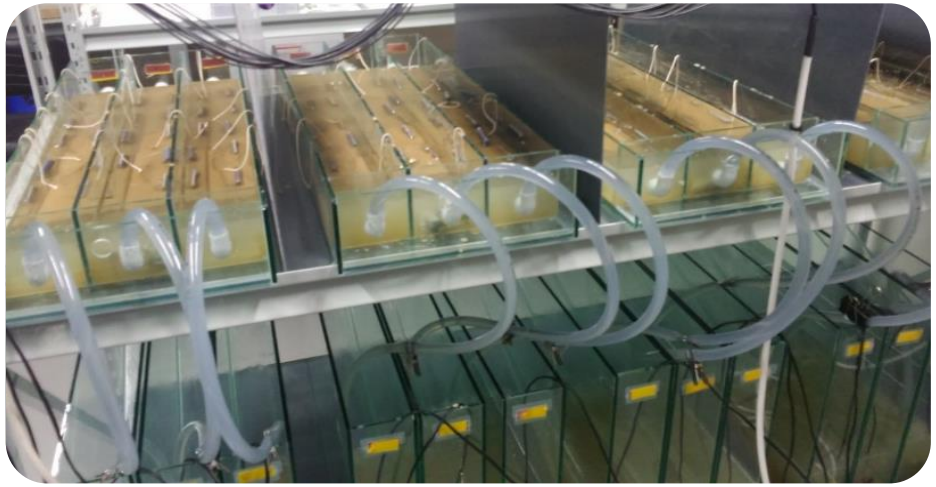
Combined effects of environmental concentrations of copper and arsenic on natural river sediment microbial communities

Ayanleh Mahamoud Ahmed

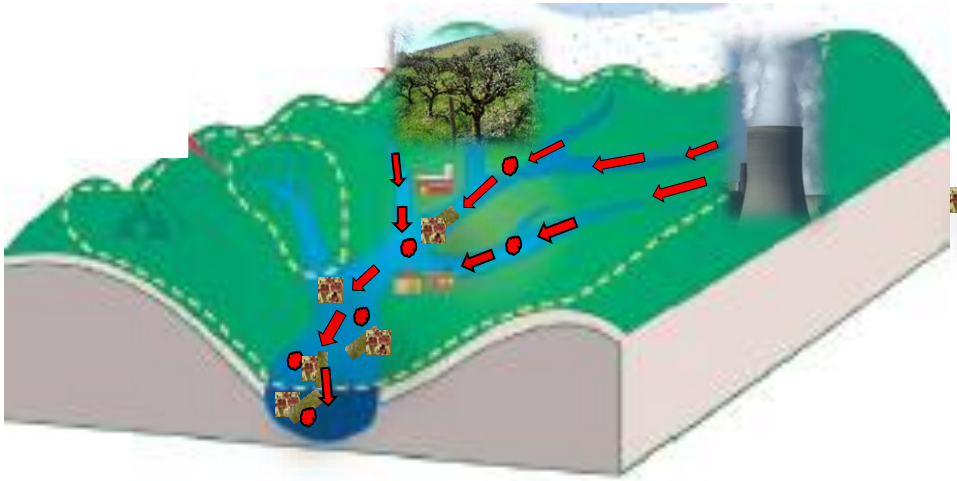


Supervisors:
Emilie Lyautey & Stéphane Pesce

Sediment

➔ **Essential component of aquatic ecosystems**

➔ **Natural reservoir of contaminants**



contaminant deposition

sediment communities,

contaminant flow

(From Water Agency)

Sediment

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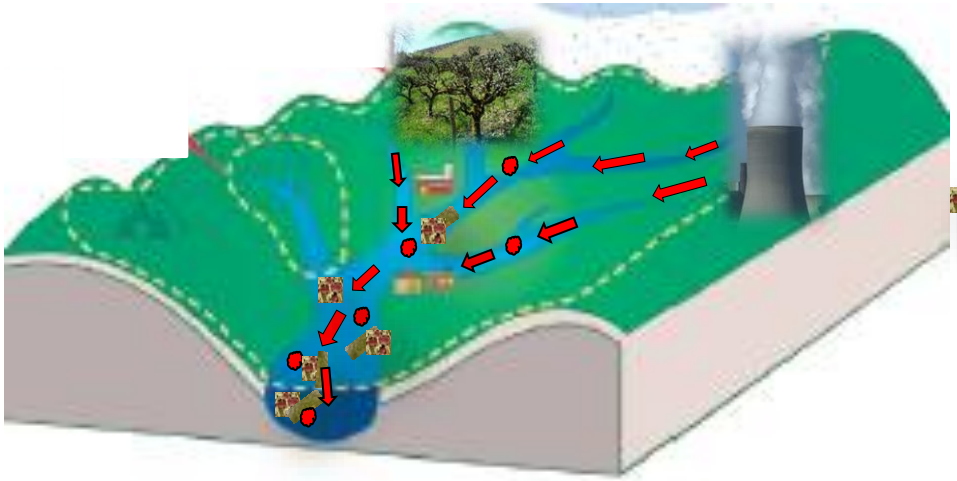

➔ **Little is known about the impact of contaminants on communities**

Context

Sediment

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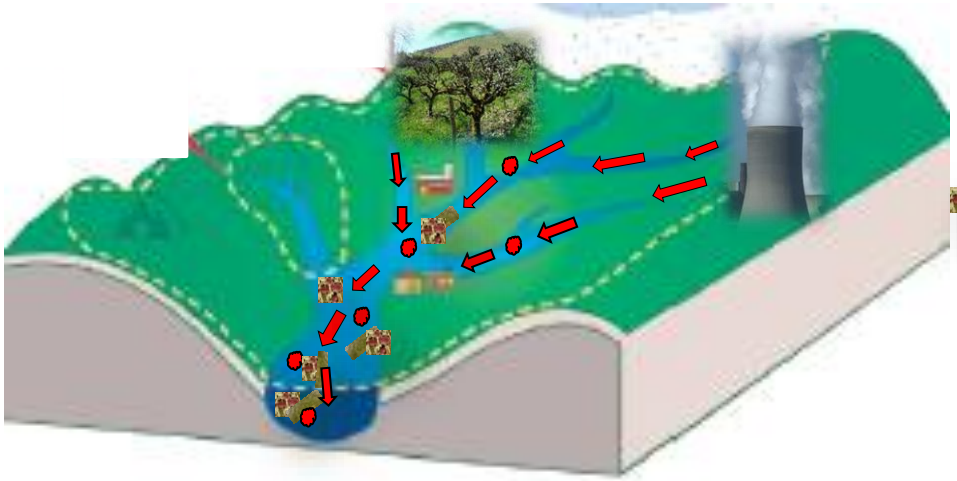
➔ Natural reservoir of contaminants



➔ Little is known about the impact of contaminants on communities

Microbial communities

➔ Metabolic diversity

➔ Major players of all biogeochemical cycles

Trace metals

Arsenic (As) and Copper (Cu)

