

# POLLUTION INVESTIGATION BY TREES (PIT)

## METHODOLOGICAL GUIDE

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## Final report



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<http://dendroremediation.univ-fcomte.fr/>

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## ABOUT ADEME

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ADEME provides expertise and advisory services to businesses, local authorities and communities, government bodies and the public at large, to enable them to establish and consolidate their environmental action. As part of this work the agency helps finance projects, from research to implementation, in the areas of waste management, soil conservation, energy efficiency and renewable energy, air quality and noise abatement.

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## A. Pollution Investigation by Trees (PIT)

### A.I. Introduction

Pollution Investigation by Trees (PIT) is an international research program that is funded by Ademe, the French Agency for Environment and Energy Management. The research focused on two different approaches to pollution investigation by trees. The approaches were phytoscreening, which uses plant chemistry to assess current conditions, and dendrochemistry, which uses plant chemistry to assess historical conditions.

Compared with conventional methods that are used to investigate polluted sites and soils, tree-based methods in which trees act as bio-indicators or proxy recorders of current and past pollution are significantly less costly, can be implemented rapidly and with minimum equipment, have minimal environmental effects, and can be used even in areas that are difficult to access (urban areas or topography). Although these methods, called phytoscreening and dendrochemistry, were known to only a few scientific experts worldwide in the early 2000s, this research program was established to further test the methods on well-documented sites and on new contaminants and to communicate relevant information to render the methods accessible to users.

PIT, which officially began in 2011 and has been operating for 3 years, is funded by Ademe and managed by CNRS. Although this program was initially planned for a team of 16 scientists from 5 nations (Canada, France, Germany, Sweden, and the USA) and 13 sites, an additional 35 scientists have joined PIT, adding Switzerland and the UK to the list of participating countries, and 11 sites have been added to the program, including 6 recycled sites. PIT's team represents 16 public and 15 private organisations. Because of the availability of well-established conventional data, twenty-four polluted sites (14 in France, 7 in the USA, 2 in Germany, and 1 in Switzerland) have been selected by PIT to document chlorinated solvents, fossil fuels, polycyclic aromatic hydrocarbons (PAHs), alkanes, PCBs, and metals.

PIT uses the following state-of-the-art analytical methods: AFS, EDXRF, GC/ECD, GC/FID, GCxGC/MS (all the GC methods are coupled with micro-extraction methods), and ICPMS, depending on the contaminant being sought. In total, the data that are gained by PIT extend to 54 cores for dendrochemistry, when only 40 were initially planned, 306 samples by GC/ECD and GC/FID, 221 samples by CVAFS, 80 samples by ICPMS, and 106 samples by GCxGC/MS. All the results are available to potential users upon request. As part of this program, PIT has gathered more than 460 published references, all of which are included in an Excel database (available on this website).

A variety of publications have been generated or are in progress from the PIT project. PIT has published 3 articles in ES&T on phytoforensic methods and one in Chemosphere on tree physiology in relation to major elemental tracers. One article was submitted in December 2013 to Elsevier (environmental modelling and software). Another article is pending at INEF, and four manuscripts are currently being drafted (PAHs and n-alkanes at the SG site, halogenated volatile organic compound (HVOC) cases at the PL and PO sites, and mercury at the SR site). PIT also has attended and presented at the following international scientific conferences: AEHS, AquaConSoil, Battelle, ChloroForum, CNEJE, EPA, Environmental Forensics, Europol, ICCE, INEF, INTERPOL, Intersol, IPS, Nicole, Sediments conference, SETAC, and TRACE and at organised presentations at the University of Missouri (Rolla) for Swiss environmental authorities, HPC in Germany, a field demonstration in Grand Lyon, and multiparty meetings for the USGS and US Forest Service. The documents and data generated by PIT are available in electronic format from the Université de Franche Comté, <http://dendroremediation.univ-fcomte.fr/spip.php?rubrique19>.

### A.II. Phytoforensic principles

#### A.II.1. What trees do

Because plants evolved to be extremely proficient in mass transfer with their surroundings and have survived as Earth's dominant biomass, plants also accumulate and store certain contaminants from their surroundings, acting as passive samplers. Novel applications and analytical methods have

been used to gain information about a wide range of contaminants in the biosphere soil, water, and air, along with available information on both past (dendrochemistry) and present (phytoscreening) contaminants. Collectively, these sampling approaches provide rapid, inexpensive, ecologically friendly, and overall “green” tools that are termed “phytoforensics” (Balouet et al., 2007; Burken et al., 2011; Shetty et al., 2014; Sorek et al., 2008).

Plants interact intimately with their environment, extracting water, CO<sub>2</sub>, and macro- and micronutrients from their surroundings, even when these life essentials are present at minute concentrations. These extraction processes also collect contaminants from the surrounding water, air, and soil.

Innovative sampling and analytical approaches have been developed to assess the extent of contamination and document pollution history. The sampling is rapid and inexpensive and causes little or no discernible damage to personal property or to the ecological systems that we protect.

Vascular plants develop an extensive subsurface root system (Figure 1) and an expansive aerial network of leaves, each with a tremendous surface area. The surface area of both the roots and leaves of the Earth’s plants is greater than the footprint of Earth’s land surface.

The extensive network linking the subsurface and atmosphere provides all the nutrients, water, and CO<sub>2</sub> that are necessary for the growth of the vast majority of Earth’s terrestrial biomass. The link between the subsurface and the air that is provided by vascular plants is the largest biologically mediated energy transfer process on terrestrial Earth, and this transfer is certainly not passive. The active transport of groundwater to and into root systems is fuelled by evapotranspiration and driven primarily by solar and wind energy (Figure 1). More than 60% of the annual precipitation is evapotranspired back into the atmosphere; thus, plants remove more terrestrial water than does the combination of all the river flows. Based on an understanding of plants’ interactions with their surroundings, plants have been used as indicators of their environments for millennia; as far back as Roman times, willow and poplar stands were used to indicate the presence of a shallow groundwater table and a favourable location for the placement of drinking wells.

As plants transpire water, many dissolved constituents are concurrently translocated. Thus, the water and wood of a tree partly reflect the groundwater and soil chemistry.

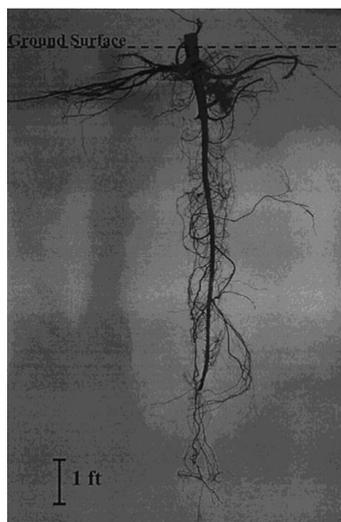


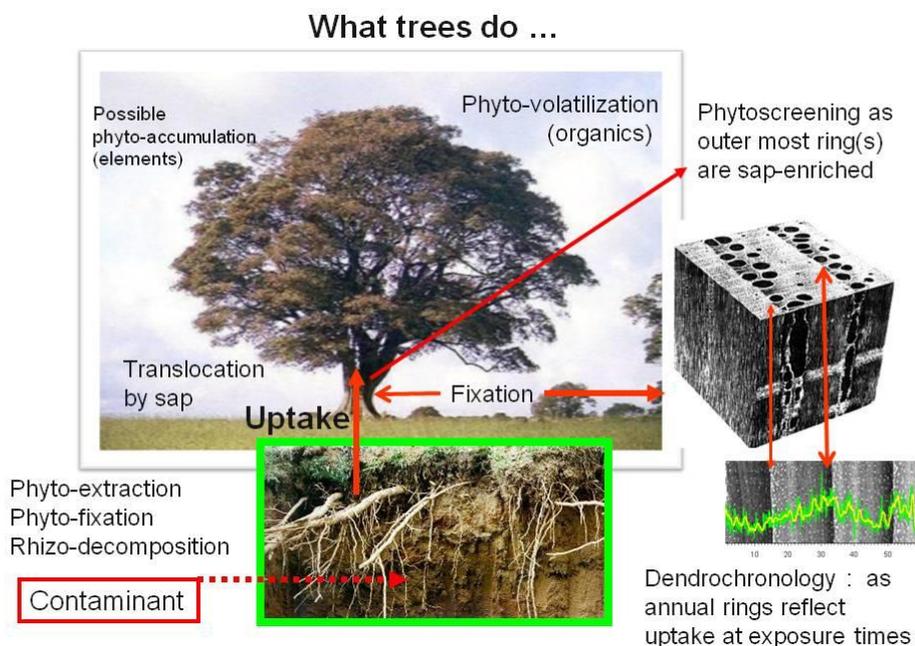
Figure 1. Photograph of tree roots by Jerry Schnoor

### A.II.2. Trees as bio-indicators/proxy recorders

Contamination can be atmospheric, involving gases, aerosol particles and precipitation, impacting the leaves or needles and bark. Alternatively, underground contamination exposes the roots to the soil, groundwater (saturated and unsaturated zones), and soil gases.

PIT focuses on analysis of xylem tissues from trees at polluted sites, because the xylem represents water and contaminants taken up by the roots from the soil and ground water. Volatile organic contaminants also may be present in the air at polluted sites, but the contaminants from the air primarily leave a chemical signature only in the leaves, needles, and bark, and are not transported into the xylem. Roots are capable of taking contaminants up from both the saturated and unsaturated zones. In the unsaturated zones, roots that primarily are used for horizontal anchoring are capable of capturing infiltrating rainwater and soil gas. Roots extending to the saturated zone act as pumps to provide a secure water supply from zones 0.5 to 2 m and deeper into the saturated zone. Roots in karst have been found at depths exceeding 100 m. A PIT case tested the uptake of HVOC contaminants from a groundwater table located at a depth of 35 m in chalk.

Samples collected from trees can represent a mixture of water collected from multiple depths and soil gases. Therefore, tree samples can represent a much larger sampling zone than a typical monitoring well. The presence of contaminants within a tree can be used to gain information on subsurface contamination. The lack of contamination in a tree, however, cannot be used to definitively state that the contamination is not present beneath the tree because a variety of factors can prevent the tree from uptaking a signature of the contamination.



**Figure 2. Dendrochemistry concept.**

Once taken up by the roots, contaminants are translocated by the sap (Figure 2). In ring-porous trees, the contaminants that are in the outermost rings where the sap is the most active reflect the current contamination of the rhizosphere. This process forms the basis of phytoscreening.

Inorganic and nonvolatile contaminants and their nonvolatile tracers or biodegradation by-products can bind to wood tissues that formed at the time of exposure. This process is the basis for dendrochemical methods.

Phytoscreening methods allow for the documentation of volatile and nonvolatile contamination, whereas dendrochemical methods do not allow for the documentation of volatile compounds during their organic phase because these compounds diffuse in wood tissues and are essentially evaporated through the bark and leaves with time. The dendrochemical analytical methods commonly address the elemental or multi-elemental tracers.

The decomposition of organic substances occurs in the rhizosphere but also takes place within tree tissues, especially for chlorinated solvents (Vroblesky et al., 2004).

### A.II.3. A brief history

The earliest mapping of a groundwater plume by phytoscreening methods (Vroblesky et al., 1999) was at a site in in South Carolina site where conventional methods were difficult because much of the plume was beneath a forested swamp. Since then, phytoscreening methods have been deployed at dozens other sites. It was a main reconnaissance method used for investigating subsurface contamination in New Heaven (MO, USA), where the USGS systematically surveyed 100 trees to map an HVOC plume at superfund site in 2000 (Schumacher et al., 2004) (see also sites NH1 and NH2). In 2008, Graber and Sorek randomly investigated trees in Tel-Aviv, providing evidence of past contaminant releases. Vroblesky published his USGS user's guide in 2008, generating international interest for phytoscreening methods (Vrobleski, 2008).

The earliest dendrochemical investigation (Sheppard and Funk, 1975) was an attempt to relate history of metals contamination in surface water from mining activities in the Spokane River (Coeur d'Alene, Idaho) to heavy metal concentrations in nearby trees. The earliest use of dendrochemistry to map the groundwater contamination history (Vroblesky et al., 1999) was at a chemical and unexploded ordinance burial site in Maryland where conventional methods could not be used because of buried explosives.

A variety of different analytical methods have been used to measure major compounds including EDXRF line-scanning methods developed by COX analytical systems, in the early 2000 (Balouet et al., 2009a). Such pioneering work at polluted sites has been accompanied by fundamental research on trees' physiology and capacity to uptake contaminants, and by development of phytoremediation research and perspectives.

### A.II.4. Factors influencing uptake and fixation by trees

Many of the important factors and processes that control the uptake of contaminants in trees have been investigated in phytoforensic studies conducted in greenhouses and in field experiments. These mechanisms are chemical, physical, or physiological in nature; can be climatic or seasonal; and can be affected by natural attenuation mechanisms, including eventual HSB retranslocation and the following:

- Absorption coefficients, such as the ratio of the outside contamination to that inside tree tissues. The influencing parameters include contamination depth (and thus root access), eventual physiological differences between tree species (no such evidence has been found by PIT, even when comparing angiosperms and gymnosperms), climate influences on the groundwater depth, evapotranspiration (Doucette et al., 2003), and precipitation reducing the uptake. The University of Missouri Science and Technology (S&T) in Rolla has documented the HVOC seasonal annual uptake by the same tree over 2.5 years, as measured approximately every 2 weeks, showing both week-by-week variability as well as annual trends (presented at SETAC, Glasgow, by Matt Limmer).
- The log of the octanol-water coefficient (log Kow) approximates the potential uptake of neutral organic compounds, with the highest potential uptake from water occurring for compounds with a log Kow of 1.0 to 3.0. When the soil organic content is high, the optimal uptake occurs at lower log Kow values (Briggs et al., 1982; Limmer et al., 2011; Sicbaldi et al., 1997).
- Uptake also is related to solubility. The less soluble the contaminants or the larger their molecules, the less likely that the contaminants will be taken up by roots.
- Rhizodegradation in which organic compounds are actively degraded by mycorrhizae in the rhizosphere. Active rhizodegradation is especially well documented for HVOCs and the petroleum hydrocarbons of benzene, toluene, ethylbenzene, and xylenes (BTEX) (Burken et al., 2011).
- Regarding source direction, although plumes are hardly isotropic, the distribution of contaminants within trees depends on the root direction. Thus, radial/cardinal uptake principles are influenced by the source/root direction (Burken et al., 2011) (see MEA, NH1, and NH2 cases).

### A.II.5. Legal admissibility

Tree chemistry has been used for litigation purposes. Dendrochemistry has been used by the judiciary as material evidence in Belgium, France, and Germany. Dendrochemistry is the method of choice when determining the contaminant-release date (see GE, PM, PO, SG, and VE sites) and can be used as an independent forensic line of evidence.

Phytoscreening methods have not yet been used as evidence in court cases. However, the legal admissibility of both methods has been documented and tested using the Daubert criteria (Balouet et al., 2009a). Belgian authorities accepted phytoscreening methods in November 2013 for an official case (independent of PIT).

Legal admissibility does not limit the use of phytoscreening at polluted sites for phase I site assessment because in such an application, trees are used at the reconnaissance level only. Tree data cannot be used for phase II levels (quantitative) of site investigation because the data that are gained from trees are semiquantitative and, by contrast with conventional methods, do not document contamination at a given depth.

### A.III. PIT's tasks

- **Testing on different contaminants.** When HVOCs had been positively documented prior to PIT's research, PIT was assigned to document other contaminants families by tree-based methods, including fossil fuels for BTEX, PAH, n-alkanes, PCBs, and metals. Investigated contaminants are same as those investigated by conventional methods.
- **Selecting polluted sites :** Before starting, PIT's steering committee was to propose 13 sites to cover the different contaminants. 11 more sites have been added since, 9 of them recycled from earlier investigations. Many more sites were encompassed prior to final selection, Each of the retained sites was to meet the following criteria :
  - Being well documented by conventional methods, and their pollution history as well documented as possible.
  - Being well vegetated to allow for phytoscreening and dendrochemical methods, including favorable trees' age.
  - Not pose significant communication issues, including legal confidentiality, and site assessors /public organization agreeing to the release of the data. To prevent potential communication concerns, all PIT sites are referred to by 2 to 3 letters (i. e. AN through VEN). US Superfund sites being already communicated by EPA on their web pages, such confidentiality was not an issue. Aerial pictures used by PIT to describe the sites are free of geodesic coordinates, street, river or city's names.
- Several sites happened to be contaminated by more than one family of contaminants (the case in PL, PO, SG, SU). Most of the sites were investigated by both phytoscreening and dendrochemical methods.
- Focus was to document underground rather than atmospheric contamination. So some precaution was taken at sites such as SR, where atmospheric mercury contamination was tested prior to site selection.
- **Establishing an international team** to include research scientists, experts specialized in polluted sites, as users, and analytical laboratories, both from public and private organizations (see section A.II), with specific attention to scientists experience, and complementarity such as to be joined by tree physiologists, computer modelling professionals and statisticians.
- **Selecting state of the art analytical methods and reference laboratories :** where methods used to analyze trees are intrinsically same as conventional methods, such as GC / MS, XRF, AFS, ICPMS, some equipment available propose significantly :
  - enhanced detection limits such as when GC coupled with Solid Phase Extraction (SPE), or combustion/trap cold vapor AFS for mercury, by the ppt.
  - high resolution to analyses multiple contaminants in one run as offered by GC xGC / MS, at ppb level. Note that PIT has been first user of these methods on wood samples.
  - high resolution when line-scanning such as COX's Itrax core scanner by COX, where beam and increment are by 50 µm, at ppm level.

- Methods needing the minimum amount of sample, lesser preparation (i.e. extraction), at reasonable costs, as offered by most referenced labs, were selected by PIT in 6 laboratories from Canada, France, Sweden, UK and USA.
- Important development efforts went to the use of GC x GC / MS as novel analytical method for wood samples, including all spike testing for over 60 organic compounds, as performed by Strathclyde University.
- Tests were to dozens of analytical operations and to some controls.
- **Adapt sampling methods** such as by using spiral augerbit to generate woodchips in sufficient mass for ICPMS or GC x GC / MS methods.
- **Fulfill administrative, contracting and accountancy tasks.** Whilst to also invest in new sampling, preparations tools and worldwide collaborations.
- **Communicating at international conferences and publishing in peer reviewed scientific journals (see section A.IV.3).**
- PIT has also attended and presented at the following international scientific conferences : AEHS, Aquaconsoil, Batelle, CNEJE, EPA, Environmental Forensics, Europol, ICCE, INEF, INTERPOL, Intersol, IPS, Nicole, Sediments conference, SETAC, TRACE and organized presentations in University of Missouri (Rolla), for Swiss environmental authorities , HPC in Germany, a field demonstration in Grand Lyon, and multiparty meetings in USGS and US Forest Service.
- **Organize meetings** with steering committees and Ademe's independent evaluation board.
- **Develop extensive bibliography** as available in pdf format and organize it in an Excel database, over 630 having been gathered with the assistance of all PIT members.
- **Sampling for phytoscreening and dendrochemistry** at the 24 contaminated sites (of which 9 are recycled from independent investigations) for 54 dendrochemistry cores, and 610 samples for phytoscreening.
- **Computerize** all gained phytoscreening analyzes and sites' conventional data in Excel spreadsheets. Dev elop an Excel template to treat dendrochemical data, so as to ease and standardize procedures, save time for each core by using 25 developed Excel and VBA macros in a dedicated template called Scanchem©, saving over 10 hours computer work per core.
- **Identify potential limitations** to the methods
- **Interpret** all data, as gained from trees or from conventionnal methods using site **modélisation** methods (such as Sufer 10), and **statistical** tools including PCA, linear and non linear regressions (see PL and OB cases).
- **Prepare site reports.**
- **Edit and exchange** all gained evidence and texts with PIT's members, site referees so as to reach agreement over data, its interpretation and consecutive reports.
- **Organize and Structure** the hereby report for its **web** release and international access.

