other localities: Rhynie, Scotland, and Alken an der Mosel, West Germany (*2*). Paleoecological data on these two sites and on Gilboa are summarized in Table 1. Modes of preservation differ. The Alken fossils appear as carbonized films in a fine-grained black shale, though some of the eurypterids are represented by thicker pieces of carbonized cuticle. These classic fossils, although well preserved, lack detail. The Rhynie fossils are found embedded in a brittle, glassy chert, thoroughly mixed with silicified plant material. The preservation is excellent but the fossils have not been removed from the matrix, and most of them have been discovered accidentally while chipping specimens for studies of plants. The Gilboa fossils are the youngest of the three assemblages, but their unusual mode of preservation provides more detail than the others and the fauna appears to be richer in undoubted terrestrial animals.

Devonian paleogeography has been variously reconstructed (*17*). One interpretation (*18*) shows a continental mass composed of the present North America and northern Europe (Laurussia), located with the equator variously situated across either its northern or southern quarter, and transgressed by extensive epicontinental seas. Reconstructions of the paleoclimate and lithofacies of western Laurussia (*19*) suggest that during much of the Devonian, the three known localities for terrestrial arthropods were near the equator and on either side of a range of mountains (the Acadians) from which extensive sediments weathered to build the massive noncalcareous rocks of the Devonian system in this area.

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15. This does not affect the status of the collembolan *Rhyniella* from the Rhynie Chert of the Middle Devonian Eifelian Stage, preceding the Givetian of the Gilboan assemblage. Springtails, primitively wingless hexapods, together with Protura and Aptera form the sister group to the Insecta. The most generalized Insecta are the primitively wingless Archaeognatha. The earliest record of the Archaeognatha is in the Muscovian Stage of the Upper Carboniferous, where they appear as members of the specialized suborder Monura. The earliest record of Machilidae is in the Carnian of the Upper Triassic [W. Hennig, *Insect Phylogeny*, A. C. Pont, Transl. and Ed. (Wiley, New York, 1981), pp. 87–119].
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Trace Elements in Tree Rings: Evidence of Recent and Historical Air Pollution

Abstract. *Annual growth rings from short-leaf pine trees in the Great Smoky Mountains National Park show suppressed growth and increased iron content between 1863 and 1912, a period of smelting activity and large sulfur dioxide releases at Copperhill, Tennessee, 88 kilometers upwind. Similar growth suppression and increases of iron and other metals were found in rings formed in the past 20 to 25 years, a period when regional fossil fuel combustion emissions increased about 200 percent. Metals concentrations in phloem and cambium are high, but whether they exceed toxic thresholds for these tissues is not known.*

Our investigations with short-leaf pine (*Pinus echinata*) in East Tennessee have shown increasing concentrations of trace metals in annual growth rings since the 1950's and relatively high concentrations in the cambial area. For most elements, ring content was serially correlated with growth; however, since the 1970's metals content in rings increased while growth rate decreased. In the Great Smoky Mountains National Park (GSMNP) iron not only increased since the 1950's, as did regional increases in fossil fuel combustion emissions, but also between 1863 and 1912 when trees may have been exposed to SO_2 and combustion products from copper ore smelting at Copperhill, Tennessee. These observations suggest that multielement analysis of tree rings can provide information on temporal changes in air pollution, acid deposition, or both.

Tree rings have been used to construct records of climate (*1*), document heavy metal pollution (*2–4*), and study the relation between growth and air pollution (*5*). Now they are being used to examine the relation between growth and acid rain (*6*). Ulrich (*7*) proposed that acid precipitation increases concentrations of Al and Fe in the soil solution. If such an increase is reflected in the chemical composition of wood, then patterns of change of these and other metals in tree rings could be used to infer temporal changes in rain acidity and associated increased metals deposition from burning of fossil fuels in recent years (*8*). We looked for evidence of increased regional atmospheric pollution in eight hardwood and six coniferous species in East Tennessee.

