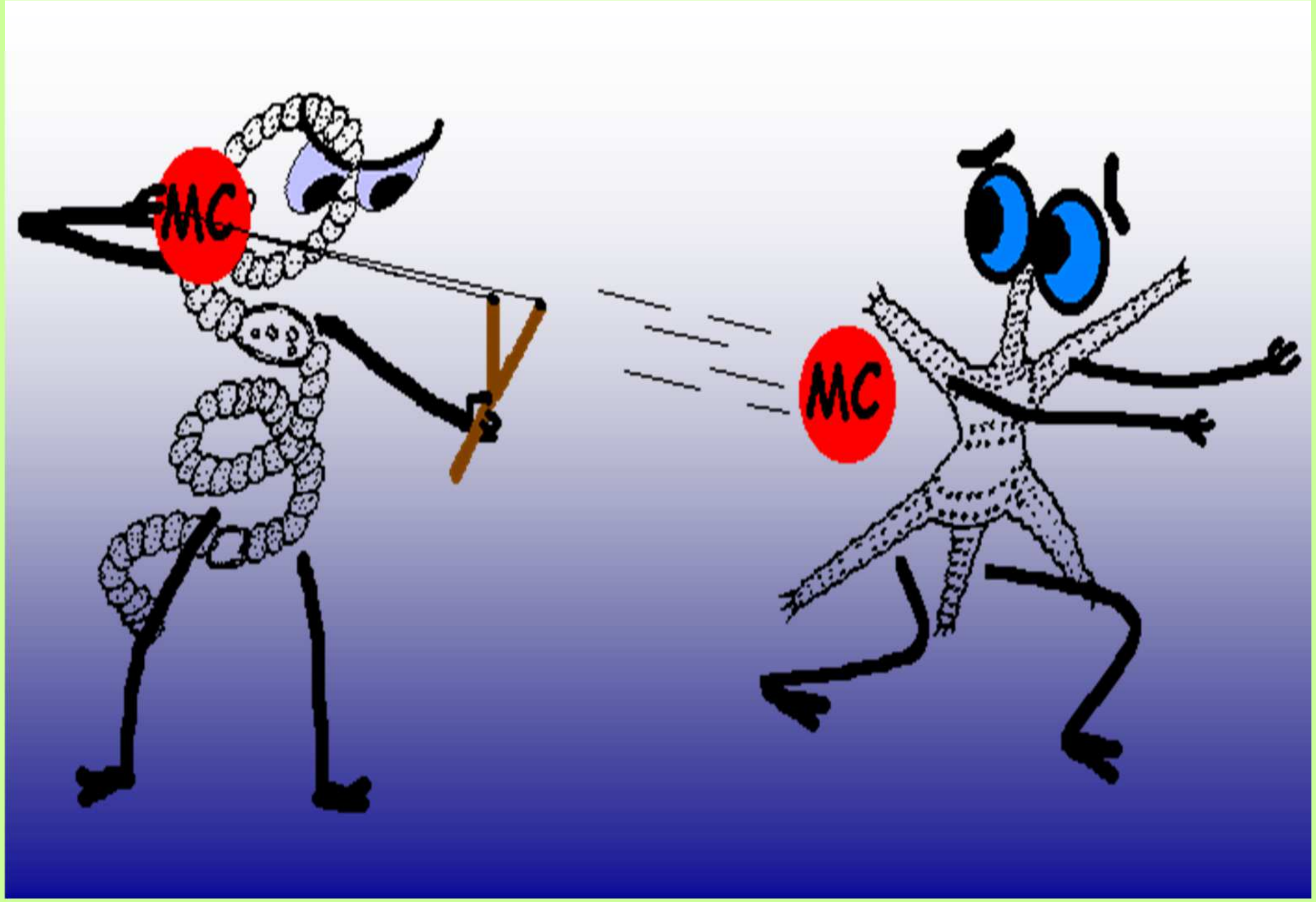
EFFECTS OF MICROCYSTINS ON PHYTOPLANKTON:EXPERIMENTAL DESIGN

Detection of ***in vivo* chlorophyll *a* fluorescence**

- fluorimeter with CCD camera
- sensitive and no-destructive endpoint
- sensitive for quick toxic injury detection



EFFECTS OF MICROCYSTINS ON PHYTOPLANKTON



Cyano vs. green??

