Quantification and Localization of Elements in Nanoplankton Cells Using an X-ray Fluorescence Microprobe

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Introduction

The development of "clean" techniques has led to a sharp increase in studies on the geochemistry of trace metals and their roles as limiting nutrients for oceanic primary production. These studies require the use of bulk size-fractionation techniques to isolate organisms or particles from their surrounding liquid medium for analysis. Typically, whole water is collected and filtered either serially or in parallel through 0.2, 2.0 and 20 µm membrane filters to divide the particles into the corresponding biological size classes of pico-, nano-, and microplankton.[1] The filters are then rinsed and analyzed for metal content. This "bulk chemistry" approach can only provide information about the metal contents of the trapped particles in aggregate and cannot distinguish metal concentrations in different organisms of the same size or between living and abiotic particles. We have attempted to overcome these limitations through the use of the hard x-ray fluorescence microprobe at the Advanced Photon Source (APS) at Argonne National Laboratory.

Materials and Methods

Natural plankton samples were preserved with 0.25% (final concentration) buffered glutaraldehyde, mounted on carbon/Formvar-coated Au finder-grids, rinsed with deionized water, and allowed to dry. The cells of interest varied in size from 20 µm to 3 µm in diameter. They were identified and located with light and epifluorescence microscopy, and the coordinates of the cells relative to a common reference point on the grid were recorded with a light microscope equipped with a digital stage micrometer. The grids were then mounted in the microprobe, and the same cells identified previously were targeted and scanned with coherent, monochromatic x-ray radiation (9.87 keV). Step sizes ranged from 0.2 µm for the small flagellated cells to 1 µm for the larger diatoms; dwell times were generally either 1 or 2 seconds. Two zone plates with focal lengths of approximately 10 cm and 40 cm were used to adjust the resolution and sensitivity as needed. The focused spot size was approximately 1.2 µm (horizontal) x 0.3 µm (vertical) with the 10 cm zone plate.

Fluorescence spectra were recorded at each pixel by a 3-element solid-state detector. The three spectra for each scan were summed, and the summed spectra from the pixels representing the target cell were then averaged to produce a single spectrum for the entire cell.[2] We found that Cl (Kα 2.622 keV) worked well as a proxy for cellular biomass, though we also used Si (Kα 1.740 keV) as an indicator of the diatom cells. The cell spectrum was then fit with a multiple Gaussian peak model; peak midpoints were constrained to match theoretical expectations and a sigmoidal baseline function used to account for systematic variations in electrical noise. An iterative Gauss-Newton search method was used to find the least-squares optimal model solution. The Kα peak heights were then normalized to incident energy flux and dwell time and converted to element densities though the use of NBS standards. Background element concentrations were similarly quantified by averaging pixels from the scan not representing the cell and subtracted from the cellular element densities. Finally, the cellular elements were summed over the area of the cell and normalized to cellular C and dry weight, which was estimated with published C:volume and C:dry weight ratios.[3-5]

Results and Discussion

The cellular Fe, Cu, and Zn concentrations are shown in Table 1 for the three types of cells analyzed. These are the first reported results, to our knowledge, of trace metal concentrations in individual phytoplankton and protozoa cells. The concentrations of Fe and Zn in the ciliates, diatoms, and flagellates are within the range of values reported for plankton from bulk chemical measurements taken during a diatom bloom off Monterey, CA,6 but the Cu concentrations we measured were higher. Our results might be expected to be higher since the other study included all plankton-sized particles, including detritus that may have had lower metal concentrations. This technique also shows promise as a tool for studying the internal distributions of elements within individual plankton cells. The elemental concentrations vary spatially within the cells. For example, in the diatom in Fig. 1, Si is primarily found in the exterior test of the cell, while

Table 1. Comparison of Fe, Cu, and Zn concentrations (µg/g) in plankton measured with bulk size-fractionation6 and x-ray microprobe (this study).

	Monterey Canyon	**This Study**		
	(n=28)	Ciliates (n=3)	Diatoms (n=6)	Flagellates (n=6)
Fe	49 - 3120	340 ± 61	1519 ± 2073	69 ± 35
Cu	1 - 45	380 ± 138	288 ± 113	122 ± 121
Zn	3 - 703	139 ± 60	214 ± 103	44 ± 25

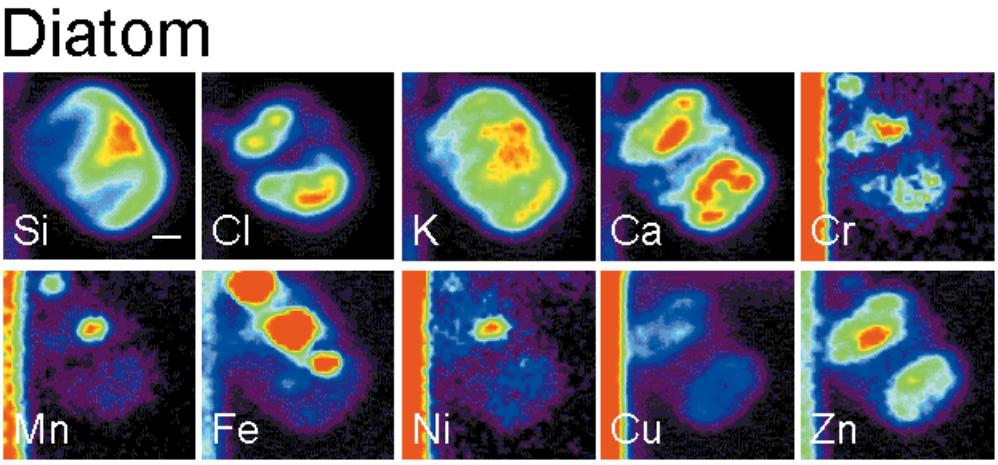

FIG. 1. Element maps of a marine centric diatom collected from the North Atlantic Ocean (scale bar = 6 μm). The color scales correspond to fluorescence intensity, not element concentration.

Cl, Ca, and Zn are concentrated in the organic biomass of the cell, as shown by their density in the two frustules of the cell.

We have shown that the synchrotron x-ray fluorescence microprobe can be a valuable tool for the study of plankton-trace metal interactions. This technique will allow us to determine the elemental composition of cells with different trophic roles and examine the variation in cell composition across environmental gradients. For example, we plan to analyze samples collected during the upcoming Fe fertilization experiment in the Southern Ocean. We will be able to compare the Fe concentration in cells from within and outside the enriched area.

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