Introduction

Trees are natural proxy recorders and can act as reliable archives for historical environmental information. Trees can document the impact of soil and groundwater contamination from both a fingerprinting and an age-dating perspective. Any contaminant that enters a tree in a sufficient concentration to affect growth will be reflected in the annual ring width. Xylem, as it builds, keeps track of the growth effects of these contaminants. The annual rings may also record contaminant exposure at concentrations low enough to not even affect growth.

Dendroecology is broadly defined as the temporal study of ecological and environmental changes depicted in dated rings (Schweingruber, 1996). Due to the ability of rings to record the contaminant history surrounding a tree, dendroecology has become a forensic tool for characterizing environmental releases of pollutants. This tool may be used to:

- estimate the age of contamination (based on the tree impact);
- characterize the elemental composition of the contamination;
- assess the release mode (abrupt versus progressive); and
- map the extent of contaminant plumes.

The age-dating of environmental impacts is based on dates supplied by tree rings and contaminant concentration anomalies found within the rings. Modern spectroscopic methods allow for line scanning of tree-core samples for elemental tracers, whereas mass spectroscopy can be used for measuring its organic and isotopic composition. Tree-ring composition can be correlated with ring-width anomalies, pointing out when contamination entered the tree.

The contaminant concentration detected in a wood sample is a function of exposure to the root system, selective uptake and discrimination, and fixation mechanisms. Such mechanisms include: 1) phyto-volatilization, 2) rhizo- or phyto-degradation, 3) hyper-accumulation of contaminants in selective parts of the plant, 4) compensation in the rhizosphere that reduces contaminant uptake, 5) retranslocation due to infection or the conversion of sapwood to heartwood, and 6) binding affinity due to tree or wood maturation. These mechanisms can all differ based on the plant species, the type of contaminant release, and the soil conditions.

The evidence supporting dendroecological methods as a forensic application is available from studies in dendrochemistry,
phytoremediation, and dendrochronology, with several hundred publications available in the scientific literature. The resolution of dendroecological methods as an age-dating tool compares favorably with other forensic methods, with a precision of 1 year, and sometimes to a seasonal scale. Dendroecological methods can also be used to distinguish asynchronous releases or “commingled plumes.” This method is one of the few that can be used when contamination is no longer available after complete remediation of a site.

Part I of this article describes the fundamental principles of dendroecology as a forensic tool and proposes a methodical approach for dendroecological investigations at contaminated sites. The purpose of these investigations is to age date contaminant releases and/or characterize the resulting plumes. Part II of this article presents and discusses six case studies for which the method was successfully applied and illustrates important observations and limitations.

**Part I: Fundamentals**

**Scientific Background**

Trees develop consecutive annual rings that can be dated, and the dating of tree rings is known as “dendrochronology” (Schweingruber, 1988; Baillie, 1982). When contamination affects the soil and groundwater near a tree, the following may occur:

- contaminants may be taken up by the tree, circulated with the sap, and fixed to the tree tissue, and
- ring growth may be altered.

Whereas dendroecological methods pertain to the use of tree rings to document environmental impacts, there are several complementary disciplines as well, such as:

- **dendrochronology**, or the use of tree ring series to date archaeological sites (Baillie, 1982, 1995), to reconstruct past climates (Fritts, 1976; Baillie et al., 1995; Nash, 1999; Cook and Kairiukstis, 1990; Cook et al., 1999), and date other events such as insect plagues, fires, or even in criminal cases (Baillie, 1982; Nash, 1999; Abrams et al., 2000);
- **dendrochemistry** (Cook and Kairiukstis, 1990), or physiology and chemistry as applied to trees and their rings; and
- **phytoremediation**, the use of plants that accumulate and sequester or uptake and evaporate contaminants to reduce environmental pollution (Fiorenza et al., 2000; McCutcheon and Schnoor, 2003).

Dendroecological methods may also be used in site assessments for plume delineation (Schumacher et al., 2004; Vroblesky et al., 2004; EPA, 2005), or to assess the efficiency of remedial efforts.


**Phytoremediation**, with more than 500 publications. Limitations or restrictions, however, do exist in such applications and are generally related to tree availability, tree suitability, tree-age compatibility, and proper use of analytical methods.

**Dendroecological Principles**

Within the root environment or rhizosphere, organic compounds decompose through microbial activity. Some microorganisms are facultatively or obligately symbiotic with tree roots. This process, called “phytodegradation,” can contribute to the availability of the contaminants for uptake by the tree. Depending on the contaminant, roots preferentially take up the contaminant, a mechanism called “rhizofiltration.” Sap will then be enriched in the contaminant, a process known as “phytoextraction,” and may vertically circulate the contaminant through the plant tissues, a mechanism called “translocation.”

When both organic compounds and elements are transported, a fraction may evaporate from the stem or the foliage through a mechanism known as “phytovolatilization.” The remaining contaminant will be fixed in plant tissues, wood, or leaves, as a result of “phytofixation” mechanisms. As such, contaminants present in heartwood are fixed, whereas sapwood is characterized by both fixed (xylem) and unfixed, sap-transported elements.

Different plant species react differently to contamination because of dissimilar physiology mechanisms (Smith and Shortle, 1996), including variable sensitivities to toxins. They also differ in metabolic processes, with possible variations in sap flow. A further mechanism, *phytoaccumulation*, is essential when selecting the correct species for phytoremediation; some plants fix contaminants at much higher concentrations than the corresponding concentrations in soil or water. This enrichment mechanism leads some plants to accumulate and fix contaminants in their tissue better than other plant species, justifying their selection in phytoremediation projects.