➔ **Ubiquitous, persistent**

➔ **Present in the environment from natural and anthropogenic sources:**

- ✓ Geochimical, mining, agricultural application, industry

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➔ **Toxicicity**

	Threshold Effect Concentrations (TEC, mg Kg^{-1} DW)	Probable Effect Concentrations (PEC, mg Kg^{-1} DW)
Arsenic	9.8	33
Copper	31.6	149
Lead	35.8	128
Zinc	121	459
Cadmium	1	5

according to MacDonald *et al.* (2000)

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Objective

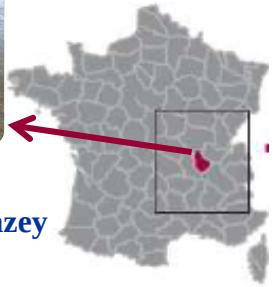
Assessing the effects of chronic exposure to copper and arsenic (alone and mixture) on river sediment microbial communities

Material and Methods

Experimental design

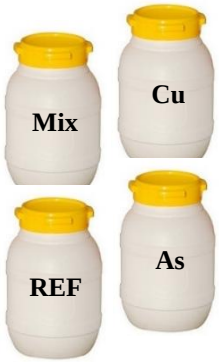
Surface sediment: uncontaminated sediments from the Ain River

Natural sediment



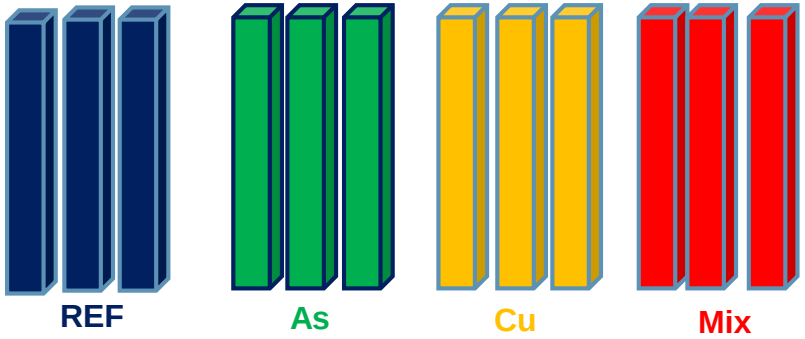
Ain River
Pont of Chazey

Spiking



6 hrs agitation
overnight decantation

Microcosms



- **12 artificial streams. 4 treatments:**
  - **REF**: without metal
  - **Cu**: 40 mg Cu/kg
  - **As**: 40 mg As/kg
  - **Mix**: 40 mg Cu/kg + 40 mg As/kg
- **Water recirculation: 6 L Renewal per week**
- **21 days, 4 sampling times (days 0, 7, 14, 21)**

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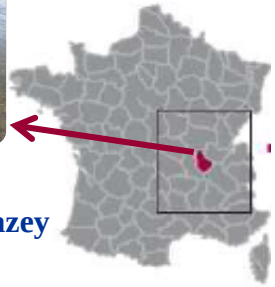

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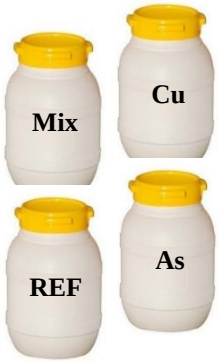

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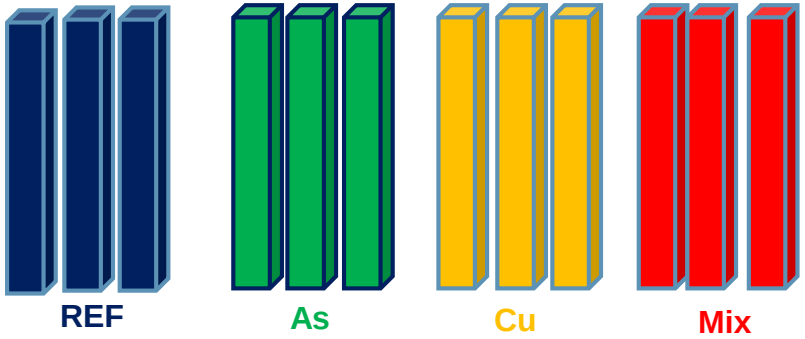

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- **Studied parameters**

Sediment characterization

Real Cu and As concentrations
In water and sediment
Sediment descriptors
DW, AFDW, particle size

Microbial communities

Functions (C, N, P cycles)
Enzymatic and metabolic activities, genetic potential

Structure
bacterial abundance (qPCR) and community composition (ARISA)

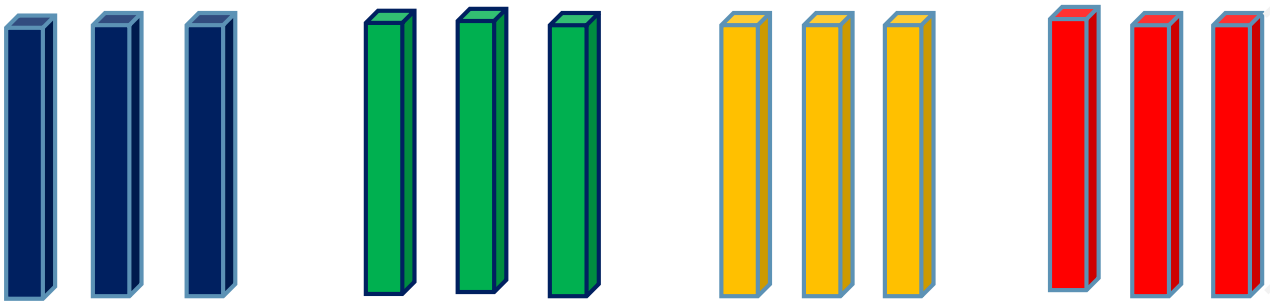
Tolerance acquisition
PICT (Pollution Induced Community Tolerance) approach

Results

Spiking efficiency

Cu and As concentrations in sediments (mg Kg $^{-1}$)

Day 0



	REF	As	Cu	Mix
Cu:	1.8	1.7	56.5	55.1
As:	2.8	31.3	3.24	31.2

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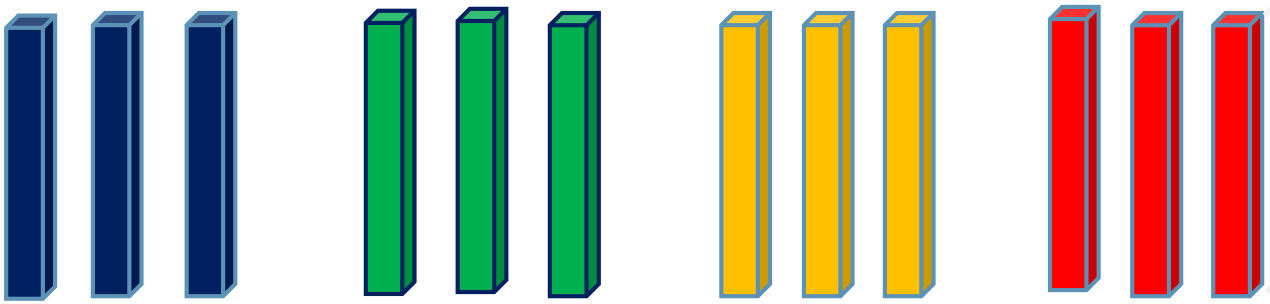