## A.IV. Summary of PIT's activities :

### A.IV.1. Field sites

Eighteen sites have been sampled since PIT's launch in 2011. Six sites independently studied by Chris Balouet have been added, with clients' authorization (Table 1).

Out of these 24 sites, 11 were investigated for HVOCs, 10 sites for metals including 2 for Mercury, 8 for petroleum hydrocarbons (BTEX, n-alkanes and PAHs), and 2 for PCBs (see table below). Fifteen are investigated by phytoscreening methods, 18 by dendrochemistry, whilst 11 sites of the 24 sites are investigated by both phytoscreening and dendrochemical methods. Twelves of the 24 sites are in France, 9 sites in USA, 2 are in Germany, 1 in Switzerland.

### A.IV.2. Summary of sample collection

**Table 1. Site locations and number of samples collected for phytoscreening (PS) and dendrochemistry (DC) analyses [F, France; USA, United States of America; CH, Switzerland; G, Germany]**

#	Site name	Contaminant	PS	DC	Sampling date
1	AC : Achères (F)	Metals	7	3	2011/04/22
2	AN : Confidential site (F)	HVOCs	10		2011/07/18
3	CR : Creusot (F)	Metals		3	2009/11/09
4	CU : Confidential site (F)	HVOCs	13		2012/06/07
5	EC : Confidential site (USA)	HVOCs		2	2004/08/08
6	FR : Confidential site (USA)	Heating oil, mining		1	2005/11/17
7	GE : Geneva (CH)	HVOCs	8	6	2011/12/23 (DC) 2012/05/ (PS)
8	HA / Confidential site (USA)	Hydrocarbons		1	2005/03/27
9	LA : Prémery (F)	BTEX	29	3	2011/04/29
10	MEA : Confidential site (F)	HVOCs, Metals	20 (HVOCs), 6 (metals)		2011/04/27
11	MEU : Noyelles-Godault (F)	Metals	22	5	2011/04/06 (DC) 2011/04/19 (PS)
12	NH1 : New Heaven Riverfront (USA)	HVOCs	16	1	2010/10/09
13	NH2 : East New Heaven (USA)	HVOCs	6	1	2010/10/09
14	OB : Confidential site (G)	Hydrocarbons	26		2012/07/23
15	PL : Ploufragan (F)	HVOCs, hydrocarbons, PCBs	20 + 6 (PAHs), 34 (HVOCs), 6 (BTEX)	3	2012/07/4-5 & 2013/06/17-18
16	PM : Confidential site (USA)	Hydrocarbons		1	2005/07/04
17	PO : La Possonière (F)	HVOCs, hydrocarbons	17 (HVOCs), 3(BTEX)	7	2011/05/10
18	SC : Saint Cyprien (F)	PCBs, hydrocarbons	7	2 (40)	2012/04/19 2013/04/20
19	SG : Confidential site (F)	HVOCs / BTEX	17 (HVOCs), 17 BTEX	1	2011/04/30
20	SR : Confidential site (USA)	Mercury	215 + 6 controls	5	2010/10/11
21	SU : Confidential site (USA)	HVOCs		1	2010/10/09
22	TA : Confidential site (F)	Mercury	90	25	2012/06/27 2013/06/23
23	VE : Verl (G)	HVOCs	51	4	2009/08/04
24	VEN : Confidential site (USA)	Fossil fuels		2	2005/09/22
25	France, Sweden, USA	Control sites		3	2012/05/
Total of samples			323 for organics (including 204 for HVOCs) 311 for Hg (SR + TA)		56 cores (including one spiked and one run at 10 & 20 sec)

### A.IV.3. Communication (publications and conferences)

In addition to publications, PIT has also attended and presented at the following international scientific conferences : AEHS, Aquaconsoil, Batelle, Chloroforum, CNEJE, EPA, Environmental Forensics, Europol, ICCE, INEF, INTERPOL, Intersol, IPS, Nicole, Sediments conference, SETAC, TRACE and organized presentations in University of Missouri (Rolla), , HPC in Germany, a field demonstration in Grand Lyon attended by 16 environment professionnals, and multiparty meetings in ADEME, USGS and US Forest Service.

General articles on PIT have also been published by UNESCO (Planet Science), Environnement Magazine and CNRS.

#### A.IV.4. Others

PIT has received an award in June 2012 from the Network of Industrially Contaminated Land in Europe (NICOLE) for Innovative Technology (Figure 3).



Figure 3. Communication on the NICOLE Technology Award 2012 for PIT.

#### A.V. Key results

PIT has developed new sampling methods to meet mass requirement for GC x GC / MS and ICPMS using spiral drill / auger bit (see section B.I.1). An example is a new extraction method for xylem by ASE for organics (PAHs, n-alkanes, PCBs) and first testing GC x GC / MS for phytoscreening applications: along with spikes and real samples (attached report by University of Strathclyde). PIT also worked to overcome GC x GC / MS limitations for gymnosperm analysis (here tested on *Pinus sylvestris*), which contain too high aromatic concentrations, potentially resulting in detector damage.

PIT has completed the first forensic Phytoscreening application for Hg (SR and TA), n-alkanes, PAH (OB and PL), PCBs (PL and SC). However, PCBs were not found in PL nor SC site's tree's xylem, when has been found in bark in SC and during tests.

Adapted sampling and analytical methods presented here prove to be reliable, cost effective, easy and rapidly developed. Statistical analysis has turned possible from the large sampling campaigns conducted by PIT, including PCA. Such methods prove useful when to indentify and distinguish apparently comingled plumes (see OB and PL cases).

The research data collected by PIT is the largest data set compared to any earlier investigation, in number of sites and samples, diversity of contaminants, comparison of conventional and phytoforensic data. It answers important questions including that:

- phytoforensic methods allow to map plumes and address source location issues, since maps gained by conventional methods match maps established from trees data.
- PAHs are not naturally occurring in trees,
- contaminant concentrations increase in trees with increased GW (and soil) concentration: this is the case for HVOCs (GE, PL, PO, VE, ..), n-alkanes and PAHs (OB, PL), and for Hg (SR, TA).
- Less soluble contaminants and with high partitioning coefficient such as mercury or PCBs deserve high resolution analyses.
- N-alkanes are found in most trees (C25-C30) (more testing needed on controls -willows). However, their concentrations significantly increase with fuels contamination.
- Differences exist in trees uptake depending on tree family for metals, including Lead and Zinc, consistent with recent developments in trees enzymology. Poplars and willows are pertinent

choice for metals, especially for lead. There is no identified difference in trees uptake of organic solutes.

- Hg background in unexposed trees is established at 4 ng/g. Hg is uptaken by the root system (in absence of atmospheric contamination, and 80 % of the samples made from apparent roots were more contaminated than higher in bolewood (SR site).

The field studies in this document show the application of tree chemistry for pollution investigation in a variety of applications. At some field sites, contamination was identified by phytoforensic methods, and not by conventional methods. An example is at the VE site, where the tree data showed evidence for a TCE release from the landfill, but the plume was not detectable from the existing groundwater well network. In the PO case, a solvent release in the mid 1950s caused an HVOC groundwater plume was not mappable by conventional methods because of topography and site ownership. In some instances, dendrochemical methods allow the determination of whether the contaminant release was progressive or a rapid onset (see the GE, SG, VE cases). The GE case, where phytoscreening and dendrochemical methods were combined, provides evidence as the relative contributions of two adjacent industries which both used TCE, causing underground contamination.

This study did not find PCBs in trees' xylem, even in areas where PCB contamination was present. A potential reason is that the high octanol/water coefficient likely limits uptake of this compound by the trees. Further investigations are being conducted by Prof Gwen O'Sullivan of University of Calgary, testing enhanced extraction and analytical protocols."

Although tree chemistry is a potentially powerful tool for investigating subsurface contamination, there are some limitations to the methods. An example is that when uncontaminated groundwater is perched above the contaminated target zone, the roots may not take up contaminated water because they have a shallower source. Depth, however, is not necessarily a major limitation, as evidenced by the AN case trees showed evidence of a 35 m deep HVOC contamination plume. In addition, trees near the ocean can be influenced by sea spray. Oceanic influences can cause the S and Cl background concentrations to be too high to use those constituents to age date HVOCs or fossil fuel releases.

The field studies presented here provide substantial evidence supporting the use of phytoforensic methods. In addition, PIT also examined the use of EDXRF in application to chemical anomalies in tree cores (Smith et al., 2014a). These studies increased the understanding of the methods and indications applicable to pollution investigation using trees.

## B. Methodological guide

The analytical methods used for each family of contaminant and whether phytoscreening or dendrochemical methods are used are listed in Table 2. The detection limit (DL) range, sample material and minimum mass required and associated sample preparation or extraction methods are also included.

**Table 2. Analytical methods used for the quantification of volatiles, semi-volatiles and inorganics using phytoscreening (PS) or dendrochemistry (DC)**

	HVOCs*	BTEX	PAH** & n-alkanes*	Cd, Cr, Cu, Ni, Pb, Zn	Hg	PCBs Dioxins
<b>PS</b>	GC/ECD SPME	GC / FID SPME	GC/MS GCxGC/ToF MS	ICP/MS ***	CV AFS	HiRes GC/MS GCxGC/MS
<b>Detection limit</b>	1 ppt	1 ppt	0.5 ppb	0.1 ppb	0.01 ppb	Ppb to Sub ppb
<b>Sampling material</b>	5 mm Ø core, 1.0 g	5 mm Ø core, >1.0 g	wood chips from auger bit, > 2 g	wood chips from auger bit, > 2 g	5 mm Ø core, >0.05 g	wood chips from auger bit, > 2 g
<b>Preparation Extraction</b>	HeadSpace	HeadSpace	Freezing / Grinding / ASE	Grinding / combustion Digestion	Combustion trap	Freezing / Grinding / ASE
<b>DC</b>	EDXRF, Cl as tracer	EDXRF: Co- tracers to fossil fuels, S, Cl, Pb, Ni, V...	GC MS/MS on isolated or combined rings	EDXRF ***	CV AFS on isolated rings	GCxGC/MS on isolated or combined rings
<b>Detection limit</b>	ppm	ppm	ppb	ppm	0,01 ppb	Ppb to sub- ppb
<b>Sample material</b>	1 cm Ø core	1 cm core	1 cm Ø core, > 1 g	1 cm Ø core	5 mm Ø core, > 0.05 g	1 cm Ø core, > 1 g
<b>Preparation Extraction</b>	Lath	Lath	Freezing / Grinding / ASE	None / lath	Combustion trap	Freezing / Grinding / ASE

HVOCs stand for Halogenated Volatile Organic Compounds. As for Hydrocarbons, BTEX (Benzene, Toluene, Ethylene, Xylen), n-alkanes and PAHs (Polyaromatic Hydrocarbons) are distinguished because their sampling and analytical methods do differ.

Main ISO standards applicable to sampling polluted sites are ISO/WD 18400 (101 through 107, and 201 through 204), **ISO 11464 for chemical analysis and ISO 17025 for testing and calibration. US EPA (1983) provides a list of other applicable standards**

\* Dendrochemistry cannot be used to measure the volatile organics (HVOCs or petroleum hydrocarbons) because they evaporate over time. It can however measure its tracers such as Cl for HVOCs, or Cl, S, Pb, V or Ni for fossil fuels.

\*\* Only the less volatile PAHs are detected.

\*\*\* Cr cannot be measured in EDXRF because of the use of Cr as the X-ray source (which can be replaced by a Cu tube). As is not included because the detection limit using ICP MS is too high for practical application in dendrochemistry.

The sample material should exclude bark unless the current atmospheric exposure can be documented. The masses indicated in the above table are the minima required to achieve satisfactory analytical confidence or the enhanced detection limit if the sample mass was doubled.

The analytical methods selected by PIT share the same universally accepted scientific principles as the conventional diagnostic methods used at polluted sites, such as gas chromatography and mass and fluorescence spectroscopy. The methods comply with or mimic international standards such as those of the EPA, ISO or ASTM. Enhanced methods such as GCxGC/ToFMS that allow for improved detection limits were chosen or coupled with enhanced extraction methods (e.g., SPME). The sampling method and preparation and extraction methods were dependent on whether a phytoscreening or dendrochemical approach was used as well as the target contaminants and analytical methods.

## B.I. Sampling methods

Sampling methods differ depending on whether phytoscreening or dendrochemistry is used as the analytical approach. Because phytoscreening addresses the current contamination, only the outermost ring(s) need to be sampled. Dendrochemical methods require longer cores with as many rings as possible. Depending on the investigated contaminant, the sample mass differs, ranging from 0.05 g for Hg to 4 g for PCBs. The dendrochemical samples are extracted at breast height, and phytoscreening samples are extracted closer to the ground.

For all methods, however, it is important to collect as much information as possible about the trees. The trees are referenced, numbered and measured in the field to calculate the diameter at breast height (DBH) and then geolocalised by GPS and photographed.

### B.I.1. Sampling tools

The following sections describe the sampling tools (see website for videos showing the field sampling<sup>1</sup> methods). The cost of these tools ranges from 30 € (spiral drill) to 87 € (sampling hammer) but reaches 1000 € for 50 cm long 10 mm Ø Pressler borers. A phytoforensics tool box must have two of each tool as well as replacement parts in the field because the tools can break.

#### B.I.1.1. Phytoscreening

Three different tools are used (Figure 4) in phytoscreening:

- a sampling hammer or increment borer for 5 mm cores from the outermost rings
- a spiral drill/auger bit; PIT used an 18 mm diameter spiral drill to generate wood chips. After removing the bark, a 20 mm diameter tube is inserted around the drill to prevent the loss of wood chips. This drill diameter allows for the extraction of approximately 1 g wood for each cm deep drilling.
- a Pressler borer of 5-mm diameter for trees with thick bark (e.g., certain old-growth pines, locusts or giant sequoias).



**Figure 4. Phytoscreening tools. (a) Sampling hammer and spiral drill/auger bit. In planta method: (b) spiral drill/auger bit with PDMS fibre and (c) detail of the insertion**

For the in planta method, phytoscreening research was conducted at the University of Missouri in Rolla and consisted of the development of an in planta system in which a metal

device was screwed into the wood to allow for the insertion of an SMPE fibre and repeated sampling of the same tree without having to make new wood samples.

In all cases, the bark is removed. The samples are usually directed towards the source except in directional phytoscreening investigations (i.e., NH OU1, NH OU4 and ME) in which samples are made in each cardinal direction. The phytoscreening samples can be taken from the tree trunk, and the height at which the sample was extracted is documented on the core (i.e., 30 cm versus 60 cm). The sampling requires several minutes per sample.

### B.I.1.2. Dendrochemistry

**Pressler borers are the preferred sampling tool at 10 mm Ø for all investigations, although 5 mm Ø is preferred for mercury. The dendrochemical samples are extracted at breast height (Figure 5).**



**Figure 5. Pressler borer at (a) 10 mm and (b) 5 mm with the borer starter**

Older trees are preferably selected so that a longer historical time span can be covered. In trees such as pine, the age can be determined in the field by counting the number of branch series growing on the trunk from bottom to top because each series of branches accounts for one year of growth. The sampled cores are secured within grooved wooden holding kits to prevent them from becoming distorted during the drying operation.

For the samples dedicated to GC x GC MS analysis, which is used for PCBs, it is important to sample each tree 2 to 3 times so that the annual rings, once segmented, can be combined to produce a higher sample mass for each ring year. When possible, a 12 mm Pressler borer should be used instead of a 10 mm diameter borer because the former enables the collection of a sampled mass that is 40% higher.

Sampling with a Pressler borer requires substantial physical force. Some tree species can have extremely hard wood, such as the Osage orange (*Maclura*). For such species, a special tool known as an increment borer starter (Jim-Gem brand by Haglöf, <http://www.forestry-suppliers.com>) that is made of a belt and screw device allows the drill bit threads to enter the wood.

In all cases, the use of long borer handles (i.e., 60 cm) is recommended to reduce the required force, and two people should be present in the field.

Tree slabs are rarely available, as in the PM case. The slabs were available in the PM case because the trees fallen two years before the phytoforensic investigation and only the stump remained. Slab material, once it is marked with pencil to show compass orientation and top, can be sectioned to produce samples for EDXRF analysis or to obtain additional material for analysis of individual growth rings.

### B.I.2. Selecting trees

Trees are selected for their known or suspected contamination; controls are selected from areas that were not exposed, and they are preferably of the same species as the exposed trees. The sampled trees must be as healthy as possible, and the scarred, infected or decayed zones of the trees must be avoided along with areas of branch interference.

The phytoscreening samples should be taken when the trees are leafed out (late spring to early fall) because contaminant concentrations are higher in the sap during these seasons. The samples to be examined using dendrochemistry can be sampled at any time of the year.

The samples should be handled with gloves or forceps and saved in plastic sampling tubes for short cores or within sample holders for long cores. Glass headspace vials are used for phytoscreening organics. If it is raining on a sampling day, all precautions should be taken when extracting the samples from the sampling tool and placing them in the sample holder so that they are not touched by raindrops. Car interiors allow for such safe handling.

### B.I.3. Phytosanitary considerations

Although phytoscreening and dendrochemistry sampling do not cause substantial damage to the trees, and the trees rapidly scar from these techniques (Kersten and Schwarze, 2005), the major fungus pathologies, cankers and diebacks must be acceptable to the samplers (<http://www.iowadnr.gov/portals/idnr/uploads/forestry/fungalcanckers.pdf>):

- ash dieback disease (*Chalara fraxinea*), which is a fungus infection in ash trees, especially *Fraxinus excelsior* in Europe (<http://www.forestry.gov.uk/chalara>);
- *Hypoxylon* canker (<http://www.na.fs.fed.us/spfo/pubs/fidls/hypoxylon/hypoxylon.htm>); and
- canker stain of plane (*Ceratocystis fimbriata*) ([http://na.fs.fed.us/spfo/pubs/pest\\_all/cstain/cstain.pdf](http://na.fs.fed.us/spfo/pubs/pest_all/cstain/cstain.pdf)).

Trees growing at a contaminated site may show signs of stress from the contamination. These trees can still be sampled, although the most impacted areas must be avoided because these infections can greatly alter the physiological functioning of the xylem and phloem. Tools can easily be sterilised after sampling, and alcohol is preferred to household bleach (which contains chlorine that could alter DC data) for cleaning the coring bits and preventing disease transmission.

Although the samples are mailed to foreign countries for laboratory analysis, there is minimal to no risk of transmission of such fungal pathogens because the sample mass is small and the pathogens cannot survive the sample treatments; for example, the PS samples are digested or destroyed after the study and the DC samples are preserved in sealed plastic bags and kept in the office.

As for the pollutant content, the sample mass ranges from 0.1 g (PS) to approximately 10 g (DC) and does not contain more than a few ng of contaminant in the most contaminated samples, which is significantly below toxic thresholds or transportation requirements. Chemical treatments or additives are not required before the samples reach the laboratory.

Whether to caulk the tree where a sample has been removed is an additional consideration: private owners often require such caulking to be performed; however, caulking can slow cambium repair. Sprays can also be used to sterilise the wound.

