Intracellular and Extracellular Cyanotoxins: Implications for their Environmental Significance and Health Risk Management

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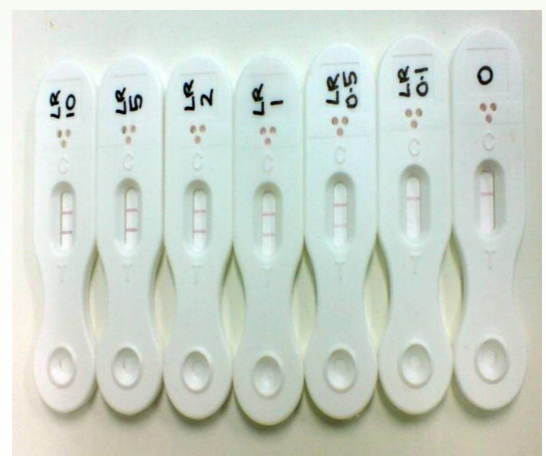
Heritage Park, E. Kilbride, Glasgow: cancellation of national swimming competition, Aug-Sept.. An example of cyanobacterial bloom impact, 2010.

The compartmentation /localisation of cyanotoxins: why do we need to know?

1. Improved cyanobacterial bloom and cyanotoxin hazard characterisation and risk assessment with reference to:
   (a) human health
   (b) wildlife and environmental protection.
2. Increased understanding of cyanotoxin functions and fates.
3. Identification of appropriate prevention, remediation and treatment methods for cyanobacterial blooms and cyanotoxins

Determination of cyanotoxin compartmentation by physicochemical and immuno-methods



Localisation of microcystins into “soluble” and “particulate” phases. [Particulate = Total *minus* Soluble]: Donkmeer.