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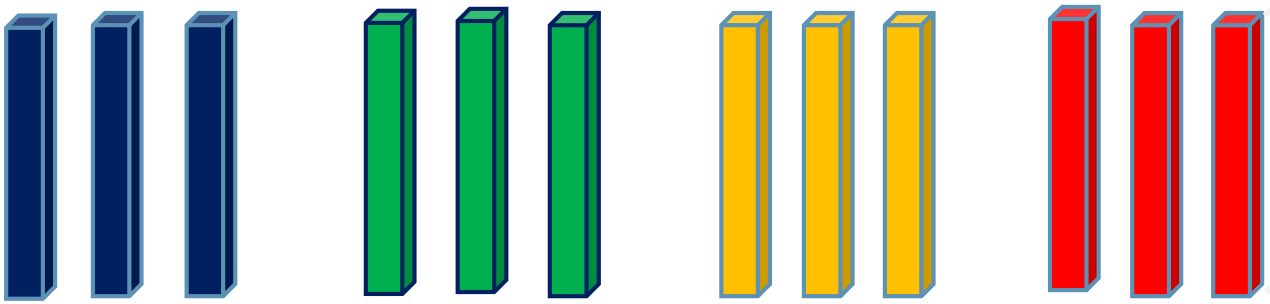

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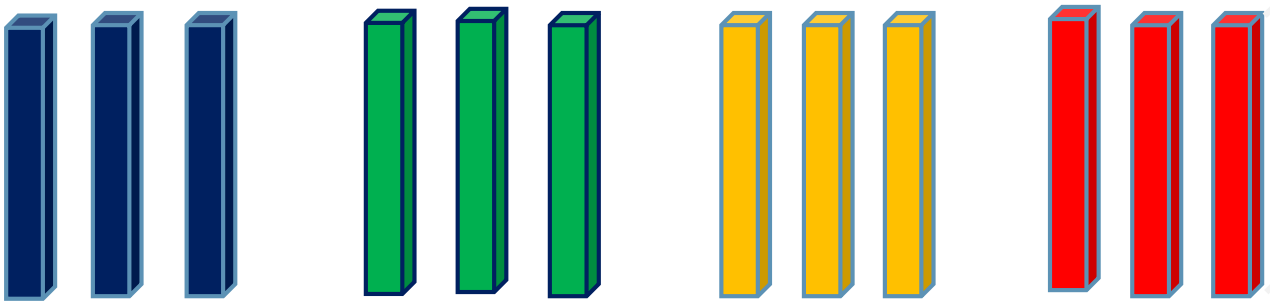
➔ **Low-contaminated reference sediment**

➔ **Initial concentration close to the targeted nominal concentrations**

Spiking efficiency

Cu and As concentrations in sediments (mg Kg $^{-1}$)

Day 21



	REF	As	Cu	Mix
Cu:	1.3	2.6	43.6	47.8
As:	3.1	26.2	2.9	24.6

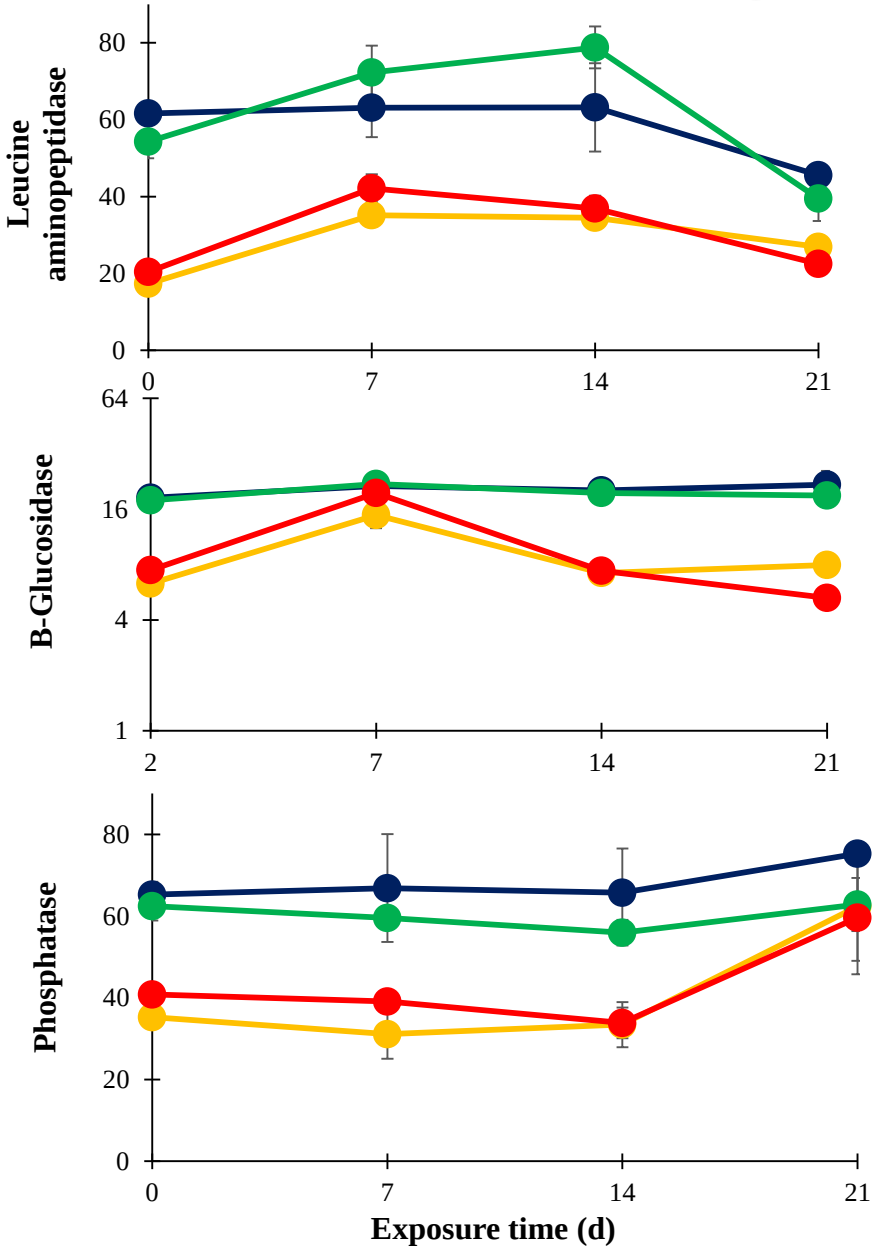
➔ Low-contaminated reference sediment

➔ Initial concentration close to the targeted nominal concentrations

➔ Rather stable concentrations between days 0 and 21

Results

Effect on Functions: enzymatic activities (nmol/h/g DM)



