

*X-ray fluorescence microscopy allows geochemists to map the distributions of many different elements simultaneously in chemically heterogeneous natural samples. This is an X-ray fluorescence micrograph of a 5 x 6 mm<sup>2</sup> section of a degraded white oak leaf taken from the forest floor in the Pine Barrens, New Jersey. Calcium is highlighted in green, manganese in red, and chlorine in blue. (Lighter color corresponds to more intense fluorescence, i.e. greater concentration.) Manganese appears concentrated in the large veins and in a more diffuse background concentration throughout the leaf tissue. Calcium is localized primarily in the smaller leaf veins. Chlorine occurs in particularly high concentration in the center of the image, near the delta formed by the leaf veins. Optical microscopy revealed that the area of high chlorine concentration coincided with the presence of a mass of fungi colonizing the leaf surface, implicating the fungi as important players in the natural chlorination of decaying plant material. Special thanks to Matthew Marcus, Lawrence Berkeley National Laboratory, Advanced Light Source, Berkeley, CA.*

## **Insights into natural chlorination processes in decaying plant material**

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Recent research has revealed extensive natural production of organochlorine ( $\text{Cl}_{\text{org}}$ ) compounds, shattering the widespread assumption that  $\text{Cl}_{\text{org}}$  in the environment derived solely from anthropogenic sources. The appearance of natural  $\text{Cl}_{\text{org}}$  in soils has been linked to the decomposition of plant litter.<sup>1,2</sup> Although laboratory studies have demonstrated that both enzymatic and abiotic processes are feasible modes of  $\text{Cl}_{\text{org}}$  production under environmental conditions,<sup>3,4</sup> the dominant mechanisms of  $\text{Cl}_{\text{org}}$  formation in the environment remain undefined. Robust measurements of  $\text{Cl}_{\text{org}}$  fluxes in soil systems will help complete the description of the natural Cl cycle, potentially illuminating the sources and sinks of naturally and industrially produced  $\text{Cl}_{\text{org}}$  in the environment.

To probe Cl dynamics in natural systems, we have developed analytical methods based on synchrotron X-ray spectroscopy. Our X-ray studies indicate that  $\text{Cl}_{\text{inorg}}$  and soluble aromatic  $\text{Cl}_{\text{org}}$  molecules leach from plant material during initial weathering stages, leaving refractory aliphatic  $\text{Cl}_{\text{org}}$  in the solid phase that persists through further degradation. Contact with soil microorganisms promotes production of additional, stable aromatic  $\text{Cl}_{\text{org}}$  in leaf tissue.

X-ray absorption spectroscopy is highly sensitive to the bonding state of Cl, allowing distinctions to be drawn between  $\text{Cl}_{\text{inorg}}$  and  $\text{Cl}_{\text{org}}$  in heterogeneous natural samples without subjecting them to strenuous isolation procedures. The dramatic increase in X-ray absorption around 2,822 eV (Cl K-absorption edge) and the surrounding spectral features correspond to electronic transitions from the Cl-1s shell to unoccupied molecular and atomic orbitals.  $\text{Cl}_{\text{org}}$  compounds display discrete absorption maxima corresponding to 1s to  $\pi^*$  or  $\sigma^*$  electronic transitions that vary in energy depending on C-Cl bond length (Figure 1a-b), while aqueous  $\text{Cl}_{\text{inorg}}$  exhibits broader spectral features at higher energy (Figure 1c). We have also applied X-ray absorption spectroscopy to the *in situ* determination of bulk  $\text{Cl}_{\text{org}}$  concentrations in environmental samples (Figure 2).<sup>5</sup> Previously established quantification methods for natural  $\text{Cl}_{\text{org}}$  require chemical isolation of organohalogen fractions and are prone to partial recoveries and/or chemical alterations of halogens.

When examined with X-ray spectroscopy, unweathered senescent white oak leaves exhibit characteristic  $\text{Cl}_{\text{inorg}}$  speciation (Figure 1d), which, after a month or more of decay on the forest floor, changes to reveal aliphatic and aromatic  $\text{Cl}_{\text{org}}$  along with some residual  $\text{Cl}_{\text{inorg}}$  (Figure 1e). Our studies revealed three major Cl fractions in senescent oak and maple leaves after abscission: soluble inorganic chloride and aromatic  $\text{Cl}_{\text{org}}$ , ~75% of which is leached from the leaf tissue within the first 50 days of weathering, and refractory aliphatic  $\text{Cl}_{\text{org}}$ , the concentration of which remains steady with decay time. Contact of plant material with soil microorganisms further increases the stable aromatic  $\text{Cl}_{\text{org}}$  concentration in decaying leaf tissue. We are currently monitoring these variations.

