# Copper bioaccumulation and internalization during freshwater biofilm development using stable Cu isotope experiment

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# Following copper bioaccumulation and internalization during freshwater biofilm development using stable Cu isotope

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## Context and aim of the study

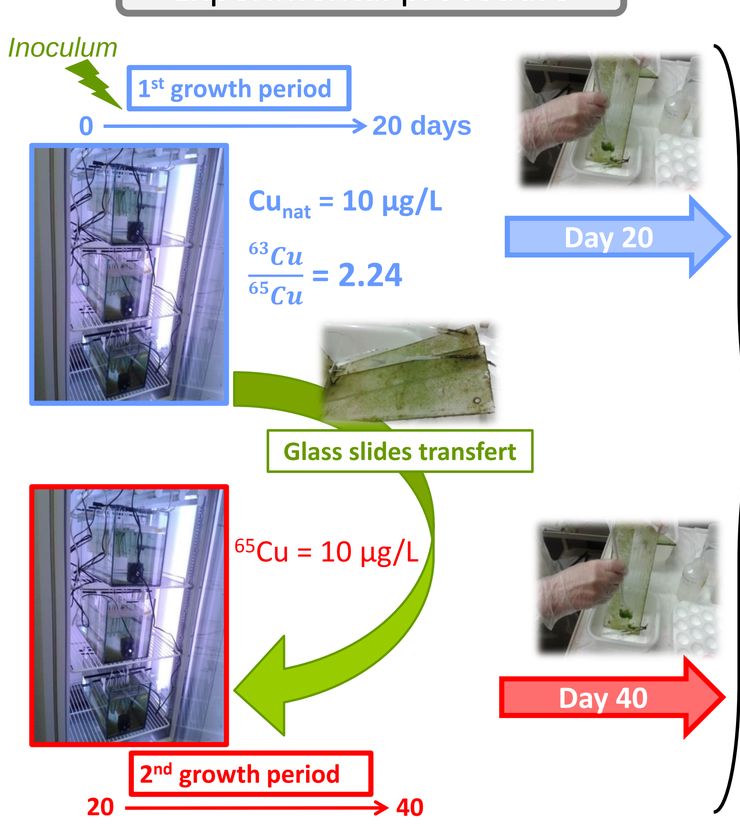
In small streams, microbial communities form river biofilms attached to solid substrates by producing extracellular polymeric substances (EPS). This matrix may act as a protective layer by limiting cellular contact with surface water contaminants. Thus, several studies (i.e. Ivorra et al. 2000) have suggested that during biofilm growth, biofilm and EPS matrix thickness could limit cellular bioaccumulation.

## Aim of the study

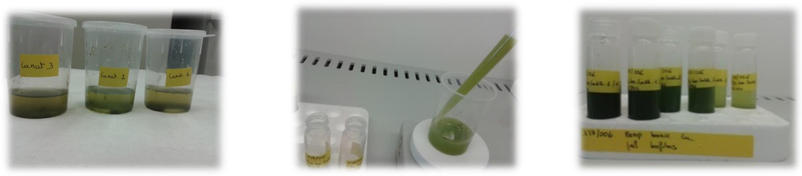
To test the hypothesis that biofilm is more exposed to Cu in early colonization stage than a mature biofilm , we conducted an experiment under controlled conditions to follow the bioaccumulation of two Cu isotopes in the different biofilm fractions throughout biofilm growth and maturation. After a first period of development (0 to 20 days) in natural dissolved copper medium, biofilm was transferred to a mono-isotopic ($^{65}$Cu) copper-enriched medium for 20 additional days. After 20 and 40 days, a sequential extraction was applied to recover Cu from the intracellular fraction, and from the colloidal and capsular EPS fractions. Copper concentrations and isotopic ratios were determined by ICP-MS in water collected at various times of the experiment and after 20 and 40 days in the different fractions of the biofilm.

