	<b>Experiment title:</b>  Coordination and speciation of aqueous Cd(II) xanthan complexes, a model system for studying the interaction between metals (Cd) with soil bacterial surfaces and biofilms	<b>Experiment number:</b>  32-02-907
	<b>Beamline:</b>  BM30B	<b>Date of experiment:</b>  From 02/07/2008 to 06/07/2008
<b>Shifts:</b>  12	<b>Local contact(s):</b> Isabelle Alliot	
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## Report:

### Aims of the experiment and scientific background

Sorption of bivalent metals on organic matter involves complexation on carboxylic, phenolic, amine, thiol and possibly phosphate functional groups. This is valid for almost any type of organic matter; dissolved or colloidal, living or degraded. The bond strength linking the cation to the functional group is comparable to those of moderately chelating model ligands, such as for example malonate or glutarate (1-4). For metal sorption on microorganisms, it is difficult to draw a 3D view of metals sorption and to determine the location of metals sorbing on bacterial cells. The major cell wall components (teichoic acids, peptidoglycan, phospholipids, lipopolysaccharides) were described early by Sherbert (5) and later by Beveridge and Murray (6) as the major metal binding sites, and this concept was the basis of the interpretation of numerous bacterial reactivity studies (for example 1, 2). But we evidenced recently combining Zn K edge EXAFS and Zn and proton titrations that these cell wall components cannot account for the measured metal loading (7).

On a more molecular level, the coordination of metals (especially copper) to organic matter starts to be well understood. On humic and fulvic acids it was shown that copper coordinates predominantly forming 5- (at low Cu surface loadings) or 6- (at high loadings) ring chelates, in essentially, malate, malonate or lactate like environments (8). Thus, two to three functional groups bind Cu simultaneously. This fits the macroscopic thermodynamic findings, which predicted stabilities accounting for chelating complexes. But the investigated organic matters do not have a sufficiently high site density to form the required high number of ring chelates, especially at high metal loadings. These molecular findings thus do still not allow to draw a comprehensive 3D view of metals coordinating on complex biopolymers. These results indicate in fact the necessary bridging of side chains to form the required high coordination numbers. We consequently decided to address these questions through triple-levelled observation of coordination, stability and conformation of copper complexing on Xanthan, a bacterial exopolysaccharide. In terms of site density, xanthan compares to natural organic matter such as humic acids. Moreover, exopolysaccharides, to which xanthan belongs, may fix large amounts of metal ions, and may thus explain the astonishingly high metal loads determined on bacterial substrates.

### Experimental method

All samples were recorded in wet state, flash frozen in liquid nitrogen, inserted in a He cryostat, and Cu K-edge EXAFS spectra were recorded in fluorescence mode using a 30-element Canberra detector. 4 to 10 scans of 40 min each were recorded, and averaged. EXAFS data treatment included linear combination fits and shell simulations.

## Results

Spectra of xanthan at pH 3.5 and 5.5 at 140 (XA3 and XA5) and 1400 (XB3 and XC5) mg/L dissolved copper concentrations were recorded. The spectra of the two xanthan constituting acids, pyruvic and glucuronic acid and their stoichiometric mixture were recorded at similar copper concentration conditions at pH 5 (Pyr5, GICA5, Mix5, Figure 1a). Pyr5 compares to the low Cu loading samples XA3 and XA5, which indicates that Cu preferentially binds to pyruvate type sites, as is expected from the pyruvate-Cu stability constants.

From Cu titrations, circular dichroism experiments and molecular dynamics calculations we know that xanthan is in a disordered state at pH 3.5, whereas it is in a more ordered state (parallel or anti-parallel chains) at pH 5.5. Molecular dynamics calculations suggest that more chelating complexes form in the disordered state, as the degree of freedom and thus the possibilities for forming inter-molecular chelating conformations naturally increases. Slight differences in the amplitude of the first RDF peak are observed as a function of the pH (Fig. 1b-c). The same trends are observed for the RDFs calculated by

molecular dynamics (Fig. 1d). These findings can be interpreted in light of conformational changes and formation of chain-crossing chelates.

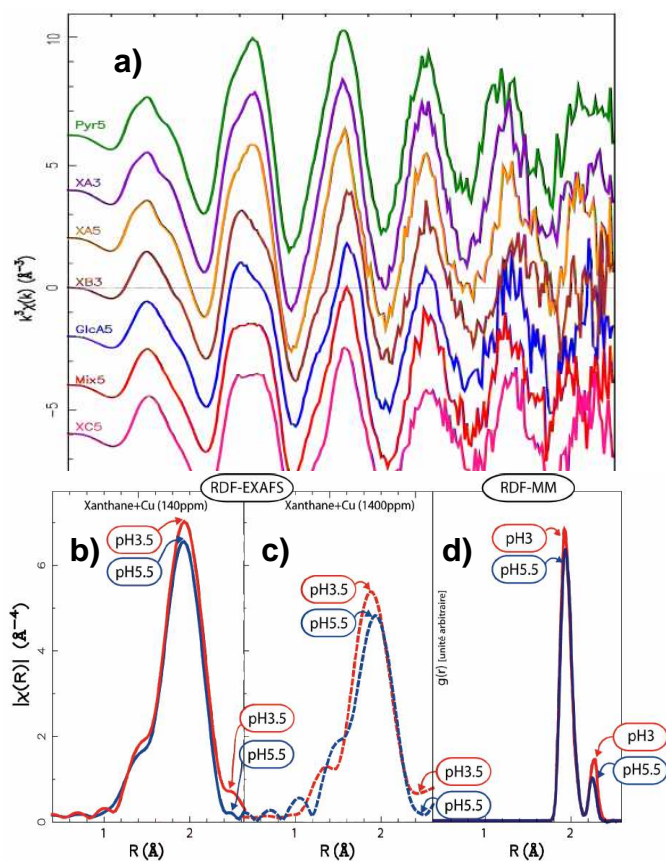


Figure 1 a) recorded EXAFS spectra, b) and c) experimental RDF recorded at the given Cu concentrations and pH values, and d) the theoretical RDF spectra obtained from molecular dynamics calculations.

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## Scientific production

### Communications

- Desaunay A., Guiné V., Causse, B., Muris M., Sarret G., Spadini L., Delolme C., Vince E., and Martins J.M.F., Heavy metal sorption onto Gram-negative bacteria: a combined approach of solution chemistry, TEM-EDS and EXAF Spectroscopy, *10th International Conference on the Biogeochemistry of Trace Elements (ICOBTE)*, 13-18 July 2009, Chihuahua, Mexico.
- Desaunay A., Guiné V., Spadini L., Sarret G., Causse B., Delolme C., Hellmann R., Vince E., Martins J.M.F., Interactions des métaux lourds avec les cellules bactériennes: une approche couplée de chimie de solution, TEM-HR et EXAFS, *Deuxièmes rencontres nationales de la recherche sur les sites et sols pollués : pollutions locales et diffuses*, Paris, 20-21 Octobre 2009.
- Causse B., Spadini L., Delolme C., Sarret G., Martins J., Heyraud A. & Mazeau K. (2008) Proton and Cu<sup>2+</sup> Reactivity of Xanthan, a Model Bacterial Exopolysaccharide. Goldschmidt 2008, 13-18 July 2008, Vancouver, Canada.

### Thesis

Causse, B. PhD thesis, «Mécanismes d'adsorption des métaux lourds par les exopolysaccharides bactériens : Le système Xanthane-Cuivre comme modèle d'étude», 11 Dec 2009, Grenoble.

### Two articles in preparation.