Micro-X-ray imaging of weathered oak leaf surfaces reveals micron-scale heterogeneities in Cl speciation that provide clues as to the different pools of  $\text{Cl}_{\text{org}}$  in weathering plant material. For example, Cl appears at highest concentration in localized “hotspots” several hundred square microns in area in the micro-X-ray fluorescence map of a weathered chestnut oak leaf from the topmost mulch layer (estimated weathering time of approximately five months) (Figure 3A). These Cl hotspots are scattered among diffuse areas of relatively low Cl concentration in the bulk of the leaf tissue. Cl hotspots similar to these in size and fluorescence intensity are observed in oak leaves at all weathering stages, although they are far less common in unweathered leaves harvested from trees, in which Cl appears more in diffuse areas of low to moderate Cl intensity.

Most Cl hotspots in this sample produced identical aromatic Cl<sub>org</sub> X-ray spectra (Figure 3B). The well-defined structural features in these spectra correspond to a polychlorinated aromatic ring. A small fraction of the Cl hotspots displayed strongly aliphatic Cl<sub>org</sub> features, and diffuse areas of relatively low Cl concentration exhibited a combination of Cl<sub>inorg</sub> and aliphatic Cl<sub>org</sub> (Figure 3B). Aromatic Cl<sub>org</sub> hotspots were often found to coincide with Fe or Mn hotspots in weathered oak leaf samples. The presence of fungi was also noted at these locations on weathered leaf surfaces. Correlations between Cl<sub>org</sub> and metals are potentially quite relevant to our interest in natural chlorination mechanisms, because metals such as Fe are key cofactors in enzymes that catalyze chlorination reactions.

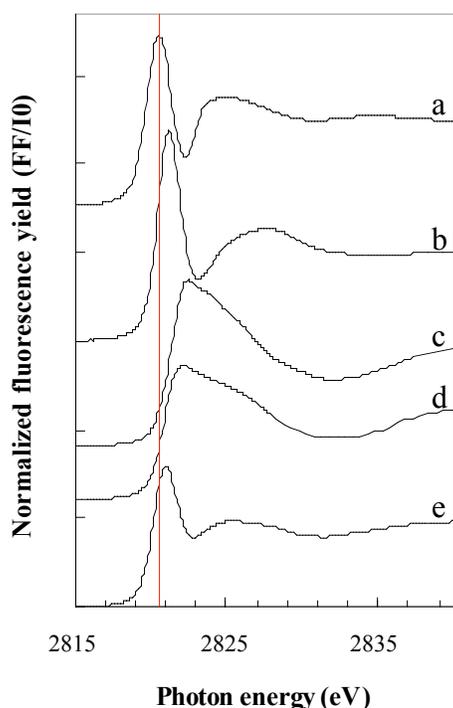
Detailed spectromicroscopic analyses of numerous oak leaf samples at different weathering stages enabled us to identify several distinct Cl fractions: 1) concentrated solid phase Cl<sub>inorg</sub> and H-bonded Cl<sub>inorg</sub> in tree-harvested oak leaves that diminish in initial weathering stages; 2) soluble aromatic and aliphatic Cl<sub>org</sub> in oak leaves during initial weathering stages; 3) insoluble, concentrated aromatic Cl<sub>org</sub> in oak leaves at advanced weathering stages; and 4) low background concentrations of Cl<sub>inorg</sub> and aliphatic Cl<sub>org</sub> that persist through advanced weathering stages. These micron-scale observations complement the bulk quantitative results outlined above, providing new insight into the mechanisms of natural chlorination in soils.