## Material and methods

### Experimental procedure



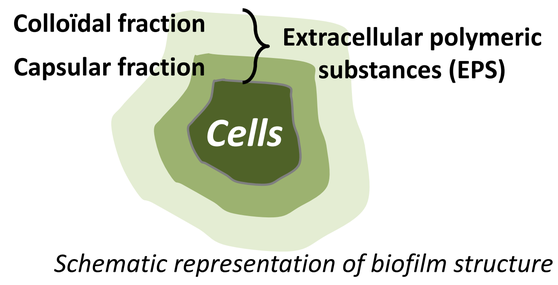

### Sequential extraction of EPS on biofilms



**Colloïdal fraction**
- 5 mL of demineralized water
- Agitation 20 min
- Centrifugation, 4000 g, 5 min
- Supernatant **C1**

**Capsular fraction**
- 5 mL EDTA 4 mM
- Agitation 3 hours
- Centrifugation, 16000 g, 20 min
- Supernatant **C2**

**Cells**
- Mineralization with Aqua regia (microwave oven)




*Schematic representation of biofilm structure*

### Cu analyses

- Analysis of 63Cu and 65Cu by ICP-MS with CCT (Thermo X7 Series II)
- Mass bias correction for isotopic ratio measurement ($^{64}$Zn/$^{66}$Zn)
- External calibration for Cu concentrations measurements
- LQ = 0.05 µg/L for water samples
- LQ = 2 µg/g d.w. for biofilms samples