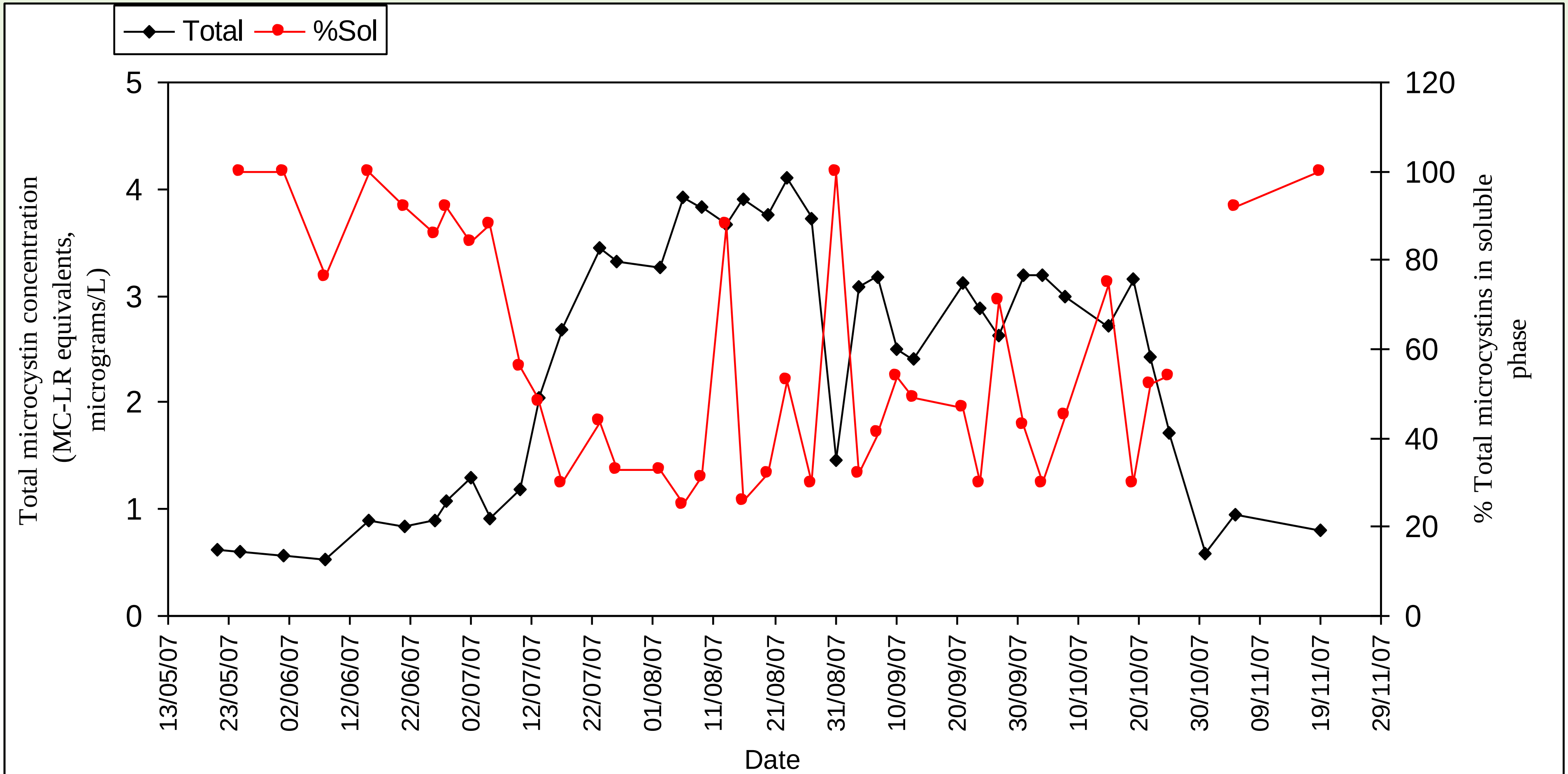


Figure 8. Total microcystin concentrations (determined by ELISA) and % total microcystin pool in soluble (dissolved) phase, for Donkmeer, 2007. Each point is the mean of triplicate determinations.

Variation in compartmentation of microcystins between particulate and soluble fractions: Brussels lakes (samples from Dr. A. Peretyatko, 2010)

	Sample	MC-LR equivalents ug/L)		% distribution	
		soluble	particulate	soluble	particulate
1	VUB 12	3.2	1.1	75	25
2	VUB 13	2.7	1.2	70	30
3	VUB 6 (scum)	307.1	151.9	67	33
4	VUB 10	3.5	2.1	62	38
5	VUB 11	1.7	1.5	54	46
6	VUB 8	0.80	0.76	51	49
7	VUB 2	1.8	2.4	43	57
8	VUB 7	< 0.5	>0.8	< 39	> 61
9	VUB 9	0.8	1.5	35	65
10	VUB 5	< 0.5	>1.1	< 31	> 69
11	VUB 4	0.8	3.2	21	79
12	VUB 3	0.7	3.4	18	82
13	VUB 1 (scum)	9.9	1639.1	1	99

All values are means of triplicate determinations

Cyanotoxin compartmentation: “particulate” and “soluble”toxin

Senescent bloom, most MC not associated with cyanobacterial cells.

Growing cells: >90% MC “particulate”

Cyanotoxins: where are they located in the waterbody ?

1. “Soluble”, due to:
   (a) extracellular release by intact, toxigenic cyanobacteria
   (b) extracellular release due to cyanobacterial cell breakdown e.g. by:
      (i) lytic bacteria
      (ii) cyanophages
      (iii) autolysis
      (iv) physical forces (e.g. during water abstraction/treatment)
      (v) chemical agents during drinking water treatment
   (c) release of cyanotoxins from other aquatic biota (excretion)
   (d) release from abiotic particulates (sediments) ?

Cyanotoxins: where are they located in the waterbody ?

