Characterization of ecotoxicity and phytotoxicity of a cyanobacterial extract containing microcystins under realistic environmental concentrations and in a soil-plant system

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Ecotoxicity and phytotoxicity of a cyanobacterial extract containing microcystins under realistic environmental concentrations

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Basel 2014
SETAC Europe

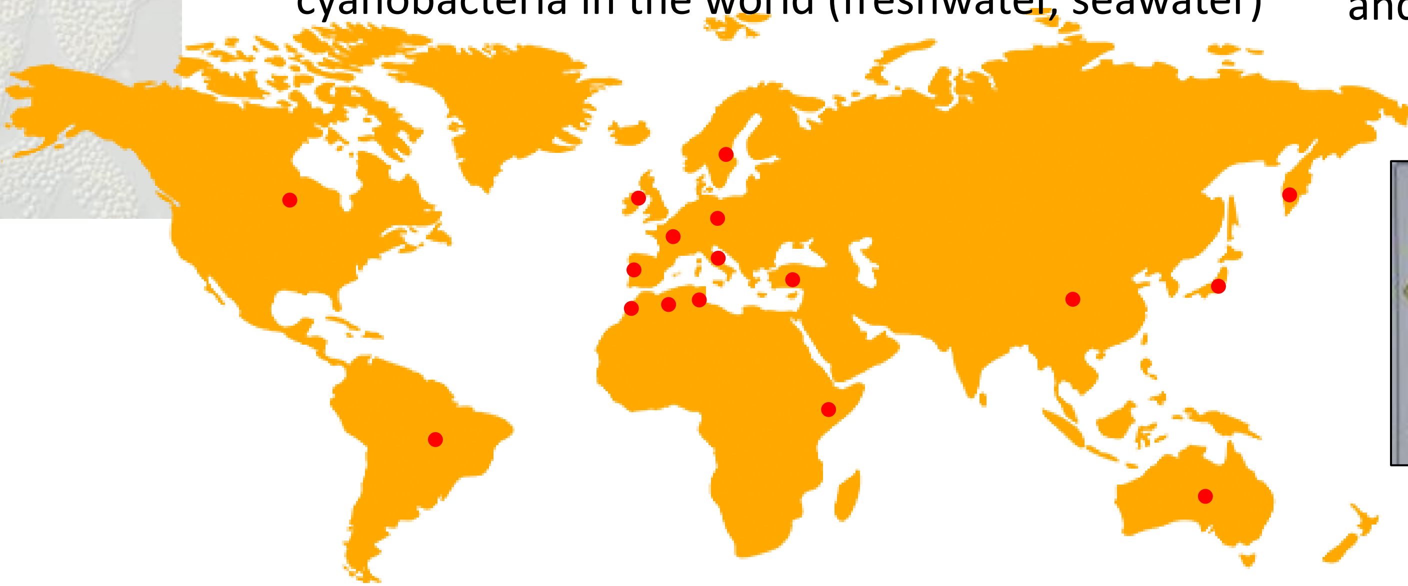
Context

excess of fertilization

global warming

eutrophication

cyanobacteria in the world (freshwater, seawater)



and their cyanotoxins

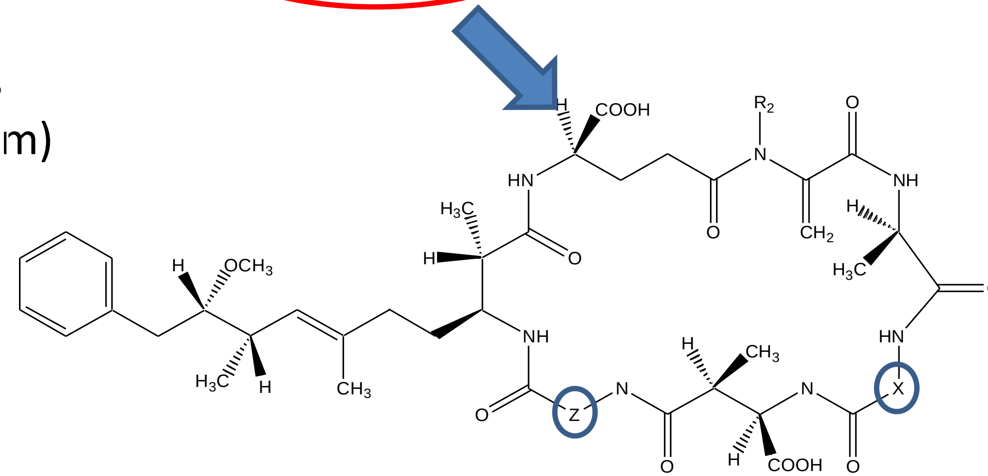
The main preoccupation: their toxins!

cyanotoxins

dermatotoxins (allergy)

neurotoxins (nervous system)

hepatotoxins (liver)



structure of microcystins MCs (with 80 variants)

Sanitary risks

Pouria et al., 1998
Žegura et al., 2011
Mankiewicz-Boczek et al., 2011

1996: Caruaru (Brazil), **60 patients** died in hemodialysis center

1 µg L^{-1} **in drinking water** (WHO)

irrigation water 100 µg L^{-1}

Economical risk and Sanitary risk (contamination of the food chain)

Our objectives

The main objective of our research program: to study **fate of microcystins** (MCs) contained in irrigation water **in soils, and their impact** on edible **tomato** (*S. lycopersicum* var. MicroTom) and **soils communities**

The specific objectives of the present presentation are to show our first results concerning the:

-characterization of the cyanobacterial extract

-phytotoxicity on tomato plants *Solanum lycopersicum* var. MicroTom and ecotoxicity of cyanobacterial extract, brought by irrigation, on soil microorganisms

