Formation of Stable Chlorinated Hydrocarbons in Weathering Plant Material

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Though several chlorinated organic compounds produced by humans are carcinogenic and toxic, some are also produced by the biotic and abiotic processes in the environment. In situ x-ray spectroscopy data indicate that natural organic matter in soils, sediments, and natural waters contain stable, less volatile organic compounds with chlorinated phenolic and aliphatic groups as the principal Cl forms. These compounds are formed at rapid rates from the transformation of inorganic Cl during humification of plant material and, thus, play a critical role in the cycling of Cl and of several major and trace elements in the environment and may influence human health.

Organo-Cl compounds have been widely used for years in agricultural and in several industrial applications, and recent investigations showed that they persist in the environment (1). Several living organisms, such as phytoplankton, wood-rotting fungi, microorganisms, and plants, produce certain enzymes (e.g., chloroperoxidase) that can convert Cl^- into organo-Cl compounds for use in cell adhesion and in defense processes (2–6). Researchers have identified several natural small-chain volatile chlorinated alkanes, alkenes, and aromatics in soil and marine systems at trace concentrations, but there is little direct evidence for persistent and less volatile organo-Cl compounds. The biogeochemical processes that lead to their formation are also poorly understood. Here I provide evidence for the existence of different forms of chlorinated organics and their formation in natural systems and show that organo-Cl compounds constitute the major Cl fraction in weathering plant material of soils.

Cl in humic substances isolated from different sources. To understand the biogeochemical reactions of Cl in the environment, I examined the chemical forms of Cl in fresh, senescent, and humified plant material (leaf, stem, bark, rootlet) of different plant species and soils collected from redwood (Big Basin Redwood State Park, California) and pine forests (Lebanon State Forest, New Jersey; Pine Forest, Corozal, Puerto Rico). These sampling sites have not been disturbed by recent anthropogenic activity, and most Cl found at these sites is of natural origin. In addition, I examined the chemical forms of Cl in humic substances isolated from soil, peat,

lignite (Leonardite), and river waters of several sites and in sediments from a dry valley (Lake Fryxell) in Antarctica. These humics were isolated at acidic and alkaline pH and are referred to as the humic and fulvic acids, respectively (7). The forms of Cl in all these samples were determined with the use of near-edge x-ray absorption fine structure (NEXAFS) spectroscopy and Extended x-ray Absorption Fine Structure Spectroscopy (EXAFS) (8) at the Stanford Synchrotron Radiation Laboratory (SSRL) (9). The Cl absorption edge and its NEXAFS spectral features are sensitive to the Cl redox state and coordination environment (Fig. 1). Free and metal-complexed inorganic Cl and different organo-Cl compounds have distinct spectral features, which are used to distinguish them in heterogeneous natural organic molecules without subjecting them

to any chemical treatment. For example, the absorption edges of Cl (absorption maximum) connected to aliphatic and aromatic carbon and inorganic Cl^- are at 2820.2 ($\pm$ 0.3), 2821.1 ($\pm$ 0.1), 2822.3 eV, respectively [(10–12); details in (11)]. The chemical forms of Cl and their relative concentrations in these samples were identified using least-squares fitting of sample spectra with those of different synthetic Cl compounds for which the coordination chemistry of Cl is known (10).

