A marine sink for chlorine in natural organic matter

Alessandra C. Leri1,*, Lawrence M. Mayer2, Kathleen R. Thornton2, Paul A. Northrup3, Marisa R. Dunigan1, Katherine J. Ness1, and Austin B. Gellis1

1 Department of Natural Sciences, Marymount Manhattan College, 221 E 71st St., New York, NY 10021, USA

2 School of Marine Sciences, University of Maine, Walpole, ME 04573, USA

3 Stony Brook University, National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY 11973, USA

*Correspondence: aleri@mmm.edu

Contents

1. Sample preparation
   1.1. Sediment trap samples
   1.2. Phytoplankton samples
   1.3. Tetraselmis treatments

2. X-ray measurements
   2.1. X-ray absorption near-edge structure (XANES) spectroscopy
      2.1.1. Cl XANES data collection
      2.1.2. Cl XANES spectral analysis
   2.2. X-ray spectromicroscopy

3. References
1. Sample preparation

Cl XANES spectroscopy is an element-specific technique that measures all forms of Cl in a sample. Therefore, a crucial factor in the analysis of marine samples by this method is removal of inorganic Cl\(^-\) to prevent it from overwhelming the Cl XANES signal. All particulates were subjected to six successive rinse-centrifuge-decant cycles in 50 mL deionized water per cycle. After this, particulates were air dried, weighed, and compressed into 13-mm pellets without dilution for bulk XANES analysis. Additional details for each type of sample appear below.

1.1. Sediment trap samples

Particulates from traps deployed in the Arabian Sea Joint Global Ocean Flux Study\(^1,2\) had been sieved (1 mm) and were preserved in formalin, which was removed prior to the rinsing procedure described above.

1.2. Phytoplankton samples

Microalgal cultures were either grown in artificial seawater (containing Na\(^+\), Cl\(^-\), Ca\(^{2+}\), Mg\(^{2+}\), K\(^+\), SO\(_4^{2-}\), and HCO\(_3^-\)) or purchased from Reed Mariculture. Algal cells were concentrated by centrifugation at 1,300 RCF. Algal cultures were sonicated and again centrifuged to remove soluble cell components. This procedure reduced inorganic Cl\(^-\) and isolated membrane components, the likely sources of particulate detritus in the ocean. The effects of cell lysis and rinsing on Cl speciation in \textit{E. huxleyi} are demonstrated in Fig. S7.

1.3. \textit{Tetraselmis} treatments

The treatment of microalgal material from \textit{Tetraselmis} sp. has been described in detail\(^3\). Briefly, \textit{Tetraselmis} membranes isolated as described in section 1.2 were used as substrate, at 500 mg·L\(^{-1}\), in treatments conducted in artificial seawater. For the Fenton-like treatments, 0.2 mM H\(_2\)O\(_2\) and 10 μM FeSO\(_4\)·7H\(_2\)O were added initially and renewed on days 0, 3, 6, 15, 17, and
20 over the 23d experimental period. To test for the effect of solar irradiation, detritus suspensions were exposed under a Suntest XLS+ solar simulator at 760 W·m\(^{-2}\) for 30 min on the same treatment days. Irradiation was performed with and without the Fenton reagents. Particulates were recovered from the reaction mixtures by centrifugation and rinsed as described above in preparation for X-ray analysis.

2. X-ray measurements

2.1. X-ray absorption near-edge structure (XANES) spectroscopy

2.1.1. Cl XANES data collection

Bulk Cl XANES spectra were collected at beamline X19A at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (Upton, NY, USA). The beamline has a fixed-exit double-crystal Si (111) monochromator, and the X-ray beam is collimated and focused with Rh-coated mirrors and steered through a 10 µm Be window. Sample pellets were mounted on halogen-free XRF tape in Cl-free polypropylene sample holders, then exposed open-faced at a 45° angle to the X-ray beam. Sample fluorescence was measured over an energy range of 2800-2900 eV using a Canberra PIPS detector. The ion chamber (I\(_0\)) and the sample chamber downstream were both purged with He to maximize photon flux at these energies. The monochromator was detuned 50% to reduce interference from high-order harmonics. Energy alignment was achieved using a chlorophenol red standard, with the absorption maximum of its spectrum assigned at 2821.24 eV.

Most XANES spectra were collected using a 0.08 eV step size around the Cl K-edge and 0.1-0.5 eV step sizes above and below the edge, with dwell times of 1.0 sec. However, minor radiation damage was observed in certain samples (the sediment trap material and the \textit{E. huxleyi}}
sample), in the form of X-ray beam-induced dechlorination, a phenomenon we have addressed in our previous work\(^4\). To minimize the duration of beam exposure, spectra of the samples in question were collected using step sizes of 0.25 eV close to the edge and 0.5-2.0 eV above and below the edge, with 0.5-sec dwell times. Thus, all data shown for *E. huxleyi* and the sediment trap samples come from single scans with a cumulative beam exposure of five minutes.