Cyanobacterial extract

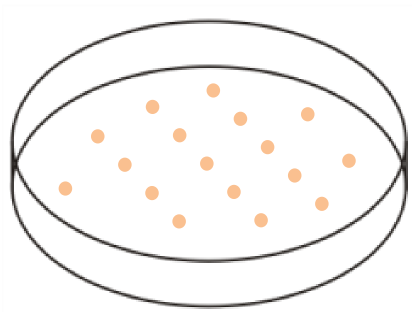
- From a **culture** of *Microcystis aeruginosa* (PCC 7820) from Pasteur Institute (Paris, France)

Conditions: **3 weeks** in BG11 + $NaNO_3$ (2 mM) + $NaHCO_3$ (10 mM)
25 °C, light: 16 h between 5-10 µmol of photons m^{-2} s^{-1}
continuous sparged 0.23 L min^{-1}

- **Harvesting of cyanobacteria**: centrifugation (25,000 *g*, 5 min) and freeze-dried
- **Toxin extraction** 3 times with aqueous MeOH (75%, v/v)
- **Quantification** of cyanotoxin in cyanobacterial extract with Protein Phosphatase 2A assay (Bouaïcha et al., 2001)
- **Identification** of cyanotoxins by UPLC-MS/MS

Phytotoxic assays *in vitro* [1]

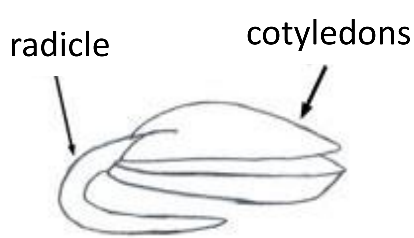
4 Seed samples: *Solanum lycopersicum* var. MicroTom and Saint-Pierre
Lactuca sativa var. *capitata*
Triticum aestivum Bio (Attlass)



20 seeds/Pétri dish covered with filter paper
3 Petri dishes/treatments

Conditions: 7 days in the dark (25 °C) with 5 mL of **cyanobacterial extract** or **cadmium chloride**, CdCl (positive control)

Treatments: **0-20 mg eq. MC-LR L^{-1}** and **0-1 g CdCl L^{-1}**



- **germination rate**
- **lengths of radicles (ImageJ, 2012)**

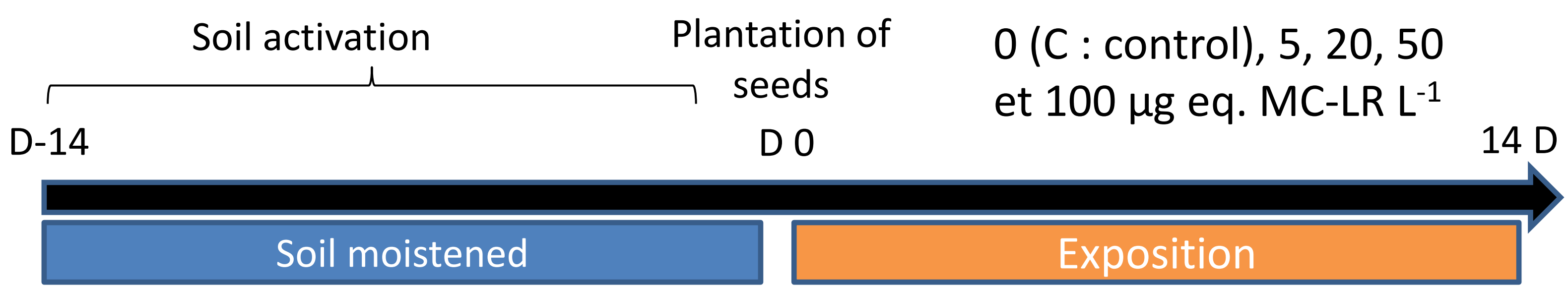
[1] from the norm AFNOR X31-201 (AFNOR, 1986)

Assays of inhibition growth of *S. lycopersicum* var. MicroTom seedlings[1]

Soil used: silty-sandy soil of experimental site Pierre-Plate (Versailles), 350 g/replicate

Seed used: *Solanum lycopersicum* var. MicroTom, 10 seeds/replicate

Conditions : 16 h of light at 160 µmol of photons $m^{-2}\ s^{-1}$, 26/21 °C (D/N) and a relative humidity <70%



- **dry biomasses of seedlings**
- **global soil enzymatic activities[2] and potential nitrification[3] of soils**
- **quantification of soil microorganisms[4]**

Clay (%)	11
Silt (%)	13
Sand (%)	**76**
Organic carbon (‰)	21.9
Total nitrogen (‰)	1.2
C/N ratio	17.5
Organic mater (‰)	37.8
pH	5,6
WHC (%)	35,5