Trees were sampled with increment corers at several sites near Oak Ridge, Tennessee, and in the GSMNP, and multielement analysis was performed by inductively coupled plasma optical emission spectroscopy (*9*). For most elements, analytical accuracy was at least 80 percent (*10*), but our recovery of Fe (46 percent) and Al (71 percent) from National Bureau of Standards (NBS) orchard and citrus leaves and pine needles was incomplete; however, analytical precision for all metals was very good. Because precision was good and because temporal patterns of trace metals in wood were very similar among trees of a stand, confidence in temporal patterns is high. However, based on the NBS standards, the metals concentrations that we report may underestimate actual levels.

At all sites, the highest trace metals concentrations were found in living phloem plus cambium tissues. Such concentrations of Al (200 to 690 ppm), Cd (0.47 to 7.5 ppm), Mn (150 to 450 ppm), and Zn (27 to 120 ppm) in aboveground tissues of agricultural or herbaceous plants are reported to be toxic (*11*), but whether such concentrations are toxic in these tree tissues is not known. At all sites short-leaf pine are now growing at 0.6 to 0.8 mm/year, compared to initial rates of 1.6 to 7.3 mm/year (Fig. 1). However, no statistically significant (*12*) correlations between current tree growth rate and trace metals concentrations in the phloem plus cambium were found. Whether metals concentrations of such magnitude in living tree tissues are potentially of concern is not yet clear.

The highest ring concentrations were found in the most recently formed rings, and a roughly twofold increase in Fe and Ti concentrations since the 1950's at Cades Cove in the GSMNP (Fig. 1) and similar increases of Al, Cu, Mn, and Zn at all sites since the 1970's was evident. Reconstruction of SO_2 emissions upwind of Cades Cove shows a roughly 200 percent increase since the 1950's (Fig. 1). We found no statistically significant site differences in concentrations of Al, Cd, Cu, Mn, and Zn in phloem plus cambium, but found significantly higher Cu (twofold), Fe (20-fold), and Zn (40-fold) in 1978 through 1983 rings 1.4 and 16 km downwind of the 1700 MW Kingston Steam Plant than at the other Oak Ridge or GSMNP sites. This latter observation and the similarity in SO_2 emissions and increases of Fe and Ti concentration at Cades Cove underscore the potential importance of fossil fuel combustion emissions as a source of trace metals in recently formed rings.

At Cades Cove a strong growth suppression occurred from about 1863 to 1912, with the lowest rate of 0.55 mm/year around 1895. The recent (post-1960's) growth declines have been observed elsewhere and are widespread in the Northeast (*13*), but the 1863–1912 decline in the GSMNP appears to be a local occurrence. This 50-year growth suppression was also observed in the other species sampled in the GSMNP but not in short-leaf pine (*14*) at Norris, Tennessee (Fig. 1), nor in any trees near Oak Ridge. Temporally, the decline is coincident with uncontrolled emissions of SO_2 and combustion products from smelting of ore containing Fe and Cu sulfides at Copperhill, Tennessee, which contributed to the destruction of all vegetation within 16 km of Copperhill (*15*). Prevailing surface winds entering the GSMNP during the growing season follow the northeast-tending valleys of the Appalachian Mountains from the Copper Basin to the GSMNP (*16*).

Periods of growth decline, both recent and historical, have often corresponded to increased concentrations of metals such as Fe and Ti (Fig. 1). Because of this relation and the variation in growth among sites, we factored out growth by multiplying elemental concentration and growth rate (microgram of element per gram of wood × gram of wood per year = microgram of element per year), yielding "xylem accumulation rates" of elements into ring segments (Fig. 2). This measure represents annual ring content or burden (total annual accumulation) and was useful in further analyzing historical patterns.

