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Combined titration, modelling, and EXAFS study for the complexation of Zinc and Cadmium by three Gram negative bacteria

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The bioavailability, toxicity, and mobility of trace metals depend on complexation reactions with functional groups of bacterial cell walls. The acid-base exchange and Cd and Zn sorption properties of three Gram negative bacteria of environmental interest (*C. metallidurans* CH34 (formerly *Ralstonia metallidurans* CH34), *Pseudomonas putida* (ATCC12633), and *Escherichia coli* K12DH5α) were investigated through an original combination of extended X-ray absorption fine structure (EXAFS) spectroscopy and equilibrium titration studies (Guiné et al., 2006 and 2007). EXAFS data for the bacteria were modelled using carboxyl and phosphoryl sites. Unexpected additional sulfhydryl sites were required to model the data from the *C. metallidurans* CH34 under similar experimental conditions. This was the first time sulfhydryl sites were identified in metal complexation by bacteria. The proportions of reactive sites depended on metal loadings and bacterial strain. The affinity of metals for these groups followed the order sulfhydryl $>$ phosphoester $>$ carboxyl.

Acid-base titration curves were fitted with a model accounting for three and four conceptual reactive sites: a carboxyl (and/or phosphodiester), a phosphomonoester, and an amine (and/or hydroxyl) reactive groups for *E. coli* and *P. putida*; and a neutral sulfhydryl group was added for *C. metallidurans* bacteria. Calculated proton, Cd and

Zn equilibrium constants and total site densities compared with literature data of numerous Gram positive and negative bacteria. These findings were compared to the structure and site density of the major cell wall components of our bacteria. It appeared that the cumulated theoretical site density of these structures was much lower than the total site density of the investigated strains. These results suggested a dominant role of extracellular polymeric substances in metal retention processes, although metal uptake and further binding to inner cell components cannot be excluded.

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