- 2. “Particulate”, associated with:

(a) cyanobacterial cells

(b) other aquatic organisms (prokaryotes, eukaryotes)

(c) abiotic particulates

Example of extracellular release of microcystins by intact cyanobacterial cells

- *Microcystis* PCC 7806
- Axenic
- Steady state continuous culture

Irradiance (µmol.m^{-2}.s^{-1})	Microcystin conc. (µg.l^{-1})
10	0.5 to 1.0
40	15 to 18

Wiedner, Visser, Fastner, Metcalf, Codd & Mur (2003) Appl. Env. Microbiol. **69**, 1475-1481.

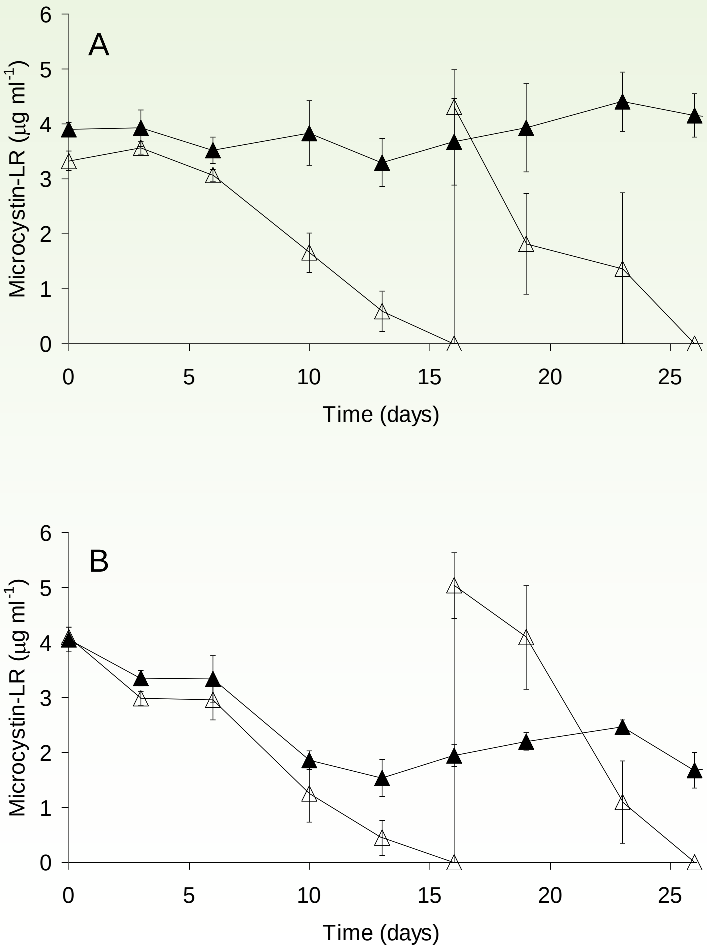
Environmental/biological fates of dissolved cyanotoxins

- Susceptibility to sensitized photo-oxidation.
- Biodegradation by aquatic bacteria.
- Uptake by other aquatic microbes, grazers, fish and plants.
- (Formation of microcystin-metals complexes).
- Sorption onto sediments.