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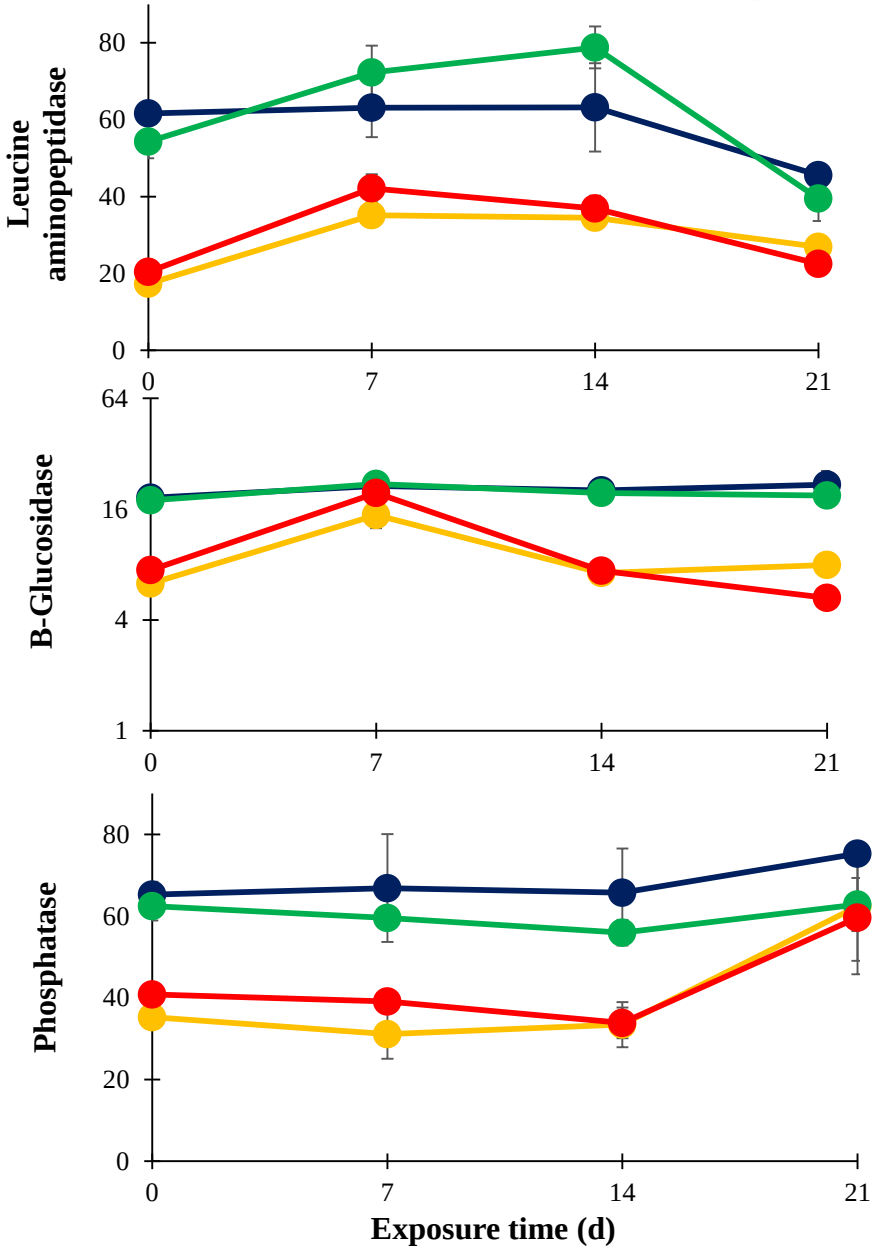

➔ **No effect of As on enzymatic activities**

➔ **Fast and significant inhibition of activities under Cu and Mix exposure**

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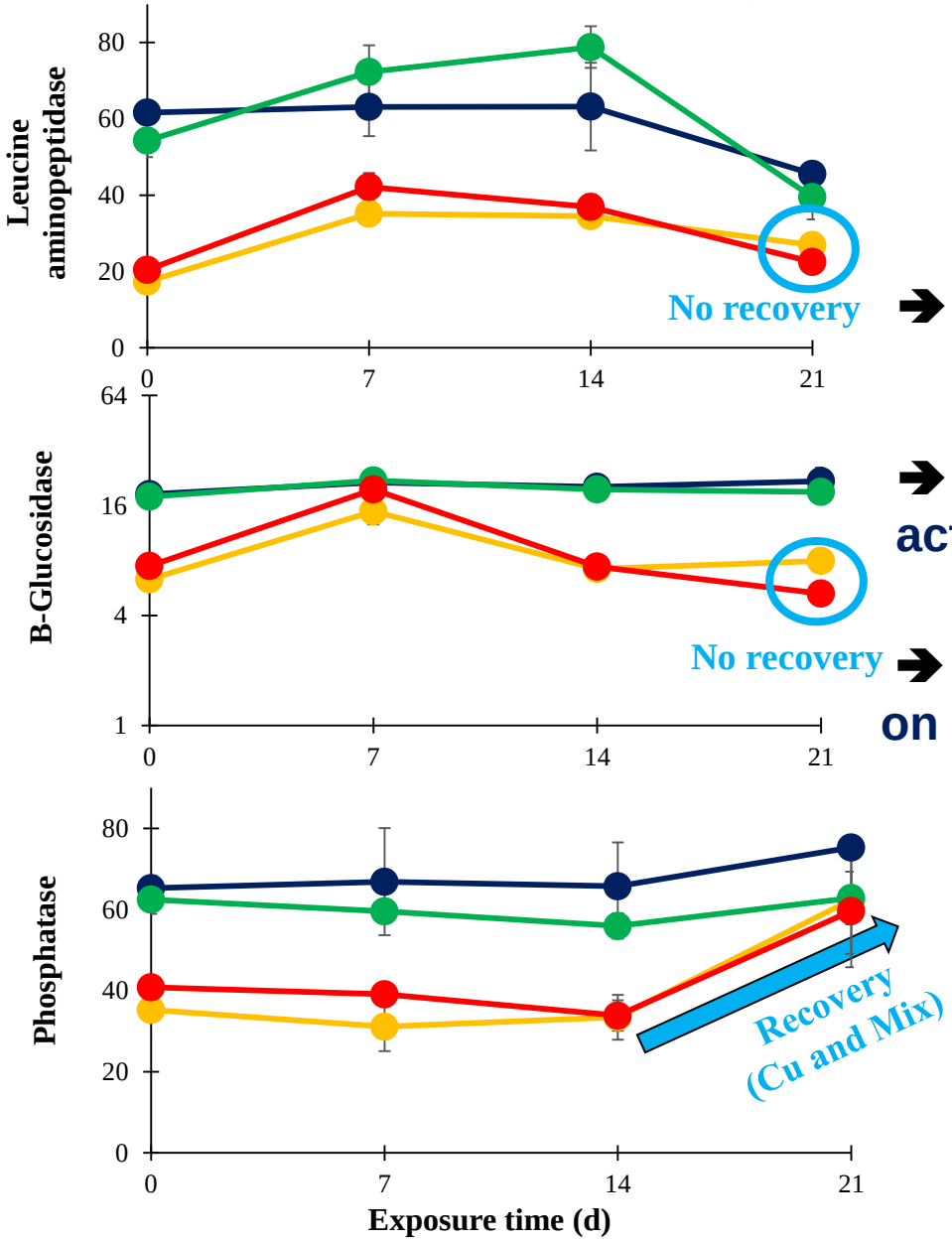