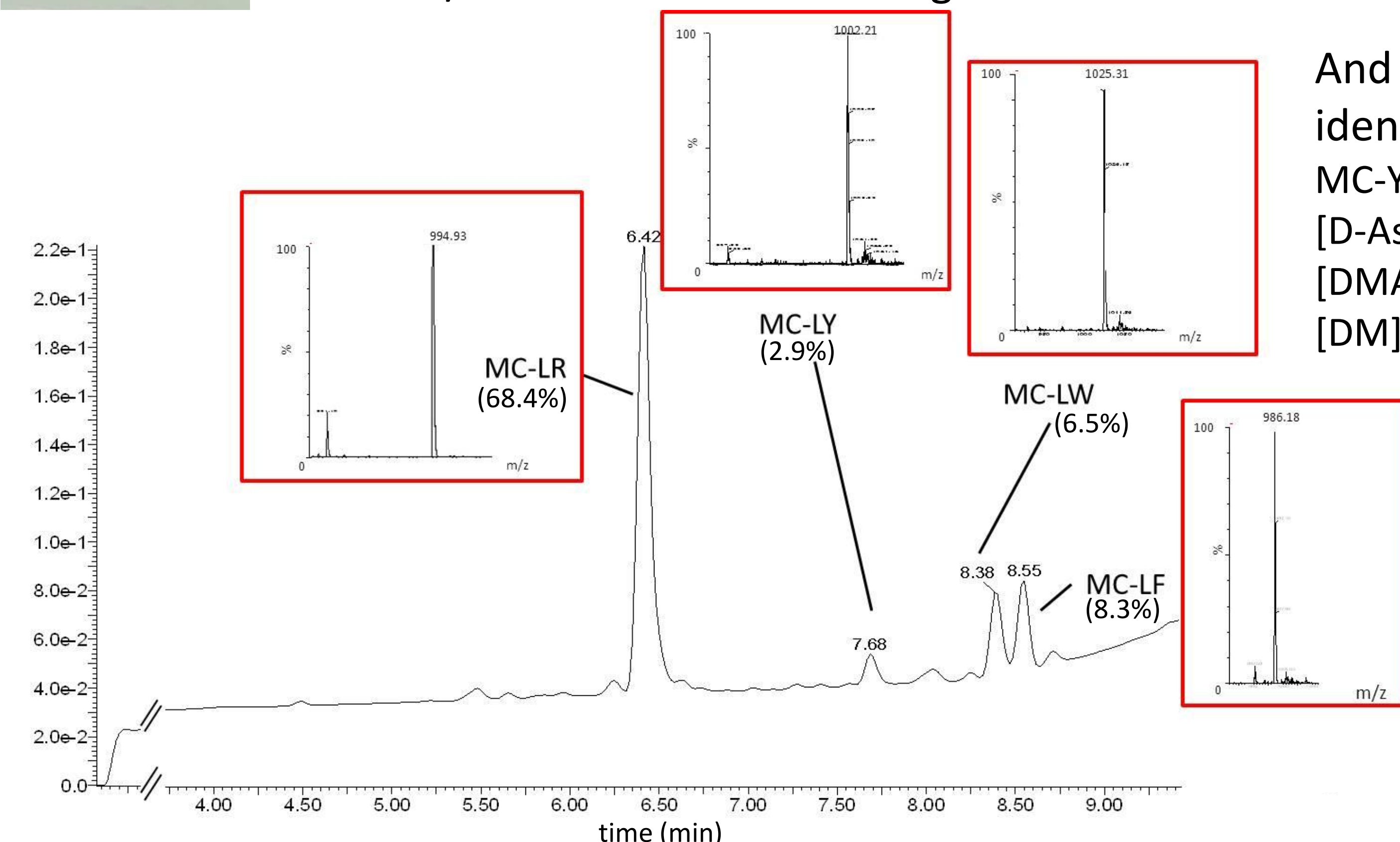
soil characteristics

[1] norm AFNOR X31-206 (AFNOR, 2011)
[2] Santiago-Martin et al., 2013
[3] ISO NF ISO 15685. (ISO 2012)
[4] Bru et al., 2008 ; Wessén et al., 2011 ; Ochenreiter et al., 2003

Characterization of cyanobacterial extract from *M. aeruginosa* (PCC7820) culture

Toxicity of this extract with PP 2A assays was: **21,6 mg eq. MC-LR L^{-1}** corresponding to **6,77 mg eq. MC-LR g^{-1} dried cells**.

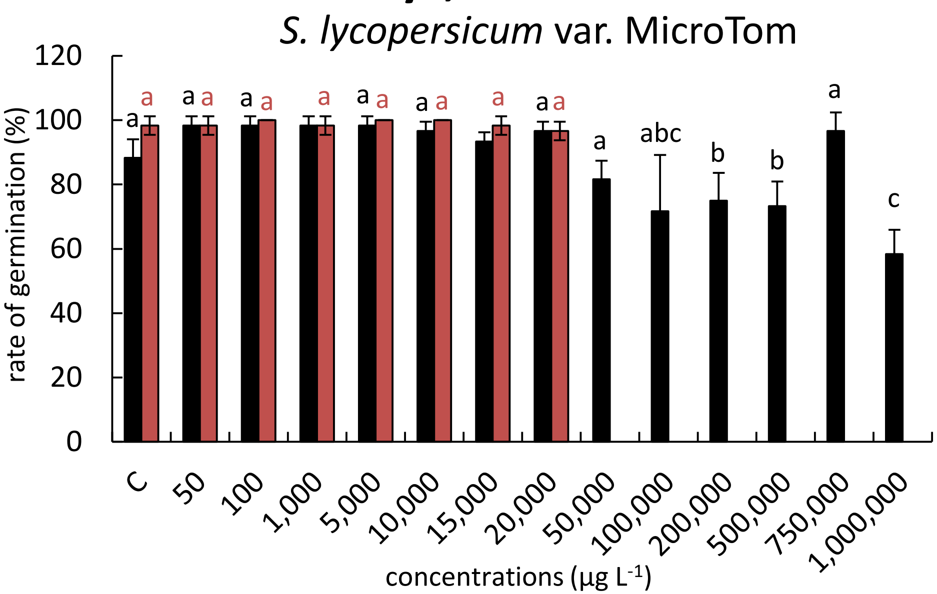
UPLC-MS/MS revealed 4 main congeners of MCs