There are additional physiological compensation mechanisms that occur in exposed trees, such as directed growth of the root system into less contaminated soil, resulting in partial reduction of contaminant uptake and fixation. Also, chemical transformation of contaminants (into less mobile and bioavailable forms) in the rhizosphere area is possible. Uptake mechanisms have been demonstrated in phytoremediation studies for many contaminants (Garbisu and Alkorta, 2001), organic compounds (Newman and Reynolds, 2004) such as petroleum fuels, chlorinated solvents, or polychlorinated biphenyls (PCBs) (Macek et al., 2004), polynuclear aromatic hydrocarbons (PAHs) (Huang et al., 2004), methyl-tertiary-butyl ether (MTBE), or benzene, toluene, ethylbenzene, and o-, m-, and p-xylene polymers (BTEX), and explosives (Snellinx et al., 2002), as well as metals (Jian et al., 2004) and radionuclides (Meagher, 2000; Edmands et al., 2001) or stable isotopes (Kawamura et al., 2006). Other compounds present at trace concentrations in the environment have also been documented (Kramer and Chardonnens, 2001). It is noteworthy that, although at first glance dendroecology may not seem applicable to some organic contaminants such as pure hydrocarbons due to their...
metabolization within plants (Newman and Reynolds, 2004), their detected presence in plants is an indication that at certain concentrations, basically any contaminant can be found undegraded in plant tissue and subsequently impact ring growth. In this respect, research is still evolving and the potential for practical application is high.

The uptake of contaminants has been documented either by analysis of plant tissues or by measuring transpiration at the foliage level. Uptake is associated with contaminant enrichment in the sap as a function of its concentration in the root environment. For example, the concentration of chlorinated solvents in sapwood is proportional to their concentration in adjacent soils and ground water (Vroblesky and Yanosky, 1990; Schumacher et al., 2004). It is the phyto-uptake and fixation mechanisms that are important when using dendrochemistry to characterize or age date contamination events.

In summary, contaminant releases may be documented in tree rings through two procedures:

- the tree-ring widths, with the assumption that widths will decline in the presence of significant contamination, and
- the contaminant concentration anomalies (at elemental level) that can be discerned in the tree rings through chemical analysis, with increases depicting the time frame of impact to the tree.

Field and Laboratory Procedures

Field Sampling

With the previously noted principles in mind, field investigative work consists of documenting and sampling exposed and control trees. Trees are selected based on their location, distance from the contaminant source, and resulting plume, age, condition (size, health), and species. The tree species should always be selected based on the availability of exposed and control trees of that species. Species identification can be aided through the use of numerous field guides (Fergus, 2002; Barnes and Wagner, 2004; Rhoads and Block, 2005).

Sampling is typically performed at chest height with a hand-operated increment borer with a minimum diameter of 10 mm. If the wood is dense and coring becomes difficult, it may be necessary to use a 5-mm borer or more powerful electrically driven drill bits to secure long enough cores. In some instances, cross sections or stem disks can be obtained if the tree is to be destroyed or when only a stump is left.

Two or more cores should be collected per tree, usually at chest height, and if site conditions allow, several trees should be sampled inside and outside of the area of the plume. In simple cases, fieldwork represents 4 hours for sampling and documenting, with four trees sampled and documented (eight cores). However, experience has shown that some sites are not easily sampled, especially if trees exhibit dense wood, such as locust trees.

Samples are maintained and preserved in grooved wooden blocks to allow them to dry without twisting. The sampling and sample preparation methods are extensively described in Wilford et al. (2005) and Balouet and Oudijk (2006).

Preparation of Samples

Samples are glued onto grooved blocks or holding kits with the transverse wood surface upright. Holding kits are dried under ambient conditions for at least 4 to 5 days before being sanded or planed to produce the flat surface necessary for optical reading and line-scanning microanalysis. The procedure is commonly performed by progressive sanding with a graded series of sandpaper from 80, 200, 400, and 600 grits. It is important to avoid excessive heat, especially with samples possibly containing organic compounds that could volatilize. Hand processing over a flat surface, such as a chilled marble tile, is a preferred method. Surfacing is terminated when the core surface is sufficiently polished to reflect light; the surface is then cleaned with either an aerosol dust cleaner or a vacuum cleaner.

Ring-Width Measurements

Optical reading or microdensitometry imaging of the core samples is necessary to obtain the ring-width data. These data will be needed to assess ring-growth anomalies, as well as to calibrate the chemical data obtained from within the annual rings. Measurement precision is commonly a few tens of microns ($\mu$m), can be as low as 1 $\mu$m in slow-growth trees (inframillimetric rings) or as high as 100 $\mu$m in fast-growing trees (centimetric rings), and the measurement precision is approximately 1%.

In the case studies described herein, the annual rings were optically measured on surfaced samples (Balouet, 2005; Balouet and Oudijk, 2006) to approximately 0.01 mm with a precision of ±30 $\mu$m. A digital camera was coupled to a video monitor with a magnification of 30 to 40 times and inter-ring distances were measured with a digitalized translation stage. Video-imaging software, which is often used in such cases, is a valuable tool for enhanced distinction of ring boundaries. The raw data are then entered into spreadsheets, allowing for easy statistical analysis and graphing.

Chemical Analysis

Chemical laboratory procedures are chosen based on the needs of the forensic investigation. The investigator needs to choose the most appropriate analytical method, giving due consideration to:

- the searched compounds, whether elemental, molecular, isotopic, organic or inorganic;
- the detection limits, which are compound and method dependent: wood-core samples are physically different and behave differently compared with other materials such as soils or fluids;
- the analytical resolution, such as line scanning to observe intra-ring resolution versus annual ring or several consecutive rings;
- the minimum sample size for micro-analysis;
- sample destructive versus non-destructive methods; and
• other limitations driven by the selected method, such as the vacuum method, which may remove some if not all of the volatile or semi-volatile searched compound or tracers.

The wood-core samples collected for the case studies that are presented in Part II of this study were analyzed with an ITRAX® wood scanner equipped with an energy-dispersive X-ray fluorescence (EDXRF) device, developed by Cox Analytical Systems of Mölndal, Sweden (Lindeberg, 2004). The laboratory analyses were performed by the Dendrolab at Stockholm University (Stockholm, Sweden). This method was chosen for its high resolution and speed of analysis: a standard beam size at 100 µm, at an increment of 200 µm or 125 dpi, and a counting time at 30 s per spot; it is a non-destructive method and a vacuum is not needed.