⬇

FURTHER RESEARCH IS REQUIRED

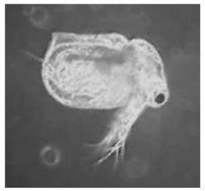
Effects of cyanotoxins on aquatic fauna:

- Mortality
- Biodiversity
- Embryotoxicity
- malformations

Daphnia magna

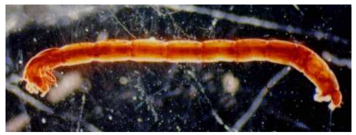
Artemia salina

Ceriodaphnia dubia



Gammarus

Chironomus riparius



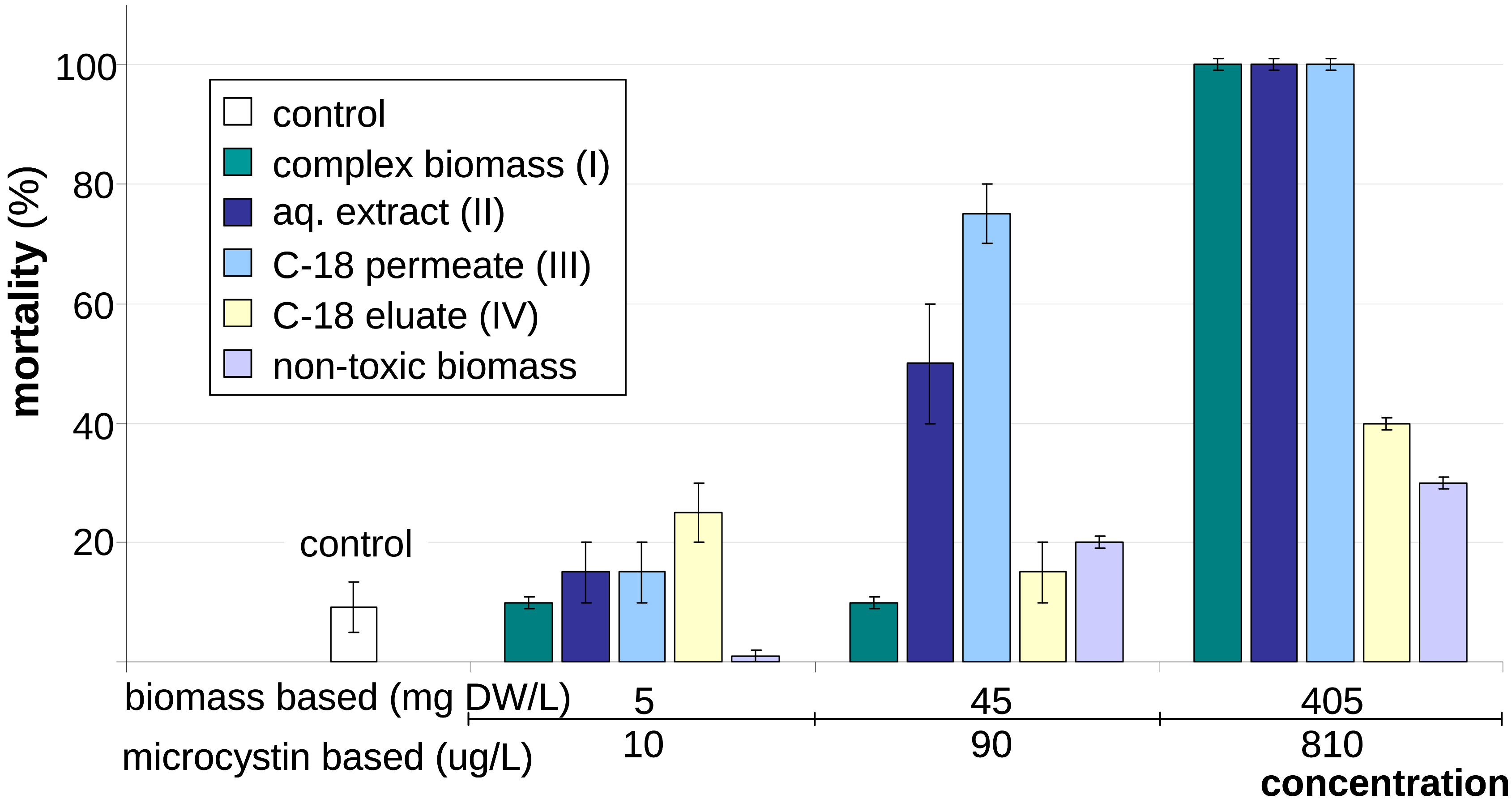


Fig: Effects of fractionated cyanobacterial biomass on
***Daphnia magna* – mortality** after 21 days