Fluorescence intensities of beam-damaged spectra do not change as a function of beam exposure, meaning that radiation does not volatilize Cl species; it only converts some of the organochlorine to inorganic Cl\(^-\). Thus, although our abbreviated beam exposure greatly mitigated the extent of beam damage, actual organochlorine proportions for certain samples may be slightly higher than our fitting results suggest.

**2.1.2. Cl XANES spectral analysis**

Cl XANES spectra were processed using the Athena software package\(^5\). Background subtraction and normalization were achieved using a straight line regressed through the pre-edge region and a quadratic function regressed through the post-edge. Peak positions, widths, and relative areas in the spectra vary according to the bonding state of Cl and can be used as an indicator of Cl speciation\(^6\). Normalized spectra of representative Cl standards illustrate how inorganic and organic forms of Cl can be readily distinguished (Fig. S1). Compared with inorganic Cl\(^-\) compounds (Fig. S1; bottom six spectra), organochlorine compounds have discrete low-energy maxima corresponding to electronic transitions from the 1s orbital to π* and σ* molecular orbitals (Fig. S1; top four spectra). The C-Cl bond length/energy determines the position of the absorption maximum: Cl atoms bound to aromatic carbon, as in chlorophenol and chlorobenzoic acid, have absorption maxima occurring 0.6 eV higher than those of aliphatic organochlorines such as chloroglucose and chloropropanol. Cl XANES spectra can also
differentiate various types of inorganic Cl\(^-\). Spectra of Cl\(^-\) in solid matrices tend to display distinct structural features because the compounds are highly ordered, as becomes evident in spectra of NaCl (s) and KCl (s) in Fig. S1. By contrast, aqueous Cl\(^-\) produces spectra with broader features, as in the NaCl (aq) and HCl (aq) spectra in Fig. S1. The spectrum of hydrated ferric chloride in Fig. S1 has a sharp, low-energy pre-edge feature at 2817.2 eV. Similar pre-edge features are often found in Cl XANES spectra of transition metal-chloride complexes due to a forbidden 1\(s\) \(\rightarrow\) 3\(d\) transition that becomes partially allowed due to mixing of ligand \(p\)-orbitals with metal \(d\)-orbitals\(^7\), \(^8\). Further information on Cl XANES features can be found in Reina et al. 2004, in which representative organochlorine and inorganic Cl\(^-\) compounds were deconvoluted into their component Gaussian and Lorentzian peaks to summarize their near-edge features\(^9\).

The substantial variations in spectral features depending on the coordination environment of Cl allow proportions of inorganic Cl\(^-\) and aliphatic/aromatic organochlorine in natural samples to be ascertained via linear combination fitting of sample spectra. LC fitting of sample spectra was performed iteratively with a library of more than thirty Cl-containing compounds using WinXAS software\(^10\). Different combinations of aliphatic, aromatic, and inorganic standards may indicate variations in Cl speciation of up to 8-10\%, which we report as error in the quantitative measurements. Overall, the LC fitting procedure gives high-quality fits with low residuals\(^11\).

Beyond the near-edge oscillations in a Cl XANES spectrum, fluorescence intensity levels out, becoming independent of Cl speciation and directly proportional to Cl concentration in the sample. The absolute fluorescence intensity, or edge step, of the spectra provides the basis for total Cl concentration measurements. Calibration curves obtained from NaCl standards in an organic matrix allow the Cl concentration in samples with similar absorption properties to be deduced with high precision\(^11\). Briefly, the edge step of each XANES spectrum was measured as
the difference at 2820 eV between the two regression lines used for background subtraction and normalization. Edge steps for sample spectra were compared with a calibration line of edge steps for pellets of NaCl homogenized in a polyacrylate matrix to quantify total Cl concentration measurements for each sample. At these energies, X-ray penetration depth into the sample matrix is minimal (~40 μm), and we found no dependence of edge step on sample mass.

2.2. X-ray spectromicroscopy

X-ray spectromicroscopy was conducted using the NSLS-II Tender-Energy Spectroscopy endstation during its commissioning at NSLS beamline X15B. Beamline X15B collects synchrotron radiation from a bending-magnet source. The beam is vertically collimated and low-pass filtered by a Pt-coated mirror, energy is selected (or scanned) using a double-crystal Si(111) monochromator, and the beam is focused by a toroidal mirror to a secondary source aperture (SSA). Beam diverging from the SSA is then re-focused by a pair of mirrors in the Kirkpatrick-Baez geometry to a microfocus at the sample. This compound focusing geometry delivers at least $10^{11}$ photons/s to the sample.

