Metal speciation and bioavailability: Revisiting the “big questions”

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Environmental context

Four decades of research on metal speciation and bioavailability have failed to answer the “big questions” of the ecological consequences of metal stress. Important, though still insufficient, insights have been gained from analytical approaches derived from inorganic environmental chemistry (targeting the quantification of free metal ion or “labile” metal concentrations) and from organic environmental chemistry (focusing on the structure of ambient metal species). The “omics” approach, not yet widely applied to this topic, offers the possibility of providing sufficient information to identify a quantitative signature of metal stress.

Historical background

The past four decades have witnessed an explosion of studies of trace metal speciation and its effects on the bioavailability and toxicity of trace metals. These studies were initially motivated by some intriguing observations, for example, that freshly-upwelled seawater did not support phytoplankton growth without some “conditioning” despite providing sufficient macronutrients (i.e., nitrogen and phosphorus)¹. This “conditioning” occurred during residence of the upwelled water at the surface, but could be mimicked by the addition of synthetic metal-chelating agents or zooplankton extract. Experiments in well-defined chemical media have demonstrated that metal toxicity is related to the free metal ion activity². It was also observed that phytoplankton species from the open ocean are more sensitive to trace metal toxicity than coastal species, suggesting that the phytoplankton community itself may reflect the selective pressure exerted by metal stress³.

At about this time, it was recognized that anthropogenic inputs of trace metals into the environment had profoundly altered their pre-industrial (and even pre-historical) concentrations; the signature of lead mining and smelting by ancient Greek and Roman civilizations, for example, is preserved in the Greenland ice core record ⁴. The reversal of such inputs, in response to the phase out of leaded gasoline, has resulted in detectable decreases in lead concentrations in surface waters of the North Atlantic Ocean ⁵.

Articulating the “big questions”

The observations that motivated the last four decades of research on trace metal speciation can be framed by a set of “big questions” related to the ecological consequences of metal stress, specifically:

- What level of stress does a given metal loading exert on an aquatic community?
- Does the composition of an aquatic community reflect current (or a past history of) metal stress?
- How would an aquatic community respond to increases in metal loading and/or stress associated with pollution or decreases associated with remediation?

The investigation of these “big questions” is predicated on the ability to define and quantify bioavailable metal concentrations. But leaving aside this issue for the moment, the possible effects of changes in the bioavailable concentration of toxic metals can be considered as illustrated in Figure 1. In cases (a) and (b), increases or decreases in the bioavailable metal concentration that occur in a metal-tolerant ecosystem do not significantly change the community (represented by the green circle). In case (c), decrease in the bioavailable metal concentration allows the recovery of a metal-sensitive community (represented by the blue circle). In case (d), increase in the bioavailable metal concentration results in a degraded ecosystem (represented by the red circle). Note

that this schematic illustration neglects the possibility that extremely low bioavailable metal concentrations might also limit phytoplankton growth and productivity⁶.

Figure 1. Schematic representation of aquatic communities (circles) subject to varying levels of metal stress. Arrows (a)-(d) represent changes in the levels of metal stress that can shift the community from tolerant to degraded conditions (d), allow recovers of a sensitive ecosystem (c), or not change the community composition (a, b).

The recognition of the importance of these “big questions” is pervasive in the scientific literature on metal speciation and bioavailability. Yet they have not been answered in four decades of research. This is partly attributable to the variety of biochemical mechanisms that organisms have evolved to regulate internal metal concentrations, to localize metals within the cell, and to control metal speciation both internally and externally⁷. As the early observations of seawater “conditioning” indicated, the dynamic interactions between aquatic organisms and their environment can mitigate the effects of external conditions on the biota.

Another major stumbling block, however, relates to the determination of metal speciation in the aquatic environment and to understanding the relationship between metal speciation and metal bioavailability.