For some elements there were strong relationships between growth and xylem accumulation rate. For Ca, Mn, and Zn at Cades Cove, the correlations between the 1833 through 1983 xylem accumulation rate and growth were positive (r^2 = 0.85, 0.90, and 0.85, respectively); Cd and Cu had weaker positive correlations between the two (r^2 = 0.51 and 0.39, respectively); and for Al, the relationship was positive before 1953 (r^2 = 0.64) but not after 1953 (r^2 = 0.026). For Fe, Mo, and Ti, poor correlations between growth and accumulation rates (r^2 = 0.062, 0.28, and 0.0004, respectively) indicated either alternative influences

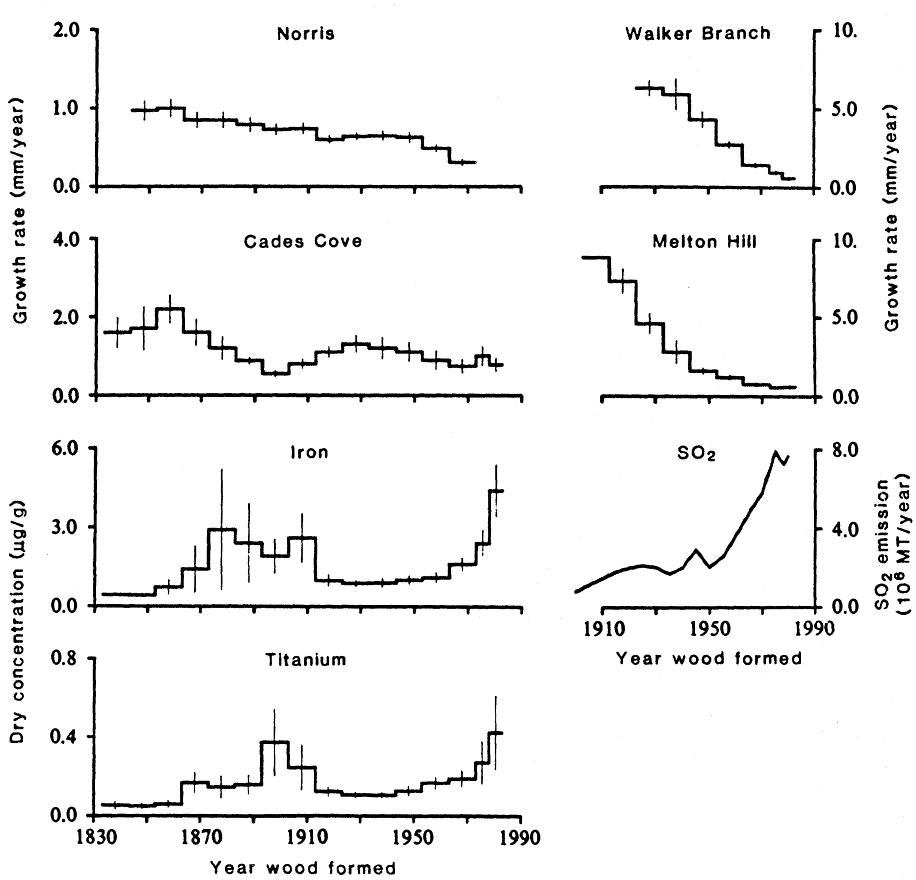
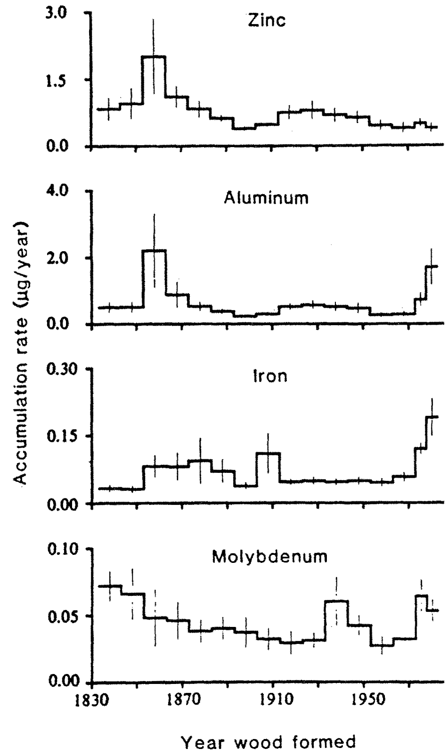


Fig. 1. Mean growth rate of short-leaf pine at four sites in East Tennessee, mean Fe and Ti concentrations in short-leaf pine at Cades Cove, Great Smoky Mountains National Park, and estimated SO_2 emissions from within 900 km southeast-southwest of Cades Cove (*8*). Error bars represent the standard error of the mean (of eight trees at Cades Cove, Walker Branch Watershed, and Melton Hill and ten trees at Norris, Tennessee); *MT*, metric tons.