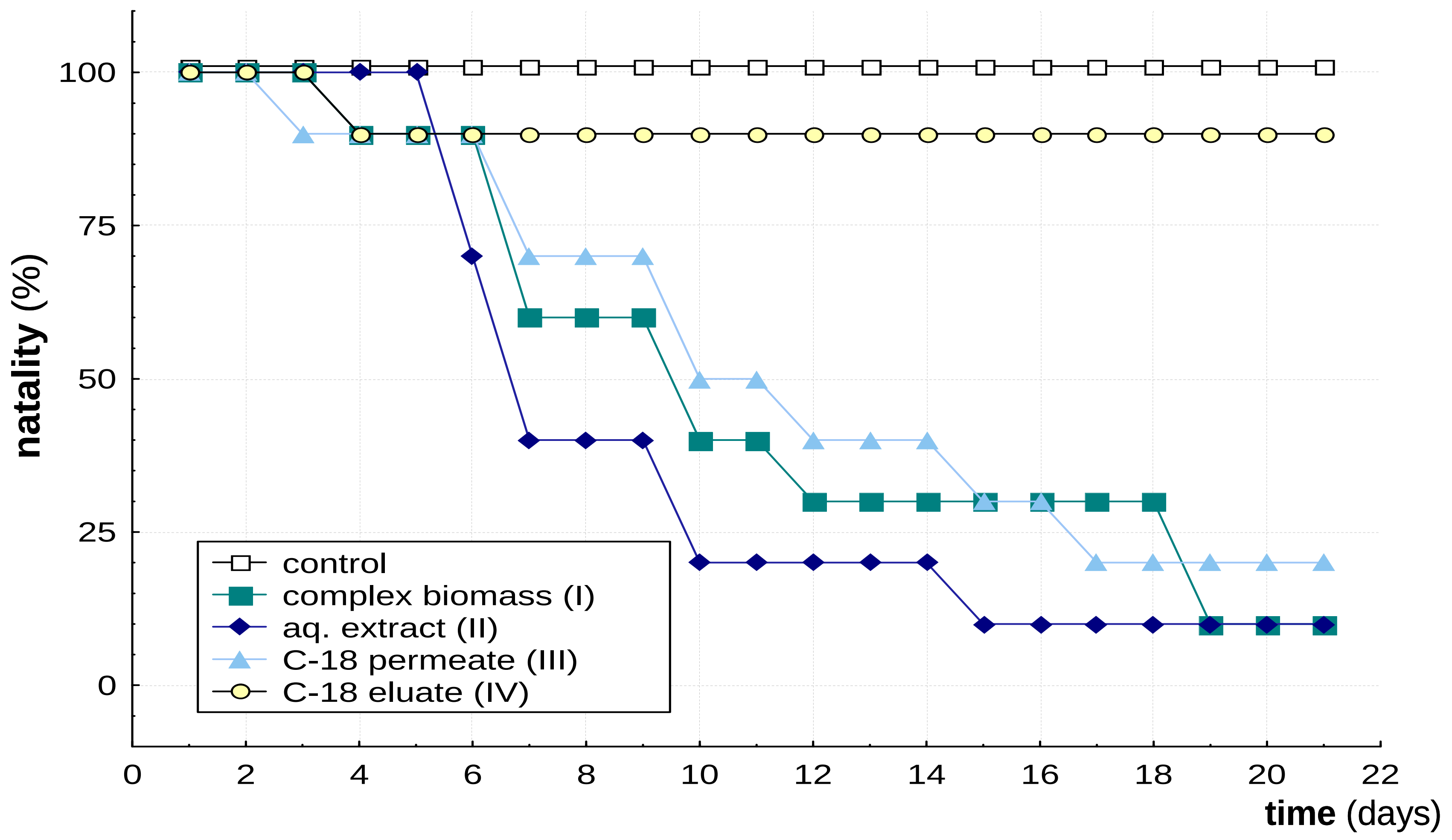


Fig.: Effects of fractionated cyanobacterial biomass on *Daphnia magna* - natality at the concentration 135 mg DW/L of each fraction (corresponding to 270 ug microcystin/L in