Different in comparison to mass spectrometry analyses, the data generated by this spectroscopic method (Figure 1) is expressed in counts, not in parts per million (ppm) or milligrams per kilogram (mg/kg). These counts, if not expressed in such absolute units, are absolute numbers of counted photons and after calibration express absolute concentrations. However, because changes in wood density along radial transects may influence the number of counts, including at ring boundaries, it is important to carefully evaluate the patterns and amplitude of relative changes. Accordingly, the term “concentration” in its subsequent uses in this article refers to the number of counts and its significant relative changes. As a note, such absolute concentrations may be transformed into mg/kg with appropriate calibration.

EDXRF detection limits (DLs) are higher for lighter elements and have been identified, for example, at approximately 40 ppm for Cl and 10 ppm for Ca. Repeatability testing with an EDXRF on wood samples, for elements present above the DL, is in the percent range.

Although EDXRF provides information for many different elements, the focus in this article is given to the following elemental markers in the wood-core samples:

• lead (Pb), which was formerly present in the Western countries in gasoline as an additive. The source of the Pb would be organic compounds such as tetraethyl Pb or tetramethyl Pb;
• chlorine (Cl), which is present in chlorinated compounds such as industrial solvents or PCBs and is also naturally present at low concentrations in fossil fuels; and
• sulphur (S), naturally present in fossil fuels; for example, S-containing petroleum compounds consist of the dibenzothiophenes.

The laboratory provides a spreadsheet depicting contaminant concentrations; for example, Pb versus distance from the sample end (in this case, the bark representing the outside of the core sample). These data can then be correlated with the ring-width distribution to assign contaminant concentrations to specific years. These data may also be coupled with microdensitometric images (Figure 4, 15) and images of the sample (Figure 8) in addition to dated rings sequences.

Data Evaluation

Raw data, either gained from ring-width measurement or chemical microanalyses, normally must be evaluated in some manner before interpretation. Small-scale variability is expected, especially at low counts, although the type of elements and climatic factors will also play a key role.

Figure 1. Chemical analysis and line-scanning principles.
Statistical tools can be employed to further interpret the data. A moving, or dynamic, average is one of the most useful tools. Each data point is replaced by the average among a selected number of surrounding data points. Because the contaminant concentration data are a time series, centered and weighted moving averages are often preferred. In Eq. (1), the moving average for data \( n \) is corrected for data over the two earlier and two later points where

\[
x_{wa} = \frac{0.2x_{n-2} + 0.5x_{n-1} + x_n + 0.5x_{n+1} + 0.2x_{n+2}}{2.4}
\]

where \( x_{wa} \) is the resultant weighted average value, \( x \) is the value for a specific data point, and \( n \) is the location of that data point. Alternatively, the chemical data may be averaged for each annual ring.

### Ring-Width Data

Core samples are normally measured several times to ensure appropriate identification and measurements of ring-widths. It is normal to measure several samples, at least two from each tree, and from a reasonable number of exposed and control trees. Because ring interpretation can depend on false or missing rings, cross-checking is performed on ring series by identifying pointer rings on exposed and control cores and by cross-checking with tree-growth databases using the quality control and cross-checking software COFECHA (Laboratory of Tree-Ring Research, University of Arizona, Tucson, AZ) (Holmes, 1983) as well as with chemical data. This free software can be downloaded from http://www.ncdc.noaa.gov/paleo/treeing.html courtesy of the Laboratory of Tree-Ring Research.

False rings occasionally occur when a spring or late summer season is punctuated by an unusually cool period. Growth is halted and, in this case, it would appear as two more or less distinct rings. Missing rings could occur when the summer season was cool and growth was halted during the entire period. Tree-ring databases are available to compare ring-width data and assess whether false and missing rings are potentially present. Two main databases are available in the public domain: the International Tree-Ring Databank (ITRDB) maintained by the National Oceanographic and Atmospheric Administration (NOAA) Paleoclimatology Program and World Data Center for Paleoclimatology (Boulder, CO; available at http://www.ncdc.noaa.gov/paleo/treeing.html) and the Dendro Database (available at http://www.wsl.ch/dendro/dendrodb.html) maintained by the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) (Zurich, Switzerland). These databases are established from mean ring widths over the years for selected species and sampling areas. Other (local) databases may be available from research organizations specializing in dendrochronology. The Palmer Drought Severity Index and other climate data are available for all of the United States through the National Oceanographic and Atmospheric Administration websites.

Cross-checking, a comparison of ring-width and chemical or elemental marker concentration data, eventually coupled with microdensitometric images, can further help identify false rings, or contribute to enhanced calibration of the chemical data to each of the annual rings (Figures 4 and 7).

### Interpretation

The dendroecological methods described here may allow:

- an assessment of the age of a contaminant release based on both ring-width and chemical data;
- a characterization of the contaminant plume based on its elemental composition, extent, and magnitude; and
- an assessment of the type of the release.

### Fundamental Principles

The fundamental principles governing ring growth are termed “the principle of limiting factors,” which states that the rates of plant processes are constrained by the primary environmental variable that is most limiting. For example, variations in precipitation or the influx of pests may adversely impact tree growth (Fritts, 1975; Schweingruber, 1996; Davidson et al., 2001).

The “principle of aggregate tree-growth” states that any individual tree-growth series can be “decomposed” into an aggregate of environmental factors, both anthropogenic and natural, that affect the patterns of tree growth over time (Cook, 1990). The model was developed to identify a common climatic signal within a forest stand or region, although it may be applied to other environmental factors that affect tree growth. The model acknowledges different parameters influencing tree growth resulting in the following equation:

\[
R_t = A_t + C_t + \delta D_{1t} + \delta D_{2t} + E_t
\]

where \( R_t \) is the measured tree-ring series; \( A_t \) is the age-size-related growth trend; \( C_t \) is the recorded climate signal; \( D_{1t} \) is the disturbance pulse within the forest stand; \( D_{2t} \) is the disturbance pulse outside of the forest stand; and \( E_t \) is the processes not accounted for by these other terms. \( \delta \) is a binary indicator of the presence or absence of a disturbance pulse.