When compared with the structural models, all of the isolated humic materials (except for the lignitic humics) exhibit an intense absorption edge at $\sim$ 2821.0 ($\pm$ 0.3) eV (Fig. 2). The resemblance of all the spectra indicates that the forms of Cl in high-molecular weight humic and low-molecular weight fulvic acid fractions are similar. Their analyses indicate that these humics primarily contain aromatic organo-Cl, with <30% aliphatic-Cl. The exceptions are that in the lignitic (Fig. 2H) and peat (Fig. 2G) humic substances, Cl^- was the dominant form, and that in the Lake Fryxell fulvic acid, the aliphatic-Cl was the dominant form (Fig. 2E). These differences may have been caused by the procedures used to isolate the humics, the coalification of organic molecules, and the dissimilarity in parental material of humics. The humic acid from lignite exhibits a broad absorption edge at 2824 eV with a pre-edge feature at 2817.4 eV, which indicates that this sample primarily contains free and metal-complexed Cl^- (up to 24% of total Cl^- is present as Fe^{3+} complexes). Although the presence of polychlorinated aliphatic compounds can produce such low-energy features, this pre-edge is too low to indicate such a coordination environment (Fig. 1) (13).
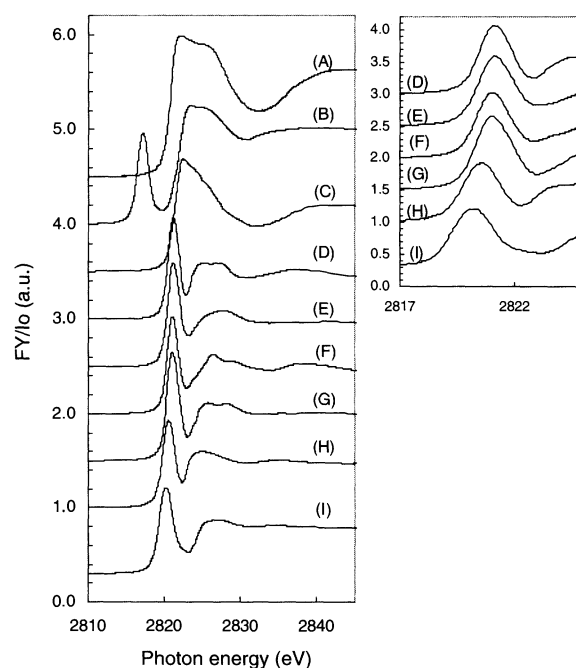

Fig. 1. Cl K-edge NEXAFS spectra of Cl in different coordination environments in inorganic and organic molecules. The inset shows an enlargement of the absorption edge of organo-Cl compounds. **(A)** Aqueous Cl^-, **(B)** solid $FeCl_3 \cdot 6H_2O$, **(C)** solid glycine·HCl, **(D)** monochlorodimedone, **(E)** chlorophenol red, **(F)** 2-chlorobenzoic acid, **(G)** tetrachlorophenol, **(H)** chlorodecane, and **(I)** trichloroacetic acid.

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Soil organic molecules not isolated from their surrounding mineral matrices also showed spectral features similar to the isolated soil humic materials (Fig. 3). Finely powdered soil from pine plantations (organic matter ~ 1.5%) exhibited a broad absorption edge, with a sharp low-energy shoulder at ~ 2821 eV. The spectral fits indicate that these samples primarily contain Cl⁻ and minor amounts of organo-Cl (Fig. 3F). The powdered sample represents a homogeneous mixture of soil minerals and organics of a ~12-cm-deep soil core from the pine Ultisol. However, topsoil (Fig. 3E), soil aggregates rich in organic molecules (Fig. 3G), and the soil plant remains [humified needles and pieces of bark, trunk, and rootlets (Fig. 3H)] exhibit an intense peak corresponding to the organo-Cl compounds. The ratio of aliphatic- to aromatic-Cl content of the soil aggregates ranged from <1 to 1.3, and that of humified plant remains was well below 1 (Figs. 3 and 4). The differences in the spectral features of powdered soil (Fig. 3F) and the organic-rich aggregates (Fig. 3G) indicate that organo-Cl is associated preferentially with the organic fraction rather than the inorganic mineral oxides. Chloride showed the opposite trend. Topsoil collected adjacent to eucalyptus, redwood, and maple trees also showed organo-Cl as the dominant fraction of Cl (spectra not shown).

Cl in living and weathered plants. In contrast to the weathered plant remains of soils and the isolated humics, the living tissue of leaves and stems and the woody parts of different plant species exhibited Cl⁻ only (e.g., Fig. 3A). The Cl⁻ in these samples can exist as free aqueous species [referred to as hydrated Cl⁻ (14)], H-bonded to organic molecule functional groups [referred to as H-bonded Cl⁻ (14)], and complexed to metal atoms. However, the spectral analyses of fresh leaves or needles indicate that hydrated and H-bonded Cl⁻ are the primary forms of Cl⁻, with the concentration of the H-bonded Cl⁻ in the range of 8 to 52% (Figs. 1 and 3). Metal-complexed Cl⁻ and organo-Cl may exist in these samples, but at a concentration below the detection of x-ray spectroscopy. In contrast, the senescent plant leaves made visible different Cl species. The dominant forms are as follows: (i) hydrated Cl⁻ in young and partly yellow colored senescent leaves, (ii) H-bonded Cl⁻ in reddish brown senescent leaves connected to plant, and (iii) organo-Cl in reddish brown senescent and humified leaves on soil. The concentration of H-bonded Cl⁻ increased as the leaves became senescent and the ratio of H-bonded to hydrated Cl⁻ increased from <1 to ~ 4.0. These changes may be caused by the dehydration of plant material associated with senescence (15). The senescent and humified leaves collected from the leaf litter showed aliphatic and aromatic Cl as the dominant forms, respectively (Figs. 3 and 4). The total Cl⁻ con-