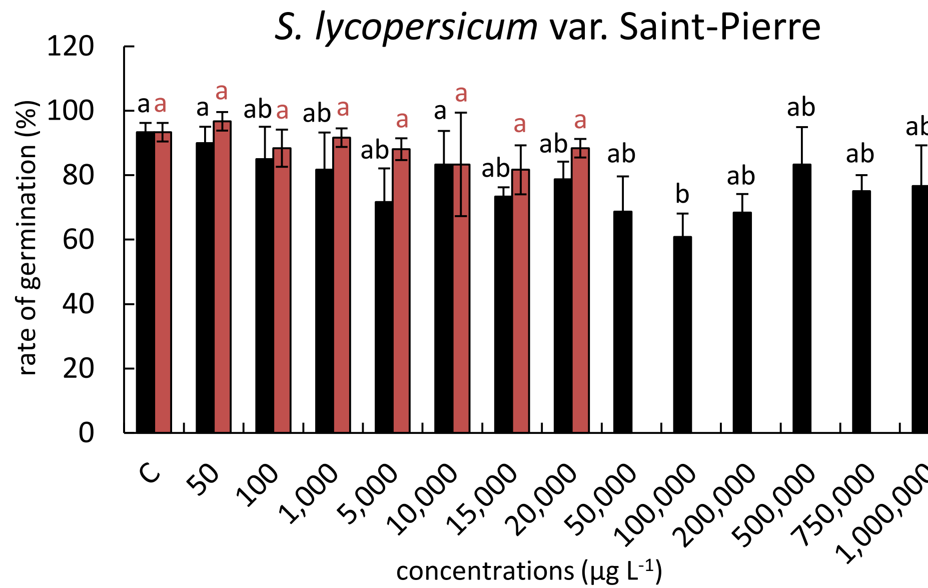


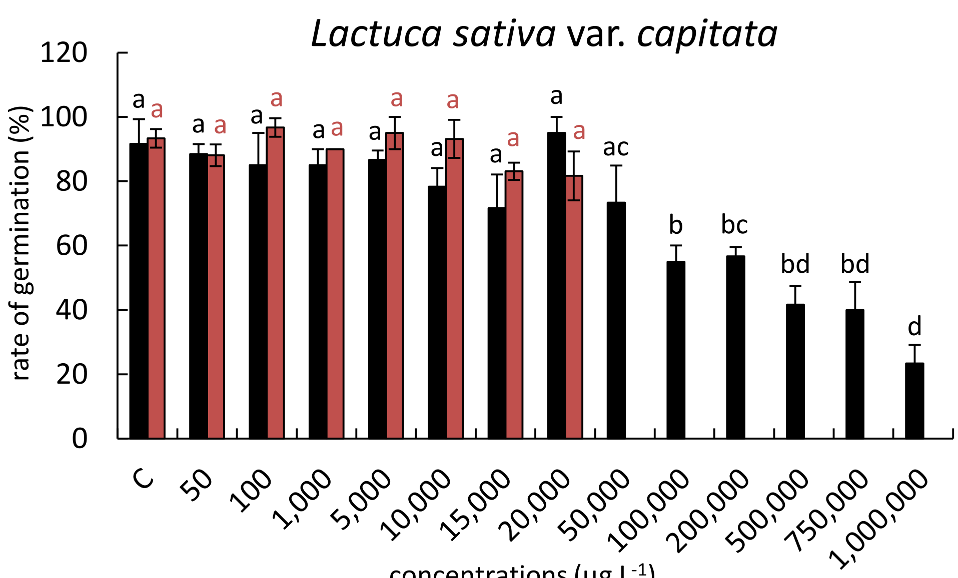
And 9 other congeners identified:

MC-YR, MC-LM, [DMAdda[5]]MC-LR, [D-Asp[3]]MC-LR, [L-MeSer[7]]MC-LR, [DMAdda[5]]MC-LF, [DM]MC-LF, [DM]MC-LW, [DMAdda[5]]MC-LW