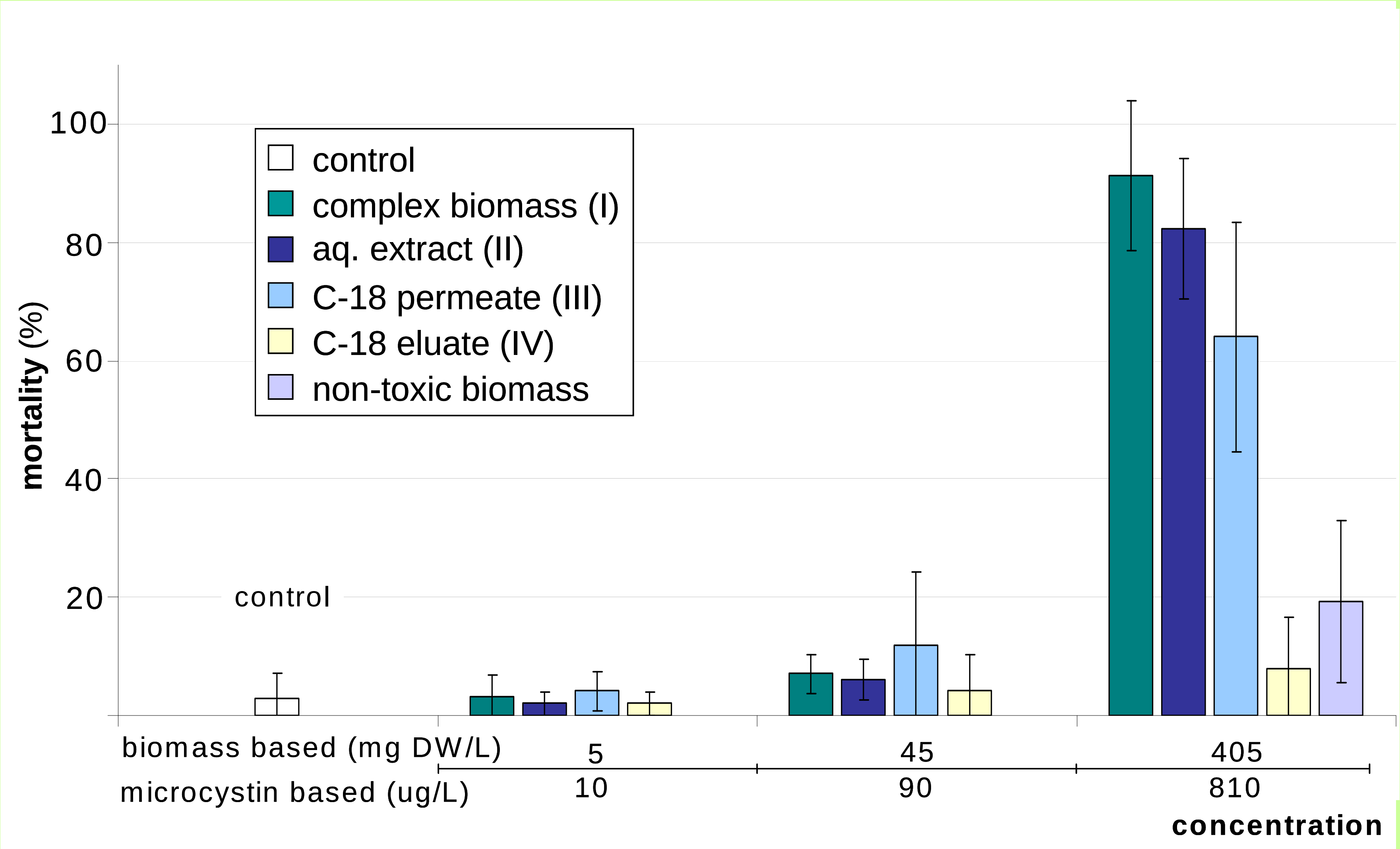


Fig.: Effects of fractionated cyanobacterial biomass on *Xenopus laevis* – 96 hour FETAX: mortality