These two principles are important to forensic investigations when disturbances caused by a local pollution event \( (D_{1t}) \) impact tree-growth. However, climate and age-size growth trends normally remain in an exposed tree’s ring series. It is essential to distinguish climate trends in exposed trees by comparing the ring series to control samples and/or climate-related databases such as the reconstructions of drought and temperature change that can be obtained via the National Climate Data Center or the Global Historical Climate Network (Asheville, NC) or the World Data Center for Paleoclimatology.

The principle of cross-dating states that matching the ring-width distribution or other ring characteristics, such as ring density patterns, among several tree-ring series allows the
identification of the exact year in which each tree ring was formed (Fritts, 1976).

The principle of replication states that the environmental signal being investigated can be maximized, and the amount of “noise” minimized, by collecting more than one core sample per tree and more than one tree per site. Obtaining more than one core per tree reduces the amount of “intra-tree variability”; in other words, the amount of non-desirable environmental signal peculiar to only one tree (Fritts, 1976; Cook and Kairutskis, 1990).

Interpreting Ring-Width Anomalies

Limitations in interpreting ring-width anomalies primarily arise from two key situations:

- no clear growth anomaly because the contamination event did not seriously or abruptly impact tree growth, as apparent in four of the six case studies presented herein; and
- a climatic anomaly causing a ring-growth decline over the same years during which the contamination occurred. In such a case, it is difficult to distinguish which part of the growth anomaly is caused by climate versus contamination; however, the chemical detection of a certain marker compound (contaminant) in the particular ring may be sufficient evidence of impact time.

It is noteworthy that sensitive species may eventually not survive the contamination, as is the case even for large trees.

Whenever ring-growth anomalies are observed, it is essential to determine climatic anomalies through the review of independent tree-ring databases or local climatic records. As observed in the case studies described in Part II of this article, there is a time lag between the onset of the chemical impact and the growth decline. In most cases, the time lag varies from 2 to 4 years. This delay is normally shorter in severe cases.

Site-specific ring-growth anomalies not matching climatic or other environmental stresses, based on a review of control samples and databases, can be attributed to the contaminant impact. It is the departure from the normal growth trend that is used to establish the impact (Figures 2 and 5). The ring year a growth decline begins, in response to contamination, is the minimum age of the impact. If chemical data are available, the impact may be traced further into the past.

Interpreting Chemical Anomalies

Data evaluation proceeds through two steps. The first step is to detect chemical anomalies, natural pulses or trends, and eventual ionic stress or retranslocation due to infection or the transformation of sapwood into heartwood. The second step is to detail data in and around these anomalous rings (Figures 8 and 9).

Graphing tools are used for data interpretation and presentation. They are often available from spreadsheet software. Interpretation should focus on the raw data and avoid unnecessary transformations that may lead to errors and the loss of information. For example, one may be tempted to compute the annual concentration mean across a ring; however, such a calculation could result in significant distortion, with the difference between the scale of observation of annual ring width and intra-annual chemical precision.

Natural Chemical Fluctuations Versus Contamination Anomalies

The long-term trend in chemical concentration, over the core, is associated with the tree age and its growth. EDXRF data provides us with a linear, or almost linear, versus asymptotic description of that trend from pith to bark. From available data, elemental concentrations can be almost stable or progressively decrease from pith to heartwood/sapwood boundary (HSB), as if the elements are progressively depleted within the rhizosphere by uptake. In the absence of pollution, injury or infection, the elemental trend lines remain basically parallel.

Annual fluctuations are commonly visible over the core for several elements, such as Ca and K, for both angiosperms and gymnosperms. These fluctuations represent naturally occurring inter-ring or intra-ring variations and differ, usually by a few percent, although sometimes in the tens of percent for gymnosperms. These annual fluctuations can be used to check on false rings, to assess significant retranslocation, or provide for ring calibration of chemical data.

Naturally occurring elemental variations can also take place at the HSB (Andrews and Siccama, 1995). Such variations have been observed at a magnitude of tens of percent, normally limited to both sides of the HSB, attributed to retranslocation and associated with chemical imbalances at the stand, such as excessive or depleted amounts of major ions.

Contaminant-related anomalies are often caused by changes in chemical availability. These anomalies may be associated with the release of elements present in a contaminant, such as Cl in chlorinated solvents. The presence of these elements alters the ion availability caused by changes in soil pH (as shown by Fe versus Zn availability in acidified soils). This last effect is known as “ionic stress.” Significant changes are also documented for some elements, such as Ca, Mg, or K, in relation to infection by microorganisms or as a tree responds to injury. Such decaying rings cannot be used for documenting plume impact. For contamination cases, the focus of this article is on elemental markers. They are specifically searched for when the type of contaminant is known (such as fossil fuels or solvents), although many elemental profiles are checked on each individual core (Figure 6). Contaminant-related anomalies clearly differ in amplitude from possible annual fluctuations or age/size variations. Pre-contamination profiles of tracers can be viewed as background concentrations and the anomalies are commonly found at two to four times the background, but obviously this variation is case specific (Figures 4, 6, 9, 12, 14, 15, and 16). Contaminant-related anomalies are commonly found over several rings. If retranslocation has not occurred on both sides of the HSB (as characterized by significant differences in concentrations), rings where peaking elemental marker concentrations exist represent the time frame when contamination first impacted the tree.
Left of the peak maxima, pithward, concentrations progressively depart from background to reach the maxima. This increase can be associated both with sap enrichment mechanisms for earlier sapwood rings and with progressive impact. The inflection point where concentrations start increasing from background does not necessarily mean that the ring in which it is observed is the ring of plume impact because this inflection point can also be related to sap-enriched rings. During that phase, in ring-porous woods, pore areas of the earlywood are enriched in contaminant when latewood is less enriched.

Right of the peak, cambiumward, a decrease can also be observed, generally progressive, commonly returning almost to background level and dependent on soil or groundwater concentrations of pollutants in the following years. Such a phenomenon occurs even when a plume is still present at stand and attributed to tree compensation mechanisms, as can be seen in cases from Part II of this study. Alternatively, this observation may highlight the ability of a tree to reduce the pollution within its rhizosphere. Such repeated observations are relevant to phytoremediation as such compensation mechanisms imply that uptake and/or fixation do vary with time.