Germination assays, *in vitro*





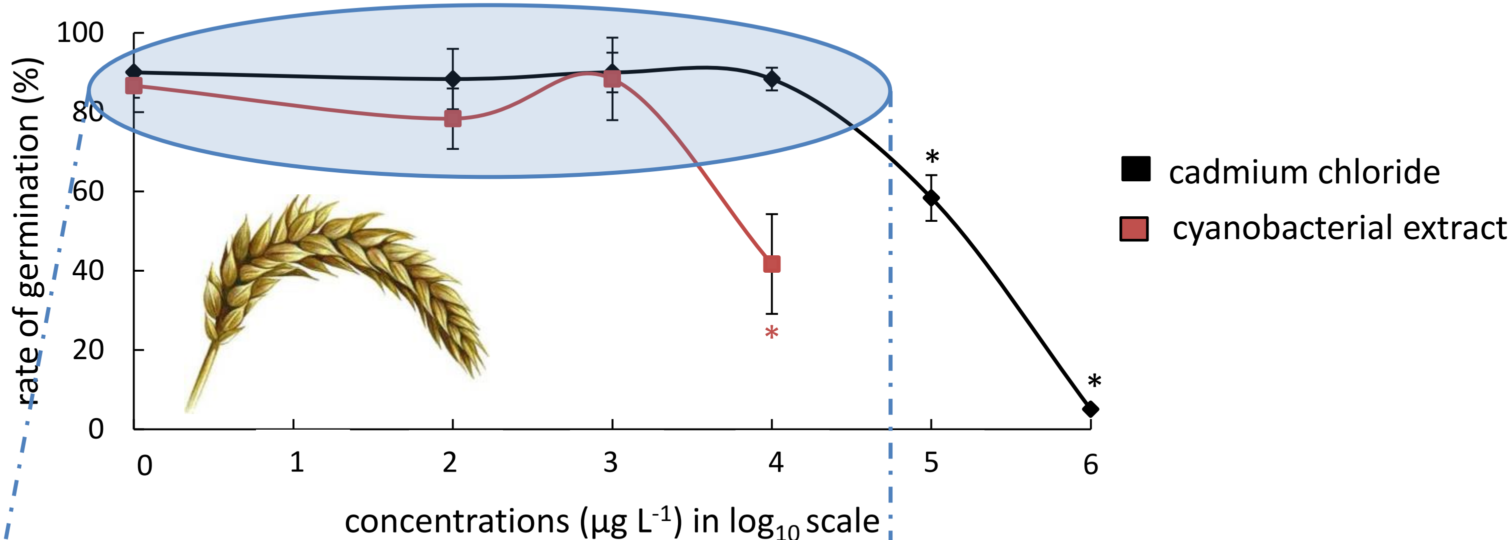


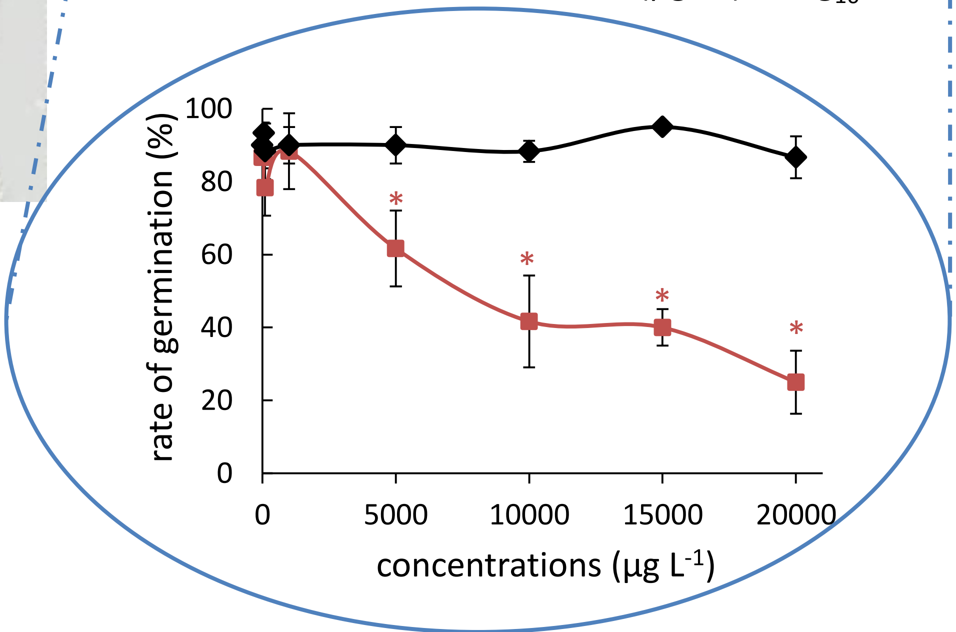
■ cadmium chloride

■ cyanobacterial extract

No significant effect of MCs on germination process on these plants

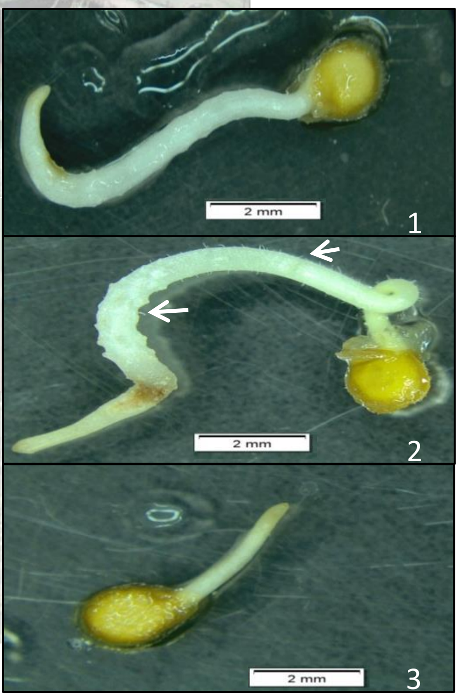
Germination assays on wheat, *in vitro*

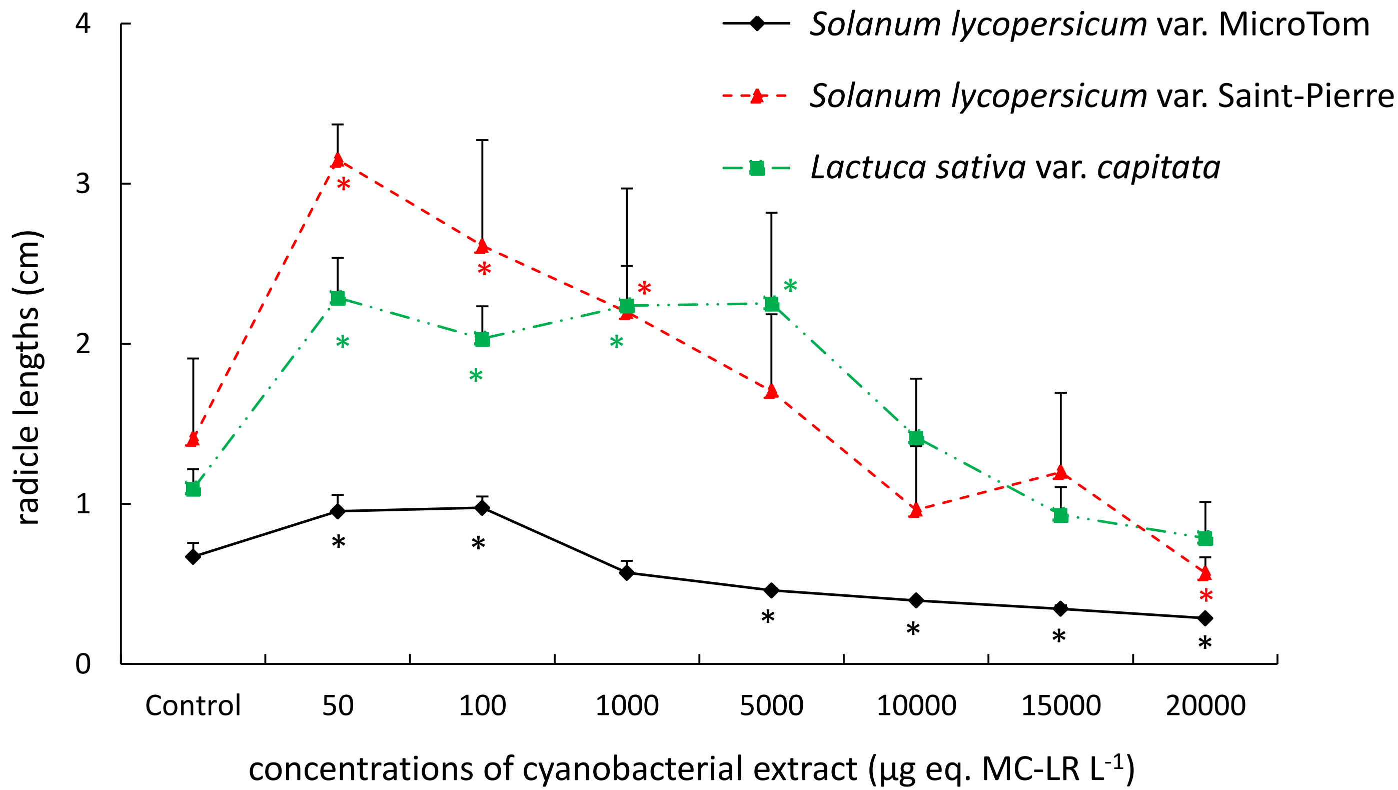




Significant decrease of the germination rate of wheat after 7 days of cyanobacterial exposure (EC_{50}=11 mg eq. MC-LR L^{-1})
BUT the EC_{50} of CdCl=145 mg L^{-1}