➔ No effect of As on enzymatic activities

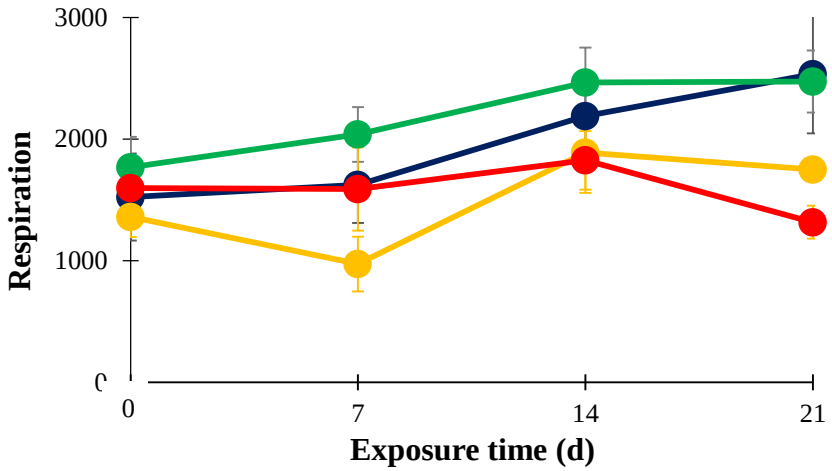
➔ Fast and significant inhibition of activities under Cu and Mix exposure

➔ Recovery or no recovery depending on tested activity

- ✓ Leucine aminopeptidase and β-glucosidase: no recovery
- ✓ Phosphatase: recovery at 21 days

Effects on Functions: Respiration (ng CO_2/h/g DM)

REF
As
Cu
Mix



➔ No effect of As on respiration

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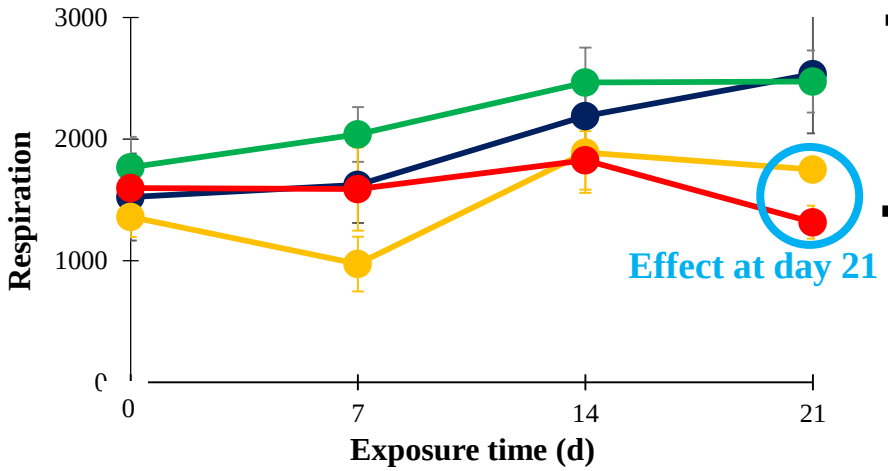


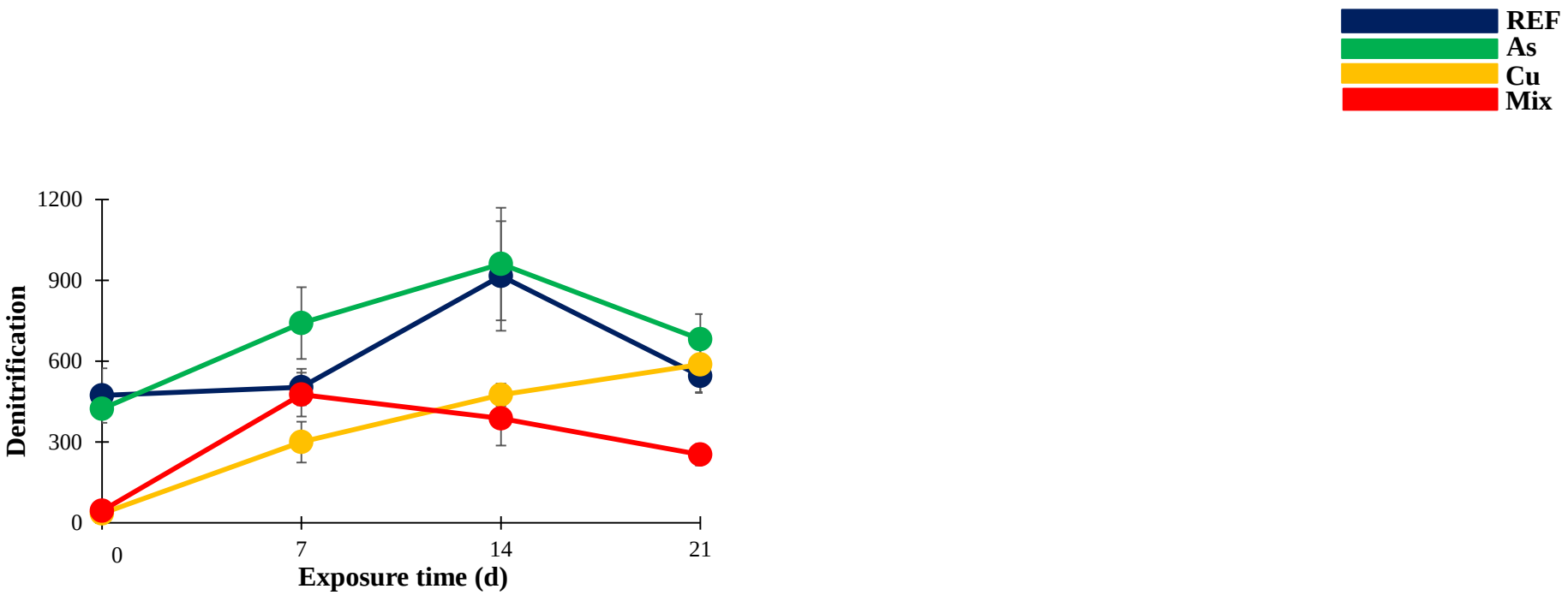
➔ No effect of As on respiration

➔ Cu and Mix exposure

- ✓ No effect from day 0 to day 14
- ✓ Inhibition at day 21

➔ No effect of As, Cu and Mix on bacterial 16S rRNA gene abundance

Effects on Functions: Denitrification (ng N_2O/h/g dw)



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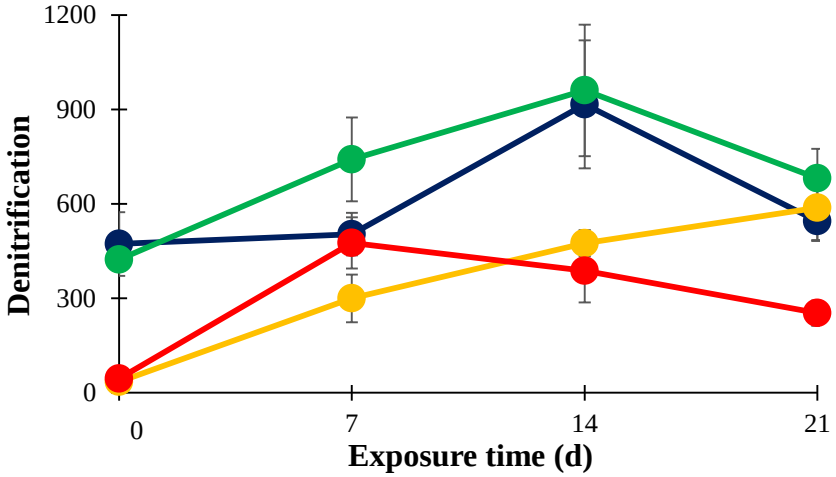