Assessing metal speciation, metal bioavailability, and the effects of metal exposure

The analytics of metal speciation has traditionally derived from inorganic environmental chemistry, specifically the disparity between total, dissolved metal concentrations and the free metal ion or “labile” metal concentrations^{8,9}. For a number of metals (though initially observed for copper), most of the total, dissolved metal is present in a form that is not detected by bioassays or the electrochemical techniques most often used to quantify the “labile” metal fraction. Such observations motivated attempts to develop analytical methods for the *in situ*, real-time, direct measurement of free metal ion concentrations (or even of some defined fraction of dissolved trace metal species). Such methods would avoid known complications associated with sample collection, preservation, and handling and allow the dynamic aspects of metal speciation to be addressed. Despite recent advances toward this goal¹⁰, it remains elusive.

Other analytical approaches derive from organic environmental chemistry and focus on elucidating the composition and eventually the structure of the dominant metal species contributing to the measured total, dissolved metal concentration. Mass spectrometric methods, usually coupled with electrospray ionization¹¹⁻¹⁴, have shown promise but are still far from allowing the identification of ambient metal species. Spectroscopic techniques (e.g., based on X-ray absorption¹⁵ or nuclear magnetic resonance¹⁶) have also been applied but generally lack the sensitivity required for ambient metal concentrations.

In an alternative approach, biomarkers have been used to characterize levels of metal stress. Both classical biomarkers, such as fatty acids^{17,18} and metal-specific biomarkers, such as glutathione endpoints¹⁹ or phytochelatins²⁰ have shown some

correlations with metal exposure, but these analyses are very labor intensive and low-throughput. In contrast, so-called “omics” revolution (i.e., from genomics to transcriptomics and proteomics and, ultimately, metagenomics) exploits high-throughput and automated analyses and offers the possibility of providing sufficient information to identify a quantitative signature of metal stress, which could be directly related to metal speciation and mechanisms of toxicity in well-defined media and compared with metal speciation determined in field samples. The opportunities of advancing marine ecology through the application of genomic tools to ecological questions are illustrated schematically in Figure 2.

Figure 2. Links between genomic tools and ecological questions. Abbreviation: EST: expressed sequence tags. Source: Dupont et al. (2007)²¹.

Since much of the early work on metal uptake and bioavailability focused on bacteria and algae, it is perhaps surprising that the “omics” approach has been only marginally exploited in the study of metal bioavailability and toxicity in these target organisms²²⁻²⁴. Some applications have, however, focused on higher organisms for which gene expression induced by metal stress can be related to higher level effects²⁵⁻²⁸.

Regulatory implications

Even in the absence of complete understanding of trace metal speciation, bioavailability, and toxicity or of completely satisfactory answers to the “big questions”, it is still necessary to enforce water quality standards for trace metals in receiving waters and limits on the discharge of trace metal-containing effluents into receiving waters or sanitary sewers. The effects of trace metal speciation have been incorporated in the U.S. regulatory framework by the inclusion of the Biotic Ligand Model (BLM) in the 2007 revision of the Aquatic Life Ambient Freshwater Quality Criteria for copper²⁹. The BLM is based on the model of action that relates toxicity to the free metal ion activity.

In the context of the “big questions”, the water quality criteria derived from application of the BLM should be carefully scrutinized for possible confounding factors that might lead to an underestimate of toxicity, such as the presence of lipid-soluble (hydrophobic) complexes that could diffuse through cell membranes³⁰, the effects of multiple stressors³¹, or kinetic effects leading to transient elevated concentrations of free or labile metals³². Also, it should be recognized that the BLM does not address the ecological (i.e., community-related) aspects of the “big questions”.

In conclusion, the “big questions” provide a basis for assessing the accomplishments of the last four decades of research on trace metal speciation and for identifying future, potentially-promising research directions, such as the “omics” approach. The “big questions” also provide a basis for scrutiny of regulatory frameworks, though such criticisms must be tempered by the recognition that regulatory decisions will necessarily be made with an incomplete knowledge base.

129

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