tent of the humified leaves was less than 30% of the total Cl in the sample. Among the plant samples, only weathered bark surfaces (Fig. 3D) and senescent leaves from the soil surface (Fig. 3B) exhibited a relatively high concentration of aliphatic organo-Cl.

Transformation of Cl during plant weathering. The results presented here document that organo-Cl compounds are the dominant forms of Cl in the organic fraction of soils, sediments, and aquatic systems and in humified organics of all examined plant samples (Fig. 4). Combinations of chlorinated phenols and mono- and dichlorinated aliphatic compounds produced the best spectral fits for the organo-Cl fraction in humics and weathered plant materials; polychlorinated acetic acid, alkanes and phenols, and mono-chlorinated cyclic compounds resulted in poor fits. However, the occurrence of these latter compounds may not be ruled out. They may exist at trace concentrations. These results are in direct contrast to the previous ex situ studies on leaf litter (16). On the basis of the spectral intensity, the concentration of organo-Cl compounds in humified plant material is estimated to be <10 mM Kg⁻¹ (common range ~0.5 to 2 mM Kg⁻¹), and the concentration varied with plant species, and the type of plant material (e.g., leaf or bark, versus trunk). My results show that aromatic organo-Cl compounds are the most abundant

forms of Cl in the weathered plant material and that their concentration increased with humification (Fig. 4). The concentration of aliphatic Cl remained constant or started to decline relative to the aromatic Cl as the humification of plant material continued. When compared with the Cl forms in humified plant material, the live plant tissue of plants has hydrated and H-bonded Cl⁻ without any detectable chlorinated organics (Figs. 3 and 4). However, their occurrence at increasing concentrations in the recently fallen plant leaves and in humified leaves in the leaf litter suggests that Cl⁻ of living plant tissue is rapidly converted to organo-Cl. To evaluate their rates of formation, I examined forms of Cl in the leaves of *Liquidambar* sp. collected in different seasons. The samples of green leaves collected before the Fall of 2000 showed Cl⁻ as the primary Cl form. The sampling at the end of following Winter showed primarily H-bonded Cl⁻ in reddish brown senescent leaves connected to the plant and organo-Cl and Cl⁻ in senescent leaves accumulated on the soil surface. These studies suggest that halogenation of organic molecules was pronounced in samples collected from the soil surface and that the transformations began within 6 months after the
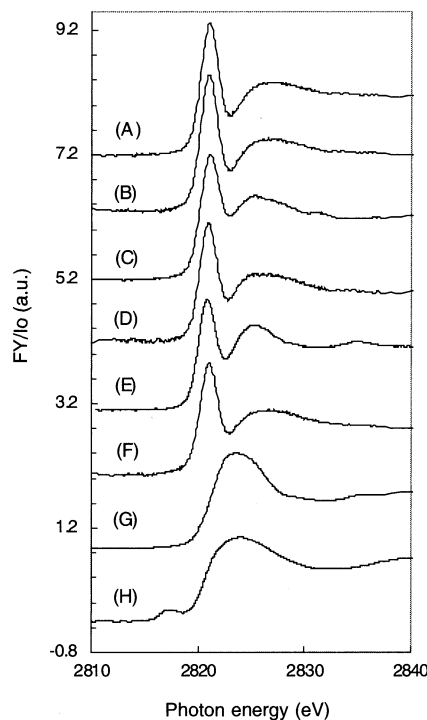
Fig. 2. Cl K-edge NEXAFS spectra of humic substances (HA, humic acid HA; FA, fulvic acid) isolated from soil, river water, peat, and lignite. (**A**) Suwannee River FA, (**B**) Suwannee River HA, (**C**) soil FA, (**D**) soil HA, (**E**) Lake Fryxell FA, (**F**) peat HA, (**G**) peat FA, and (**H**) Leonardite HA.
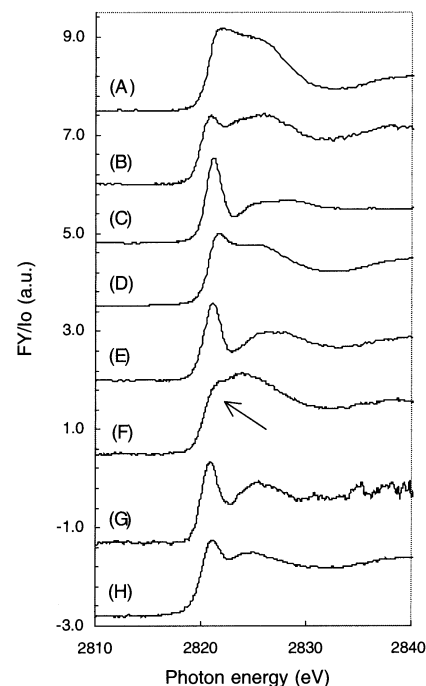
Fig. 3. Cl K-edge NEXAFS spectra of live, senescent, and highly humified plant material (pine forest samples). (**A**) Green pine needles, (**B**) senescent reddish brown pine needles attached to the tree, (**C**) senescent leaves from soil surface, (**D**) moss collected from pine tree bark surface, (**E**) top soil in the vicinity of a pine tree, (**F**) finely powdered pine Ultisol, (**G**) organic-rich soil aggregate near pine tree, and (**H**) plant remains (needles, rootlets, wood) of pine Ultisol. The arrow indicates the sharp low-energy shoulder in (**F**).