Elements that are highly mobile within the cytoplasm of living cells, such as K and N, translocate readily and are less suitable as markers of changes in environmental exposure than S or Pb. From the case studies described herein, the lack of significant elemental retranslocation can be seen by the marked and contrasted annual cycles and the sharp shape of pollution-related peaks. If significant radial retranslocation had taken place, the peaks would have been smoothed.

Elemental marker concentrations in manufactured products change with time, such as the mandatory reductions of S in fossil fuels (ASTM, 1998), Pb in gasoline (Gibbs, 1990), and Cl in all fossil fuels (Karaulova et al., 1981; Howard and Vocke, 2004). Therefore, during a continuous impact to a tree over several years, a lessening in the elemental marker concentration may be observed. In contrast, enrichment of the marker may occur in the sap, keeping the concentrations within the rings steady or even increasing, especially in the outermost rings when pollution is still impacting the tree.

With regard to the concentrations of these elemental markers, S concentrations in crude oil and its resulting distillate fuels had been in the percent range. Refineries have recently put more effort into the removal of S. The same holds true for Cl concentrations, which, depending on the crude oil, can be rather high. In the past decade, refiners have drastically reduced the Cl content of their products. With Pb, the content of organic lead in automotive gasoline was never greater than approximately 4 g/gallon (or slightly more than 1 g/L) (Kaplan et al., 1997). However, by the late 1970s, these concentrations had also been drastically reduced. Accordingly, S and Cl are likely to be excellent markers for petroleum contamination because of their high concentrations. Pb can also be used as a marker, but its presence was substantially reduced beginning in the 1970s, and by the mid-1980s, it was essentially nil.

Cl should be considered an excellent marker for chlorinated solvents, especially tetrachloroethene (PCE) and trichloroethylene (TCE). For example, the molecular makeup of TCE (CCl\_2) is 80% Cl by weight, whereas for PCE the percentage of Cl is even higher.

Characterizing the Environmental Release

The chemical signature of a contaminant anomaly relates to an elemental marker such as Cl for chlorinated solvents, Pb for leaded gasoline, and S for fossil fuels or heavy metals for mining or electroplating wastes. S and Cl may be used as joined markers of fossil fuels. Even if Cl is found at very low concentrations in fuels, its profiles match the S profiles in all of the fossil fuel cases in this study. The match in S and Cl anomalous profiles is considered a signature of fossil fuel plumes. In leaded gasoline cases, anomalous Pb profiles can be associated with S and Cl anomalies. In chlorinated solvent cases (Figure 4), anomalous profiles are found for Cl only, while other elements may be unaltered.

The increase in the elemental marker concentration in the tree rings relates to the increase in the marker's availability in the root environment or simply a comparison of natural background and the contaminant source. Elemental markers found in the heartwood are fixed, whereas anomalies found in the sapwood may be related to active sap enrichment.

The amplitude of contaminant-related chemical anomalies commonly exceeds the inter-ring and intra-ring variation range. Elemental markers are often found at concentrations of two or more times background in significant pollution cases, although this multiplier will depend on the site-specific conditions.

If a discontinuous release of minimal amounts occurred, the anomaly may only impact a small number of rings and may not be followed by ring-growth anomalies. If a continuous and severe release occurred, the anomaly will extend over several rings and be followed by a reduction in ring widths. If the pollution relates to volatile compounds such as chlorinated solvents or fossil fuels, and the plume is still impacting the tree at time of sampling, the outermost sapwood rings will show, cambiumward, an increase in tracers (as in cases 2, 4, and 5, and Figures 4 and 12). Such a progressive profile in the outermost rings may not be associated with a recent spill but instead may indicate that the plume is still active in the area. Such cambium-sided progressive profile indicates the presence of sap-transported elements, not yet fixed, and can be used to determine the number of rings where sap enrichment may take place.

When a contaminant contains more than one elemental marker, such as Cl, Pb in leaded gasoline or Na and Cl in salt, these markers may show parallel profiles over several rings. In some rare instances, some elements that are not present in the release may also show altered profiles. This phenomenon suggests a change in ion availability related to the release, such as soil acidification, versus a physiological mechanism in response to the impact.

The precision in age dating of a contaminant release will always be case specific. The precision will depend on
the magnitude of the release, the length of time it occurred (continuous versus discontinuous), the distance from the tree, the subsurface geochemical conditions including groundwater depth, and specific conditions of the tree and its species.

Age-Dating Impacts Based on Chemical Data

Increasing elemental concentrations in the environment, such as during a contaminant release, will result in increased elemental concentrations in the exposed ring series. With time, a tree's physiological responses and adaptation do result in uptake and fixation control mechanisms, finally resulting in a relative decrease in the marker's concentrations, even when the release is still impacting the tree. As such, the forensic investigator can reasonably estimate the time frame of an impact at a tree's stand through the dated ring where the tracer(s) peaks. However, an investigator also has to consider:

- Xylem sap flows through all of the open pores of sapwood. The width of sapwood with respect to absolute radial distance and numbers of rings varies between tree species and among individual trees within a species. Consequently, sapwood rings formed prior to the exposure of the ring to pollutant-containing sap may also be enriched with the marker chemical. Particularly in the case of ring-porous wood, this enrichment of previously formed wood would likely be restricted to the surfaces of the cell walls of open pores in the earlywood. The marker would likely not be incorporated or evident in latewood formed prior to exposure. The presence of the marker across the entire ring width is evidence that the ring formed when the marker was present in the sap.
- Radial retranslocation can occur at the HSB (Andrews and Siccama, 1995). Infection can cause elemental anomalies and readily identified by a concomitant increase in K concentration (Smith and Shortle, 1996). Unless significant radial retranslocation can be documented at the HSB, the rings with the highest marker concentration represent the time of peak exposure. Because of compensatory responses after impact, concentrations tend to decrease in subsequent rings.
- If the release is recent and only impacted the outermost sapwood rings, the age-dating of impact would be less precise, primarily depending on the number of sapwood rings in the sampled core.