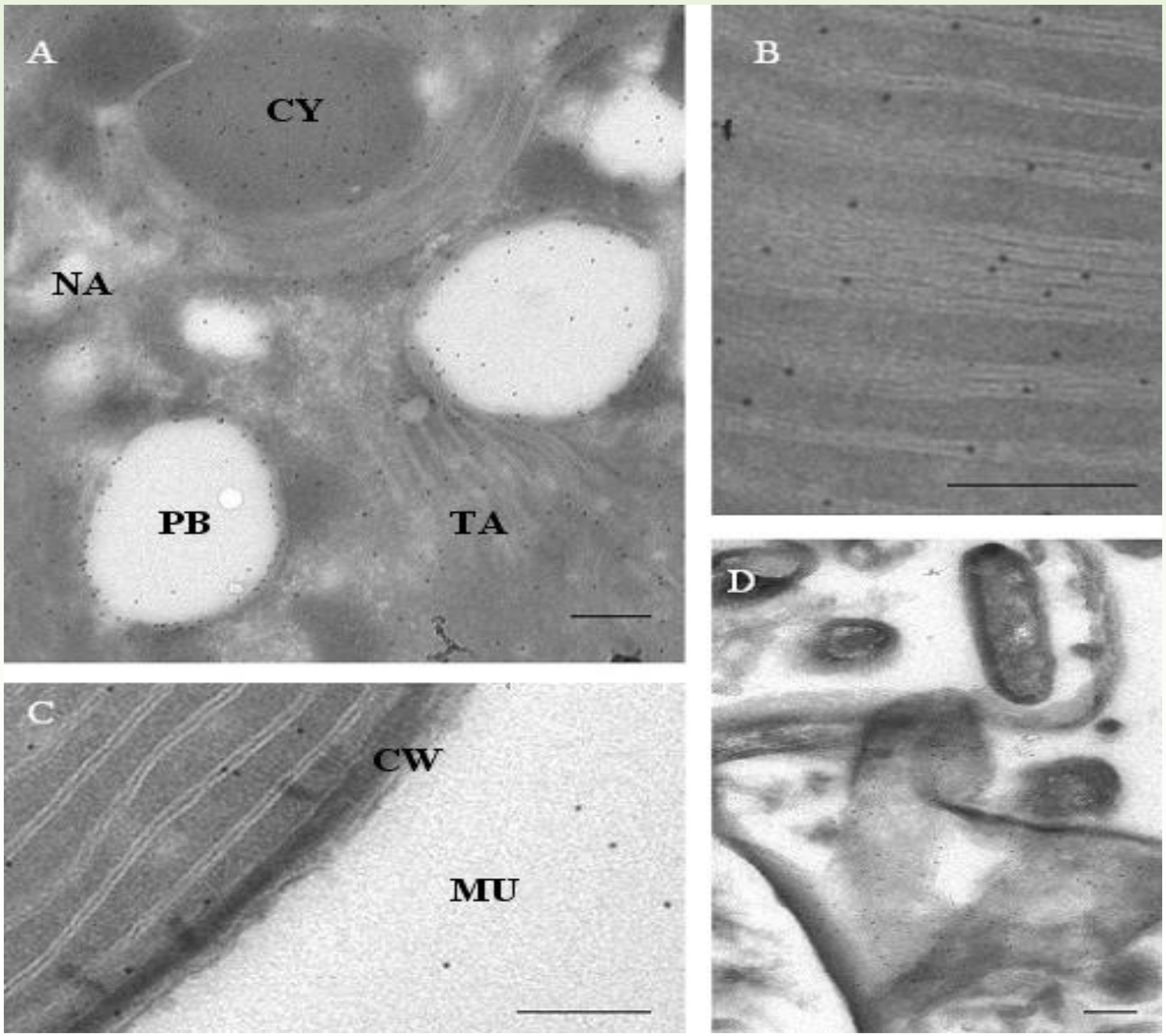
Biodegradation of microcystins by bacteria: commonly reported, but incompletely investigated