leaves became senescent. These results may indicate that soil fauna and flora influence halogenation reactions.

Environmental significance. Though several Cl metabolites produced by bacteria, fungi, and phytoplankton that can halogenate organic substrates have been identified from soils and oceans, only haloperoxidases were isolated and studied extensively for their role in organohalogenation reactions (*17*). However, their reaction products and the geochemical conditions conducive to the chlorination reactions are not yet well understood. Although the concentrations are smaller, recent studies have also shown that Fe-oxyhydroxides commonly found in soils and sediments can catalyze the reactions of Cl⁻ and organic substrates and form aliphatic volatile organo-Cl compounds (*18*). The low volatility and high biological stability of organo-Cl forms in humics isolated from different sources suggest that they are part of large biomacromolecules in weathered plant material, such as lignins and tannins. On the basis of the Cl forms found in plant material, I propose that halogenation of organic molecules in plants begins with the dehydration of Cl⁻ and the simultaneous formation of H-bonds with the organic molecule functional groups (*14*). The later steps involved in chlorination reactions may be coupled to the oxidation and humification of fresh organic matter. These organohalogenation reactions may also be one of the critical biochemical reactions involved in humifi-
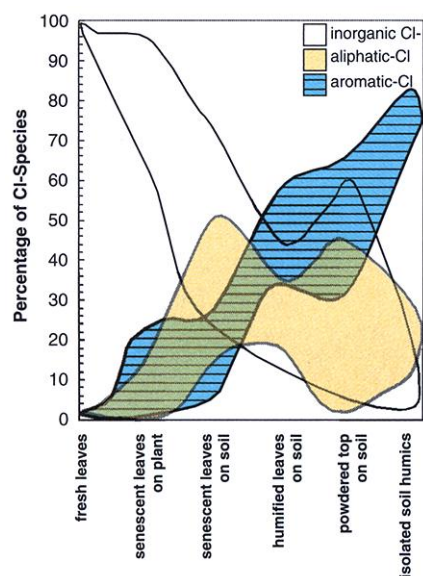
cation reactions in nature. Occurrence of organo-Cl compounds as the major Cl fraction in humic substances derived from simple organisms, such as phytoplankton and bacteria (Lake Fryxell fulvic acid from Antarctica) and lignin-containing higher plants (e.g., Suwannee River, peat, and soil humics), also suggests that chlorination of organic molecules is universal irrespective of the organic substrate sources and the geographic location. However, differences in chlorination reactions and their rates, as well as the chemistry of organic substrates, may lead to the observed variations in the relative concentration of different aliphatic and aromatic Cl compounds (e.g., Fryxell fulvic acid and humics derived from plants). The stability and decay of these naturally formed organo-Cl compounds is still unknown.