## B.II. Sample preparation and extraction methods

Accelerated solvent extraction (ASE): ASE is also known as pressurised liquid extraction (PLE) and is described in the Strathclyde report dated November 2012, in which a Dionex ASE 350 was used. Tests conducted for PCBs determined that 100% hexane was preferred compared to blends combining hexane and toluene. The best extraction temperature was found to be 100°C. The extraction parameters were set at 1,500 psi, 5 min static time, 40% rinse volume, 60 sec purge time and one static cycle. The extracts were further evaporated under negative pressure conditions before analysis.

Combustion (pyrolysis): Combustion is performed for Hg analysis by combustion trap cold vapour analysis. This procedure can also be used before acid digestion on wood sample ashes for ICPMS.

Digestion: Digestion is performed for metals before ICP/MS on ground samples by using nitric acid solutions (H<sub>2</sub>NO<sub>3</sub>).

**Drying:** Drying is performed on all samples dedicated to dendrochemical investigations. Generally, sapwood rings contain 40 to 60% moisture, and heartwood contains less. The samples are first dried in ambient air for at least 3 days to allow for sample surfacing and verification of sample quality or isolate annual rings as required for GC, GC x GC and ICP/MS and CVAFS purposes. Sample drying should not be performed before other analytical methods. Sample heating except at the ambient temperature is not allowed because it risks evaporating the contaminants, even partially. The water content of the core is a limitation in CVAFS when measuring Hg. For all the pre-dried samples, concentrations are given in dry wood mass. For other samples run for volatiles using headspace methods, the samples are dried after analysis so that the contaminant concentrations can be given relative to the sap.

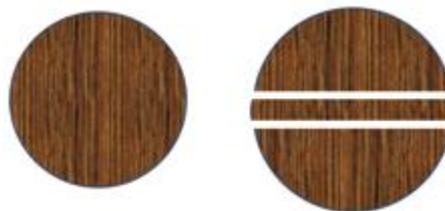
**Freezing:** Freezing of samples to be analysed for their organic contaminants by GC x GC/ToFMS or samples for semi-volatiles is best performed at -80°C upon receipt to conserve the samples before grinding.

**Note:** Frozen samples can also be used in a new extraction method known as “freeze – centrifuge”, which was developed by the Missouri University of Science and Technology (Rolla, Missouri, USA) to extract explosives before analysis by ultra-fast liquid chromatography (UfLC) (Ma et al., 2012). PIT is not required to use this freeze-centrifuge method.

**Grinding:** Grinding is performed before digestion for ICP/MS and accelerated solvent extraction GC/MS x MS. Once ground, the wood cores or chips are turned to  $\mu\text{m}$ -sized particles to ease digestion or solvent extraction. Over 100 PIT samples were milled before analyses by ICPMS, and another 100 were milled before GC x GC-MS. The grinding of samples dedicated to organics for use in the GC x GC/MS method was performed when frozen by using liquid nitrogen.

**Headspace:** Headspace gas chromatography is the preferred method for phytoscreening volatile organics, including volatile aromatic compounds (VACs), BTEX and HVOCs. Over 400 such samples have been processed for PIT at the Missouri University of Science and Technology.

**Laths:** Cores to be run by EDXRF for dendrochemistry purposes are first dried in ambient air conditions, and then two-mm thick laths are cut from the 10 mm cores perpendicular to the wood grain using a twin disk saw to jointly allow for EDXRF line scanning analyses and microdensitometric images of the sampled tree (Figure 6).



**Figure 6. Laths are cut perpendicular to the grain**

**Laser ablation:** Laser ablation is commonly associated with inductively coupled plasma mass spectrometry (ICPMS) in the analysis of metals. This method has not been used by PIT.

**Ring segmenting:** For the dendrochemical investigation of mercury and semi-volatiles, the segmentation of dated rings is required before CT CVAFS or GC x GC/ToFMS. Such segmenting is performed under optical magnification because the ring boundaries are rarely perpendicular to the axis of the cores.

**SPME:** Solid-phase micro-extraction is used by PIT to phytoscreen the HVOCs, BTEX, PAHs or PCBs, and it is used in GC/ECD, GC/FID and GC x GC/ToFMS methods. Polydimethylsiloxane (PDMS) and carboxene/PDMS composite (Car/PDMS) grain are used to retain the target organic contaminants. SPME grain are used either in the lab to capture the volatile organics or in the field, which is a method known as “in planta” that was developed by Missouri University of Science and Technology. The in planta method can be applied for monitoring as well as screening.

**Surfacing:** To verify the quality of the dendrochemical core and inspect cores with digital microscopes at low magnification (10x to 40x) for decayed samples, those with branch interference or reduced parallax and older samples, trees are pre-surfaced using different sand papers from 50 to 600 grit made of silicon carbide to reduce possible contamination by other minerals. To prevent the evaporation of organics (e.g., PCBs) by heat, this surfacing is performed on a frozen marble slide. Surfacing is a required step when segmenting individual rings for dendrochemical analysis, which is the case for mercury or PCBs.

### B.III. Analytical methods

The analytical methods used by PIT are based on the same principles that are used in conventional site assessment methods and criminal forensics. The analytical method depends on the investigated contaminant and may depend on whether the phytoscreening or dendrochemical approach is used.

**Footnote on the phytoscreening analysis:** to provide a statistical assessment of accuracy, 10% of the samples should be analysed in duplicate. The duplicate samples should be collected from the same tree as the initial sampling and taken only a few cm from the initial sample. Concentrations can change significantly at different diameters of the tree. Gradients in the subsurface pollutant concentration can also be apparent in trees, and up to 10-fold different concentrations can be found on opposite sides of a tree (Limmer et al., 2013).

The following sections successively describe the phytoscreening dendrochemistry methods.

#### B.III.1. Phytoscreening chlorinated compounds: HVOCs and PCBs

HVOCs and PCBs contain chlorine and can be analysed by GC ECD techniques.

Because HVOCs are volatiles, they are measured from headspace vials by GC/ECD/SPME. These compounds are too volatile for analyses by GC x GC/ToFMS.

PCBs must be extracted and can be analysed by GC/ECD methods after Soxhlet extraction or by GC x GC/ToFMS after ASE.

##### B.III.1.1. GC/ECD/SPME for HVOCs

The samples are placed in headspace vials (2 cl) in the field, and the caps must be tightly screwed closed. The time period before analysis should be limited to 5 days maximum, and the samples should be chilled if possible.

All analyses of PIT HVOC samples have been performed at the Missouri University of Science and Technology. Although the GC ECD (Gas Chromatography/Electron Capture Detection) is the preferred method for HVOC detection, the combination of solid-phase micro-extraction (SPME) sampling in vivo using PDMS grain (polydimethylsiloxane) or composite carboxene/PDMS grain can also be used. SPME in vivo sampling allows for considerably reduced detection limits from ppb to sub-ppt levels for compounds such as TCE and PCE. Such concentrations are given for the initial sapstream or transpiration streams after the wet and dried wood masses are calculated for mass correction.

The following chlorinated solvents were identified and measured by PIT from the 204 analyses performed at the Missouri University of Science and Technology on the 11 HVOC sites: tetrachloroethene (PCE), trichloroethene (TCE), *cis*-1,2-dichloroethene (cDCE), trichloroethanol (TCEOH), chloroform or trichloromethane (CF), carbon-tetrachloride or tetrachloromethane (CT), trichloroethane (111TCA) and dichloromethane (DCM). The measured concentrations ranged from several ppt to over 400,000 ppt for individual compounds, up to 800,000 ppt for total HVOCs (PL case) and as little as 0.5 ppt as a method detection limit (Limmer et al., 2012). The analytical method is described in the journal article and supporting information appearing in *Environmental Science and*

*Technology (Limmer et al., 2012)*. This method is not applicable in dendrochemistry because HVOCs evaporate over time or diffuse throughout the rings.

GC methods, used in this investigation can document highly volatile contaminants, such as dichloromethane. This is because headspace GC methods do not require solvent extraction, such as by hexane, which is commonly for semi-volatiles. Hexane extraction limits the ability to detect volatile compounds in headspace because even with minor heating, there is little tendency for highly volatile compounds to leave the solvent and volatilize (the boiling point is 71°C for hexane and only 40°C for DCM).

An issue was raised with regard to PIT's PL case, which was the only case out of 11 in which DCM was documented. In this case, very high concentrations of DCM of up to 300,000 ppt (300 ppb) were found in trees in the 2012 sampling campaign, whereas the groundwater, soil and soil gas data from dozens of samples were less positive for DCM (although 2 groundwater samples had similar concentrations of 100 s µg/l (i.e., ppb). The 2013 test campaign at the same site failed to find DCMs in any of the trees, soil, groundwater or soil gas samples. See the PL case for further discussion.

A regular GC/ECD that is not coupled to SPME was applied in earlier investigations conducted at the CU site. Although the method is applicable, the detection limits are approximately 10 to 1000 times higher and are not sensitive enough for phytoscreening investigations.

#### **B.III.1.2. GC/ECD/SPME for PCBs**

PIT has used the GC/ECD/SPME method at the University of Kingston (ON) after SOXHLET extraction of SC. However, PCBs, which are also referred to as persistent organic compounds, can be highly volatile and readily evaporate when the samples are heated or within weeks, even at ambient temperatures.

#### **B.III.1.3. GC x GC/ToFMS for PCBs**

The recent advent of a comprehensive two-dimensional gas chromatography method coupled to time-of-flight mass spectrometry (GC x GC-ToFMS) has led PIT to test these methods for their unique capacity to discriminate a broad range of volatile and semi-volatile organic contaminants at ppb detection limits.

Therefore, PIT has contracted with the University of Strathclyde to develop extraction and analytical methods for phytoforensic applications and run 100 samples for PCBs/dioxins, PAHs and other volatile organics from 3 sites (OB, PL, SC). The testing work and protocols are described in the Strathclyde report. Two PIT sites (PL and SC) have been tested by this method.

For all tests conducted on spiked samples, no PCBs were found in PIT's PL or SC cases; however, they were found in a bark sample taken from a Glasgow street. A new campaign independent of PIT is currently being conducted by Gwen O'Sullivan at the University of Calgary in connection with PIT's team and the University of Strathclyde.

### **B.III.2. Phytoscreening hydrocarbons**

#### **B.III.2.1. GC/FID/SPME for BTEX, hydrocarbon VOCs and PAHs**

The GC/FID/SPME sampling method is the same as that used in GC/ECD flame ionisation detection (FID), which is the preferred method for BTEX. When coupled with the SPME, the method detection limits are lowered to approximately the 10 ppt level. This method has been successfully tested in the laboratory on spiked trees placed in greenhouse drums by the Missouri University of Science and Technology.

In the 100 GC/FID analyses performed at the Missouri University of Science and Technology, only benzene and toluene were found (SG and LA site), and their concentrations were less than 50 ppt for toluene and 4 ppt for benzene. Ethylbenzene and xylene never exceeded the detection limits, even at sites where contamination by petroleum hydrocarbons was positively documented (OB, PL and PO). In the case of PO, the trees within the pollution plume were very young poplar, and they might have been prevented from uptaking contaminated GW and soils by a concrete slab. Previous

research has shown that biodegradation in the rhizosphere is a predominant fate if aerobic conditions are maintained in the subsurface (Wilson et al., 2013).

Contrary to the HVOCs, the time before analysis of BTEX should be limited to 2 days maximum, which accommodates the faster natural attenuation of BTEX. To allow for longer delays between sampling and analysis, PIT has attempted to make the microsamples inert by adding a few drops of sulphuric acid diluted at 0.1 N. This method has been used at the PL site when the samples were not positive for BTEX (although the site was known to be slightly contaminated by BTEX). Preservation methods have been assessed, and the immediate freezing of samples or addition of sulphuric acid addition can preserve the samples before analysis.

Note that total hydrocarbons (THCs) are common at sites polluted by fossil fuels, and a conventional GC/FID analysis is commonly performed. Because of the abundance of naturally generated aromatics, such as phenols, toluene or other VACs, the positive measure of the total hydrocarbons in trees might be misleading as representing the natural physiology of these plants rather than anthropic contamination by petroleum hydrocarbons in the environment. When sampling US cedar trees (not Lebanon cedar), toluene produced from the plant is routinely detected.

BTEX compounds do not appear to be readily detectable by GC x GC/ToFMS methods. The reason is that the compounds are too polar for the method.

#### **B.III.2.2. GC x GC/ToFMS for PAHs and n-alkanes**

The GC xGC/ToFMS method cannot analyse volatile organics that have boiling points below that of hexane (71°C). However, test results have shown the method's ability to reveal the presence of approximately 1000 different organics. These organics are either naturally occurring or the result of environmental exposure. Naturally occurring organics should be verified from the controls of the same species because such compounds cannot be used in phytoforensic investigations.

Three sites were tested by PIT (OB, PL and SC), and GC x GC/ToFMS was able to identify and quantify n-alkanes from C14 to C39 and the 16 US EPA PAHs and other PAHs from m/z 242 to 264.

Volatile organic compounds are not applicable for dendrochemistry because they evaporate over time, often before sampling or before analysis, or diffuse within the stem, as shown in the NH case. Semi-volatile organics such as PCBs and dioxins and less volatile PAHs can be analysed by dendrochemical approaches after isolating the individual rings by GC x GC/ToFMS, which was performed in the SC case.

### **B.III.3. Phytoscreening metals**

#### **B.III.3.1. ICP/MS**

One hundred PIT samples were ground and digested by nitric acid solutions before the analysis by inductively coupled plasma mass spectrometry at Missouri University of Science and Technology, Rolla, for the metals (As, Cd, Cr, Cu, Ni, Pb, Zn). In this method, a solution is injected into a plasma torch, and the ions are separated by a mass spectrometer according to their mass-to-charge ratio. Calibrations are conducted with standard solutions and produce a detection limit that varies greatly (from low ppm to sub ppb) for different analytes. Over 50 tests were conducted prior to running the 100 samples on standard solutions, and they showed that the arsenic analysis requires digestion using a sealed digestion process, such as in a high-temperature microwave. Mercury concentrations in wood tissues are naturally too low to be readily analysed by ICP/MS methods.

Pyrolysis can be an alternative preparation method when coupled with acid digestion; however, As and Zn would be lost at the temperatures reached with this method.

#### **B.III.3.2. AFS for PS and DC for Mercury**

The samples are dried 5 mm cores made either from a sampling hammer for phytoscreening or a 5 mm Pressler borer for dendrochemistry after the annual rings have been segmented. The samples are placed in 6 mm tubes, where they are combusted. The sample's mercury content is directed in the gas phase to a gold sand amalgamator, where it is trapped (combustion trap). The gold

sand is later desorbed and analysed by gas phase atomic fluorescence spectroscopy (AFS). The minimum sample mass is 50 mg, and units are in dry wood with detection limits of 0.1 or 0.01 ppb depending on the equipment. A total of 221 samples were analysed by PIT from contaminated and non-contaminated environments in France and the USA. The background noise is approximately 2 ppb. Concentrations at contaminated sites exceed 4 ppb and range in the dozens of ppb when significant soil contamination is found, with maximums found at 1 ppm. Such concentrations are too low for dendrochemical EDXRF methods (see section B.III.4.1)

### **B.III.3.3. Other methods**

**Portable GC/MS for HCs and HVOCs:** The recent development of GC/MS portable units allows samples to be analysed in the field; however, such analyses are at the ppb level and have questionable quantification accuracy. Such portable units were previously used in the field for conventional site investigation methods and phytoscreening (e.g., the NH case).

Gathering data by in-field analysis is rapid and can provide a new tool for plume delineation; however, the detection range in ppb limits the usefulness of such analyses in phytoscreening applications because of issues with accuracy and quantification (see, also, the CU case).

For the analysis of ammunition (Karnjanapiboonwong et al., 2012), the University of Missouri has developed and tested new freeze/centrifuge extraction methods to measure explosives at the ppb level by ultrafast liquid chromatography.

## **B.III.4. Dendrochemistry**

Dendrochemical methods cannot document volatiles, although they may work for semi-volatiles. The main dendrochemical methods use the fluorescence of elemental pollutants or their tracers and are performed using the following techniques:

- line-scanning (i.e., the ITRAX equipment by COX or LA ICPMS; see below);
- individual rings once they are segmented and dated, which is the method used for Hg dendrochemistry.

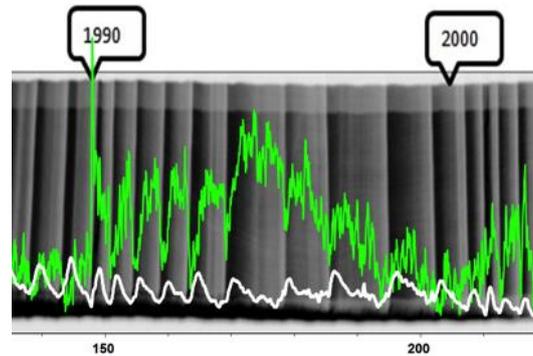
Elemental compounds and pollutant co-tracers can be detected and used to determine multi-elemental signatures (Balouet et al., 2012, 2009b).

### **B.III.4.1. EDXRF**

The 10 mm diameter cores are dried before a 2 mm thick lath is cut over the core's length perpendicular to the grain using a twin disk saw. Thirty-one elements are simultaneously analysed by energy-dispersive X-ray fluorescence (EDXRF), which is described in (Balouet et al., 2012b). The ITRAX Multiscanner is a "flat-beam" X-ray scanner in which samples are exposed to a precisely defined, thin X-ray beam with a vertical dimension of 50 µm. An X-ray line camera records the transmitted radiation that passes through the sample. Therefore, a full-scale two-dimensional radiographic image with 50 µm resolution is obtained by moving the sample relative to the beam in 50 µm steps (**Erreur ! Source du renvoi introuvable.**). Unlike optical photographs, dense material in the radiograph appear light in tone and less dense material appears dark. At each step, a silicon drift chamber detector (SDD) records the characteristic fluorescent X-ray radiation (XRF) from specific alkaline earth and other metal elements with the atomic weight of Mg and higher. The collimator system within the SDD confines the XRF recording to a sample area of 0.05 × 2 mm<sup>2</sup>. The SDD functions as an energy-dispersive X-ray detector with a capacity of 100,000 counts per second (cps) and a resolution of approximately 135 eV at 5.9 keV. Equipped with the standard 1.9-kW chromium-tube X-ray source, the SDD records approximately 20,000 cps for wood tissue. An exposure time of 10 s is normally sufficient to record element concentrations above 10 ppm but not below 2 ppm, although the exposure time is dependent on the atomic mass and other possible mathematical calculations such as the time-weighted centred moving average (TWCMA).

Analytical concentrations are given in counts (cts) of fluorescent photons for the following 30 elements: Mg, Al, Si, P, S, Cl, Ar, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Mo, Cd, Cs, Ba, Hg, Pb and Bi. The elemental data for Cr and Mo cannot be used in dendrochemistry because these elements are used as the X-ray source and detector, respectively. Other analytical parameters such as the mean square error (MSE) and total coherent and incoherent photons are also recorded.

Other elements at extremely low concentrations, such as Hg (which does not exceed 1 ppm in most polluted trees), cannot be used in EDXRF dendrochemical investigations.