Water | Colloïdal C1 | Capsular C2 | Cells

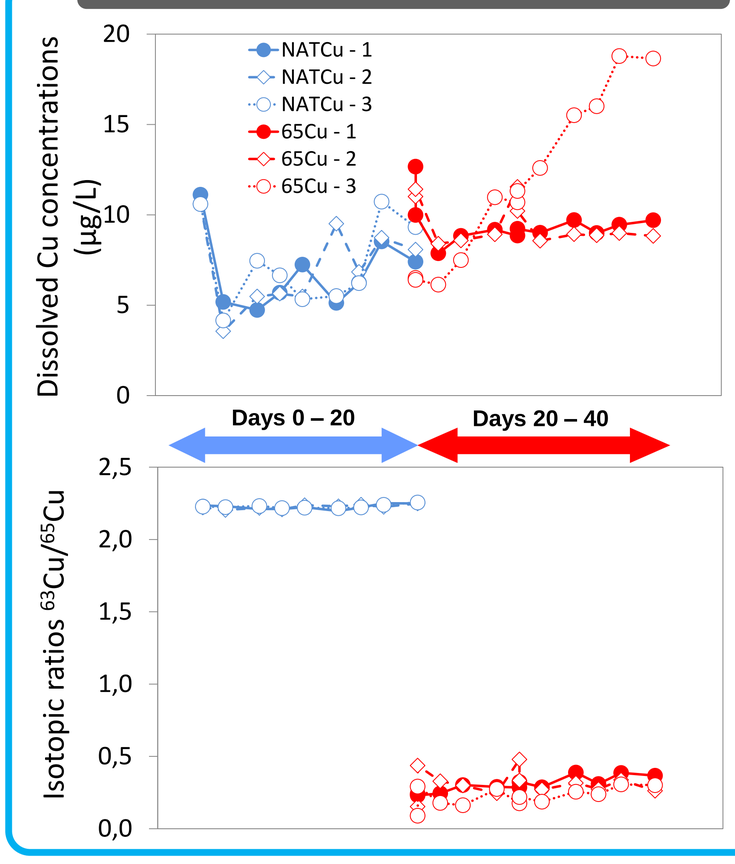
### EPS Characterization

-Polysacc
Prot
HPSEC

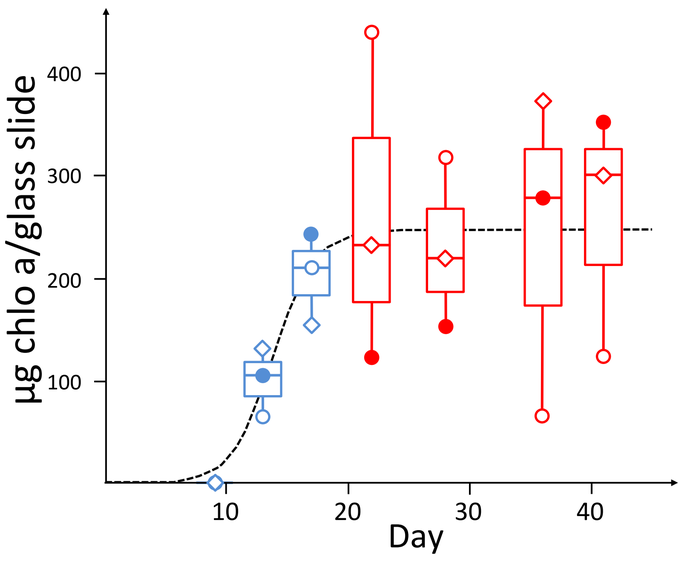
### Biofilm growth

xxx

## Cu in dissolved medium



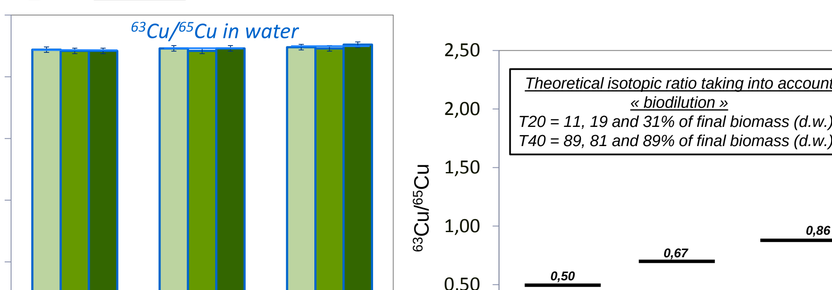

## Biofilm growth



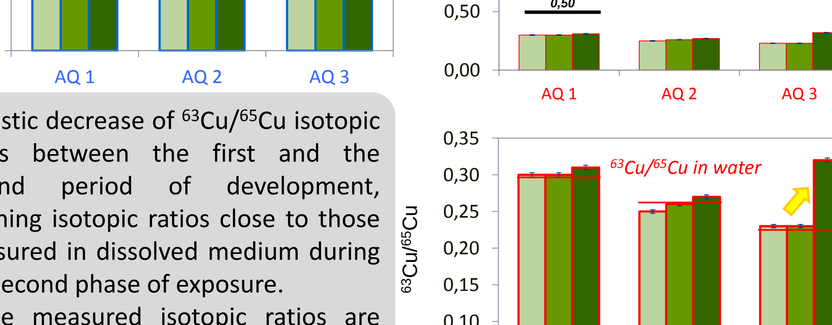

- Mean growth rate was 0.6 ± 0,4 µg chl *a*/day.
- Maximum growth rate was reached after 13.8 ± 1.5 days.
- Great variability between each aquarium during the second period of exposure.

## EPS characterization

Polysaccharids



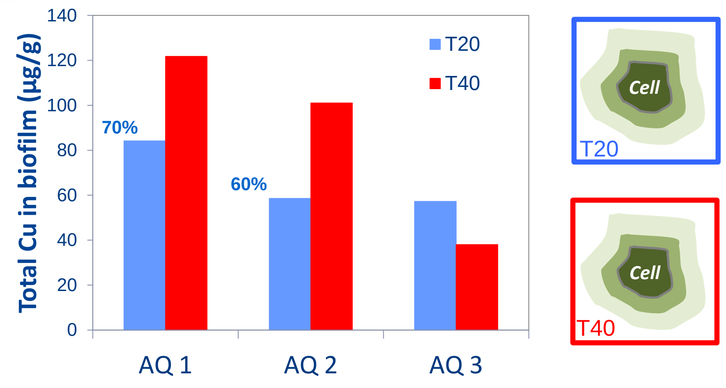

Proteins

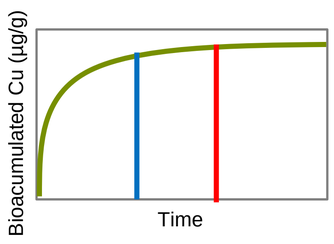



- Polysaccharids and proteins concentrations are 2.7 and 2.0 times higher in colloïdal fraction than in capsular fraction.
- No change of polysaccharids and proteins concentrations between the early stage of biofilm development and the second phase of growing (AQ 1 and 2).
- Decrease of polysaccharids and proteins concentrations in the third aquarium.