Fig. 2. Mean xylem accumulation rates of four elements in short-leaf pine from Cades Cove, Great Smoky Mountains National Park. Error bars represent the standard error of the mean (of eight trees). Zinc shows a strong correlation with growth, as does Al until recent times, which may be indicative of a recent change in Al availability. Iron shows very poor correlation with growth but may be responsive to the influences of SO_2 from Copperhill. Molybdenum shows a pattern suggestive of translocation from younger to older rings.



on uptake or across-ring translocation.

At Cades Cove, an increased Fe xylem accumulation rate coincided with growth suppression during the Copperhill era and since the early 1950's. Similar increases occurred for Al and Cd since the 1960's and for Cu, Mo, and Ti since the 1970's. At the Oak Ridge sites, the same historical relation between the xylem accumulation rate and growth rate were observed, except the increases in xylem accumulation rate that are coincident with decreases in growth rate began after the 1970's for all elements, including Ca, Mn, and Zn. Such a change in the relation between growth and xylem accumulation rates parallels recent changes in availability of these trace metals to trees. For Al, Cd, Cu, and Zn, combustion sources have significantly added to natural sources in recent years, both in the Oak Ridge area (*17*) and regionally (*8*). For Fe, accumulation rate increases may reflect change in soil Fe availability, from acid deposition (*18*), because the Fe accumulation rate, unlike any other element, increased during the Copperhill era and since the late 1950's.

Interpretation of trace metals patterns in tree rings would be compromised by lateral translocation subsequent to initial incorporation in ring tissues. If there was translocation from older to younger rings, then older trees should have higher concentrations or xylem accumulation rates in recent rings than younger trees. Recent work with Virginia pine (*Pinus virginiana*) has shown the opposite (*19*). Robitaille (*4*) found no evidence of translocation of Cu and Zn in balsam fir (*Abies balsamea*) near a copper smelter in Eastern Canada. In our study, the serial correlations between growth and xylem accumulation rate argue against across-ring translocation of Al, Ca, Cd, Cu, Mn, and Zn. Translocation in years following formation of historical rings would smooth out year-to-year variability in trace-metals content, and any correlation between growth and xylem accumulation rate would likely be lost. Models of translocation from younger to older rings (*20*), however, suggest that the Mo xylem accumulation rate pattern at Cades Cove (Fig. 2) includes possible translocation.

In summary, the close temporal relation between Copperhill history and reduced tree growth and increased Fe xylem accumulation rate at Cades Cove provides strong inferential evidence that these trees were affected by the same smelting emissions that devastated Copperhill. Recent increases in xylem accumulation rates of many trace metals, despite declining tree growth, suggest recent regional-scale increases in trace metal availability, possibly from increased wet and dry deposition of fossil-fuel combustion products. Although the mechanisms and pathways of metals incorporation in tree rings need to be clarified, our study suggests that multielement analysis of tree rings would be useful in determining when changes in air pollution and acid rain began to occur and in geographically mapping their extent, thereby providing key evidence of the source or sources. High concentrations of phytotoxic metals in living bole tissues signal a need to investigate toxic thresholds for individual and combined effects of trace metals on forest growth and stability.