Several organo-Cl compounds are highly toxic and carcinogenic, and the bioavailability of the naturally produced organo-Cl compounds and their influence on human health needs further evaluation. Although several organohalogens found in soils and sediments are attributed to the anthropogenic sources, their formation by natural biogeochemical processes should be considered while the sources of halocarbons in the environment are assessed. The high sensitivity of organo-Cl compounds to light and their electron-accepting ability can also significantly influence the photolytic and redox reactions of several elements found in the surface waters of lakes and oceans and in anaerobic sediments and soils. The recalcitrant nature (its stability against biological attack) of the chlorinated derivatives of different organic molecules and their complexes (e.g., biphenyls) to biotic and abiotic transformations (*19*) suggests that halogenation of natural organic molecules may play a critical role in the stability and the storage of different types of C and other biologically important elements in soils and sediments. Although the reactions of several chemical species in the atmosphere, such as ozone, are attributed to the reactions of volatile aliphatic organohalogens (e.g., chlorofluorocarbons) (*20*), the dust storms in the tropics can contribute large quantities of soil particles consisting of humic materials and associated organo-Cl to the total halocarbons in the atmosphere and can facilitate the heterogeneous reactions of the atmospheric constituents (*21*). Improved understanding of the molecular chemistry of these naturally produced organohalogens and their concentrations and rates of formation in natural systems will help in the assessment of their toxicity and their role in the biogeochemical reactions of major and trace elements and Cl cycle in the environment.

Fig. 4. Variations in inorganic Cl⁻ and organo-Cl compounds with humification of plant material in soils. The concentration of each chemical species is shown as a region, with its center and edges for any given sample corresponding to the average and the range of concentrations, respectively. The wide variations in the concentration ranges of different types of samples may be caused by the variations in the chlorination processes and related fauna and flora, and the extent of humification.

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7. Fluvial, soil, peat, and lignitic humic and fulvic acids were isolated and characterized by the International Humic Substance Society. The Lake Fryxell fulvic acid was isolated from Lake Fryxell, Antarctica, and characterized by G. Aiken (U.S. Geological Survey) and Y. Chin (Ohio State Univ.).
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9. Supplementary details of experimental procedures are available at *Science* Online at www.sciencemag.org/cgi/content/full/1067153/DC1.
10. Details of model compounds examined in this study are available at *Science* Online at www.sciencemag.org/cgi/content/full/1067153/DC1.
11. X-ray absorption spectroscopy of Cl and its electronic transitions are discussed in the supplemental section available at *Science* Online at www.sciencemag.org/cgi/content/full/1067153/DC1.
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14. The energy of absorption edge is sensitive to the redox state of Cl (*12*), and the features adjacent to the edge are sensitive to the local coordination around Cl, which includes variations in H-bonding environment. The Cl⁻ ion in aqueous solutions and in crystalline amino acids, such as glycine·HCl and Trizma·HCl, exhibit identical absorption edge energies, but with significant differences in spectral features in the vicinity of absorption edge (Fig. 1). These spectral features suggest that Cl⁻ ion exhibits different H-bonding environment in these samples, e.g., Cl⁻ H-bonded to H₂O in aqueous solutions and the organic molecule functional groups in amino acids. The Cl-EXAFS analysis of these samples indicate that the atoms in the first solvation shell of Cl⁻ are similar at 2.6 (± 0.2) Å with significant differences for the atoms in the second solvation shell. These bond distances are typical of H-bonded Cl rather than C-bonded Cl, which exhibits bond lengths of <1.78 Å (e.g., Cl-benzoic acid, Cl-decane). Chloride in senescent plant leaves and lichen on bark surfaces showed NEXAFS spectral features similar to that of glycine·HCl. Although Cl-EXAFS of leaf samples were not collected because of low Cl⁻ concentration, the closeness of the NEXAFS spectral features of lichen, senescent leaves, and glycine·HCl suggest that the coordination environment of Cl in these samples may be similar, indicating that Cl⁻ ion exhibits a similar structural environment in these samples.
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