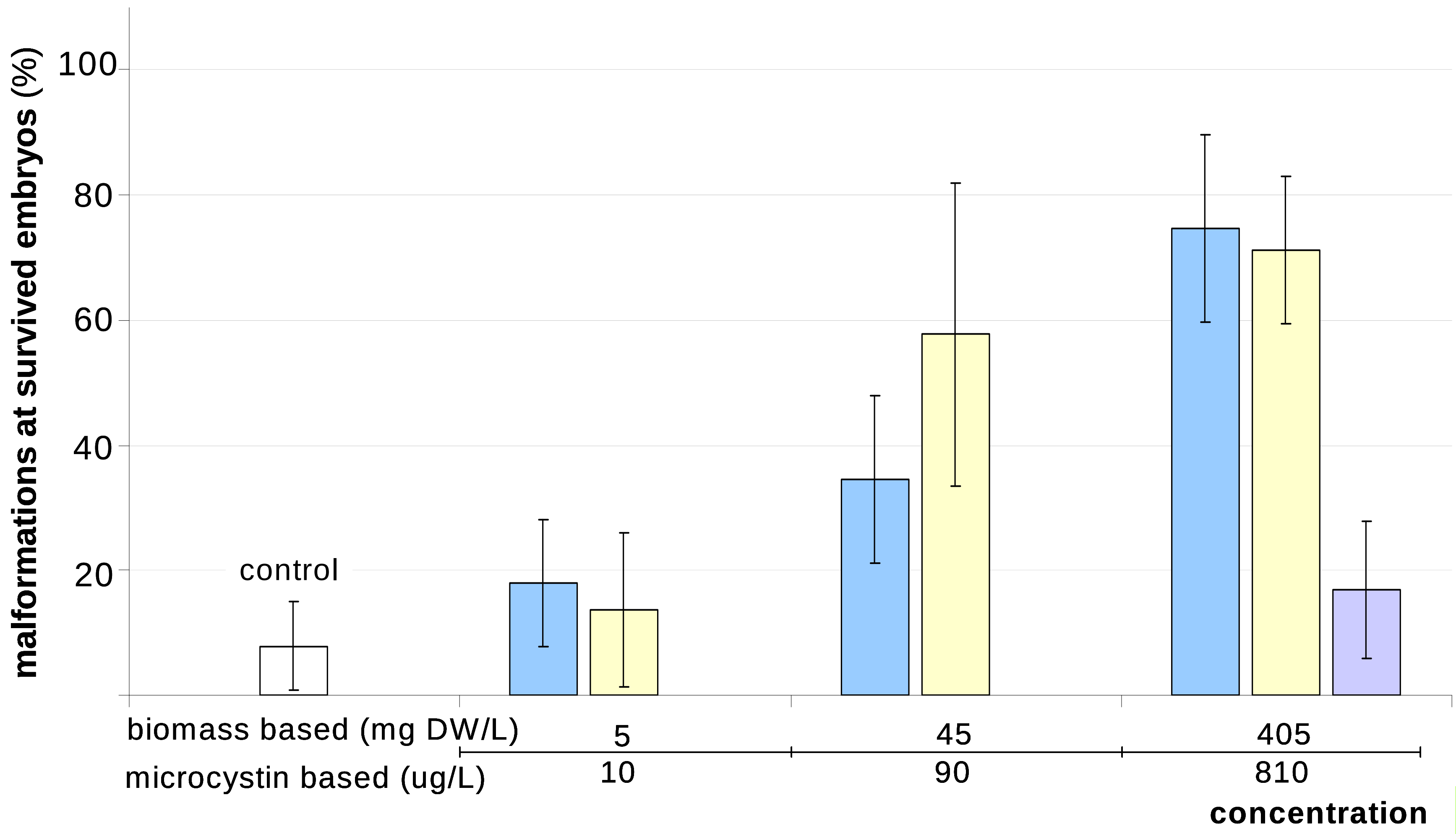


Fig.: Effects of fractionated cyanobacterial biomass on *Xenopus laevis* – 96 hour FETAX: teratogenicity