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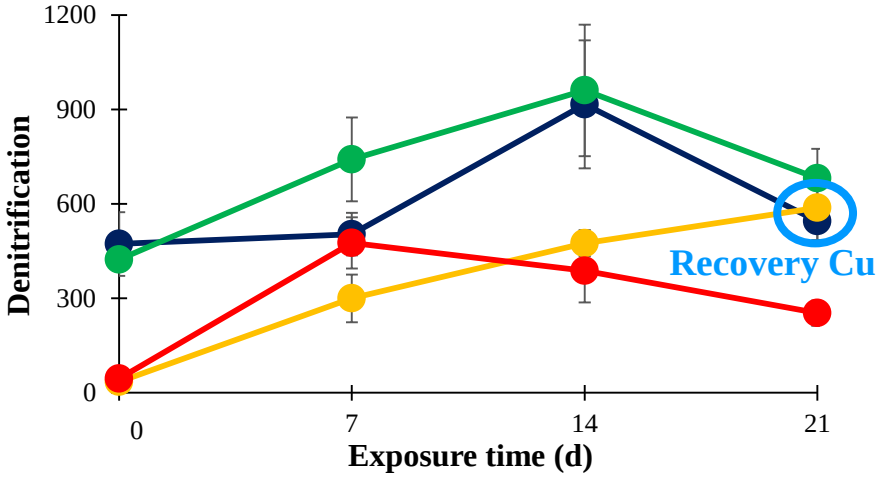

REF
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Mix

➔ No effects of As on denitrification

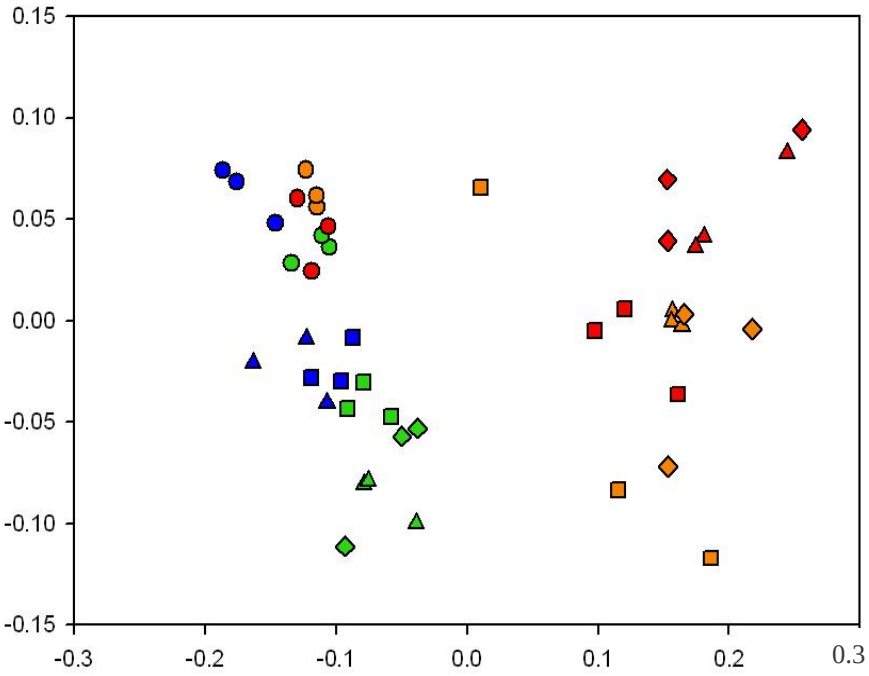
➔ Fast and significant inhibition of denitrification under Cu and Mix exposure

➔ Recovery at day 21 under Cu alone

➔ No effect of As, Cu and Mix on denitrification gene (*nirS*, *nirK*, *nosZ* clades I and II) abundances

Results

Effects on Bacterial Community Composition (BCC)



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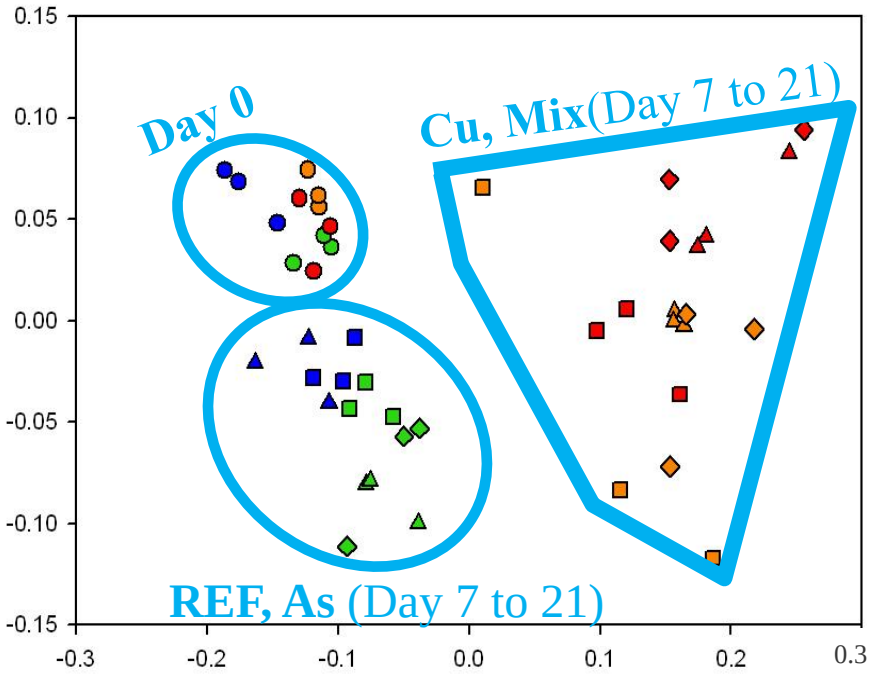


