

Metal isotope coded profiling of organic ligands by mass spectrometry in aquatic environments

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Metal isotope coded profiling (MICP) introduces a universal discovery platform for metal chelating natural products that act as metallophores, ion buffers or sequestering agents. The detection of cation and oxoanion complexing ligands is facilitated by the identification of unique isotopic signatures created by the application of isotopically pure metals.

We present a targeted analysis of low-molecular-weight organic ligands based on fast UHPLC-ESI-MS measurements. Replacement of, for example, natural iron or molybdenum with isotopically pure $^{54}\text{Fe}/^{58}\text{Fe}$ (ratio 1:1) or $^{95}\text{Mo}/^{98}\text{Mo}$ (ratio 1:1) causes easily detectable unique isotopic signatures in the mass spectra of potential metal-complexing ligands. This can be achieved under laboratory conditions not only in growth media, but also by spiking directly aqueous samples or solid-phase extracts. Importantly, as the relative affinity of the metallophores for e.g., Mo or Fe is dependent on the pH, all experiments need to be conducted under pH-controlled conditions. The improved ionization efficiency of some metal complexes helps to enhance the signal-to-noise ratio compared to the free ligand at the same chromatographic conditions. The methodology does not necessarily depend on HR-ESI-MS measurements (e.g., Q-Exactive Orbitrap) and can be applied to any mass spectrometer. With MICP, two birds can be killed with one stone: (i) the identification of metallophores (e.g., siderophores, molybdophores) for metal uptake by any organism and (ii) organic ligands which solely work as metal buffer in dissolved organic matter (DOM).

We currently address following two main research lines: First, DOM has often been used as a proxy for bio-productivity in terms of a carbon source; however, the specific impact of DOM as a “metal buffer” for biological processes is still under-investigated. Upon the administration of individual isotopes or isotopic pairs, for example, $^{54}\text{Fe}/^{58}\text{Fe}$, $^{63}\text{Cu}/^{65}\text{Cu}$, $^{66}\text{Zn}/^{68}\text{Zn}$, or $^{95}\text{Mo}/^{98}\text{Mo}$ and subsequent measurements by mass spectrometry, organic ligands can be identified that contribute potentially to the regulation of bioavailability and toxicity of metals in the water column. Secondly, metal acquisition by ligand exchange processes is vital for macroalgae-bacteria interactions. Both alga and bacteria contribute to the “soup of organic ligands” which potentially increases the bioavailability of metals due to various ligand exchanges with DOM. In particular, we will outline the analytical process resulting in the identification of ligands in the chemosphere of the marine macroalga *Ulva* (Chlorophyta) and its associated bacteria.

In summary, the identification of metal organic ligands in natural samples will pave the way for understanding the organic ligand-mediated metal acquisition shuttled from DOM to alga.

(References: Wichard T. (2016) *Front. Mar. Sci.* 3:131; Deicke M., Mohr J.M., Wichard T. (2014) *Analyst*, 139, 6096-6099)