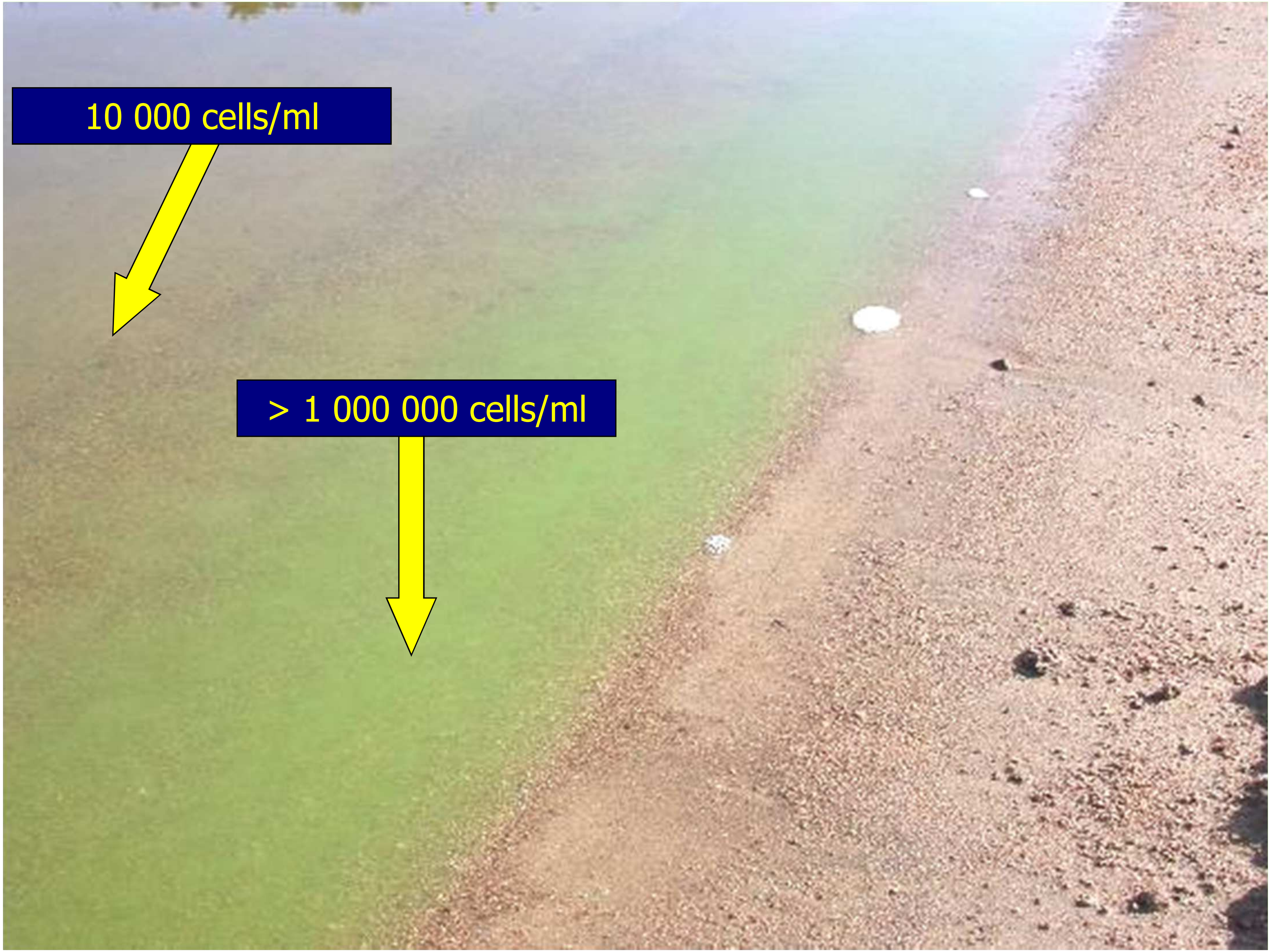
Effect of cyanobacterial biomass on Xenopus embryo malformation

… and the other effects???

- The reality shows, that there are more ecological and human health effects than are currently studied
- Trends:
  - MALDI-TOF-MS (peptide toxins)
  - From human health to health of the aquatic ecosystems
  - Detection by mode of action (teratogenity, malformations, GJIC, conjugation etc.)

Human exposure

- Acute
  - dermal contact , irritance
  - Respiratory – water skiing, fontans
  - Ingestion - during swimming and water sports
- Chronic
  - Drinking water
  - Repeteated sporting activity within water blooms
  - Dietary supplements – e.g. *Spirulina* from non-controlled sources


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

Effects of cyanotoxins on freshwater ecosystems

- Zooplankton, **zoobenthos** (reproduction, species composition, abundancy)
- Phytoplankton- total changes of structure, **diatoms are the most sensitive**
- **Fish communities** – serious changes, effects from histopathology, up to the reproduction and embryolarval development
- **Amphibians** (reproduction, malformations – FETAX)
- **Macrophytes** (photosynthesis, biodiversity, biomass,)

Conclusion

All cyanobacteria produce toxic compounds
e.g. DETECTION AND QUANTIFICATION OF CYANOBACTERIAL BIOMASS became to be dominant tool for the risks evaluation and risk management

Cyanobacterial water blooms are dynamic systems with permanent migration:

- Spacial
- Temporal
- Horizontal
- Vertical

Remote sensing –should be really great help to interpret risks for both – Humans and water ecosystems

Expected tools: a combination of satelite, aircraft sensing with in-lake measurements.