Age-Dating Impact Based on Ring Width

Ring-growth decline often follows a release's impact, within a few years, but in some cases up to 3 or 4 years (Balouet and Oudijk, 2006). This phenomenon may be caused by the low concentrations of a contaminant being an actual food source for the tree, but once the concentrations become higher, the contaminant starts to have a toxic effect. Because releases differ in time, space, and composition, trees will record a wide range of responses.

### PART II: Case Studies

Part II of this study presents original results in validation and support of the forensic use of dendroecology. Six case studies (Table 1), ranging from New Jersey to California, are described in addition to interpretative discussions and lesson learned. Previously, three studies were performed by others using a similar approach to age date environmental releases. Yanosky et al. (2001) demonstrated the applicability of the method to age date a chlorinated solvent plume based on ring width and Cl content. Punshon et al. (2003) age dated a dam failure at a nickel mine based on the nickel content of nearby tree rings. Balouet and Oudijk (2006) described the age dating of fossil fuel plumes from a maintenance yard, identifying three asynchronous releases in a commingled plume.

#### Background

Forensic dendroecological investigations have been performed by three of the authors at 20 sites within the United States. Six cases are presented here, while an seventh case was described in Balouet and Oudijk (2006). The aim of these investigations was to estimate the time frame of the releases. These cases are presented here to illustrate the method, to show that each case has its individual characteristics, and to highlight considerations for interpretation. Unlike typical dendrological studies, these forensic cases are limited to a small number of trees per site (between 2 and 14). One may not need evidence from more than one tree. In all cases, the water table was shallow, between 8 and 30 feet in depth, and all the “exposed” trees were located over the respective plumes.

The authors successfully used 33 species for ring-width measurements and 11 for chemical evidence (Table 2). Some samples, however, were discarded because the tree showed critically limiting endogenous factors (decayed rings, physical tilting) or were too young to be considered in the investigation. In the case studies provided herein, the cores are presented with the pith at the left and the bark at the right, and such an orientation of core should be a standard when presenting data.

#### Case Presentations

Six cases studies are presented. Table 1 summarizes the cases.

<table>
<thead>
<tr>
<th>Table 1. Description of the case studies</th>
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<tbody>
<tr>
<td>Case study</td>
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<td>6</td>
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</tbody>
</table>

UST, underground storage tank.
Table 2. Tree species used in the forensic dendroecological investigations performed by the authors

<table>
<thead>
<tr>
<th>Gymnosperms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fam. Pinaceae:</td>
</tr>
<tr>
<td>Pinus sylvestris: Scots Pine*</td>
</tr>
<tr>
<td>Picea glauca: White spruce</td>
</tr>
<tr>
<td>Picea pungens: Blue spruce*</td>
</tr>
<tr>
<td>Cedrus libani: Lebanon cedar</td>
</tr>
<tr>
<td>Fam. Cupressaceae:</td>
</tr>
<tr>
<td>Chamaecyparis thyoides: Atlantic white cedar*</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Angiosperms</th>
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<tbody>
<tr>
<td>Fam. Aceraceae:</td>
</tr>
<tr>
<td>Acer saccharinum: Silver maple</td>
</tr>
<tr>
<td>Acer saccharum: Sugar maple*</td>
</tr>
<tr>
<td>Acer leucoderme: Chalk maple</td>
</tr>
<tr>
<td>Acer rubrum: Red maple</td>
</tr>
<tr>
<td>Fam. Anacardiaceae:</td>
</tr>
<tr>
<td>Schinus molle: Peppertree</td>
</tr>
<tr>
<td>Fam. Betulaceae:</td>
</tr>
<tr>
<td>Birch sp.</td>
</tr>
<tr>
<td>Fam. Bignoniaceae:</td>
</tr>
<tr>
<td>Catalpa speciosa: Northern Catalpa</td>
</tr>
<tr>
<td>Fam. Fagaceae:</td>
</tr>
<tr>
<td>Quercus muehlenbergii/Quercus prinus: &quot;chestnut&quot; oak</td>
</tr>
<tr>
<td>Quercus alba: White oak*</td>
</tr>
<tr>
<td>Quercus rubra: Red oak*</td>
</tr>
<tr>
<td>Fam. Hamamelidaceae:</td>
</tr>
<tr>
<td>Liquidambar styraciflua: Sweetgum</td>
</tr>
<tr>
<td>Fam. Hippocastanaceae:</td>
</tr>
<tr>
<td>Aesculus californica: Californian buckeye</td>
</tr>
<tr>
<td>Fam. Leguminosae:</td>
</tr>
<tr>
<td>Robinia pseudoacacia: Black locust*</td>
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<tr>
<td>Fam. Magnoliaceae:</td>
</tr>
<tr>
<td>Magnolia sp.: Magnolia*</td>
</tr>
<tr>
<td>Liriodendron tulipifera: Tulip tree or yellow poplar*</td>
</tr>
<tr>
<td>Fam. Oleaceae:</td>
</tr>
<tr>
<td>Sorbus americana: American Mountain Ash</td>
</tr>
<tr>
<td>Fraxinus papillosa: Chihuahua ash*</td>
</tr>
<tr>
<td>Sorbus sp.: Mountain ash</td>
</tr>
<tr>
<td>Fam. Platanaceae:</td>
</tr>
<tr>
<td>Platanus occidentalis: Sycamore</td>
</tr>
<tr>
<td>Fam. Rosaceae:</td>
</tr>
<tr>
<td>Prunus cerasus: Plum tree</td>
</tr>
<tr>
<td>Prunus sp.: Cherry tree</td>
</tr>
<tr>
<td>Fam. Rutaceae:</td>
</tr>
<tr>
<td>Citrus lemon: Lemon</td>
</tr>
<tr>
<td>Citrus aurantium: Orange</td>
</tr>
<tr>
<td>Fam. Salicaceae:</td>
</tr>
<tr>
<td>Salix caroliniana: Coastal plain willow</td>
</tr>
<tr>
<td>Salix sp.: Willow</td>
</tr>
</tbody>
</table>

*Tree species for which chemical analyses were performed.