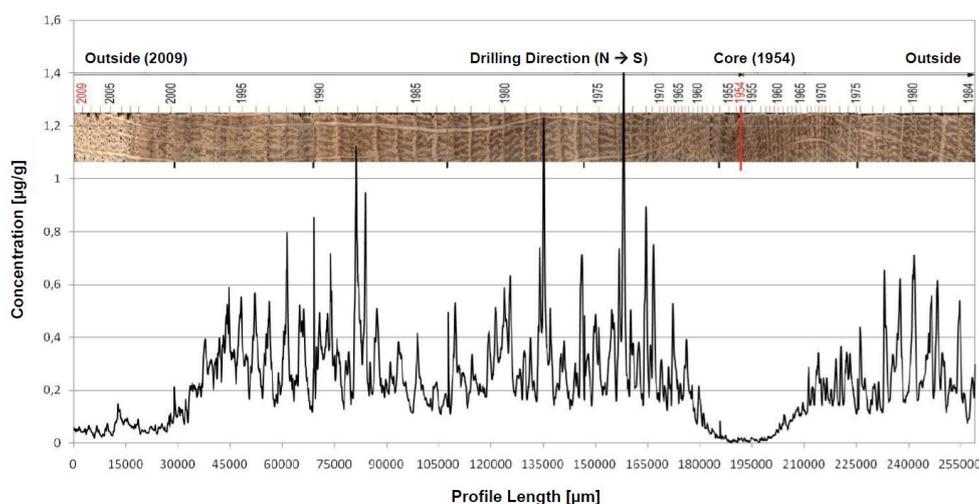
**Figure 7.** Example of EDXRF profiles for chlorine (in green) and calcium (in white) for the VE case, tree 7

The maximum core length is limited to 30 cm for the translation stage, which provides 6,000 data points for length and 180,000 analytical data points. All elemental data must be normalised for matrix-density effects so that the photon counts reflect the absolute concentrations. Because of the number of mathematical, statistical and graphing operations required before dendrochemical interpretation, a new software program called Scanchem© has been developed by PIT (refer to the Scanchem© section B.V).

Dendrochemical anomalies associated with soil or groundwater contamination (Balouet et al., 2009a) depart from the background in the annual minima, maxima and average over at least one ring. Typically, such anomalies are followed by a “recovery” after a few rings in which concentrations remain slightly higher than the previous background, even when contamination is still present in the tree. Such partial “recoveries” shown by dendrochemical records can be attributed to a compensation mechanism within the rhizosphere, such as microroot renewal and turnover (Wang et al., 2012). Dendrochemical anomalies can be monoelemental, such as with chlorine in chlorinated solvents, or multielemental, such as with leaded gasoline that is characterised by Cl, S and Pb.

#### **B.III.4.2. LA/ICPMS by line scanning**

Independent of PIT, the University of Jena has developed LA/ICPMS methods by line scanning wood cores (Figure 8). Note that LA/ICPMS can document a or Bo, which are two important elements when investigating polluted sites that are too light for EDXRF detection.



**Figure 8.** Figure courtesy of Dirk Merten, University of Jena: investigation of a uranium-contaminated site by line-scanning core LA/ICPMS at the ppb level

## B.IV. Data handling

The data used by PIT include maps or aerial photographs, brief historical summaries, pollutant data from sites derived from conventional methods and new phytoscreening and dendrochemical analyses.

Except for Hg, all the analyses used or generated by PIT covered multiple contaminants:

- \* GC/ECD: all HVOCs
- \* GC/FID: BTEX (E and X were not found in PIT's samples)
- \* GC x GC/ToFMS: 16 PAHS and 24 n-alkanes plus other organics
- \* ICP/MS: 6 metals
- \* EDXRF: 30 elements simultaneously analysed

In addition, all of the trees were identified by the species or the genus and diameter at breast height. For most sites, the latitude and longitude were processed in Excel files.

Pictures were taken of all trees. Maps and aerials are typically in .png format. Site and tree coordinates are not released through PIT's website for confidentiality reasons. Pictures of the trees and most sites are not available through PIT's website because of the file sizes and for confidentiality reasons.

For some of PIT's sites where confidentiality was not a potential issue, synthetic site reports are provided in Appendix H III.

### B.IV.1. Databases

All databases operated by PIT are in Excel format, which was chosen for its capacity to handle complex and large files and its widespread use.

All pollutant data except for EDXRF data have been combined in one single Excel file for all sites, which is called "*general PIT analyses*". This file is accessible via PIT's website, provided in Appendix H1. The EDXRF data are provided in Appendix H2.

### B.IV.2. Statistics

Some of the overall statistics of PIT's program are provided in Section B.VI. The advanced statistical page of tree species can be found in Appendix H2. For other specific statistical tools used by PIT, see Sections VI.

## B.V. Scanchem©

Scanchem© has been designed and developed under PIT as an Excel template to computerize the more than 1,500 calculations, statistical and graphic operations that are required to support dendrochemical interpretation, in an efficient and readily reproducible way. The template can be downloaded at the following INRA web site :

<https://www1.clermont.inra.fr/piaf-www1/Telechargements/telecharger.phphem>.

## B.VI. Statistical methods used by PIT

Since the first phytoscreening investigations by Vroblesky (Vroblesky et al., 1999), numerous experiments have been conducted in laboratories, especially by Burken (Burken et al., 2009), to test the uptake of volatile organics by plants and examine the physico-chemical characteristics of these compounds (see Section B VI I). Similarly, since the first EDXRF/line scanning dendrochemical investigations in 2002 (Balouet et al., 2009a, 2007), PIT has conducted many more tests, as described below in Section B VI II. The use of control samples in phytoforensics is as important as it is in other disciplines of forensics.

### B.VI.1. Testing phytoscreening methods

Phytoscreening methods have been repeatedly tested since their inception in 1999 by the following means:

- considering the physico-chemical properties of contaminants;
- testing volatile organics in lab experiments on hydroponic “reactors”;
- comparing tree cores in field tests with conventional data at polluted sites; and
- testing control samples to ensure that the contaminants are not naturally present in trees.

#### B.VI.1.1. Physico-chemical properties (Table 3)

**Organics:** The important physico-chemical properties of contaminants that are taken up by plants include their water solubility; Henry’s law constant ( $k_H$ ), which describes the solubility of trace gases; Log  $K_{ow}$ , which is also known as Log P (for partitioning) and describes the differential solubility of chemical compounds in octanol versus water and their hydrophilic versus hydrophobic and lipophilic affinities; and the soil sorption to the soil carbon. Density is also an important parameter because compounds heavier than water tend to drown in groundwater (e.g., chlorinated solvents at  $>1$ ) or surface in the groundwater, such as occurs with petroleum hydrocarbons with densities  $<1$ .

**Table 3. Chemical characteristics of major hydrocarbons.**

Common name	Henry's constant	Log Kow	Soil-sorption Log Koc	Water Solubility	Density	Dissipation half-lives	Ground water frequency rank*
<b>Benzene</b>	0.557	2.13	1.49 / 1.73	1780	0.876	< 2,0	11
<b>Toluene</b>	0.660	2.73	1.75 / 2.28	531	0.867	< 2.0	9
<b>Ethylbenzene</b>	0.843	3.15	2.22	161	0.867		12
<b>m-xylene</b>	0.73	3.2	2.11 / 2.46	161	0.864		
<b>O-Xylene</b>	0.551	3.12	1.68 / 1.83	207	0.880		42
<b>p-xylene</b>	0.690	3.15	1.68 / 1.83	181	0.861	2.2	
<b>Pseudocumene</b>	0.524	3.65	3.34	57	0.876		
<b>Naphthalene</b>	0.43	3.36	2.98	31	0.997		18
<b>MTBE</b>	0.70	0.94	1.09	36200	0.740		
<b>Tetrachloroethylene, PCE</b>	1.73	2.88	2.37	210	1.623		3
<b>TCE, trichloroethylene</b>	1.03	2.53	2.00	1280	1.464		2
<b>Carbon Tetrachloride</b>	2.99	2.64	1,78	1200	1.594	5	16
<b>DCE</b>	2.62	2.13	2.18	6400	1.213		
<b>Methyl chloroform</b>	1.76	2.49	1.78 / 2.03	4590	1.44		
<b>Ethylchloride</b>	1.11	1.43	1.62	6710	0.920		
<b>Vinyl Chloride</b>	2.68	1.38	1.75	2700	0.910		15

The petroleum hydrocarbons above and HVOCs below are based on the USGS open-file report 2006-1338 and \* were taken from the EPA report 600/R-93/140 by Marti Minnich. DCM is the n° 1 contaminant according to the EPA report.

The left columns indicate the dissipation half-lives, which indicate how fast the compounds will evaporate, and the groundwater frequency rank indicates how often these compounds are found at polluted sites.

The partitioning coefficients octanol/water and water/carbon are influenced by other physico-chemical parameters, including the pH.

Some authors (Cutter and Guyette, 1993) have tried to ascertain the compounds that can be investigated by trees solely according to physico-chemical parameters. The same criteria have been used in other guides (Holm et al., 2011) to report “definitive” conclusions, such as the inability to detect PAHs by this method; however, such strong statements are subject to criticism because even if these compounds are less likely to be taken up by trees based on their physicochemical properties, they may still be taken up; thus, selecting the proper analytical methods and appropriate detection limits is still important. As a result, PIT has been using the most sensitive methods and testing recent methods, including GC x GC/ToFMS (see the appendix in PIT’s web site), and several of the earlier established “strong” conclusions can be contradicted when using modern analytical methods.

Note that other physico-chemical properties are also essential to phytoscreening methods:

- When organic compounds are extracted by solvent methods (e.g., ASE and Soxhlet), all the compounds with boiling points lower than the solvent cannot be used for phytoscreening purposes (e.g., dichloromethane’s boiling point is at 39.6°C, and hexane’s is at 69°C).
- Even less volatile compounds, including PCBs and semi-volatile compounds, evaporate; thus, for phytoscreening methods, certain compounds must be frozen or vacuum preserved before being transmitted to the lab to prevent their loss and eventual degradation.

Three essential parameters remain:

- the form of contamination (whether in DNAPL, aerobic or anaerobic phase, and thus groundwater, soil or soil gas) because these aspects drive the fate and uptake of contaminants;
- rhizodegradation;
- translocation by sap; the tree’s enzymology is critical for metal uptake and translocation (this has been partly tested in the lab (see below) and in the field (see PIT’s cases)).

**PCBs:** This group of 209 different compounds have very low solubility that is variable depending on the compound. It is generally considered that lighter and lesser chlorinated PCBs have higher bio-availability.

**Metals:** The solubility of metals is an important parameter. However, contaminating metals are found in different ionic, oxygenation or hydroxylation statuses and can be methylated (e.g., methyl mercury). In addition, their metallic, ionic and organic forms influence their solubility and bioavailability.

### ***B.VI.1.2. Tree physiology***

Intensive research, especially from a phytoremediation perspective, has been conducted in the lab and field over the past 2 decades to verify the species that would work best for phytoremediation, and it has included clones and bacteria-inoculated roots, such as for trees contaminated by rhizoextraction or that have rhizodegradation capacities. Poplars and willows have been documented as having the highest phytoremediation efficiencies for most metal contaminants when compared to other angiosperms, and molecular investigations (Blaudez et al., 2003, 2003; Migeon et al., 2010, 2009; Pottier et al., 2015) have shown that specific genes enhance the translocation and accumulation efficiency of these trees.

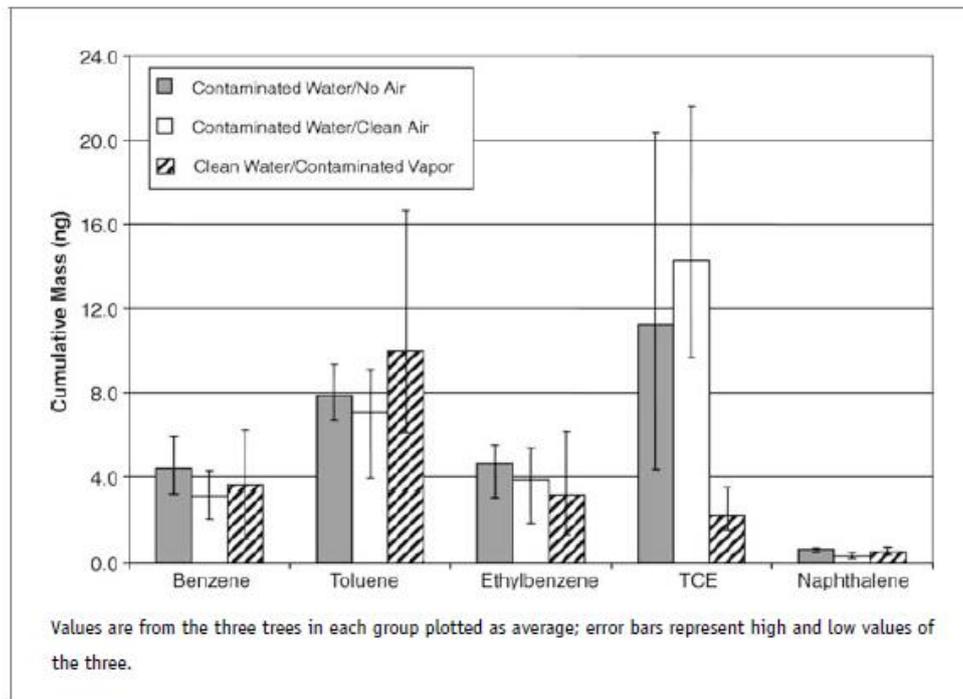
### ***B.VI.1.3. Lab (hydroponic) experiments***

“Reactors” are greenhouse-planted pots used in labs to test the uptake of trees under controlled/spike exposure and similar soil/climate conditions, also known as hydroponic conditions. The tests have primarily focused on volatile organics, including TCE, BTEX, PAH and MTBE (Burken et al., 2009), and experimental campaigns are on-going.

Experiments have examined root exposure to soil, soil gases, groundwater, DNAPL and spatial heterogeneity (radial phytoscreening). The physico-chemistry of contaminants has been considered according to partitioning coefficients, including octanol/water ( $\text{Log } K_{ow}$ ) and Henry's constant (for vapour fugacity and the air/water partitioning coefficient). Most tests have been conducted on poplars to document the sapflow concentration and eventual rhizodecomposition or rhizofiltration mechanisms.

A summary of the main results (see Burken et al. 2009) is as follows:

- The respective roles of groundwater versus aerobic environment or contaminating soil gases can be established based on their influence on sap content (Figure 9).



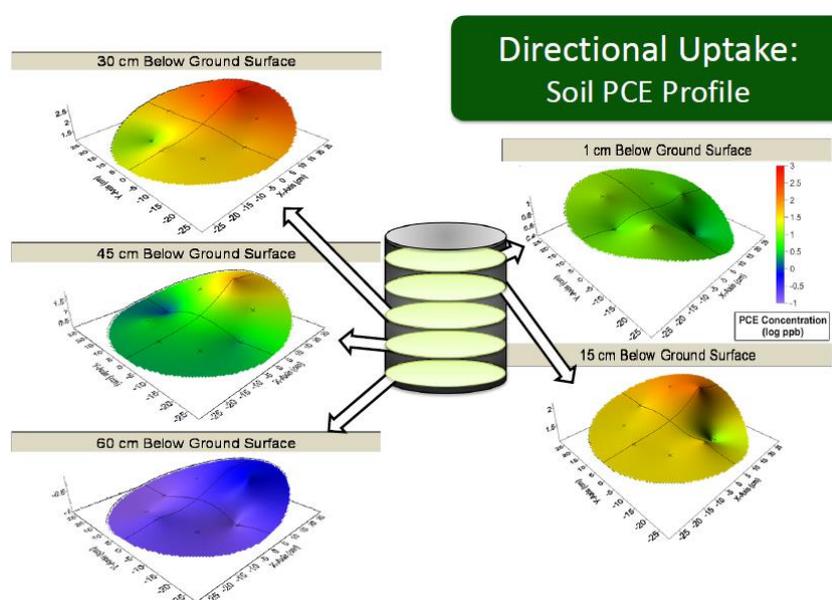
**Figure 9. Average cumulative mass of each contaminant collected in the diffusion traps for the three groups: (1) contaminated water without air exchange, (2) contaminated water with air exchange and (3) clean water/contaminated water, from (Burken et al., 2009)**

- The octanol/water partitioning coefficient ( $\text{Log } K_{ow}$ ) reflects the water solubility and hydrophobic/hydrophilic properties; it has been estimated that  $\text{Log } K_{ow}$  is optimal for VOC uptake and translocation when it is between 1.8 and 2.5.
  - The higher Henry's constant is for a contaminant, the less it is suspected of being absorbed by roots in the gas phase.
  - For example, the 3.15  $\text{Log } K_{ow}$  value of ethylbenzene makes its translocation less efficient, and its relatively high Henry's constant of 0.330 makes it a more likely candidate for binding to the root epidermis and other plant tissues. Such mechanisms may explain why ethylbenzene (and xylene) have not been found in the 4 cases by PIT for BTEX (LA, PL, PO and SG).
- Naphthalene ( $\text{Log } K_{ow} = 3.36$ ; Henry's constant = 0.018) is the least translocated compound of the 5 tested by (Burken et al., 2009)
- Experimental tests proved that trees can uptake more contaminants from GW only or soil gases when the 5 tested VOC contaminants do not cross the vadose zone.

- Error bars in figure 10 (above) show that even under hydroponic conditions, trees remain individual organisms.
- The fluctuation of the water table, which is influenced by the higher diurnal/lesser nocturnal evapotranspiration by trees, can influence the aerobic/rhizosphere degradation of tested volatile organics.

Other hydroponic tests proved that PCBs were more abundant in the root systems, when soil/GW were tested. The lightest PCBs were shown to translocate under such hydroponic conditions in poplar stems (Van Aken et al., 2010).

In 2009, Burken tested the radial/directional uptake and translocation by trees in drum reactors and hydroponically confirmed the reasonable principle under which the radially directed exposure concentrations matched the radial distribution of their stems (Figure 10). Such evidence supports the fact that phytoscreening field samples are collected on the side of the tree facing the pollutant source and that when only a limited number of trees are available from a plume of limited extension, directional phytoscreening is of practical value (see the MEA case as PIT's example application).



**Figure 10. Hydroponic tests on directional phytoscreening tests**

When directional phytoscreening is used, the samples should be taken low on the stem, at 30 cm above the ground or above the tree collar.

**PCBs:** (Liu and Schnoor, 2008) demonstrated the poplar's capacity to uptake and translocate lesser chlorinated PCBs. PCB uptake in root systems had been established previously; however, plant uptake is selective, and most soluble and lesser chlorinated PCBs are more easily taken up and translocated when the plant's rhizosphere contributes to their natural degradation, and the metabolism of PCBs has been documented within plant tissues.

#### **B.VI.1.4. Field testing**

**Chlorinated solvents:** HVOCs are the most common pollutant found in soils and groundwater worldwide and are most frequently investigated by phytoscreening methods. A comparison of conventional and phytoscreening data, which were tested by PIT at 11 sites, was conducted to complement previous investigations. There is no debate in the scientific community concerning the efficiency of these methods for HVOCs if the sampling and analyses are conducted according to state-of-the-art protocols.

An additional issue concerns the potential differences that may exist among plant genera or families with regard to their rhizosphere capacity to degrade HVOCs or selectively uptake and

translocate HVOCs from underground, which might also be influenced by the trees' ages. Such statistical investigations are limited by the availability of conventional underground site data:

- In all cases investigating both phytoscreening and underground data, the plumes were identical, and HVOC plume anomalies were not found relating to any tree genus (see PL, PO or VE case).
- There are few such investigated sites where conventional data from GW, soil and soil gases are available to establish possible field-based relationships with tree concentrations.
- Underground soils and substrate heterogeneities and their resulting hydrogeological patterns are well-established parameters that influence plume migration and fate.
- Based on the 11 sites investigated by PIT and the 204 samples, there were no data from trees that would lead to the interpretation of significant differences, even between gymnosperms and angiosperms.
- There were no identifiable or significant differences between gymnosperms and angiosperms based on their sap content in the various HVOC species (i.e., TCE, PCE and Cis-DCE) per site.

