Interactions between natural organic ligands and trace metals studied by fluorescence lifetime and fluorescence quenching

Ayoub Nouhi (1), Houssam Hajjoul (2), Roland Redon (3), Jean-Pierre Gagné (4), and Stéphane Mounier (5)

(1) University of Toulon, La Garde, France (nouhi@univ-tln.fr), (2) University of Toulon, La Garde, France (houssam.hajjoul@univ-tln.fr), (3) University of Toulon, La Garde, France (redon@univ-tln.fr), (4) Institut des sciences de la mer de Rimouski, Rimouski, Canada (Jean-Pierre_Gagne@uqar.ca), (5) University of Toulon, La Garde, France (mounier@univ-tln.fr)

Improved insight on the interactions between natural organic ligands and trace metals is of paramount importance for better understanding transport and toxicity pathways of metal ions in the environment. Fluorescence spectroscopy allows introspecting ligands-metals interactions. Time-resolved laser fluorescence spectroscopy (TRLFS) measures fluorophore lifetime probing the local molecular environment. Excitation Emission Fluorescence Matrices (EEFMs) and their statistical treatment : parallel factor analysis (PARAFAC) using PROGMEEF Matlab homemade program, can give insight on the number or nature of organic fluorophores involved in the interactions. Quenching of fluorescence by metals can occur following two processes: dynamic and static quenching (Lakowicz, 2013). In the first case, quenching is caused by physical collisions among molecules and in the second case fluorophores can form nonfluorescent complexes with quenchers. It is possible to identify the different mechanisms because each type of quenching corresponds to a different mathematical model (Lakowicz, 2013; Valeur and Berberan-Santos, 2012). In TRLFS, the study of fluorescence decay's laws induced by nanosecond pulsed laser will allow to exactly qualify the type of interaction. The crucial point of the temporal deconvolution will be the evaluation of the best fitting between the different physical models and the decays measured. From the most suitable time decay model, it will be possible to deduce the quenching which modifies the fluorescence.

The aim of this study was to characterize interactions between natural organic ligands and trace metals using fluorescence tools to evaluate the fluorescence lifetime of the fluorophore, the occurrence of quenching in presence of metal, discuss its mechanism and estimate conditional stability constants if a complex organic ligand-metal is formed.

This study has been done in two steps. First, we have examined the interactions between salicylic acid and copper in order to calibrate our assays and compare our results with literature. Several studies have shown that static quenching occurs in that case (Brun and Schröder, 1975; Lavrik and Mulloev, 2010; Ventry et al., 1991; Babko, 1968). Indeed, after processing the EEFMs and TRLFS data, we found a fluorescence intensity decay by about 50% and a constant lifetime for the fluorophore suggesting a static quenching, in agreement with the literature.

In the second step, we have studied the interactions between metal and different types of natural organic matters. In this case, EEMFs and TRLFS experiments were done on samples prepared by dissolving copper in four different fractions of organic matter extracted from estuarine water (St. Lawrence Estuary, Canada). Organic matter was obtained using DAX-8 and XAD-4 resins in series. Humic and fulvic acids are obtained following the IHSS protocol.

The results of interaction between humic substances and copper gathered after processing data on PROGMEEF have shown a fluorescence intensity decay by about 57% for the first component and 88% for the second component. The fluorescence lifetime for both components were close to 2 ns and 6 ns respectively and the pH range was stable and close to 6. This means that a static quenching takes place in this case in agreement with the literature. Our study also focused on the investigation of complexation of organic matter by other metals in particular Aluminum, Arsenic, Europium and Uranium.