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10. Detection limits are ten times the standard deviation of background noise or roughly the following concentrations in wood (in micrograms per gram, dry weight): 0.6 Al, 1.5 Ca, 0.09 Cd, 0.32 Cu, 0.2 Fe, 0.01 Mn, 0.3 Mo, 0.4 Ti, and 0.2 Zn. Replicate analyses of NBS orchard leaves, citrus leaves, and pine needles indicate good precision, with coefficients of variation for Al, Cd, Cu, Fe, Mn, Mo, Ti, and Zn of 5.4, 22, 5.0, 10, 3.9, 19, 4.0, and 2.3, respectively; and fair accuracy with recoveries of 71, 99, 73, 46, 82, 100, 100, and 88 percent, respectively.
11. Toxicity or yield reductions in plants with aboveground dry weight tissue concentrations of > 150 ppm Zn [National Research Council, *Zinc* (University Park Press, Baltimore, 1979), 2 to 80 ppm Cd [L. E. Sommers, in *Sludge-Health Risks of Land Application* (Ann Arbor Science, Ann Arbor, Mich. 1980), p. 105], > 400 to 500 ppm Mn [L. P. Gough, H. T. Shacklette, A. A. Case, *U.S. Geol. Surv. Bull. 1466* (1979)], and > 200 to 325 ppm Al [G. J. Ouellette and L. Dessureaux, *Can. J. Plant Sci.* **38**, 206 (1958)].
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15. Between 1854 and 1908, approximately 88 km upwind of Cades Cove, the Copper Basin of southeastern Tennessee was developed. The first copper smelter in the Ducktown Mining District was built in 1854, and between 1866 and 1879 there was rapid development of the basin. In 1891 heap roasting of ore was initiated. The heap roasting process released large quantities of SO_2 at ground level which destroyed all vegetation in the basin. By 1902 blast furnaces replaced heap roasting, and SO_2 emissions were from stacks at higher elevations. By 1908 in response to the high demand for sulfuric acid and litigation in the U.S. Supreme Court by the attorney general of Georgia to reduce caustic emissions, sulfuric acid recovery plants were in

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17. There are five coal-fired power plants in the Oak Ridge area. Kingston burns 4.0×10^9 kg of coal per year; Bull Run, 2.3×10^9 kg/year; Y-12, 1.1×10^8 kg/year; K-25, 3.2×10^7 kg/year; and the ORNL power plant, 2.5×10^7 kg/year; the total in the Oak Ridge area is approximately 6.5×10^9 kg/year.
18. Iron is most available to plants in the ferrous form: uptake into blades of *Macrocystis* and *Phaseolus* roots and shoots has been shown to be dependent on the prereduction of Fe(III) to Fe(II); S. L. Manley, *Plant Physiol.* **68**, 914 (1981). Iron uptake is also enhanced by reduction of rhizosphere *p*H [A. N. Sarkar and R. G. Wyn Jones, *Plant Soil* **66**, 361 (1982)].
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Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and Pre-AIDS

Abstract. *A cell system was developed for the reproducible detection of human T-lymphotropic retroviruses (HTLV family) from patients with the acquired immunodeficiency syndrome (AIDS) or with signs or symptoms that frequently precede AIDS (pre-AIDS). The cells are specific clones from a permissive human neoplastic T-cell line. Some of the clones permanently grow and continuously produce large amounts of virus after infection with cytopathic (HTLV-III) variants of these viruses. One cytopathic effect of HTLV-III in this system is the arrangement of multiple nuclei in a characteristic ring formation in giant cells of the infected T-cell population. These structures can be used as an indicator to detect HTLV-III in clinical specimens. This system opens the way to the routine detection of HTLV-III and related cytopathic variants of HTLV in patients with AIDS or pre-AIDS and in healthy carriers, and it provides large amounts of virus for detailed molecular and immunological analyses.*