Radicles lengths after exposure *in vitro*





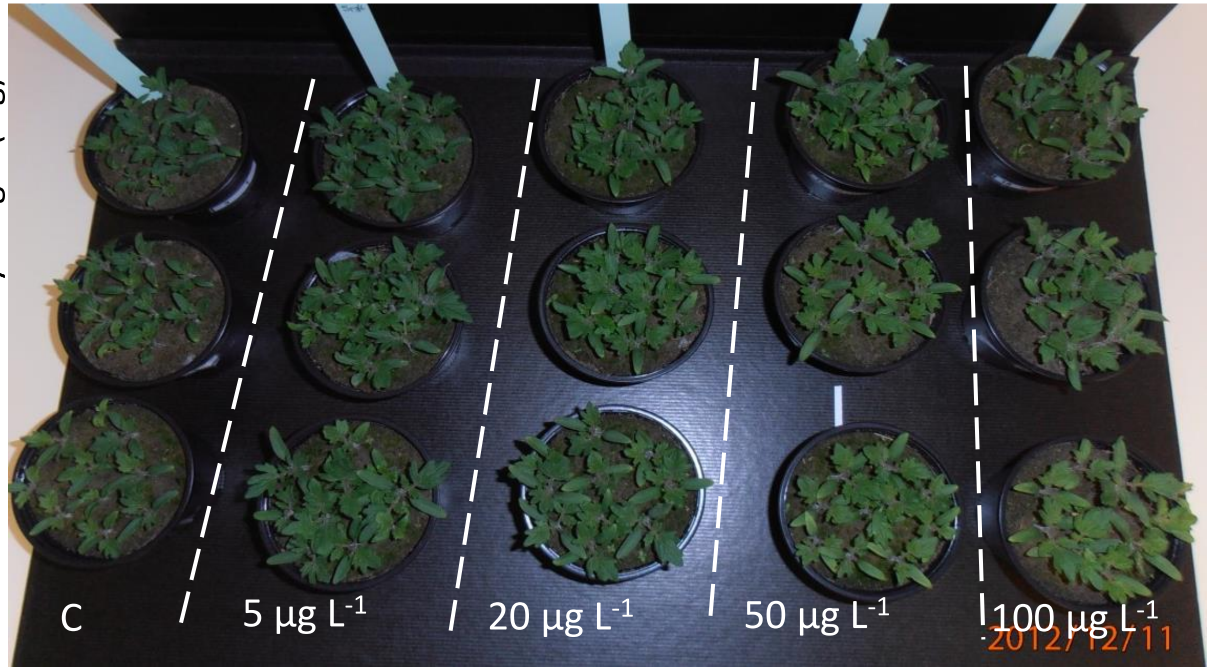
Two different effects according cyanobacterial concentrations:

- **at low concentrations (50-100 µg eq. MC-LR L^{-1}), increase of the radicle length**
- **at high concentrations (>1 mg eq. MC-LR L^{-1}), decrease of the radicle length**

Similar effects but different sensitivities, according plant species

Phytotoxicity of cyanobacterial extract on tomato *S. lycopersicum* var. MicroTom after 14 days of irrigation

biomasses: dry weight (mg)