It is apparent from all previously published records that the key factor influencing tree uptake of HVOCs is the concentration to which the roots are exposed, and it has been clearly established (and documented in PIT's HVOC cases and available analytical data) that the spatial distribution of underground plumes can vary greatly within only a few metres, even in the most simple cases such as 1 source and 1 release event. Therefore, it is PIT's view that this question is scientifically pertinent, but it cannot be properly addressed unless sites are found that meet the following criteria: the plume, soil and substrate are adequately homogenous; data on groundwater, soil and soil gases obtained by conventional methods are available for each site; and they have sufficiently dense and varied vegetation per surface unit. PIT invites its readership to propose such sites for further investigation.

**N-alkanes:** Because n-alkanes are major tracers of contamination by petroleum hydrocarbons and can be detected by GC x GC/ToFMS, PIT has analysed 54 trees from the OB and PL cases. As expected, some n-alkanes were found in all the sampled angiosperms, especially in the C24-C29 range; however, all n-alkane concentrations in trees increased with documented underground contamination by petroleum hydrocarbons. PIT also established that gymnosperms (e.g., *Pinus* in the SC case) had such high organic content that n-alkanes could not be identified, and the concentrations were too high to potentially damage the detector.

**PAHs:** In collaboration with the University of Strathclyde, PIT has investigated the PAHs in trees using GC x GC/MS methods (see the OB, PL and SC cases and the report on GCxGC/MS in appendix). More importantly, PIT's research determined that PAHs are not naturally present in trees and that their concentration in trees is correlated with underground anthropic contamination by petroleum hydrocarbons.

**PCBs:** To date, there is no data indicating that PCBs can be documented in tree xylem with field testing methods; therefore, it is not possible to support PCBs being forensically documented from xylem tissue by phytoscreening and dendrochemical methods. Unfortunately, 15 samples evaluated by PIT from the SC site, which is one of the most contaminated sites in the world, were lost after overheating them at 150°C for 8 hr. In addition, the detection limit used by PIT is at the level of ppb, thus levels below this threshold would not be detected.

**Metals:** Some authors have verified metal concentrations in eastern red cedars growing on different substrates (rhyolite, dolomite, sandstone, chert and limestone) and showed that natural background conditions, without anthropic contamination, influenced xylem concentrations (Guyette et al., 1992). Other research on metals conducted at polluted sites on hundreds of trees has been useful in comparing concentration ranges at contaminated and control sites (Table 4).

**Table 4. Metal concentrations (mg/g DWt) in various trees as determined by dendrochemical method and corresponding references.**

Metal	Reference	Range of metal concentration
<b>As</b>	(Cheng et al., 2007)	Contaminated : $0.28 \pm 0.15$ (n = 32 / 4 genera) Uncontaminated : $0.06 \pm 0.04$ (n = 30)
	(Loredo et al., 1999)	Contaminated : 2 - 13 (n = 31 / 8 genera)
<b>Cd</b>	(Brackhage et al., 1996)	Contaminated : 0.5 - 13 (n = 6) Uncontaminated : 0.25 - 1.3 (n = 3)
	(Hagemeyer et al., 1992)	Contaminated : 0.11 - 0.216 (n = 10, <i>Fagus</i> )
	(Sheppard et al., 2007)	Contaminated : 0.134 - 0.145 ( <i>Populus</i> ) Uncontaminated : 0.036 - 0.045 ( <i>Populus</i> )
	(Stewart et al., 1991)	Contaminated : 0.19 (n = 6, <i>Dacryocarpus</i> ) Uncontaminated : 0.015 - 0.021
	(Watmough, 1997)	0.03 - 2.73 (bibliographic research)
	(Watmough and Hutchinson, 2003)	Contaminated : 0.04 - 1.4 (n = 13 ; 4 genera)
	(USGS, 2004)	0.05 - 0.55 (n = 4, <i>Pinus</i> )
	<b>Cr</b>	(Brabander et al., 1999)
(Sheppard and Funk, 1975)		0.02 - 0.9 (n = 3, <i>Pinus</i> )
(USGS, 2004)		Contaminated : 5 - 15 (n = 5 ; <i>Pinus</i> )
<b>Cu</b>	(Sheppard et al., 2007)	Contaminated : 1.42 - 2.24
	(Stewart et al., 1991)	Contaminated : 4.1 - 10.6 ( <i>Dacryocarpus</i> ) Uncontaminated : 0.59 - 1.5 ( <i>Dacryocarpus</i> )
	(Watmough, 1997)	0.08 - 50.5 (Bibliographic research)
	(Watmough and Hutchinson, 2003)	Contaminated : 2.0-7.0 (n = 9, <i>Acer</i> )
	(Watmough and Hutchinson, 2003)	Contaminated : 2.0 - 15 (n = 13, 4 genera)
	(Zou et al., 2004)	Maxima : 0.8 - 6.4
<b>Hg</b>	PIT, SR & Ta sites	Contaminated : $> 0.004 < 1$ Uncontaminated : $< 0.004$
<b>Ni</b>	(Watmough, 1997)	0.18 - 10 (bibliographic research)
<b>Pb</b>	(Baes and Ragsdale, 1981)	Contaminated : max = 0.27 - 25 (n = 15 / 3 genera) Uncontaminated : max = 0.05 - 3 (n = 18 / 3 genera)
	(Barnes et al., 1976)	Contaminated : 240 - 680 Uncontaminated : 1.5 - 8.4 (n = 7 / 4 genera)
	(Bellis et al., 2004)	Contaminated : 0.2 - 2.5 (1 genus) Uncontaminated : 0.2 - 0.5
	(Bindler et al., 2004)	Uncontaminated : $0.021 \pm 0.008$ (1 genus)
	(Devall et al., 2006)	Contaminated : 1.7 - 10.5
	(Fisher et al., 2002)	Contaminated : 0.28 (n = 29) Uncontaminated : 0.02 (n = 1)
	(Hagemeyer et al., 1992)	Contaminated : 0.24 - 0.38 (n = 8, <i>Fagus</i> ) Uncontaminated : 0.15 (n = 15, <i>Fagus</i> )
	(Sheppard et al., 2007)	Contaminated : 0.10 - 0.15 (n = 22, 2 genera) Uncontaminated : 0.02- 0.043 (n = 5)
	(Stewart et al., 1991)	Contaminated : 3.2 Uncontaminated : 0.045 - 0.19
	(Watmough, 1997)	0.04 - 20 (bibliographic research)
	(Watmough and Hutchinson, 2002)	Contaminated : 2.1 - 5.2 (n = 16, 3 genera) Uncontaminated : 1
	(Zou et al., 2004)	Contaminated : 0.4 - 10 (n = 11, <i>Pinus</i> )
	<b>Zn</b>	(Brackhage et al., 1996)
(Hagemeyer et al., 1992)		Contaminated : 5.39 - 12.3 (n = 23, <i>Fagus</i> )
(Sheppard and Funk, 1975)		0.4 - 109 (n = 3, <i>Pinus</i> )
(Stewart et al., 1991)		0.3 - 280 (bibliographic research)
(Watmough et al., 1998)		Contaminated : 20 - 95 (n = 11, <i>Acer</i> )
(Zou et al., 2004)		Contaminated : 7 - 40 (n = 11, <i>Pinus</i> )

Note that Stewart (1991) and Sheppard (2007) included dendrochemical data. In addition, Hagemeyer (1975) compared seasonal concentrations (April, June, September and December), providing the first evidence of seasonal variations in metals, which has been confirmed by EDXRF dendrochemistry.

### **B.VI.2. Testing dendrochemical methods**

In typical dendrochronology studies, control trees provide documentation of the natural background conditions, such as droughts, that can influence tree growth and visual tree-ring patterns. In dendrochemistry studies, impacts of contaminants on tree growth are determined by comparing tree-ring patterns at the contaminated site to control trees of the same age under similar climatic conditions. Rings of trees at contaminated sites that substantially pre-date the pollution event can also be used to document natural backgrounds. Forensic applications of dendrochemistry also require that the chemicals can be determined and internal physiological mechanisms can be distinguished from chemical impacts associated with pollution events.

The following section addresses controls, such as non-exposed trees, as well as tests based on a total of 108,000 EDXRF elemental data points. The analytical repeatability of EDXRF line scanning analysis is within 1%.

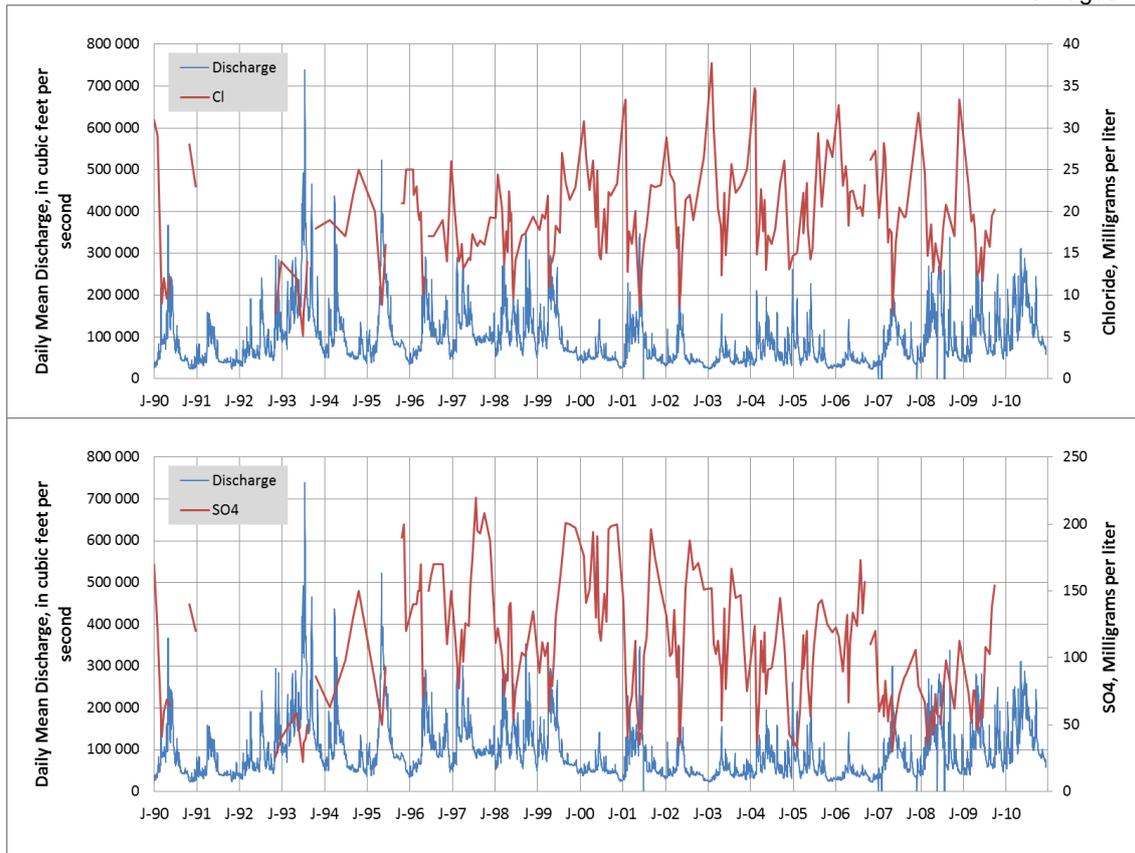
#### **B.VI.2.1. Unexposed controls**

PIT sampled three control trees (*Picea sp.*, *Quercus sp.* and *Fagus sp.*) in the forest of Compiègne, which is located approximately 60 km north of Paris. A fourth control tree (*Pinus sylvestris*) located in Swedish Lapland was also sampled. Together, these trees provide a baseline of the elemental distributions in non-polluted trees. Here, we will focus on the elements potassium (K) and calcium (Ca), which can affect the physiological function of trees, and chlorine (Cl), which is a common tracer of petroleum hydrocarbons and volatile chlorinated solvent compounds.

#### **B.VI.2.2. Major elements in unexposed controls**

Ca and K are the most abundant elements in trees and are essential tracers of internal physiological mechanisms such as decay, mineralisation, retranslocation, compartmentalisation, age/size trends and intra-ring distribution. These internal physiological processes have been recently discussed (Smith et al., 2014b).

The cyclical intra-ring patterns are not related to density because EDXRF data are normalised. This is further confirmed by research on ringless tropical trees (Poussart et al., 2006) where an X-ray microprobe synchrotron of calcium established intra-ring profiles comparable to those generated by EDXRF linescanning. In her associated research, Poussart was able to correlate Ca maxima with the amount of dry-season rainfall.



**Figure 11. Elemental distribution of Cl and SO<sub>4</sub> in the Missouri River as surveyed by the US Army Corps of Engineers from 1996 through 2009 (after Herman). Similar trends were found for Ca, Na, Mg and K.**

The intra-ring chemical cycles found in tree rings are consistent with those shown in Figure 11, in which the low flows are in the late winter and the summer flows are regulated higher than normal to allow barge traffic. Elemental concentrations are lower in spring, after winter recharge, and higher in summer, when precipitation is reduced.

Such natural discharge/recharge mechanisms are suspected in groundwater that is influenced by seasonal precipitations and provide a reasonable explanation for the cyclical seasonal pattern of elemental uptake by trees; further research in dendrochemistry and climatology may prove promising.

PIT's analyses of dated tree cores by EDXRF of Ca and K are shown in Figure 12 and illustrate some of these processes. Ca concentrations, quantified as counts (cts), are shown on the right (red) vertical axis (scale: 0 – 4,500 counts). K concentrations are shown on the left (blue) vertical axis (scale: -100 – 1,500 counts). The top two panels show normal inter- and intra-ring variations of Ca and K in *Pinus* and *Picea*. The third panel (*Fagus*) shows the enrichment of K prior to 1950 in the pith near the tree's centre, which suggests decay. The fourth and fifth panels are of a 113-year-old *Quercus* that had to be scanned in two pieces because of its length. The inner core shows normal variations of Ca and K. However, the higher concentrations of K in the outer core beginning at approximately 1997 indicate retranslocation and compartmentalisation (Smith et al., 2014b).

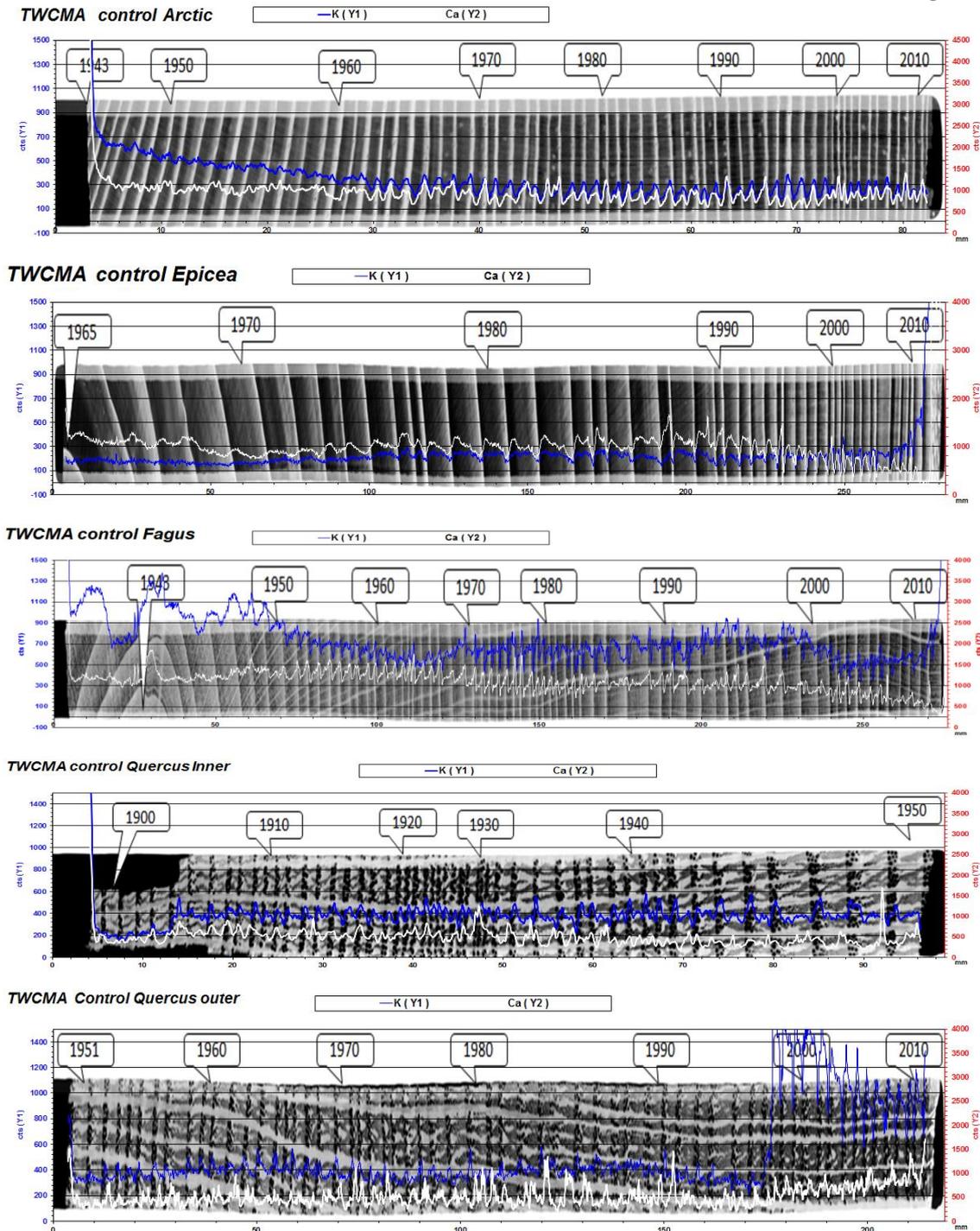
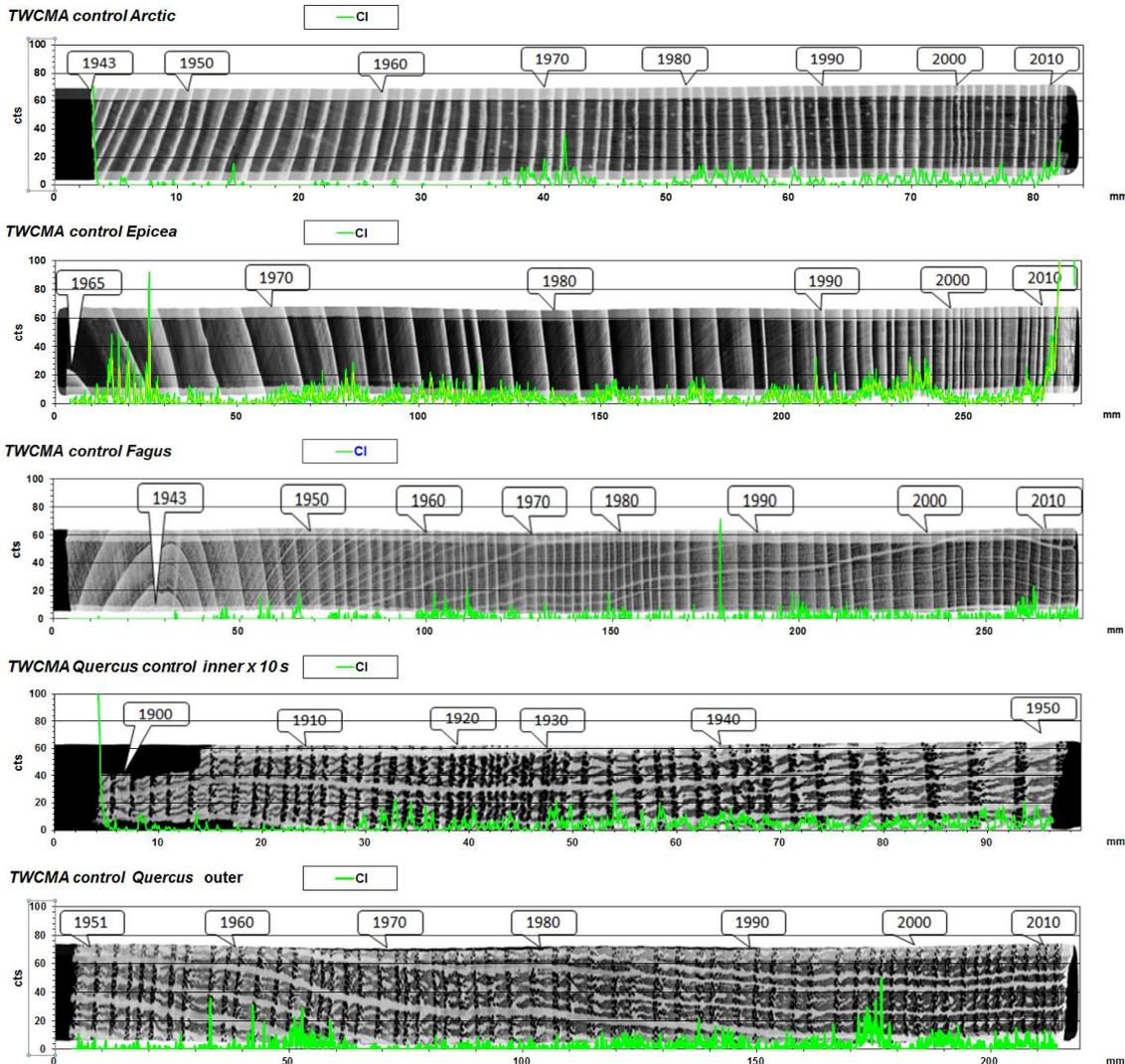


Figure 12. The five panels illustrate profiles of normal variation of Ca and K concentrations except for K in *Quercus*, which shows obvious retranslocation at the heartwood/sapwood boundary (HSB) after approximately 1997, and for *Fagus*, which shows enrichment in the pith that suggests decay in the rings prior to 1950

### B.VI.2.3. Chlorine in unexposed controls

The Cl profiles shown in Figure 13 illustrate two types of Cl anomalies: narrow high Cl concentrations (< 100  $\mu\text{m}$  in extent) and higher Cl concentrations found in isolated rings. An isolated Cl anomaly is evident in the control *Fagus* at ring year 1988 (71 cts). Another anomaly is evident in the control *Picea* at ring year 1967 that peaked at 92 cts. Both of these anomalies are limited and approximately 100  $\mu\text{m}$  and are interpreted as evidence of micro-mineralisation (Smith et al., 2014b).