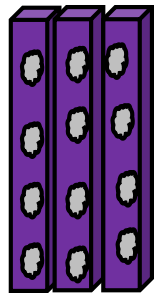
➔ No effect of As on BCC

➔ Significant changes of BCC from the first week of exposure to Cu and Mix

➔ No recovery of BCC between day 7 and day 21 under Cu and Mix exposure

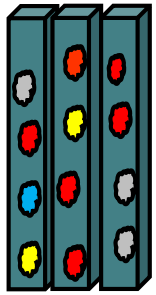
Effect on tolerance acquisition

PICT approach principle



Control

Chronic exposure



Exposed

↓

Selection of tolerant species

Tolerance Induction
Chronic Exposure Phase

* PICT: Pollution Induced Community Tolerance

Results

Effect on tolerance acquisition

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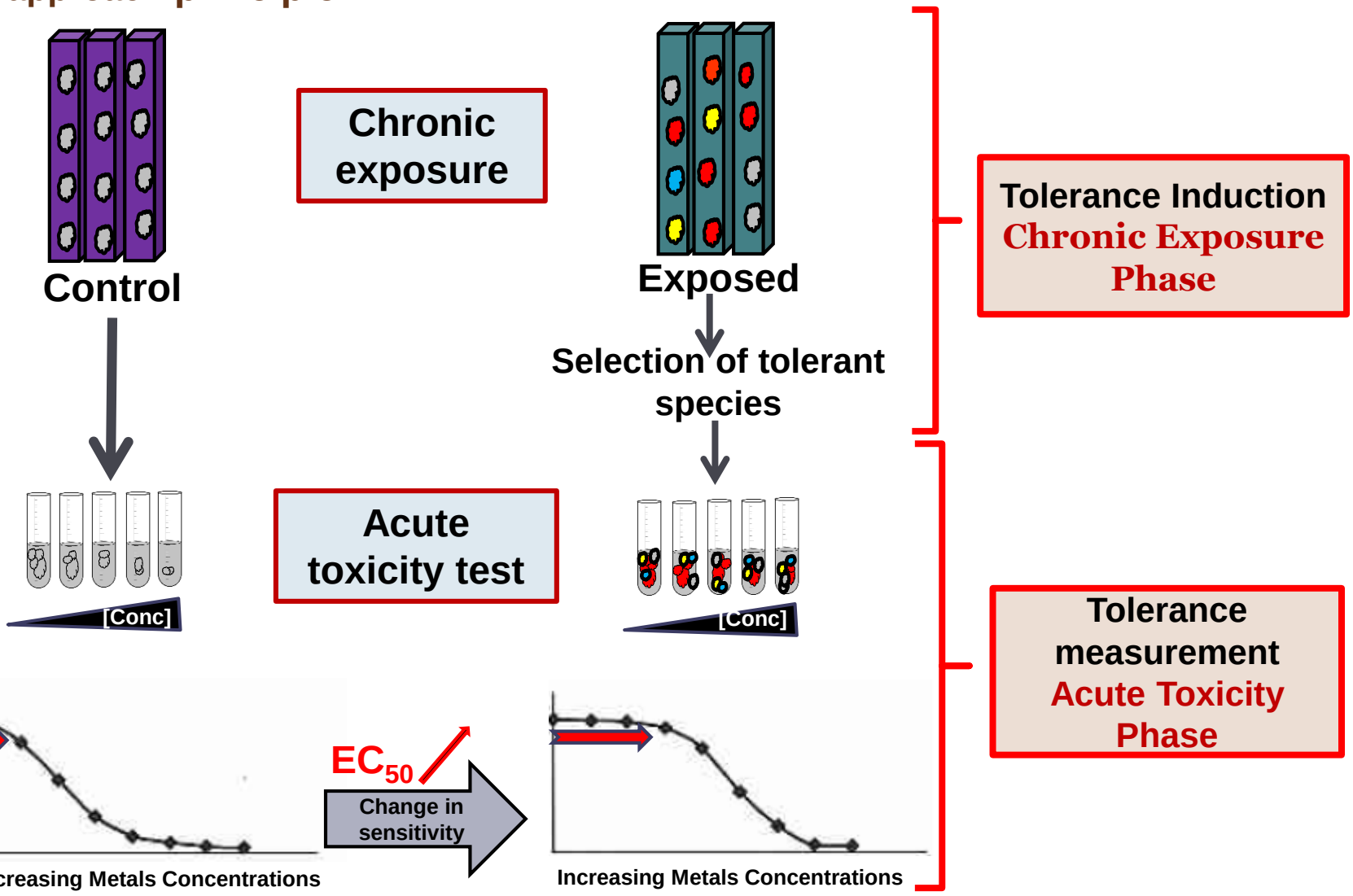

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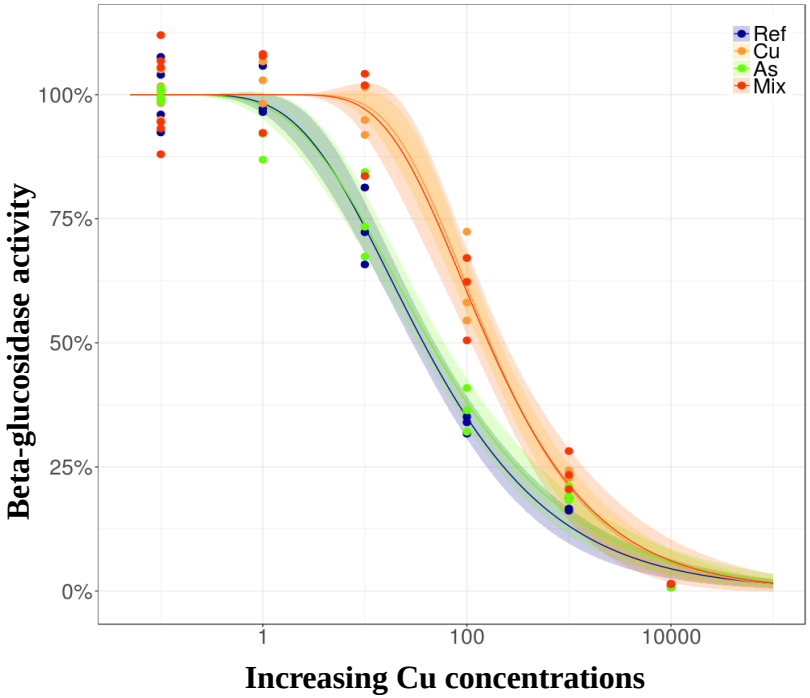
Effect on tolerance acquisition

After 21 days of chronic exposure and 4 hrs of acute exposition



➔ No effect of Cu on tolerance of As exposed communities (*i.e.* no cotolerance)

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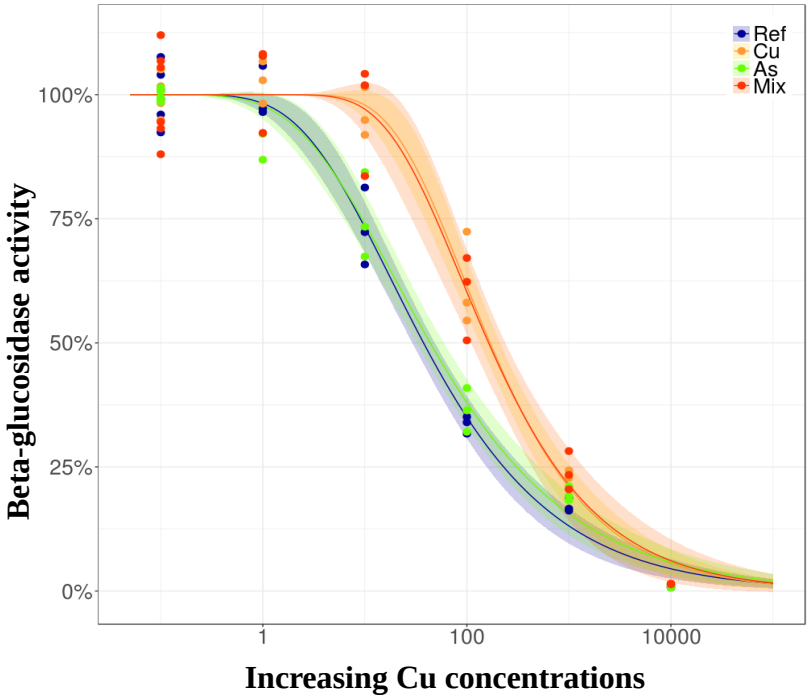