Case Study 1: Ring Width Showing Contamination by Heating Oil No. 2, Southern NJ

In this case, conclusions were reached based on the ring-width data without chemical microanalyses. A catalpa tree had been sampled at a location outside of the plume area, while a sycamore tree and oak stump were sampled within the plume. An ITRDB tree-growth database for the area was available for the tulip and oak trees, but unfortunately not for the sycamores. The results of tree core analysis (tree-ring width) for the different selected trees are presented in Figure 2.

The sycamore is located immediately adjacent to a underground storage tank (UST), which developed a leak of no. 2 heating oil. The heating oil migrated a significant distance and, based on investigative boreholes, impacted the sycamore. Separate-phase petroleum was identified within the root zone of this tree. The very marked growth decline of the sycamore, starting in the late 1940s, is attributable to an age-growth trend, whereas the second major growth anomaly starting in the late 1970s is considered to be petroleum related. The late-1970s anomaly was not apparent in the control trees or the tree-growth database. An age date of the late 1970s is reasonable considering the extent of the plume and the condition of the tank once it was removed.

Case Study 2: Chlorinated Solvent Plume, Industrial Plant, Southern California

The site is impacted by a plume of chlorinated solvents, of more than one km in length, originating from an upgradient industrial plant. The water table is present at about 10 m below ground surface (bgs). Wood-core samples were collected from Chihuahua ash trees located about 200 m downgradient of the source. A control sample was also collected in the vicinity but outside of the plume area (upgradient and lateral of the source) from the same species.

The results are presented in Figure 3 (for ring-width data) and Figure 4 (for chemical data). The core sampled from exposed tree did not extend to the pith, so only impact after 1985 could be documented. There is Cl enrichment beginning in 1985 as found in earlywood, while the latewood for that same year has not been enriched. Based on this information and on the Cl peak around point 30 mm, it could be deduced that the chlorinated

Figure 2. Ring-width thickness versus time, case study 1.
solvent first impacted the tree in late 1986. Note that the 1986 ring shows a marked latewood anomaly corresponding to a high Cl concentration in the xylem. The 1988 ring shows a clear and contrasted pattern of sap enrichment. The impact was followed by ring-growth anomalies starting in 1989. A distinct and separate anomaly is present in 1993–1994, based on peak near 107 mm, which may be attributed to a worsening of the situation (contaminant release). This second event is followed by further growth reduction starting in 1997. In this case, it may be possible to demonstrate two asynchronous events, as in a commingled plume or the worsening of a single plume. The progressive linear Cl trend beginning from point 130 (HSB) towards the cambium or bark corresponds to sap circulation of Cl as a tracer and is an indication that the tree is still being impacted by the plume.

This case study was chosen to test and validate the method accuracy for chlorinated solvents, as in this case there were available historical records indicating the timing of the main contamination events (recorded inception time of plume and releases of PCE and TCE). Our results were in accordance with the site historical data that mentioned the TCE/PCE plume inception in the mid-1980s and several additional releases in the 1990s.

Case Study 3: Unknown Plumes in Case 2
As part of investigation in the case study no. 2, a control tree has been sampled and analyzed. The ring-width data for that tree are provided in Figure 5. In this case, the profiles shown in Figure 6 show parallel trends for P and S and independent Cl anomalies. These trends reveal several asynchronous releases, with two distinct elemental signatures, Cl versus P and S, each rapidly followed by growth anomalies. A first Cl-only anomaly occurred in 1985, and a second anomaly occurred in 1986 with P and S. A third Cl anomaly occurred in late 1987 to early 1988, and two more Cl-only anomalies occurred in 1996 and 2000. The joined P and S anomalies may be related to property vegetation and the application of fertilizers, possibly in connection with recent construction work, whereas the Cl anomalies may be related to chlorinated solvents or other chlorinated compounds. Site-specific data are needed for a more accurate assessment of the contamination source and type in this particular case. Of note is that this tree (control for case study 2) was closely located to a recently built apartment complex—which may explain some observed diverse anomalies. Figure 7 is provided to illustrate how total coherent counts and incoherent...
counts, respectively, from searched fluorescence wavelengths and scattered photons, can be used to check on ring boundaries, checking on possible false rings, or to calibrate chemical data.

**Case Study 4: Leaking Underground Storage Tank, No. 2 Heating Oil, NJ**

Wood-core samples were collected from two maple trees at locations proximate to a leaking underground heating oil tank (Figures 8, 9, and 10). In this case, the impact year was estimated, based on the chemical evidence, as 1978. There is one growth anomaly, although not a major one, for the exposed maple at ring year 1982, which does not match positive years on the Palmer Drought Severity Index database. In this case again, the delay between chemical impact and growth anomaly is of approximately 3 to 4 years.

Starting in 1977, parallel anomalies for S and Cl are present. Both S and Cl are present in fossil fuels (Karaulova, 1981; Warren, 1995; ASTM, 1998) and their parallel anomalies can be considered as elemental markers. If 1977 was the year of impact, one would logically expect sap-enrichment mechanisms for the ring in the preceding year of 1976; this was not the case, however, and thus 1977 cannot be considered as the impact year. The year 1979 may also be discarded because elemental peaks are found over the first half of the ring. Accordingly, 1978 is the most probable year that the initial impact to the tree.

The 1978 peaking S and Cl anomalies are followed in 1982 by a tree-growth decline visible on X-ray and ring-width profile. This 4-year delay is according to the observations made during other dendroecological investigations.

**Case Study 5. Leaking Underground Storage Tank, No. 2 Heating Oil, NJ**

For insurance purposes, a dendroecological investigation was completed near a release of no. 2 heating oil at a residential property in a beachfront community of central New Jersey.
Wood-core samples from a scotch pine located within 5 m of the leaking tank were obtained. Two control samples from nearby trees of the same species were also collected and the ITRDB data was also consulted.

The ring width results are presented in Figure 11. There is no marked growth anomaly and exposed trees’ ring-growth profiles match the ITRDB trends for scotch pines in New Jersey. In this exposed tree, inter-ring and intra-ring annual patterns are clearly visible, which support excellent annual ring calibration (Figure 12). S concentrations show a clear, almost linear increase from the very first year (1982) until 1994. The increase in S concentration is about threefold, starting at around 500 counts to about 1,500 counts, with a very slow recovery (rings 1994–2000). The K and Ca anomalies around ring 1994 are attributed to pollution stress.