Chlorine distributions show a consistent absence of major elemental anomalies. Except for a few rings, there are synchronous Cl departures from the background in Compiègne's controls for ring year 2008 (*Picea* and *Fagus* but not *Quercus*), 1996 (*Picea* and *Quercus* but not *Fagus*) and 1964/1967 (*Picea* and *Quercus* but not as high in *Fagus*), although they are not much greater than the inter-ring variability (see the definition of a dendrochemical anomaly in Section B. III 4.1



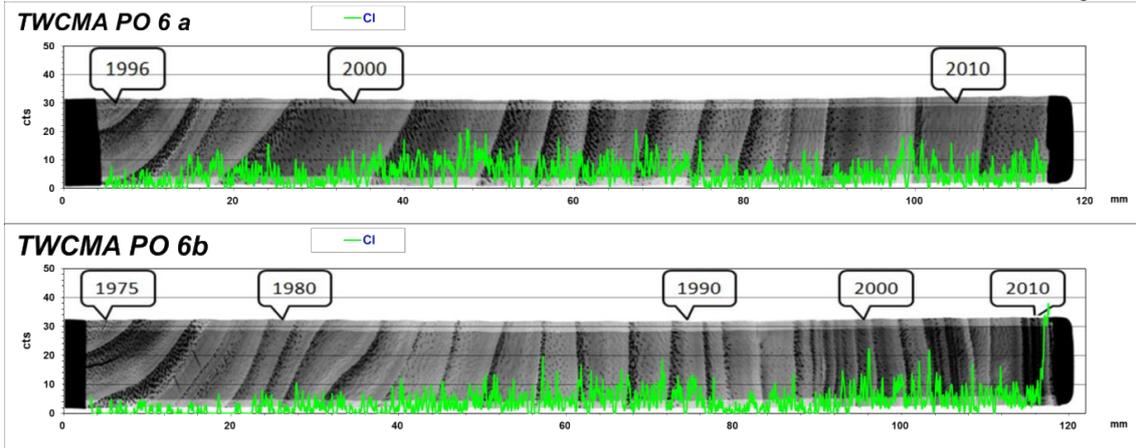
**Figure 13.** The five panels show low Cl concentrations that average 2-8 cts over the core depending on the tree species along with minor anomalies that are physiologically driven by climate. The above trees show some minor amplitude changes in chlorine in the mid-1960s in all cores, 1996/1998 in *Picea* and *Quercus* and 2008 (a well-documented heat wave in France) in *Picea* and *Fagus*. The Cl peaks found in the *Picea* ring year 1967 and in the *Fagus* ring year 1988 are very narrow and only extend over approximately 100  $\mu\text{m}$ ; they are viewed as the results of mineralisation (Smith et al., in press).

The Cl data shown in Figure 13 highlight the importance of analysing controls of the same species as those studied at contaminated sites when conducting phytoforensics investigation. It is important to distinguish internal physiological phenomena from those caused by external factors, such as impacts of contaminated soil or groundwater.

### B.VI.3. Other PIT tests

#### B.VI.3.1. Comparing 2 nearby trees of the same species

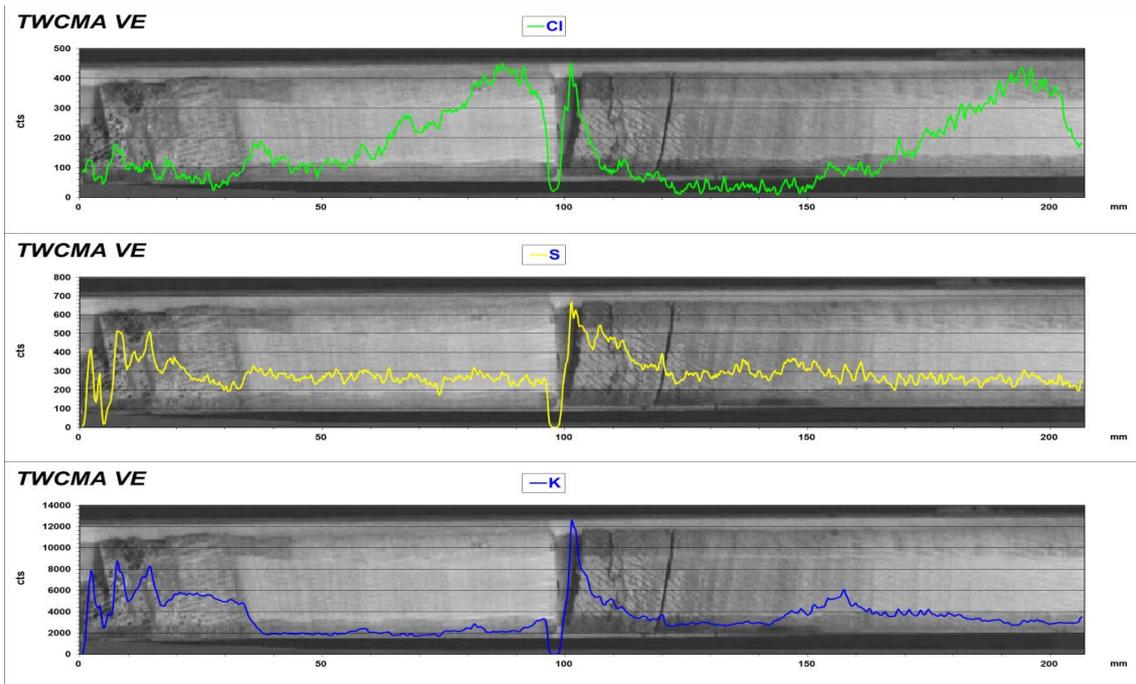
The two trees in Figure 14 are ashes (*Fraxinus excelsior*) located in an area mildly contaminated by HVOCs (PO site).



**Figure 14. Chlorine profiles for trees 6a and 6b**

The Cl profiles (Figure 14) clearly show the same background, and the two trees share a minor Cl impact, possibly by chlorinated solvents of approximately the same concentration, over the years 2000-2010.

### **B.VI.3.2. Comparing 2 cores of the same tree**



**Figure 15. Two 10 cm cores of the same sycamore tree aligned for EDXRF analysis. Elemental profiles are graphed from top to bottom for Cl, S and K.**

This sycamore was located 100 m from the oceanfront in New Jersey. The tree was sampled twice, and the two cores were aligned for EDXRF analysis in same run. Both cores show exceedingly high chlorine and sulphur concentrations at similar concentrations that peak at 400 cts and above 500 cts, respectively (Figure 15). The abundance of these contaminants is ten times above the natural background of other trees analysed by PIT, which is attributed to the major oceanic influence. Note that the cores are heavily decayed in the oldest rings, as shown by their K profiles, and this decay has also influenced the Cl and S distribution. Such overwhelming background and decay makes such trees unfit for use with chloride or sulphur as pollutant tracers.

### B.VI.3.3. Comparing 10 and 20 second counting times

The EDXRF counting time is an important analytical parameter. At 10 sec, the time required to analyse a 30 cm core is 19 hr, including counting and translation times. The counting time doubles for a 20 sec counting time. A 10 sec counting time is PIT's routine setting, and it was chosen as a compromise to allow analysis in the ppm range, even for less abundant elements such as Cl, while minimising instrument run time (Figure 16).

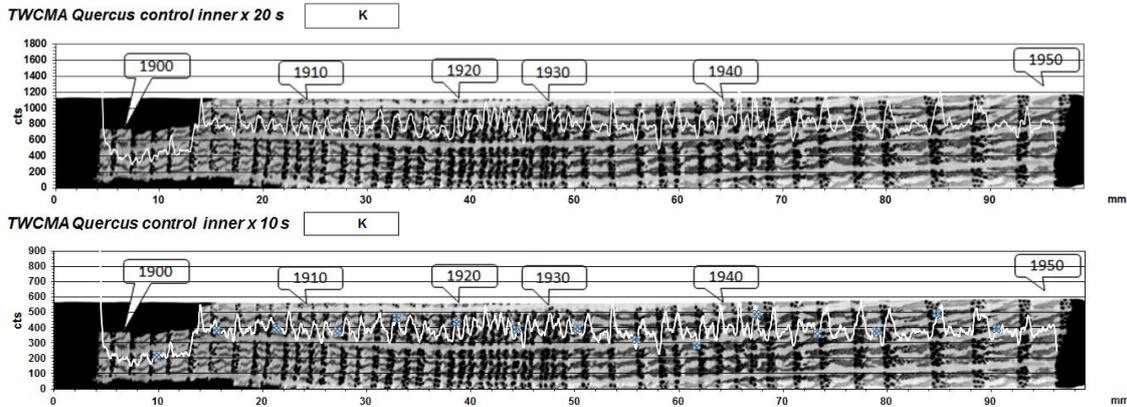


Figure 16. Comparison of the same core run at 20 sec (above) and 10 sec (below). The potassium profiles were exactly the same, but the concentrations doubled at 20 sec, as expected.

Such testing further highlights the reproducibility of EDXRF analyses (1,800 points) with less than 1% variance, which is acceptable for forensic applications.

### B.VI.3.4. Artefacts

To characterise potential artefacts by their elemental signature, a Scotch pine sample was analysed once for a baseline and then spiked and rerun after being spiked for the local Cl and S fingerprint (left, X = 3 to 20) and compared the results to the Cl and S signatures of the municipal water supply (centre, X = 35 to 55) and road salt (right, X = 70 to 82) (Figure 17).

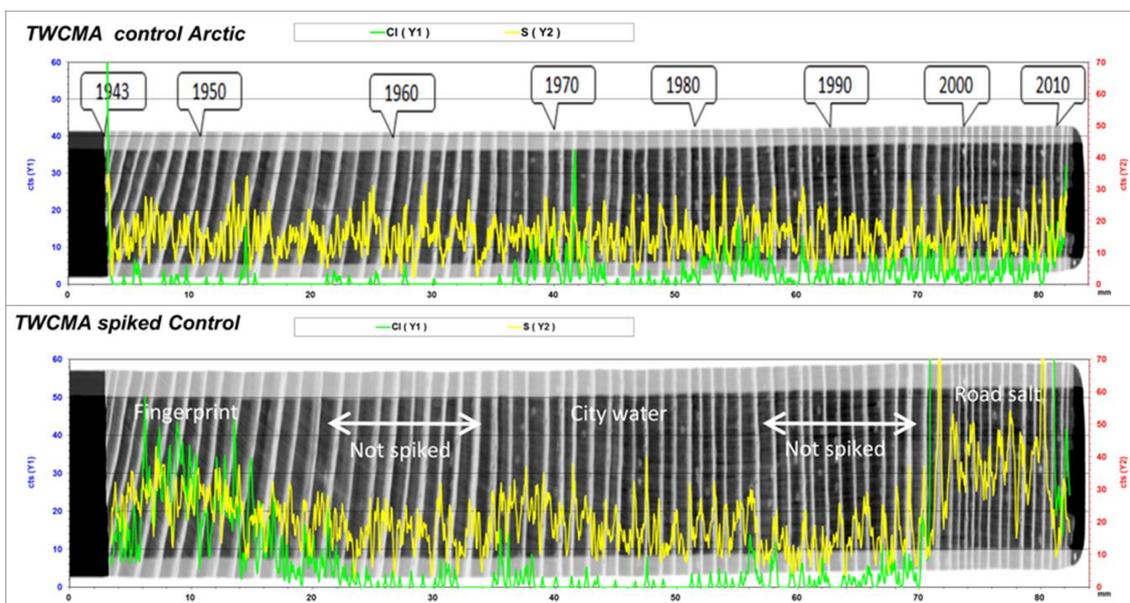
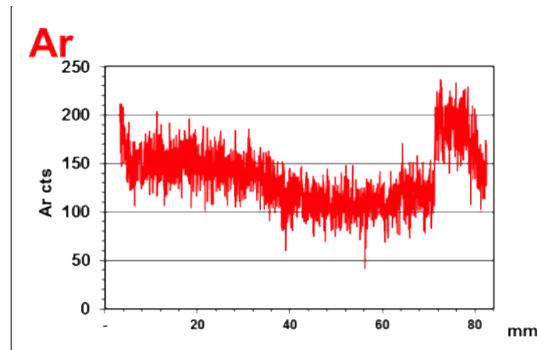


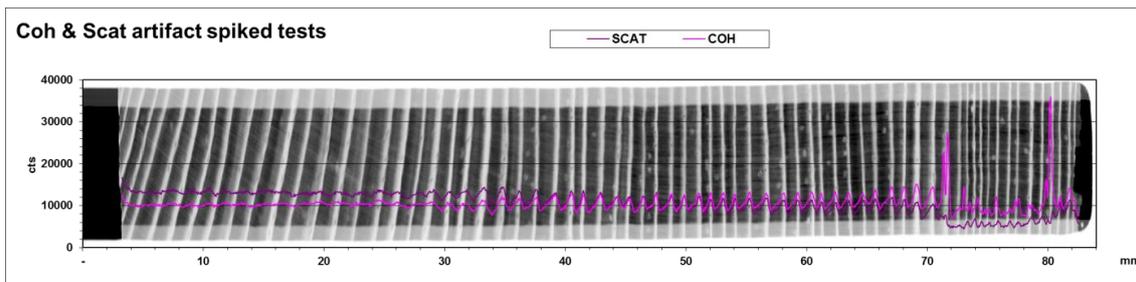
Figure 17. From left to right, the spike causes an increase in Cl and S but not P or K (not shown). The S profile is slightly increased in the municipal water but not Cl. The road salt spike causes the most significant anomalies for S and Cl (>10,000 cts) and also causes a decline of Ca and K and most elements; it also reduces the total coherent and incoherent photons and increases the argon counts.

The alteration of the argon profile over the road salt-spiked zone in the same core reinforces the role of matrix effects (Figure 18), which are likely caused by the shrinking of the spiked zone, which in turn caused a decline in the coherent and incoherent cts resulting from the increased distance between the sample and detector.



**Figure 18. Argon concentration anomalies in the road salt-spiked segment of the same core (~70 – 80 mm) in Figure 17**

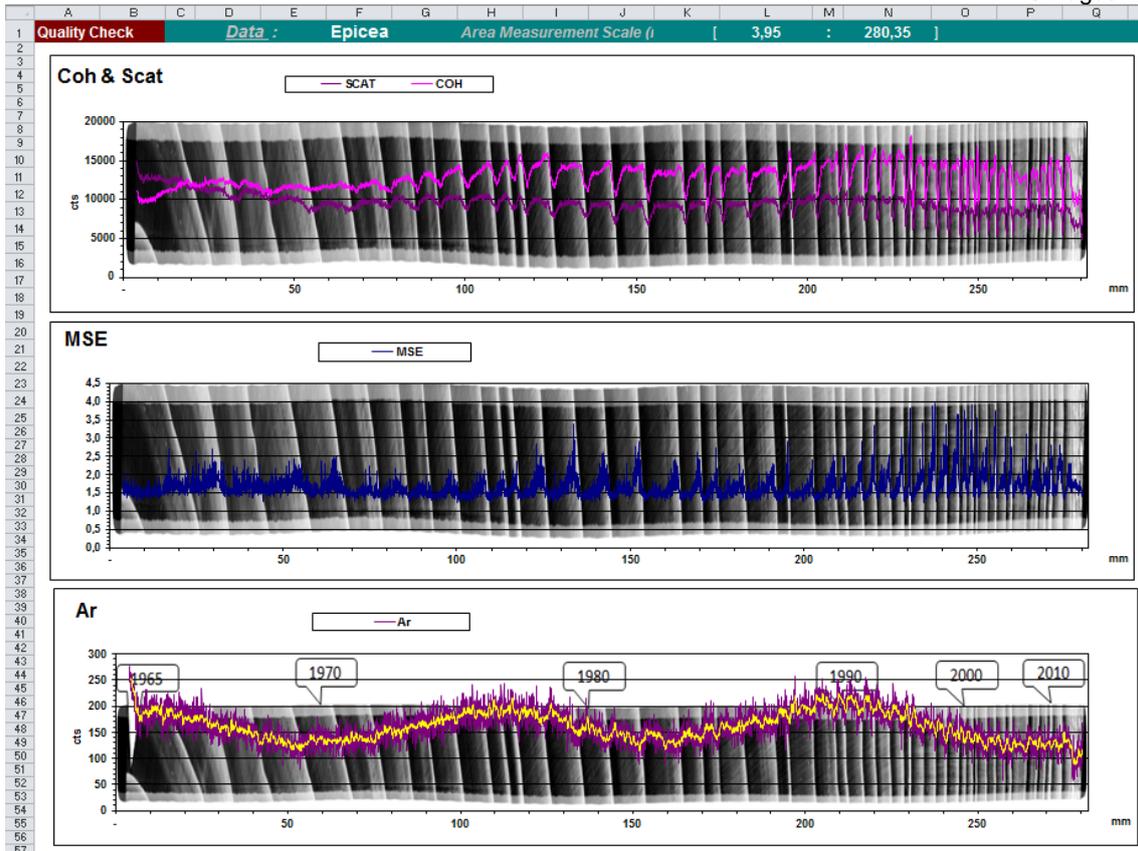
In the unspiked areas ( $X = 20$  to  $35$  and  $X = 55$  to  $70$ ; Figure 19), the CI minima are 0 and the S minima are  $<5$  cts. However, in the spiked areas, the averages, minima and maxima increase, which is consistent with the definition of the dendrochemical anomalies in Section B III 4.1



**Figure 19. Scattered and coherent counts show anomalies from the road salt-spiked area**

### **B.VI.3.5. Quality checks**

Each EDXRF PIT data set was entered into a Scanchem©.xls template that was developed by PIT. The ITRAX equipment is extremely sensitive, and very few Ar profiles are stable over a core's translation during the analysis. The above example shows a bi-modal argon profile, which is commonly found in EDXRF runs, with up to a  $\pm 50\%$  amplitude. Note that the argon profiles are not corrected under Scanchem© for the matrix effect related to intra-ring annual density changes (early/latewood) because this effect only reflects minor changes in air thickness between the sample and detector. Those changes are estimated by a few hundreds of  $\mu\text{m}$  out of a millimetric distance between sample and detector, whereas the ITRAX translation of the core can be up to 28 cm in length. PIT has previously discussed such long-trend profiles for Ar with the equipment manufacturer (Cox Analytical Systems), which encompasses possible and minor ( $10/30,000$  or less than  $1/30,000$ ) impacts on the analytical results. Such bimodal long trends for argon are not considered dendrochemical anomalies because they do not reflect the wood's elemental content, and distance changes of a few hundreds of  $\mu\text{m}$  are an acceptable variation change. Note that the Ar profiles increase at breaks in the core (see the EDXRF files on the website for the VE case).