Dried sediment trap particulates were fixed onto X-ray-clean polyfilm with petrolatum, and samples were mounted in a sample chamber purged with He. For imaging, each sample was raster-scanned in a rapid, continuous-motion mode while exposed to the stationary microfocused X-ray beam. Fluorescence data were collected using a Canberra Ultra-Low-Energy Ge detector and a Vortex Si detector, and individual fluorescence peaks were isolated from the detector spectrum. The fluorescence signal was normalized to incident beam intensity as measured using a windowless ionization chamber. Element-specific XRF maps were collected with a 10 x 20 μm$^2$ spot size and a 20-μm pixel size, with a dwell time of 2 s per pixel.
Samples were mapped at low energy (just above the Cl K-edge) to image Si and Cl, then re-mapped at higher energy (above the Ca K-edge) to image Ca and Cl. Then, using the same microfocused beam, Cl K-edge XANES spectra were collected at selected points on the image. Beam-induced dechlorination was potentially an issue with the intense microfocused beam, so XANES spectra were also measured in a rapid continuous-scanning mode. This provided 10 to 50 scans for each spot, taking only 19 s per scan; comparison of the first few scans to the last few revealed no discernible changes.

Micro-XRF maps were processed using Interactive Data Language (IDL) visualization software. Micro-XANES spectra were processed using Athena software as described above for the bulk XANES spectra.

3. References


Figure S1. Normalized Cl K-edge XANES spectra of representative Cl-containing model compounds. For additional Cl standard spectra calibrated to these edge energies, refer to Reina et al., 2004.

- $x = 2820.6$ eV (aliphatic C-Cl)
- $x = 2821.2$ eV (aromatic C-Cl)
Figure S2. Aliphatic and aromatic organochlorine concentrations in the 26 Arabian Sea POM samples examined, showing no correlation with depth of sediment trap in the water column.
Figure S3. Scatterplots associated with μ-XRF images from Fig. 2. Fluorescence counts for Cl are plotted against those for Ca (a-b) and Si (c), with each pixel in the μ-XRF map contributing one point in the scatterplot. These plots indicate no correlation between Cl and Si, and no strong correlation between Cl and Ca. Lower-signal datapoints are dominated by a small non-zero fluorescence background, which may deceptively suggest a Cl-Ca correlation in plots a-b. Higher-Cl datapoints (outside the shaded box in b) exhibit a broad range of Ca concentrations and no overall correlation (although certain subsets of Cl pixels may correlate with Ca).
Figure S4. Normalized Cl K-edge XANES spectra of rinsed phytoplankton membranes, with Cl standards at bottom for comparison. Vertical line intersects the x-axis at 2820.6 eV, the characteristic absorption maximum of aliphatic organochlorine, represented here by 1-chloro-2-propanol. Concentrations of aliphatic, aromatic, and inorganic Cl species tabulated at right. Spectral noise is indicative of low Cl concentrations in *Phaeodactylum* and *Tetraselmis* sp.; Cl concentrations in these samples fall close to the detection limit of approx. 1 mg·kg⁻¹ and are reported as approximates. Cl was undetectable in whole cells of another alga, *Nannochloropsis* sp. Errors reflect uncertainties distinguishing aliphatic and aromatic forms of organochlorine through linear combination fitting.

<table>
<thead>
<tr>
<th>Organism</th>
<th>[aliph Clorg] (mg·kg⁻¹)</th>
<th>[arom Clorg] (mg·kg⁻¹)</th>
<th>[inorg Cl⁻] (mg·kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pavlova</em> sp.</td>
<td>52 ± 4</td>
<td>4 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td><em>Phaeodactylum</em> sp.</td>
<td>~2</td>
<td>~0</td>
<td>~1</td>
</tr>
<tr>
<td><em>Tetraselmis</em> sp.</td>
<td>~1</td>
<td>~0</td>
<td>~0</td>
</tr>
<tr>
<td><em>Emiliana huxleyi</em></td>
<td>670 ± 54</td>
<td>~0</td>
<td>820 ± 65</td>
</tr>
</tbody>
</table>
Figure S5. Unnormalized Cl K-edge XANES spectra showing organo-chlorine enrichment from 56 ± 5 to 307 ± 25 mg·kg⁻¹ in *Pavlova* sp. membranes upon treatment with 20 mM H₂O₂ in artificial seawater for 100 min. Vertical line intersects the x-axis at 2820.6 eV, the characteristic absorption maximum of aliphatic organochlorine.
Figure S6. Normalized Cl K-edge XANES spectra of fatty acid chlorohydrin models (1-chloro-2-propanol and chlorinated oleic acid) juxtaposed with spectra of treated algal particulates. Vertical line intersects the x-axis at 2820.6 eV, the characteristic absorption maximum of aliphatic organochlorine, represented here by 1-chloro-2-propanol.
Figure S7. Normalized Cl K-edge XANES spectra of *E. huxleyi* before and after cell lysing and rinsing, with inorganic, aliphatic, and aromatic Cl model compounds for comparison.

- $x = 2820.6$ eV (aliphatic C-Cl)
- $x = 2821.2$ eV (aromatic C-Cl)