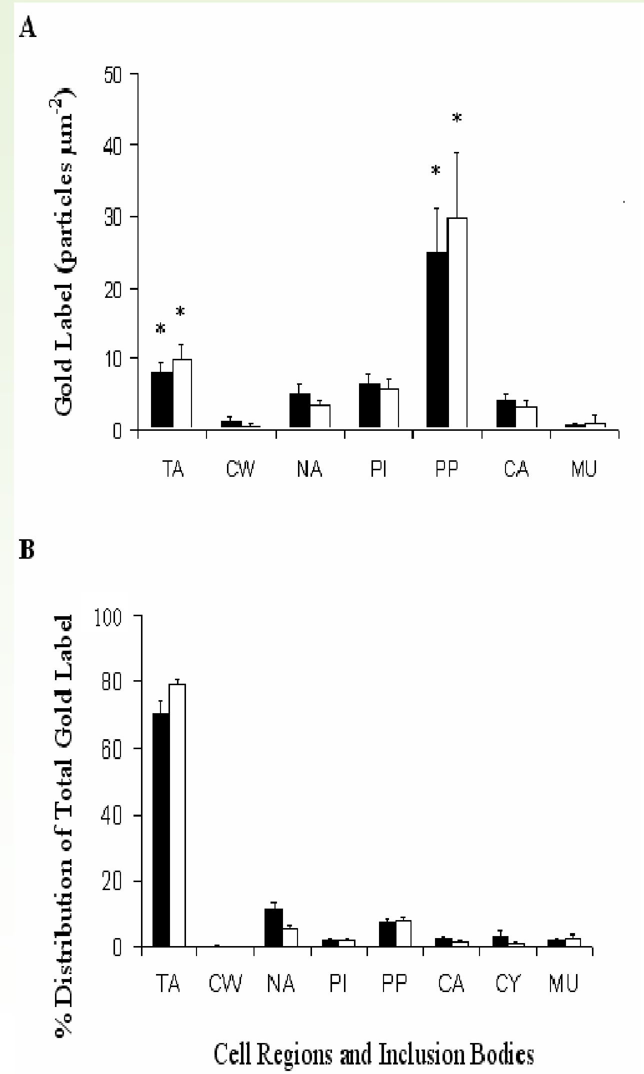
- Reports of biodegradation of MC: Australia, Canada, China, Denmark, Finland, Japan, Poland, UK
- Usually only measured as loss of MC from solution.
- Mineralisation to C02 rarely measured.
- ***KEY Questions:***
- Toxicity of cyanotoxin/cyanotoxin products after "biodegradation"
- Can cyantoxins serve as sources of nitrogen, carbon and energy for bacterial growth ?

Examples of "biodegradation" of dissolved MC-LR in lake water: L. Rescobie, Scotland



Localisation of microcystin in cyanobacterial cells (*Microcystis aerugniosa*)





Localization of microcystins [MC] in cyanobacterial cells, colonies, filaments

Microcystis aeruginosa colonies, if positive for MC:

- all cells in the colony contain the toxins
- no gradients apparent across colonies
- major assocations of MC with **thylakoids** and **poly-P bodies**
- colony mucilage also contains MCs.

Planktothrix agardhii filaments, if positive for MC:

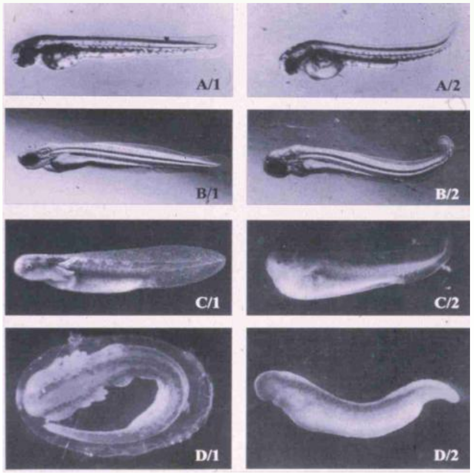
- all cells in filament contain the toxins
- no gradients apparent from tip to centre of filament

Cyanobacterial toxins in other aquatic microbiota

Cyanotoxins can be taken up by a wide range of aquatic microbiota and :

- Stored
- Metabolised
- Transferred via food chains

- Heterotrophic bacteria (cyanotoxin-degraders).
- Zooplankton
- Phytoplankton-grazers
- Fish eggs, embryos
- Juvenile forms of fish, amphibians



Examples of cyanotoxins in zooplankton:

Donkmeer and Westveld Lakes: BBlooms

Brachionus

Keratella

Brachionus, Keratella, Bosmina, Polyarthra: 23 – 118pg microcystins and/or microcystin products per zooplankton animal.