**Figure 20.** Coherent and incoherent counts (top panel) reflect the stability of the equipment settings over the analytical run as well as the intra-ring density changes, which are also known as matrix effects. The mean square error (MSE), shown in the centre panel, is automatically calculated by the ITRAX program and is typically equal to or less than 2 and logically higher at ring boundaries as a result of matrix effects. The argon profile reflects the distance between the sample and detector because the measured argon reflects the air thickness and Ar abundance as a gas present in air, but not in wood, between the X-ray source, sample and fluorescent photon detector.

In the Ar profile in Figure 20, the yellow line is a moving average of nine counts that reveal the inter-ring patterns caused by very small changes of only a few  $\mu\text{m}$  in the core's thickness. The changes are the result of the differential effects of the twin saw disk that removes less material from most of the dense intra-ring zones and more from the early wood tissues. Because all the raw data are normalised automatically under Scanchem©, the effects of such minor changes in the cores' thickness are removed.

## C. Major results and lessons learned

Key results of PIT's investigations at its 24 sites are summarized in the following four tables, arranged by main families of contaminants. Results for sites investigated for two or more contaminant families are presented in the tables for the relevant contaminant family.

- Chlorinated solvents (HVOCs) is a group of 25 compounds regulated by Directive 1999/13/EC, and include chemical species such as PCE, TCE, cDCE, TCA, DCM, CT, CF (Table 2)
- Metals, Cd, Cr, Cu, Ni, Pb, and Zn (Table 2) are presented separately from Hg (Table 2), because of differences in sites and analytical methods.
- Petroleum hydrocarbons including BTEX, n-alkanes (C-14/C-39 = 23 compounds) and PAHs (16 compounds) (Table 2)
- PCBs (209 isomers)

### C.I. HVOCs

**Table 5. Summary of HVOC site investigations, combining analytical results for phytoscreening PS (in green in ppb), dendrochemistry DC (in brown, CI concentration range in counts), groundwater GW (in blue, concentration range in µg/l) and soils (in black, as concentration range in mg/kg). Sites are listed by their acronyms (see table ; number of samples and analytical campaigns is given for each site and analyzed media. Contaminants are listed per their acronyms.**

HVOC Cases	Major results	PS concentration ranges (ppb)
		DC Max CI (cts TWCMA)
<b>AN</b> Former solvent cleaning facilities	<b>PS</b> (n=10) : Contaminated groundwater is 35 meters deep in a chalk bedrock. HVOC contamination was documented in trees, despite groundwater depth, and allowed to map the plume. Phytoscreening distinguished between two distinct releases of PCE and TCE, which was later confirmed by isotopic analysis of groundwater.	0.003<PCE<0.013 0.027<TCE< 0.058
	<b>GW</b> (n=31 x 4 analytical campaigns in 2005 and 2006)	0<PCE<2 000 µg/l 0<TCE<1000 µg/l 0<cDCE<6360 µg/l
	<b>DC</b> : no core was run for EDXRF for trees contamination too low (60 ppt maximum).	
<b>CU</b> Former repair facility + uphill landfill	<b>PS</b> (n=13): This site was earlier investigated for source identification and plume delineation by phytoscreening methods. Prior to investigation by PIT, private analysis found no contamination at the ppb level. When resampled and measured by PIT at the ppt level, low contamination levels were found, confirming the presence of an impervious soil layer preventing trees to access deeper contamination. However, distribution of the plume suggested contamination also occurred from a second source uphill.	0.002<PCE< 0.015
	<b>GW</b> (n=15 , sampled in 2007)	0<PCE<403 µg/l
	<b>DC</b> : not attempted for too low PS concentrations	
<b>EC</b> Metal cleaning facility	<b>DC</b> (n=2): This site was investigated to confirm that an historical release had taken place in 1986. Two later pollutant waves were dated to 1994 and 1997. The dendrochemical anomalies in control tree may provide evidence that earlier HVOC contamination by the industrial source extended farther to the east of the site and impacted the adjacent property. The chemically distinct dendrochemical impacts recorded in the trees may indicate that the contamination plumes differed chemically.	30<CI max< 200 cts TWCMA

<b>GE</b> 2 nearby metal cleaning facilities	<b>PS</b> (n=8): The phytoscreening methods clearly indicated distinct HVOC plumes near trees A and G, and a west to east differentiation was assumed to be associated with the two distinct plumes and related to the past releases of the two distinct nearby industrial plants.	0.004<PCE<0.523 TCE max 0.026 cDCE max 20.606
	<b>DC</b> (n=6): Dendrochemical investigation distinguished releases in 1980 (source 1) and 1957 (source 2) from two adjacent industries separated by only 30 m.	40<CI max<140 cts <i>TWCMA</i>
<b>MEA</b> Former metal work plant	<b>PS</b> (n=5 x 4 directional): The use of HVOC phytoscreening using directional methods is useful when only a few trees are available at a site or the area for investigation is limited, such as is the case here. The hotspot of HVOC contamination was identifiable by phytoscreening and consistent with the conventional site data.  Directional centroids of most exposed tree are centered ; the 4 other trees centroids confirmed plume migration from this spot.	0.002<PCE<0.037 0.060<TCE <1.132 0<cDCE< 0.075
	<b>GW</b> (n=3, years 2006, 2009 and 2010)	0.1<PCE<2 µg/l 56<TCE<8120 µg/l 140<DCE<6360 µg/l
	<b>DC</b> : no core sampled for trees too young, around 10 years only.	
<b>NH1</b> Former solvent cleaning plant	<b>PS</b> (n=2 x 4 directional): Directional phytoscreening confirmed direction to source, when samples oriented towards river showed greatly reduced concentrations, as per river influence.	0.3<PCE<24.4 1.520<TCE <7.7 0<cDCE<108
	<b>GW</b> (n=5, x 5 sampling campaigns)	168<PCE<199 µg/l 43.9<TCE<48.8 µg/l 0.1<DCE<246 µg/l
	<b>Soil</b> (n=80)	100<PCE<110,000 µg/kg 11<TCE<11,000 µg/kg
	<b>DC</b> (n=2): River's influence prevented tree to record significant CI anomalies but a small one in 1993.	CI max 20 cts <i>TWCMA</i>
<b>NH2</b> Former owner cleaned deck with HVOCs	<b>PS</b> (n=1): High HVOC concentrations. Directional phytoscreening confirming source direction.	0.3<PCE<28 .034 0.863<TCE<13.056 13.143<cDCE<146.886
	<b>GW</b> (n=4)	PCE<10,000 µg/l
	<b>Soil</b> (n=70)	PCE <1000mg/kg
	<b>DC</b> (n=1): tree was already contaminated when it started to grow in 1989. Tree published for physiological anomalies.	CI max 22 cts <i>TWCMA</i>
<b>PL</b> Former oil and solvent recycling facility	<b>PS</b> (n=34): The HVOC data, except for the DCM data, match the conventional site groundwater, soil, and soil gas data in terms of individual HVOC species and concentrations, including hot spots.  DCM was found in important concentrations in 2012, but none a year later suggesting a significant atmospheric release during the first campaign.	PCE Not quantified 0.253<TCE<296.386 78.831<cDCE<491.376 4.825<TCEOH<12.279 61.005<DCM<308.560
	<b>GW</b> n=17 (years 2008, 2011 and 2012)	TCE<20 000µg/L cDCE<20 000 µg/L CM<250 µg/L
	<b>DC</b> (n=3): tree close to buildings and tree other side of river show very limited CI impacts, typically below 10 cts <i>TWCMA</i> . Two trees show moderate impact in 2000.	CI max 15 cts <i>TWCMA</i>

<b>PO</b>  Formerly wineryard, smelter, train station, dairy, metal coating, and electrolysis facility	<b>PS</b> (n=17) The HVOCs plumes geographically match those that had been established based on trees or conventional methods, both in their extent and chemical signature. This match is especially strong when identifying the major source using tree 12. However, 5 isolated plumes that were known for their limited extension and identified by conventional methods that were performed within buildings were not found outside close to the buildings, as reasonably expected. However, the HVOC plumes that were identified down the gradient from the buildings by conventional methods were matched using the phytoscreening methods.	0.000<PCE<12.640 0.000<TCE<1.330 0.000<111TCA<3.470 0.000<cDCE<7.470 0.000<CT<0.002 0.000<CF<0.140
	<b>Soil</b> (n=6 in 2003 and 26 in 2008)	1<ΣHVOCs<5000mg/kg
	<b>GW</b> (n=14)	1<ΣChloroethenes<315 261 µg/l 1<ΣChloroethanes<60 366 µg/l
	<b>DC</b> (n=7): Oldest tree (1900) shows 10 asynchronous CI impacts. All different consecutive facilities have impacted, from 1828 to 2003 different trees, depending on their locations.	CI max 50 cts <i>TWCMA</i>
<b>SG</b>  Former garage	<b>PS</b> (n= 14): This site was investigated for plume delineation, as the use of wells was limited by private property access and surrounding roads of high traffic. Plume's extension appeared to be over 1 km, oriented by groundwater. PCE and TCE maps coincided geographically.	0.006<PCE<11.719 0.027<TCE<0.282
	<b>DC</b> (n=1): Chlorine profiles indicated a slow and progressive impact starting 1962.	CI max 40 cts <i>TWCMA</i>
<b>SU</b>  Landfill	<b>DC</b> (n=1): Important quantities of HVOCs were dumped into a karst sinkhole, contaminating deep groundwater, but not impacting sampled tree.	CI max 10 cts <i>TWCMA</i>
<b>VE</b>  Metal treatment facility	<b>PS</b> (n=46): Phytoscreening campaign confirmed groundwater contamination. Location of release source was determined/confirmed. An area containing TCE contaminated fill material was identified. This case has been published.	0.002<PCE<0.249 0<TCE<1.549
	<b>DC</b> (n=4): Five asynchronous releases have been identified, and dated respectively to 1948 (sustained by historical evidence), 1975, 1990 and 1999. This case has been published.	CI max 180 cts <i>TWCMA</i>

HVOCs are anthropic compounds, not found naturally in trees. In all above PS cases, the plumes as established from phytoscreening methods did globally match the maps established by conventional methods, and relative proportions of the individual HVOCs (i. e. PCE, TCE, cDCE...) were same in groundwater as in trees' sap. Vinyl Chloride has not been measured in trees whilst present in groundwater.

Limmer and Burken (In press) have evaluated the variability results of 1913 VOC phytoscreening analysis, performed at 39 sites, 44 tree genera, in North America and Europe, including PIT sites, along with 230 replicate samples. They concluded that tree genus, diameter and wood type (porous or non-porous) did not significantly affect tree VOCs concentration.

For dendrochemistry, maximum CI concentrations only is provided (in cts *TWCMA*). Due to seasonal changes and computer assimilation of concentrations below 2 cts to 0, In all DC cases, background concentration of CI averaged at or below 5 cts *TWCMA* regardless of species or site.

## C.II. Metals

### C.II.1. Cd/Cr/Cu/Ni/Pb/Zn

**Table 6. Summary of site investigations for metals, combining analytical results for phytoscreening PS (in green in ppb), dendrochemistry DC (in brown, concentration rang, in counts, as averaged for each core), groundwater GW (in blue, concentration range in µg/l) and soils (in black, as concentration range in mg/kg). Sites are listed by their acronyms; number of samples and analytical campaigns is given for each site and analyzed media. Contaminants are listed per their elemental acronyms.**

Metal Cases	Major results	PS concentrations ranges (ppb)
		DC core average ranges (cts TWCMA)
AC Urban sewage spreading site	PS (n=20 trees / 35 samples at different heights): The former spreading site is now plated by crops, and there is no tree but by roads. Two hotspots were identified, one for Cd, Cu, Ni, Pb and another one for Cd, Ni and Zn.	0.008<Cd<0.085 1.450<Cr<2.686 1.065<Cu<5.016 0.342<Ni<1.248 0.023<Pb<1.802 3.260<Zn<16.512
	DC (n=3): No common trend could be identified amongst the different metals in the 3 cores, nor any major anomaly, consistent with site history and release mode.	5<Cd<6 cts TWCMA 5<Cu<7 cts TWCMA Ni = 2 cts TWCMA 37<Pb<46 cts TWCMA 195<Zn<263 cts TWCMA
CR Smelter	DC (n=3): Site was investigated to assess source and age of P contamination of water reservoirs, as smelter a possible source. Although P is significantly retranslocated in sapwood, the P concentrations in sapwood were lower by presumed source (15 cts) than in other trees of the same species, same age, located 15 km from source (140 cts). Three past anomalies were correlated in time over the 3 cores for Iron and Manganese.	71<Cu<107 cts TWCMA 0.05<Ni <0.2 cts TWCMA 5.80<Pb <7.8 cts TWCMA 218<Zn<326 cts TWCMA
LA Charcoal factory	PS (n=25): Metals were more abundant by the most heavily equipped area. Cr is presumed to be contributed by the burner's stainless steel operation. Both slag heaps were enriched in Cr, at concentrations comparable to burner area. However, the western slag heap's trees were more concentrated in lead, while the southern slag heap's trees contained more Ni and Zn.	0.004<Cd< 0.921 1.881<Cr<2.359 1.599<Cu<6.726 0.230<Ni<1.252 0.165<Pb<3.158 2.321<Zn<69.462
	GW (n=15, 6 wells, sampled between years 2000 and 2004)	0<Cr<24.5 µg/l 0<Cu<35 µg/l 0<Ni<72.8 µg/l 0<Pb<694.1 µg/l 0<Zn<88 µg/l
	DC (n=3): 3 cores were run to check if the slag heaps, combustion area and former fuel station had any identifiable and datable impact on trees. No such anomaly was found but for V, by the former fuel station.	4.5<Cd<5.2 TWCMA 14<Cu<15.7 cts TWCMA 0.3<Ni<0.5 cts TWCMA 1.3<Pb<27.3 cts TWCMA 221<Zn<283 cts TWCMA
MEA Metal plating	PS (n=6): Metal debris were deposited in field after a fire destroyed a building 10 years before sampling. The 3 analyzed willows proved to be more concentrated than the 3 adjacent poplars for Cd, Cu, Ni, Pb and Zn. Zn was the most and Cd the least abundant metal in both soils and in trees.	0.079<Cd<0.596 1.887<Cr<2.259 1.305<Cu<3.326 0.489<Ni<1.524 0.124<Pb<0.833 12.309<Zn<40.304
	GW (n=2, in 2009 and 2010)	2.5<Cr<22 µg/l 180<Cu<862 µg/l 161<Ni<200 µg/l 999<Zn<1100 µg/l
	Soil (n=2)	40<Cr<190 mg/kg 1200<Cu<15,000 mg/kg 93<Ni<8000 mg/kg

		93<Zn<8000 mg/kg
	<b>DC:</b> no core were sampled for trees were too young (<10 years)	
<b>MEU</b> Smelter	<b>PS</b> (n=41): the area has been heavily impacted by 2 smelters located 4 km away. Two hot spots were documented by phytoscreening methods for Pb and Cd. Tree contamination by Cu,Ni and Zn was associated with only one of the smelters. Poplar hyperaccumulated Pb, Zn, Ni and Cd, as compared to the sycamores. Pb,Zn, Cd, Cu and Ni were also found at higher concentrations than at any other sites.	0,022<Cd<6.566 1.663<Cr<5.557 1.185<Cu<17.428 0.2209<Ni<3.970 0.026<Pb<24.926 2.287<Zn<1154.095
	<b>DC</b> (n=5): Dendrochemistry confirmed metal hyperaccumulation in control poplar located at 10 km distance, as compared to sycamore by smelters. Dendrochemical anomaly was found for Cd, dated between 1980 and 1990. Another Cd anomaly is found by and after 2000 near one plant, when the other smelter had been stopped since.	7.5<Cd<14.7 TWCMA 10.6<Cu<40.4 cts TWCMA 0<Ni<5.3 cts TWCMA 1.3<Pb<90.8 cts TWCMA 206<Zn<568cts TWCMA
<b>NH</b>	<b>PS</b> (n=1) : One split core was analyzed from this site, after grinding its 20 rings, to compare ICPMS to EDXRF data.	Cd = 0.441 Cr= 2.135 Cu = 2.380 Ni = 0.291 Pb = 0.453 Zn = 18.361
	<b>DC</b> (n=1) Where the relative metal proportions remain same for Cd, Cu, Ni and Zn by EDXRF and ICPMS ; Pb appears less abundant by EDXRF, because of its reduced sensibility towards heavier elements.	Cd = 16 TWCMA Cu = 78 TWCMA Ni = 0 TWCMA Pb = 1 TWCMA Zn = 315 TWCMA
<b>PO</b> Formerly vineyard, smelter, railroad area, dairy, metal coating, and electrolysis facility	<b>PS</b> (n=9): The trees concentrations were higher in poplars for Cd and Zn, whatever their location, confirming the bioaccumulation by this genus for these two metals.	0.009<Cd<4.368 1.991<Cr<2.416 1.644<Cu<3.983 0.649<Ni<2.517 0.439<Pb<1.512 5.027<Zn<53.042
	<b>Soil</b> (n=8, in landfill))	31<Cd <143 236<Cr<408 72<Cu<150 830<Ni<4170 Pb = 205 209<Zn<1570
	<b>DC</b> (n=5): No significant dendrochemical metal anomaly was found for the 5 surveyed metals.	4.8 <Cd<16 TWCMA 14.7<Cu<40 cts TWCMA 0<Ni<0.4 cts TWCMA 17<Pb<25 cts TWCMA 183<Zn<299 cts TWCMA

Note that Cr cannot be measured by DC methods as EDXRF equipment uses a Cr tube. As and Hg are not available after wood sample calcination performed before ICP MS.

Fifteen trees from AC, SR and MEU sites were tested for metal concentration in aerial roots, at 1' and 3' height. Data could not confirm that metal concentrations were higher at lowest sampling points.

In the MEU case, the comparison of metal concentrations in trees and soil are difficult because most of the surface sampled soils were disturbed by ploughing or contaminated by landfill.