## Bioaccumulation of Cu in biofilm

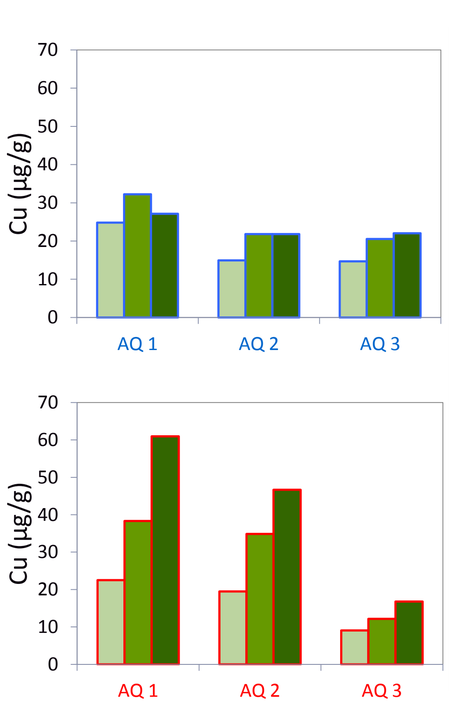
### 1 Total concentration of Cu in biofilm




- Assessment of total Cu concentration in the entire biofilm suggests that a large part of accumulated Cu would mainly related to the first period of bioaccumulation.
- In aquarium n°3, similar Cu concentrations between the two successive periods of growing.

### 2 Cu concentrations in the fractions of the biofilm




- Assessment of Cu concentrations in the different fractions of the biofilm suggests a comparable Cu accumulation in the colloïdal and capsular fractions of EPS and in the cells during the early stage of development.
- During the second period of exposure, results indicate an increase of Cu bioaccumulation in the capsular fraction and in the cells.
- In aquarium n°3 similar results between the first and the second period of development.

### 3 Isotopic ratios of $^{63}$Cu/$^{65}$Cu in the fractions of the biofilm




- Drastic decrease of $^{63}$Cu/$^{65}$Cu isotopic ratios between the first and the second period of development, reaching isotopic ratios close to those measured in dissolved medium during the second phase of exposure.
- The measured isotopic ratios are lower than theoretical isotopic ratios taking into account « biodilution »
- In aquarium 3, cells display an isotopic ratio slighlty higher than in the EPS fractions.

## Conclusions

- According to analytical procedure (total Cu concentrations, Cu concentrations in the different fraction of the biofilm or isotopic tracing), informations on Cu accumulation kinetics can be very different.
- While a total Cu concentration approach seems to indicate a Cu saturation over time, isotopic tracing approach suggests that the entire biofilm (EPS + cells) is continuously in intense renewal as well as the bioaccumulated Cu.
- In aquarium n°3, the increase of dissolved Cu during the second period of exposure has led to a less Cu accumulation. In that case, we noticed an isotopic ratio slightly higher in cells than in EPS fractions, suggesting a potential protective effect of EPS matrix

## Perspectives

- Refine kinetics of accumulation during the second period of exposure (biofilm extraction every day to follow isotopic ratios in the different fractions)
- Coupling tracing approaches with microscopie/analysis approaches (LA-ICP-MS, µ-SXRF)

*Ivorra, N., et al. (2000). "Differences in the sensitivity of benthic microalgae to Zn and Cd regarding biofilm development and exposure history." Environmental Toxicology and Chemistry **19**(5): 1332-1339.*