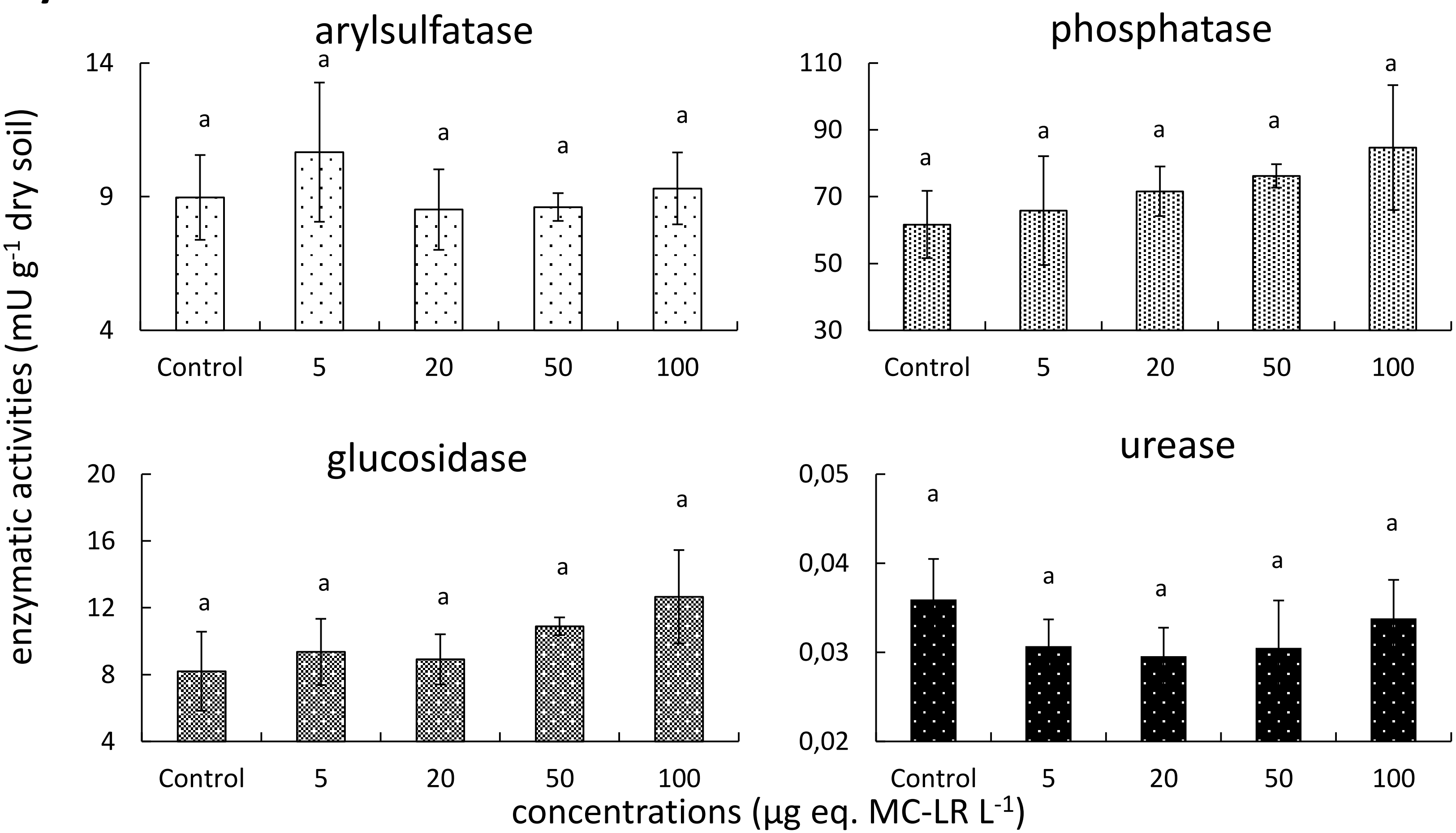


aerial parts
roots

After 14 days of irrigation of soil-plant system:

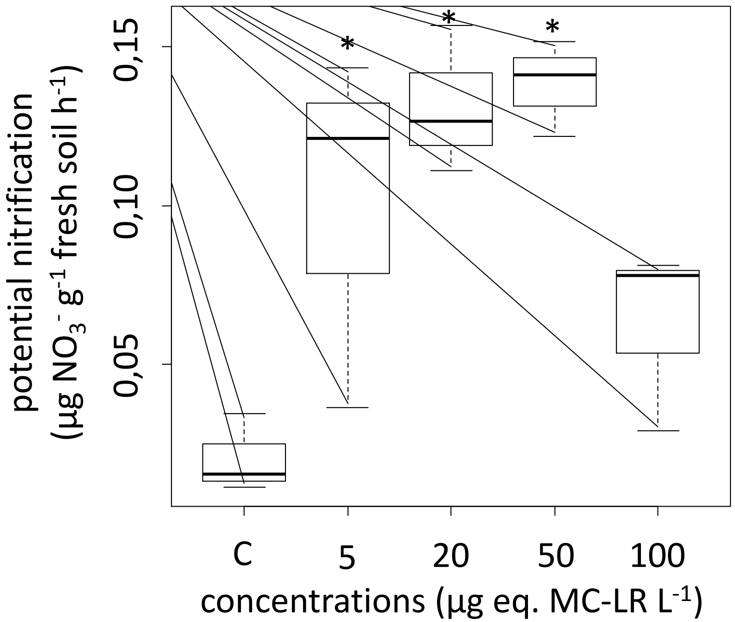
- **similar biomasses of roots between treatments**
- **increase of aerial parts biomasses for seedling exposed to cyanobacterial extract**

Soil ecotoxicological parameters followed after 14 days of irrigation with cyanobacterial extract