#### **Funding:**

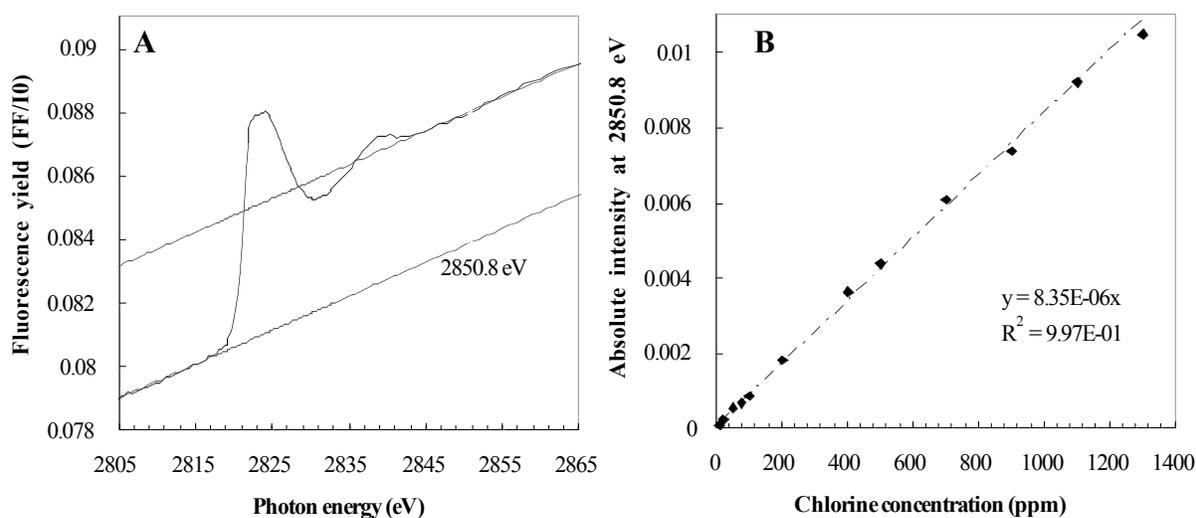
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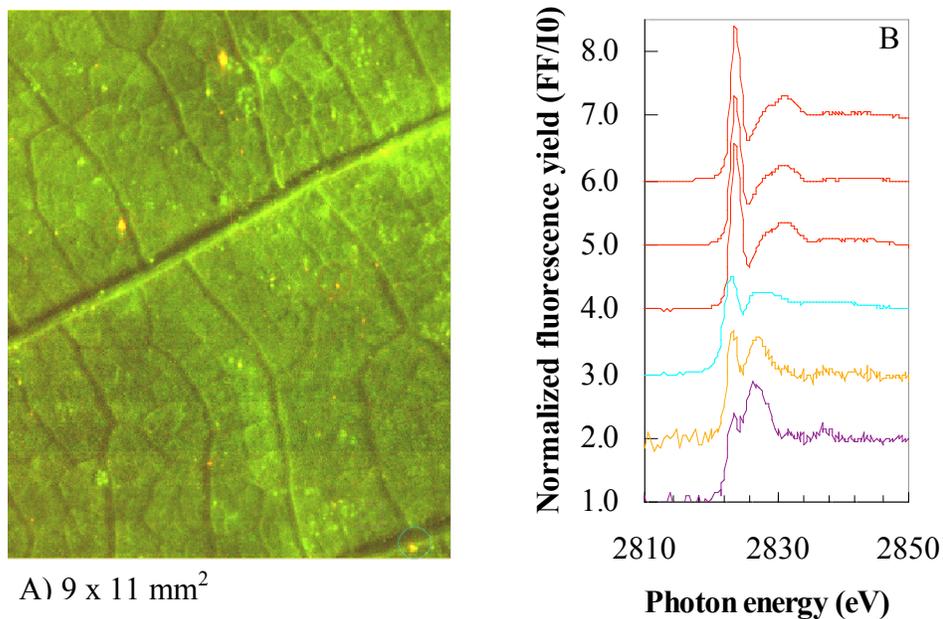
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**Figure 1.** Cl K-edge X-ray absorption spectra of model compounds and field samples: a) chlorodecane; b) chlorophenol red; c) glycine-HCl; d) unweathered senescent white oak leaves; e) effects on d after >1 year weathering on soil surface. Vertical line at 2820.6 eV intersects aliphatic  $Cl_{org}$  absorption maximum.



**Figure 2.** Quantification of Cl concentration from Cl K-edge X-ray absorption spectra. A) Unnormalized Cl K-edge X-ray absorption spectrum of NaCl standard in polyacrylic acid (PAA) matrix (200 ppm Cl). The difference at 2850.8 eV between splines through the pre-edge and post-edge regions is used as a measure of absolute Cl fluorescence intensity. By this energy, near-edge oscillations are attenuated and absorption intensity is independent of Cl speciation. B) Relationship between Cl concentration in PAA-based NaCl standards and absolute Cl fluorescence intensity. X-ray beam absorption and scattering by PAA-based standards and natural organic material are comparable, allowing for direct measurement of total Cl concentrations in natural samples. Percentage estimates of  $Cl_{inorg}$  and aliphatic and aromatic  $Cl_{org}$  in natural samples are ascertained via least-squares fitting of normalized sample spectra with spectra of representative model compounds (such as those in Figure 1a-c). Combination of these proportions with total Cl concentrations yields the concentrations of  $Cl_{inorg}$ , aromatic  $Cl_{org}$ , and aliphatic  $Cl_{org}$  in natural samples.



**Figure 3.** A) X-ray fluorescence map of a weathered chestnut oak leaf from the forest floor mulch (estimated weathering time of approximately 5 months). Green = S; red = Cl. Lighter color corresponds to greater fluorescence intensity, i.e., greater elemental concentration. B) Cl K-edge X-ray absorption spectra corresponding to the circled/boxed areas in A. Colors of circles/boxes in A match colors of associated spectra. Red spectra = aromatic Cl<sub>org</sub>; turquoise spectrum = aliphatic Cl<sub>org</sub>; orange/violet spectra = mixed aliphatic Cl<sub>org</sub>/Cl<sub>inorg</sub>.