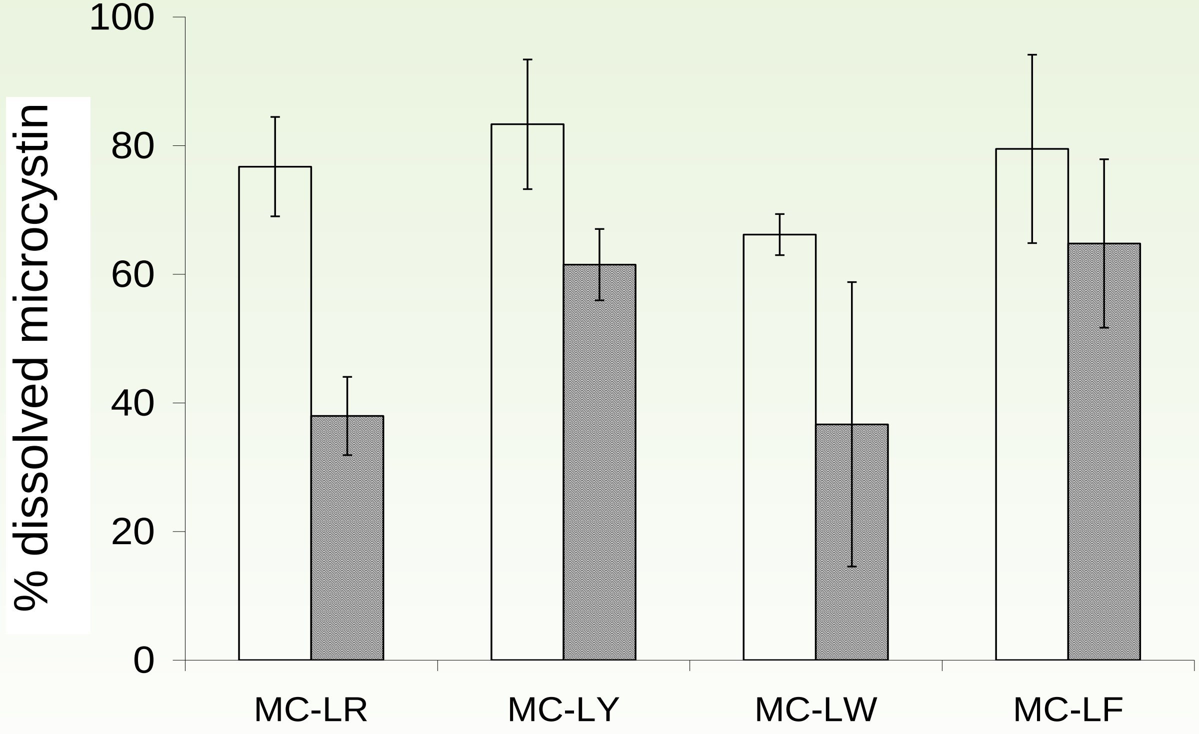
Particulate microcystins and/or microcystin products: zooplankton

- Donkmeer, 19 Aug 2010: *Daphnia* sp.
  : *Leptodora* sp.

- Fort Bornem, 13 Sept 2010: *Daphnia* sp.