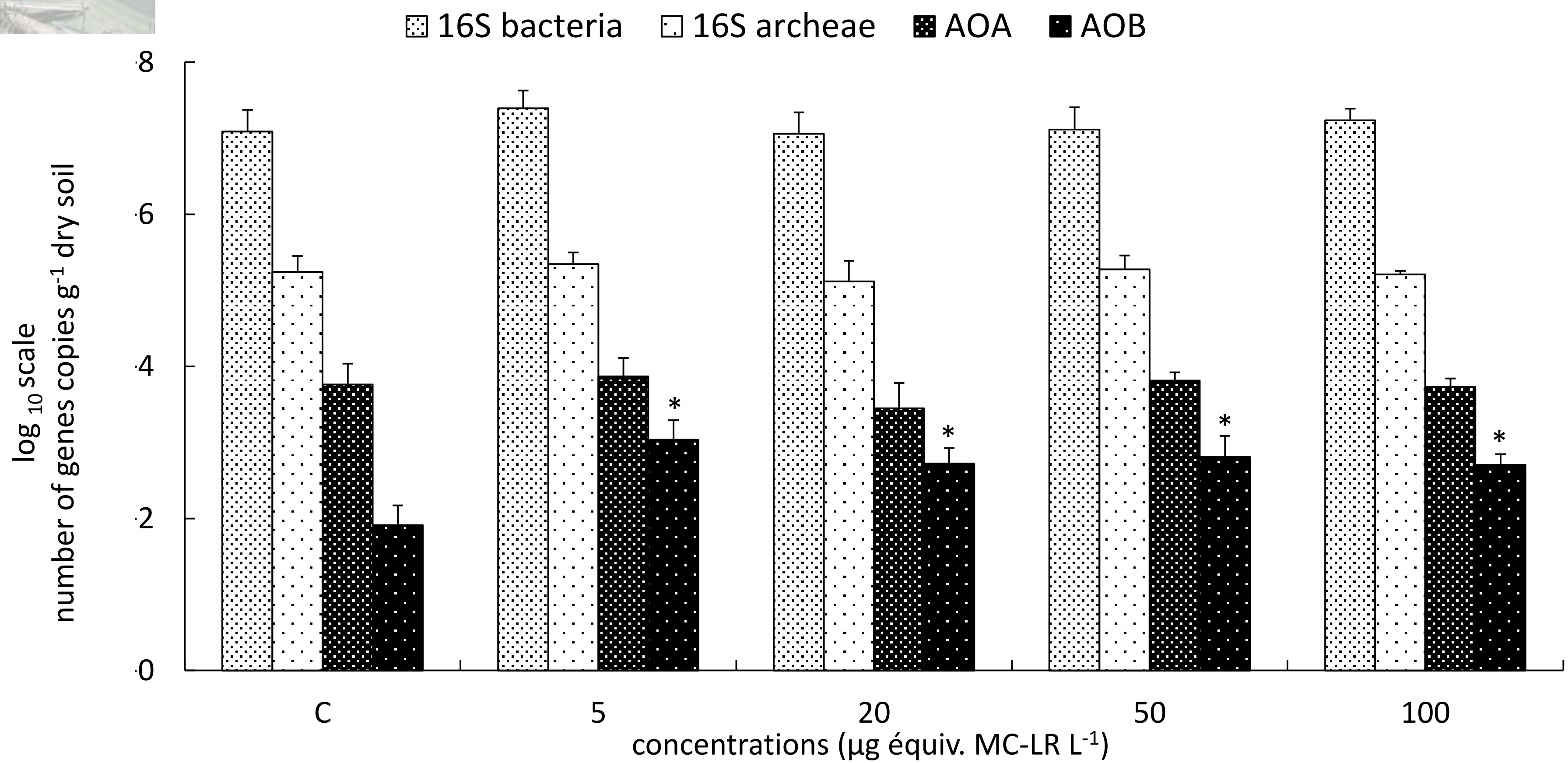
After 14 days of soil-plant system irrigation, no modification of global enzymatic activities of soil.

Soil ecotoxicological parameters followed after 14 days of irrigation with cyanobacterial extract



- After 14 days of soil irrigation, potential nitrification was significantly impacted with an increase of this activity in soils exposed to aqueous cyanobacterial extract.
- An increase was observed for soils exposed to cyanobacterial extract comprised between 5 and 50 µg eq. MC-LR L^{-1} but not for the upper concentration (100 µg eq. MC-LR L^{-1}).

Abundances of soil microbial communities after 14 days of irrigation with cyanobacterial extract



After 14 days of irrigation with cyanobacterial extract , abundances of nitrifying bacteria (AOB) increased for all treatments. This result could explain results obtained on potential nitrification with positive correlation ($r=0,56$; $p<0,05$).

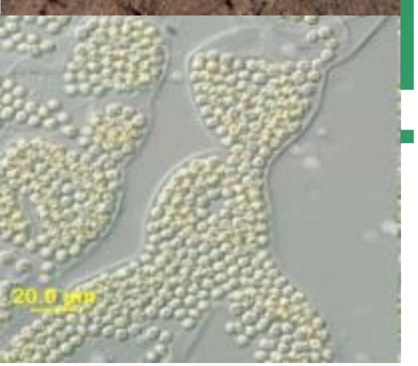
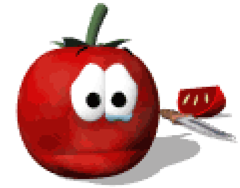
- The MCs could affect the metabolism of seeds during the germination process, but the concentrations must be very important and sensitivities differed between plant species.
- The germination of wheat seeds, a monocotyledon, was strongly impacted by MCs with an EC50 13 times lower than cadmium chloride (positive control).
- « Environmental concentrations » (50 et 100 µq eq. MC-LR L^{-1}) seemed increase radicle growth but high concentrations decreased their growth. The growth of aerial parts of seedlings were also stimulated.
- « Environmental concentrations » did not change global soil enzymatic activities but the abundances of nitrifying bacteria increased as their nitrifying activity.

MCs, at "environmental concentrations", could stimulate seedlings growth of some species but what are the effects after a long chronic exposition ?

The MCs brought by irrigation modified soil microbial activities, can you observe this response on other soil type ? And during a long exposition ?





Thank you for your attention!!

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