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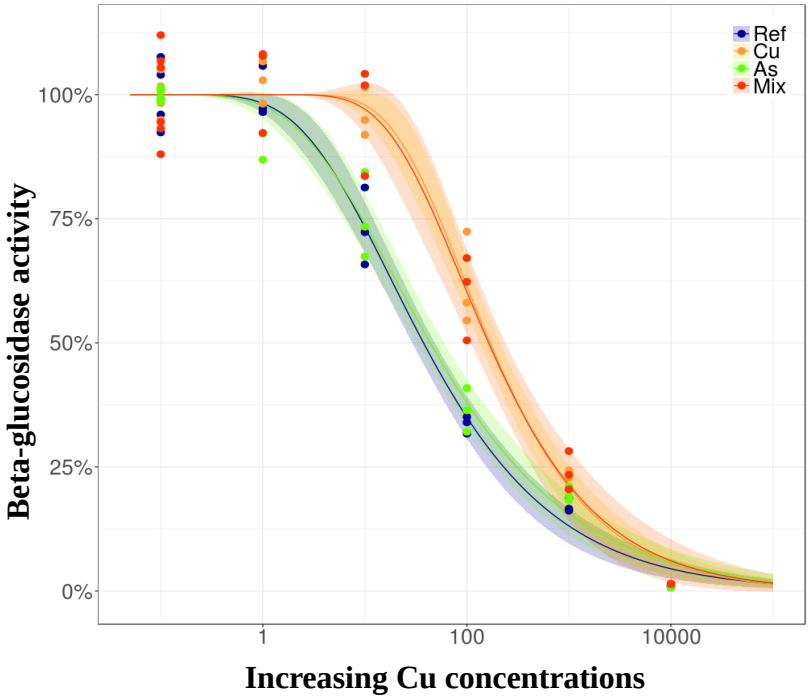

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- **Effective concentrations (EC) inhibiting 50% of beta-glucosidase activity after acute Cu exposure (4 hrs)**

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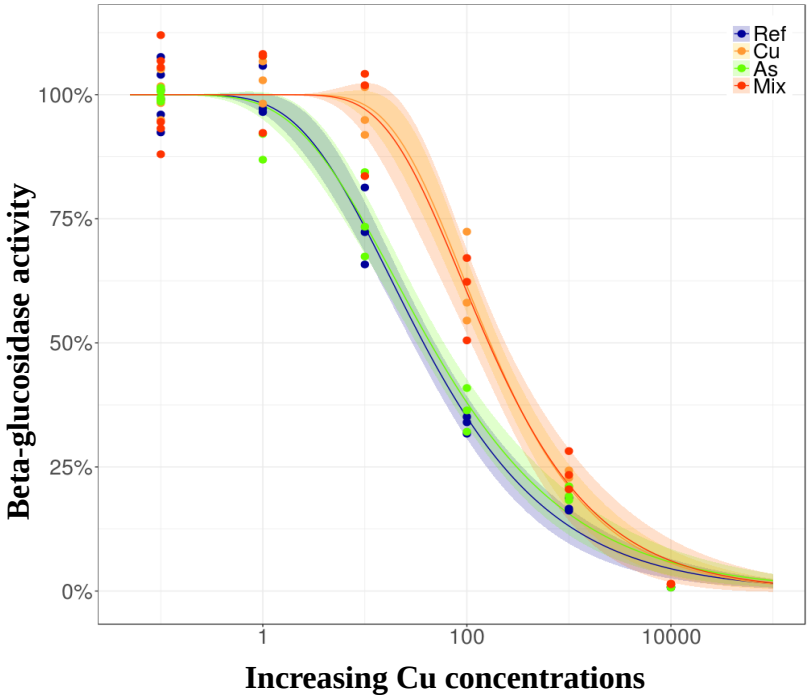

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Conclusions and Perspectives

➔ **Environmental concentrations** of **As**

- **Undetectable or very limited effect on functions, structure and tolerance**

Hyp1: low bioavailability of the contaminant?

✓ **Important complexation of arsenic with OM**

Hyp2: Arsenic speciation: less toxic form?

Conclusions and Perspectives

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Hyp1: low bioavailability of the contaminant?

✓ **Important complexation of arsenic with OM**

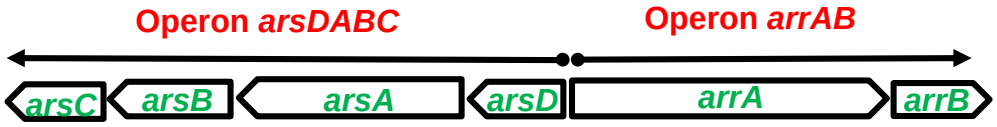
Hyp2: Arsenic speciation: less toxic form?

Hyp3: Presence of iron oxide?

✓ **Adsorption of arsenic**

Hyp4: Microbial communities tolerant to arsenic?

✓ **Quantification of resistances genes**



From Patrick Billard *et al.* (2013)

Conclusions and Perspectives

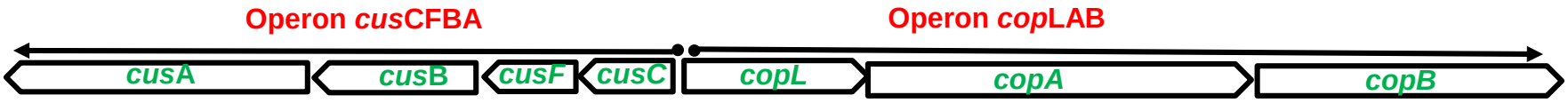
➔ Environmental concentrations of **Cu** alone and in **Mixture**

- **Fast and marked effects on the structure and most of the measured functions**

- **No structural recovery but functional recovery at day 21 depending on the tested function and exposure conditions (alone or mix)**

- **Cu tolerance acquisition (PICT) under Cu and Mix exposure is consistent with BCC changes**

  ✓ **Quantification of resistance genes: tolerance acquisition**



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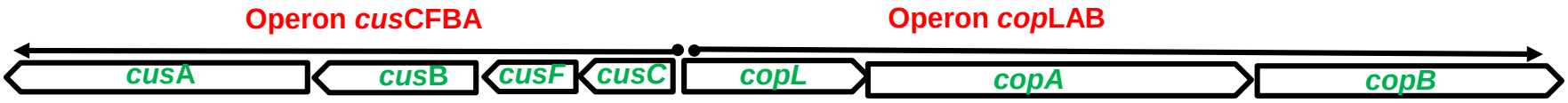

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- **Metals accumulation in sediments impact microbial communities**

  ✓ **Functional Role in Aquatic Ecosystems**

- **Application of PICT in the sediment compartment**

  ✓ **Promising biomotoring tool for environmental risk**

Thank you for your attention

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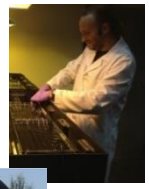


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Funding