Epidemiologic data suggest that the acquired immunodeficiency syndrome (AIDS) is caused by an infectious agent that is horizontally transmitted by intimate contact or blood products (*1–3*). Though the disease is manifested by opportunistic infections, predominantly *Pneumocystis carinii* pneumonia (*4*), and by Kaposi's sarcoma (*5*), the underlying disorder affects the patient's cell-mediated immunity (*6*), resulting in absolute lymphopenia and reduced subpopulations of helper T lymphocytes ($OKT4^+$). Moreover, before a complete clinical manifestation of the disease occurs, its prodrome, pre-AIDS, is frequently characterized by unexplained chronic lymphadenopathy or leukopenia involving helper T lymphocytes (*5, 6*). This leads to the severe immune deficiency of the patient and suggests that a specific subset of T cells could be a primary target for an infectious agent. Although patients with AIDS or pre-AIDS are often chronically infected with cytomegalovirus (*7*) or hepatitis B virus (*8*), for various reasons these appear to be opportunistic or coincidental infections. We have proposed that AIDS may be caused by a virus from the family of human T-cell lymphotropic retroviruses (HTLV) (*9*) that includes two major, well-characterized subgroups of human retroviruses, called human T-cell leukemia-lymphoma viruses, HTLV-I (*9–12*) and HTLV-II (*9, 11, 13*). The most common isolate, HTLV-I, is obtained mainly from patients with mature T-cell malignancies (*9, 12*). Seroepidemiological studies, the biological effects of the virus in vitro, and nucleic acid hybridization data indicate that HTLV-I is etiologically associated with the T-cell malignancy of adults that is endemic in certain areas of the south of Japan (*14*), the Caribbean (*15*), and Africa (*16*). HTLV-II was first isolated from a patient with a T-cell variant of hairy cell leukemia (*13*). To date, this is the only reported isolate of HTLV-II from a patient with a neoplastic disease. Virus isolation and seroepidemiological data show that both HTLV-I and HTLV-II can sometimes be found in patients with AIDS (*17*).

That a retrovirus of the HTLV family might be an etiological agent of AIDS was suggested by the findings (i) that another retrovirus, feline leukemia virus, causes immune deficiency in cats (*18*); and that (ii) retroviruses of the HTLV family are T-cell tropic (*12, 19*); (iii) preferentially infect helper T cells ($OKT4^+$) (*12, 19*); (iv) have cytopathic effects on various human and mammalian cells, as demonstrated by their induction of cell syncytia formation (*20*); (v) can alter some T-cell functions (*21*); (vi) can in some cases selectively kill T cells (*22*); and (viii) may be transmitted by intimate contact and blood products (*9*). Also consistent with an HTLV etiology were the results of Essex and Lee and their colleagues showing the presence of antibodies to cell membrane antigens of HTLV-infected cells in serum samples from more than 40 percent of patients with AIDS (*23*). This antigen has since been defined as part of the envelope of HTLV (*24*). The more frequent detection in AIDS patients of antibodies to a membrane protein rather than to HTLV-I internal structural core proteins (*25*), together with the low incidence of isolations of HTLV-I or HTLV-II from AIDS patients, also suggested that a new variant of HTLV might be present.

The original detection and isolation of HTLV-I were made possible by the discovery of T-cell growth factor (TCGF) (*26*), also called interleukin 2 (IL-2), which stimulates the growth of different subsets of normal and neoplastic mature T cells (*27*), and by the development of sensitive assays for reverse transcriptase (RT), an enzyme characteristic of retroviruses (*28*). The procedures used previously for the transmission and continuous production of HTLV-I and -II were first worked out in mammalian cells transformed by avian sarcoma virus (*29*). These methods involved cocultivation of the transformed cells with cells permissive for the particular virus strain. Normal human T cells in cocultivation experiments preferentially yielded HTLV of both subgroups. Some of these viruses showed an immortalizing (transforming) capability for certain target T cells (*9, 12*). We thought that HTLV variants that have cytopathic effects on their target cells but do not immortalize them might be more important in the cause of AIDS. In fact, such variants were frequently but only transiently detected when normal T cells were used as targets in cocultivation or cell-free transmission experiments. This transience was our main obstacle to the isolation of these cytopathic variants of HTLV from patients with AIDS or pre-AIDS. We subsequently found a cell line that is highly susceptible to and permissive for cytopathic variants of HTLV. This cell line can grow permanently after infection with the virus. We report here the estab-