In this case, sapwood only extends over five annual rings. Accordingly, the progressive and ongoing increase in S, more than 12 rings prior to 1994, cannot be interpreted as sap enrichment only. The interpretation is that plume onset has been slow and progressive, suggesting a low leakage rate. The onset of leakage from the tank, because of its proximity to the tree, is very close in time to the tree impact estimated around 1983 and followed by a continuous increase in S, until 1994 when other major chemical anomalies are observed, attributed to a worsening of the release.

Case Study 6: Former Gasoline Service Station, Northern NJ

A forensic investigation was conducted in 2005 at an abandoned gasoline service station to estimate the age of an on-site release. Soil remediation through excavation and disposal was conducted in early 2004. Accordingly, on-site contamination was no longer present. Because the site had already been mitigated, alternative age-dating methods were no longer viable. A stump, attributable to an ash, remained on-site, close to the former tank locations. Wood samples were obtained from the stump in 2005 and the results are presented in Figures 13–15. Although the tree-ring data did not demonstrate any major growth anomalies (Figure 13), chemical anomalies were found for S, Cl, V, and Pb in rings corresponding to 1977 through 1980 (Figures 10 and 11). These data reveal that the first release occurred in 1977 or sometime earlier. The lead profile is anomalous over the rings corresponding to the years 1990 through 1995 (Figure 14). The source of this contamination is not fossil fuels, as the station was no longer in service and further supported by the normal S and Cl profiles over those years.
Figure 9. S concentrations in wood-core samples versus distance from pith for case study 4. Profile for S is below profile for Cl.

Figure 10. A detail of S and Cl concentrations in wood-core sample for case study 4. Profile for S is above the profile for Cl.

Limitations in the Forensic Use of Dendroecology

Based on the literature and on the experience of the authors related to several case studies, including those cases described here, the following main limitations should be taken into account whenever using or considering dendroecological methods as a forensic tool:

- Not all contamination sites have trees or, at the very least, the distance to a tree may be significantly great so that the tree is outside of the contaminated plume. It should be noted that, even when a tree is distant from the source, it can still be of use, especially if the contaminant plume migrated in the direction of the tree. However, these methods will only provide an arrival date to the root system. A second calculation estimating the travel time from the source to the roots would be needed and...

Figure 11. Ring-width thickness versus time for case study 5. Exposed trees are pine 1 and 2. Pines 3 and 4 are controls. Exposed trees are of the same age as controls, although the exposed trees have a slower growth rate.
Figure 12. Ca, K, and S in wood-core sample versus distance from the pith for case study no. 5. Ca in light gray, K in darker gray and S × 10 in black.

Figure 13. Ring-width thickness versus time for case study 6. The mean ring width is shown for the stump in black and the Palmer Drought Severity Index in gray, by x-axis.

Figure 14. Pb concentrations versus time across the wood-core sample for case study 6.
Figure 15. S and Cl concentrations versus time for wood-core sample for case study no. 6. S profile is above Cl profile.

the longer the traveling distance the higher the uncertainty of this calculation.
- For recent releases, affecting only the sapwood, the age-dating precision will be reduced, as peak maxima will be by cambium, as the most sap enriched xylem. In this case, the age-dating is based as a minimum age when plume was not impacting tree.
- Types of trees that may not be appropriate include:
  - Trees that have been replanted: Trees that are part of a re-landscaped area may not be appropriate for this type of investigation, depending on the time of implantation. These trees would maintain the record, through ring widths and chemistry, of a previous location.
  - Trees that have been significantly pruned: Trees that have experienced pruning may have experienced significant stress that would be reflected in the ring-width data.
  - Trees that have false-ring or no-ring years: False rings often occur when a cold spell occurred during the summer that appears in the ring record as a quasi winter. Rings can be missing as well, for example, when tree growth was seriously limited as a result of environmental factors such as wind, fire, flooding, disease, earthquakes, landslides, or severe climatic conditions; in general, such conditions may be distinguished by comparing with an independent database.
- Trees in warm climates: The method may not work well in the tropics, such as Hawaii or Brazil, where seasonal change is more subtle and tree rings are less distinguishable; however, in some warm and semi-arid areas of the world, trees produce seasonal growth rings that are associated to the annual cycle of the rain period.
- Other limitations include:
  - Alternative sources for elemental markers—there are numerous sources for the elemental markers found in the core samples. Alternative sources include acid rain, surface water uptake, and air pollution. Accordingly, whenever a forensic investigation is conducted, potential, alternative sources must be assessed, as well as the contamination history of the area.
  - Species identification—in colder climates, identification of the exact tree species is limited by seasonal factors (leaves, flowers, fruits).
  - Possible retranslocation mechanisms. Retranslocation of elemental markers, as a radial migration across the annual rings or HSB, is possible depending on the species and the element.

Figure 16. V concentrations versus time for wood-core sample from case study 6.
● The availability of control trees of the same species (outside the plume) as well as the availability of an independent database for the area.

Summary and Conclusions

The tree-ring chronologies describe the pattern and history of growth suppression and release that can be associated with aging and natural disturbances. The width and the composition of rings are dependent upon the tree species and environmental factors (stresses). When significant contamination events occur, they will be reflected in the tree rings and may affect both ring width and chemical composition. Usually, the ring width is affected some years after the contamination first entered the tree, being dependent on the release mode, contaminant concentrations, and tree species. However, tracers may be detected in the wood formed prior to environmental exposure due the number of rings of conducting sapwood. There are many elements that are not transformed and do not significantly retranslocate from one ring to the other. Such elements (Pb, Cl, S, other heavy metals, including different isotopes) are used as markers for age dating environmental contamination through dendroecology with a precision to the year and, sometimes, to the growth season. Entire compounds may also be used as markers if evidence is found for their preservation in the tree without significant metabolism. The various case studies presented here support the method and have proven that the method can be successfully applied regardless of the physical presence of contamination at the time of study. Dendroecology offers an important forensic tool and, wherever applicable, can be used as an independent line of evidence in a forensic investigation.

Acknowledgement

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References