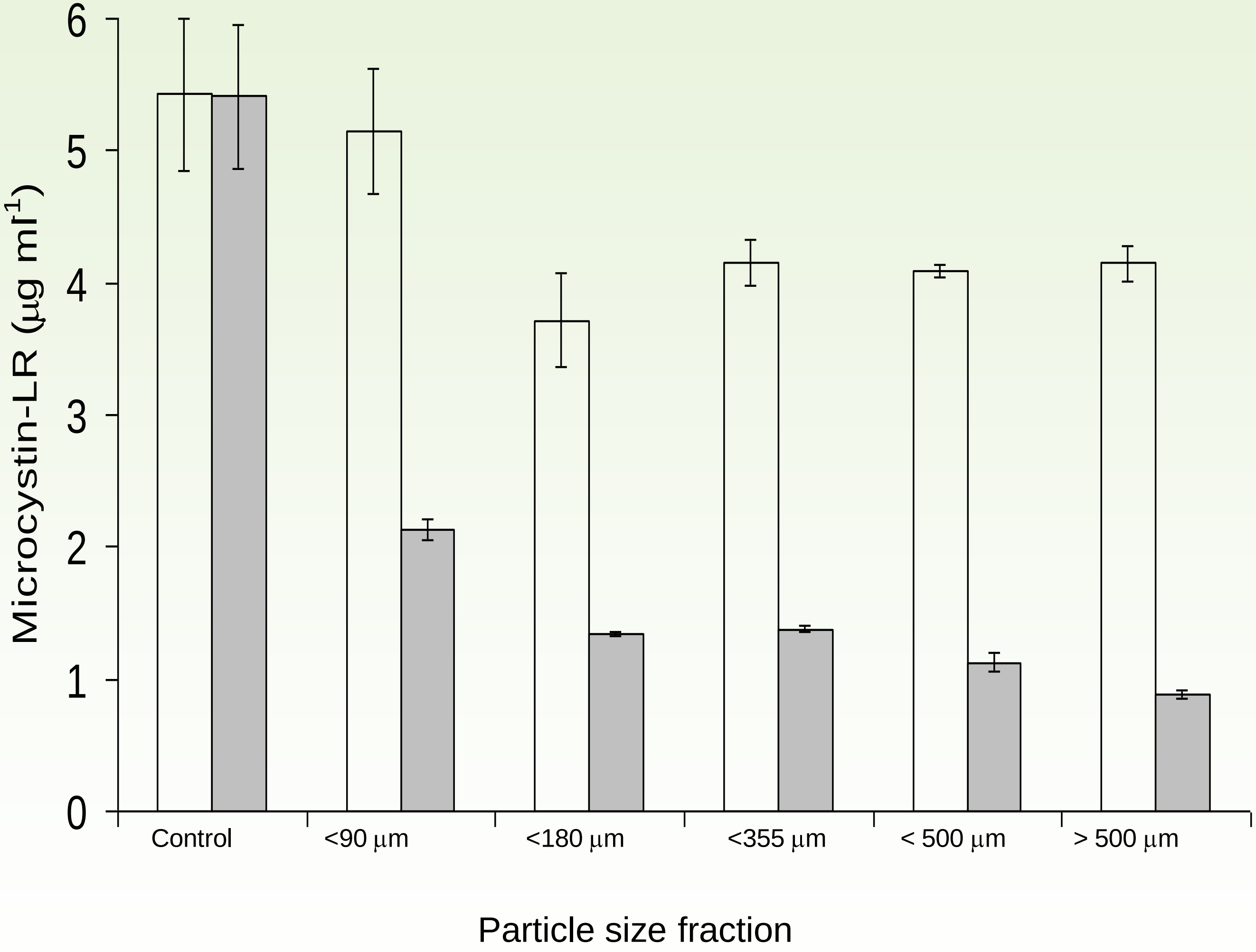
(Samples from Jeroen V. Wichelen)

Sorption of pure microcystins to 50 mg/ml lake sediment



Initial MC conc.: 2.5 µg/ml
Open columns: after one hour
Hatched columns: after 24 hours
Vertical bars: SD (n=3)

Sorption of microcystin-LR to sediment particle size fractions



50 mg/ml sediment. Open columns, I hour contact. Closed columns, 24 hours contact.

Contribution of understanding of cyanotoxin compartmentation in cyanotoxin risk management:

1. Aids further rational policy development for health and environmental protection.
2. Identifies further potential methods for cyanotoxin removal/control in waterbodies.
3. Is useful in selection of appropriate drinking water abstraction and treatment methods.

Loch Rescobie,
Angus, Scotland

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