As expected, the metal concentration ranges measured by ICPMS and EDXRF were highest in the most polluted sites, especially and by decreasing concentrations in the smelter sites (MEU> PO>CR). Manganese appears to be a good dendrochemical tracer of past activities of metal industries, Pb, V and Ni are appropriate tracers for contamination by fossil fuels.

The bioaccumulation of metals in willows and poplars is confirmed by bibliography and PIT's data. This evidence highlights the need, for comparing metals especially, to sample same tree species Hg.

**Table 7. Summary of site investigations for Hg, combining analytical results for phytoscreening PS (in green in ppb), dendrochemistry DC (in brown, Hg concentration range in ng/g), groundwater GW (in blue, concentration range in ng/l) and soils (in black, as concentration range in mg/kg). Sites are listed by their acronyms; number of samples and analytical campaigns is given for each site and analyzed media.**

<b>SR Hg Floodplain</b>	<b>PS</b> (n=24): Site is in floodplain of the South River, VA. Previous floods have deposited contamination over 40 km downstream of the Hg source. Uncontaminated land corresponded to tree samples under 4 ng/g total Hg, for all tree species tested. Highest tree ring Hg concentration was approximately 1 ppm.	1.9<Hg<143.31
	<b>Surface water</b> (n=22) over 25 miles <b>Ground water</b> (n=22) over 25 miles	37<Hg<281 ng/l 1.5<Hg<778 ng/l
	<b>Soil</b> , 0 – 15 cm (n=12)	6.3<Hg<61 mg/kg
	<b>DC</b> (n=5 / 132 rings): Because sampled areas have been subject to asynchronous contaminations episodes and flooded areas have significant differences in Hg concentrations even within a few meters, no common contamination trend could be identified within trees.	0.76<Hg<28.11
<b>TA Hg Chlor-alkali plant</b>	<b>DC</b> (n=7 / 170 rings) at landfill location <b>DC</b> (n=8 / 120 rings) at control location We used cores collected on <i>salicaceae</i> species (poplar and willow) as data logger for Hg emission to investigate the historical changes of Hg in a dredged sediments landfill from a chlor-alkali facility and a nearby control location. The highest Hg concentration was detected in cores of an old poplar, before the commissioning of the mitigation pond in 1964. Low levels of Hg concentration detected in tree cores after the shutdown of the sediments landfill, despite the current high Hg concentration in the soil, may suggest that the dredged sediments is not anymore a source of volatile Hg. Trees in direct contact with the Hg source on the sediments landfill were clearly enriched in Hg as compared to trees from the control location, over the entire period exposure.	1.4<Hg<40.2 1.9<Hg<31.1
	<b>Soil</b> , 0 – 15 cm (n=8) at landfill location	3.2<Hg<4.6 mg/kg
	<b>Soil</b> , 0 – 15 cm (n=7) at control location	0.2<Hg<0.9 mg/kg

Hg had not been researched with PS nor DC methods before PIT but for its impact on leaves and needles. The 2 sites investigated by PIT for Hg confirm that xylem's concentrations in uncontaminated areas is below 5 ng/g. The SR results reflect contamination in a flood area of irregular contamination in space and time, where the TA investigations confirms the history of site's activity. Two publications are being submitted by PI on Hg.

Mercury is at too low concentrations for EDXRF dendrochemical methods.

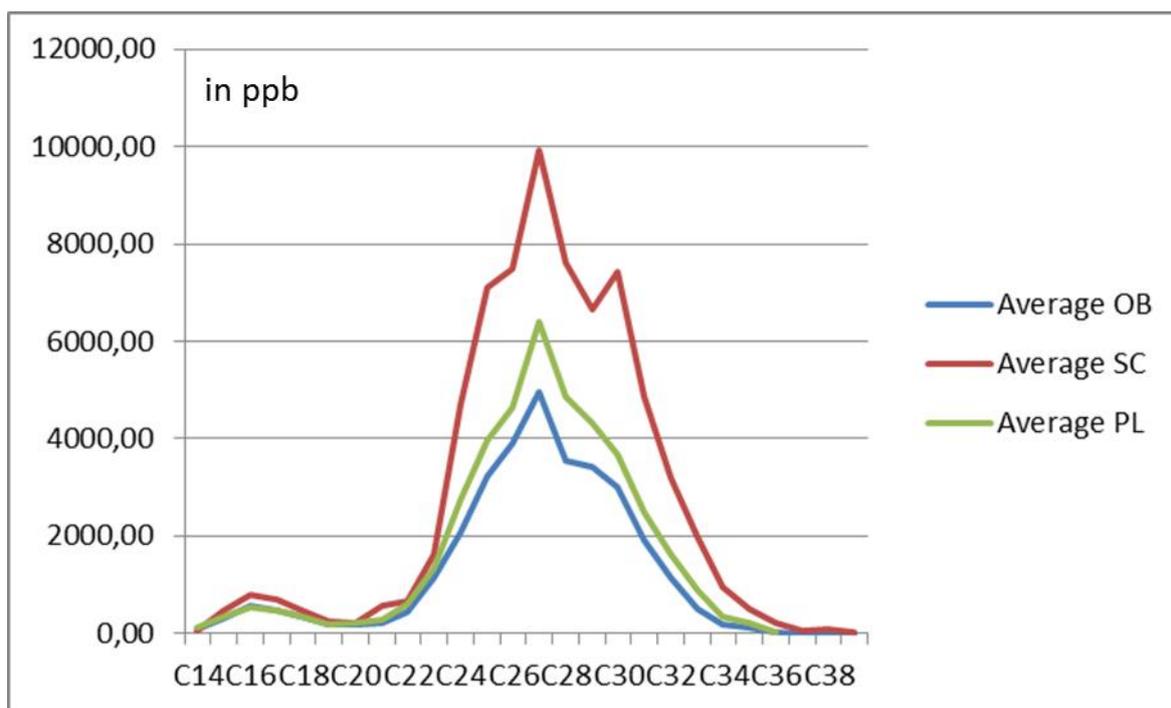
### C.III. Fossil fuels.

Eight sites contaminated by hydrocarbons have been investigated by PIT. Some of these sites, but not all, have been investigated by PS and DC methods; some only by DC. Analytical methods used for phytoscreening campaigns were either for BTEX via GC FID, or for PAHs and n-alkanes via GC x GC MS.

#### C.III.1. Fingerprint of fossil fuels.

**BTEX** : are not naturally found in trees, except for Toluene in a few trees species.

## N-alkanes



**Figure 21. N-alkanes profiles for the averages of the 3 studied sites (in ppb).**

OB site is covered by different tree species and PL sites basically by willows (*Salix*). Both are polluted by petroleum hydrocarbons ; SC site represents individual rings of one same pine tree (1973 through 2012) in an area polluted by a major wood fire in 2008.

There is an apparent similarity in average and individual samples profiles for the 3 sites, and same proportions of individual n-alkanes (C-15 to C-39). The n-alkanes concentration averages are about 10 times less at PL than OB.

For each site, the variability between n-alkanes concentrations is by 100 times-fold between the different samples. However, comparing the same tree species, the n-alkane concentrations are higher at most polluted areas, and decrease by distance to source (OB). Even the « control » trees contain some n-alkanes, of same profile. Further research is conducted by PIT members, with publication in perspective.

## PAHs

PAHs are anthropic contaminants and not naturally present in leafed trees, when occasionally found in conifers resin.

Dendrochemical methods rely on elemental tracers including Pb as a potential additive, S and Cl as well as Ni, and V as natural co-tracers, when the volatile organics have evaporated with time from tree tissues.

### C.III.2. Sites data

**Table 8. Summary of site investigations for fossil fuels, combining analytical results for phytoscreening PSX (in green in ppb), dendrochemistry DC (in brown, Cl, Ni, Pb, S and V concentration range in counts), groundwater GW (in blue, concentration range in µg/l) and soils (in black, as concentration range in mg/kg). Sites are listed by their acronyms; number of samples and analytical campaigns is given for each site and analysed media. Contaminants are listed per their acronyms**

Petroleum hydrocarbons		In ppm or ppb In µg/l
		Average cts <small>TWCMA</small>
<b>HA</b>  Leaking underground storage tanks	<b>PS</b> : Not surveyed	
	<b>DC</b> (n=1): Three asynchronous anomalies were found for Pb, S, Cl, Ni and V, associated to two releases of diesel fuel and one leaded fuel release dated respectively to 1957, 1978 and 1974 (Balouet et al. 2006).	Cl = 16 cts <small>TWCMA</small> Ni = 6.9 cts <small>TWCMA</small> Pb = 15.3 cts <small>TWCMA</small> S = 10 cts <small>TWCMA</small> V = 5.6 cts <small>TWCMA</small>
<b>LA</b>  Charcoal factory	<b>PS</b> (n=27) : BTEX : Toluene was found in 20 of the 27 trees, only in 3 of the 7 <i>Fraxinus</i> , or one of the three <i>Betula</i> analyzed. Highest B & T concentrations were found by the distillation & storage area and by sewage treatment basin. Tree 18 was the most contaminated, consistent with the data from ground water wells. The relative ratio of B/T differed between the 2 slag heap trees. No BTEX contamination was found near the former fuel station. PAHs were not found.	0<B<480 ppb 0<T<330 ppb E & X = 0 ppb
	<b>GW</b> (n=15, 6 wells, sampled between years 2000 and 2004)	0<B<48.1 µg/l 1.3<T<17.5 µg/l 0<ΣHCs<860 µg/l
	<b>DC</b> (n=3): Three asynchronous anomalies were found for S, Cl and V, one of which is found in 1980 in the two trees closest to former fuel station.	3.3<Cl <7.4 cts <small>TWCMA</small> 0.3<Ni <0.5 cts <small>TWCMA</small> 26.6<Pb<34.2 cts <small>TWCMA</small> 12.8<S<27.8 cts <small>TWCMA</small> 36.4<V = 43.5 cts <small>TWCMA</small>
<b>OB</b>  Landfill contaminated by petroleum hydrocarbons	<b>PS</b> (n= 26, sampled in July 2012) <b>Trees</b> OB1 and OB5 were the most contaminated by Σn-alkanes (respectively at 493 and 396 ppb) when adjacent to the two most contaminated wells where BTEX was respectively measured at 4178 and 587 ppb. The two same trees were respectively contaminated by ΣPAHs of 21.5 and 3.3 ppb when nearby wells were contaminated by 400 and 1100 ppb .  This site is under scientific publication along with PL case (see below).	0.8< Σn-alkanes<493 ppm 0<ΣPAHs<18557 ppb
	<b>GW</b> (n=42, 12 wells, sampled in 2010)	0<BTEX<4178 µg/l 0.19<ΣPAHs<4002 µg/l
	<b>DC</b> (n=0)	
<b>PL</b>  Former oil and solvent recycling facility	<b>PS</b> : (n=26) BTEX were not found in any of the 6 trees analyzed by GC FID. The 20 other trees were analysed by GCxGC/MS for n-alkanes and 16 PAHs. Analytical data proved that PAHs are not naturally present in trees, but are found in trees located over contaminated underground, further allowing chemical fingerprint .  N-alkanes showed a similar Gaussian distribution for C-23 to C-32 as in OB case, as possibly naturally present in trees versus a similar fossil fuel contamination. However, deviation from such observed and averaged Gaussian profile, is found in trees growing over significantly contaminated areas, and not the less exposed trees, even of same species, suggesting an identifiable and distinct chemical fingerprint that could be forensically associated to underground contamination.	0.35< Σn-alkanes<49 ppm 0<ΣPAHs<404 ppb

	<b>Soil (n=65)</b>	0<ΣVAC.<0,07g/kg 0<ΣPAHs<0,1g/kg, ΣHcs (C10-C40) <120g/kg
	<b>GW (n=17wells)</b>	0<ΣVAC.<400 µg/l 0<ΣPAHs<300µg/l, ΣHcs (C10-C40) <2400 µg/l
	<b>DC (n=3):</b> No dendrochemical anomaly has been found for S, Pb, V or Ni. Cl was anomalous for contamination by HVOCs.	0.2<Cl <1.1 cts <small>TWCMA</small> 5.5<Ni <18.1 cts <small>TWCMA</small> 0<Pb<9.0 cts <small>TWCMA</small> 6.8<S<33.4 cts <small>TWCMA</small> 22.2<V<54.4 cts <small>TWCMA</small>
<b>PM</b> Former fuel station	<b>PS :</b> (n=0) No phytoscreening	
	<b>DC (n=1):</b> One tree stump was left by Underground Storage Tank (UST) area after site clean-up 2 years before sampling. A slab from the stump was collected, , surfaced and analyzed by EDXRF. There was a dendrochemical anomaly for S, Cl and V, but not for Ni nor Pb in the tree-ring record from 1977- 1980, indicating a release of diesel fuel (not leaded gasoline) prior to 1977.	Cl = 23 cts <small>TWCMA</small> Ni = 207 cts <small>TWCMA</small> Pb = 15.3 cts <small>TWCMA</small> S =50 cts <small>TWCMA</small> V = 1.3 cts <small>TWCMA</small>
<b>PO</b> Formerly winery, smelter, railroad area, dairy, metal coating, and electrolysis facility	<b>PS :</b> (n=6) No BTEX was found. Hydrocarbons at site corresponded mainly to heavy fuels, and were limited to 3 small hotspots.	BTEX = 0
	Soil (n=5 in 2003 and 21 in 2008)	ΣHCs<32000mg/kg
	<b>GW (n=17)</b>	1.7<ΣHCs<520 µg/l, 6<BTEX<520 µg/l
<b>SC</b> Former PCB recycling site with major fire in 2008	<b>PS (n=0 for PAHs or n-alkanes)</b>	
	<b>DC (n=1; 40 rings) :</b> this pine core was analyzed, ring by ring, to check on the distribution of PAHs and n-alkanes over time. Conifers prove to be naturally rich in n-alkanes and PAHs. PAHs profile did not reflect the major fire of 2008.	2722< Σn-alkanes<704,621 ppb 0<ΣPAHs<2797 ppb
<b>SG</b> Former car repair shop	<b>PS (n=17, sampled in 2011) :</b> BTEX : The benzene plume did match the HVOC plume. There was no associated Toluene nor ethylbenzene nor xylene to that plume. However, a distinct toluene plume was found 200 m distant, by another industrial plant. A tree by another nearby fuel retailer showed no contaminated by petroleum fuels.	0,05<B<0,68 ppb 0.18<T<21.88 ppb E & X = 0 ppb
	<b>GW (n=57, 8 wells, years 2007 through 2009)</b>	ΣHcs (C10-C40) <61 µg/l
	<b>DC (n=1)</b> There was no joined anomaly for Cl and S when Cl profile was driven by HVOCs release.	Cl = 6 cts <small>TWCMA</small> Ni = 1.9 cts <small>TWCMA</small> Pb = 22.1 cts <small>TWCMA</small> S =30.6 cts <small>TWCMA</small> V = 56.8 cts <small>TWCMA</small>
<b>VEN</b> Leaking heating oil tank	<b>PS (n=0) :</b> Not surveyed	
	<b>DC (n=2)</b> Two cores were sampled from the same tree and compared for the influence of decay and local environment. The area was under direct oceanic influence and had too high Cl and S natural concentrations to allow for age dating fossil fuel release.	Cl = 166 cts <small>TWCMA</small> Ni = 2 cts <small>TWCMA</small> Pb = 161 cts <small>TWCMA</small> S =286 cts <small>TWCMA</small>

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The plumes delineated and mapped by conventional and phytoscreening methods did match. Pines should not be run for GCxGC/MS for their high organic content, which could even damage the collimator. PAHs and n-alkanes are naturally found in gymnosperms, probably as part of resin.

#### **C.IV. PCBs**

Extraction methods have been tested in full by University of Strathclyde, including spiked samples, but no PCB were found in any of the 100 trees analyzed by GC x GC/MS, including the 9 samples analyzed from PL and SC sites, which were known to be contaminated by PCBs. Lightest PCBs were however documented in poplar wood tissues (Van Aken et al. 2010).

Independent research using leaves and pine needles have however allowed to document current PCB contamination by phytoscreening methods using high resolution GC/MS, when dendrochemical methods using annual pine needles series also allowed to reconstruct past PCB contamination over a maximum of 10 years, as for the oldest live needles that can be sampled on a tree (Kylin et al. 1994).

## D. Conclusions

PIT has surpassed its initial plans by essentially doubling the number of investigated sites and the number of phytoscreening and dendrochemical analyses completed. The worldwide team of PIT contributors has also doubled and met or exceeded the anticipated goals including :

### Testing :

- Procedures established for several families of contaminants : fossil fuels (PAHs, n-alkanes, BTEX), HVOCs, metals (including Hg), PCBs,
- New analytical methods for phytoforensics, including CVAFS for Mercury and GC x GC /ToFMS,
- New sampling method of spiral drill / auger bit for wood chips, when analysis required sample mass greater than 1 g, as was the case for metals (except Hg) or PCBs ;

### Diverse geological and hydrogeological contexts,

**Comparison of various analytical methods** and operating parameters including the use of spiked samples and replicate analytical runs ;

**Established databases** to include all site data, and bibliography.

**Publication of** peer-reviewed scientific articles and this methodical guide ; available through the ADEME, CNRS, or INRA websites.

**Associating potential users of phytoforensic methods**, including governmental, academic, and private organizations dedicated to investigate and remediate polluted sites.

### D.I. Interpretation of PIT's results

PIT has compared site data gained on the 24 contaminated sites (plus control sites) by conventional methods to phytoforensic evidence. There has been no instance where they contradicted each other; instead, phytoforensic methods proved to be faster and cheaper in use than conventional methods, and provided information not readily available by conventional methods (see below).

### D.II. Limitations

Such limitations may reasonably be distinguished as being :

- dependent on analytical methods,
- related to site conditions ,
- are compound specific,
- possibly driven by internal tree physiology mechanisms,
- due to field sampling contingencies

### Analytics :

- There is no single analytical method to identify nor quantify all types of pollutants such as organics (e.g. PAH, PCB, petroleum derivatives) and inorganics (e.g. light and heavy metals, mercurials).
- State of the art analytical methods with low detection and quantification limits are essential. For example, HVOC analysis requires limits at the ppt level as provided by GC/ECD /SPME/PDMS methods, instead of the conventional GC MS methods by the ppb.
- For example, EDXRF line scanning methods allow for the ppm sensitivity on some 35 elements, while line scanning under LA/ICPMS allows detection of other elements such as Na, B, and U by the ppb level. However these methods fail to measure some critical elemental pollutant tracers such as Cl or S.
- Such analytical high resolution equipment is scarce and not readily available for wood samples in certified labs, i.e. ISO 17025 (except for PCBs in Kingston, Ontario, or Hg by Cebam in Seattle). Such certification is not necessary for preliminary Phase I investigations.

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- Conventional extraction procedures are minimal or completely unnecessary with headspace GC for HVOCs, or for Mercury under combustion trap cold vapor atomic fluorescence spectroscopy (CT CVAFS), and greatly simplified in freeze-centrifuge methods or for calcination before ICPMS for metals. However, expensive grinding and extraction is needed for conventional ICPMS or before accelerated solvent extraction (ASE) or Soxhlet extraction (i. e. PCBs).
- Calcination methods used before ICPMS prevent access to Arsenic and mercury.

**Site related :**

- As expected, contamination deep in the soil profile results in lower contaminant concentrations in trees. Similarly, interposition of impervious layers such as clay, limit root access to deeper aquifer (Cu case).
- Oceanic influences raise the background concentration of S and Cl, limiting dendrochemical investigation on chlorinated solvents and fossil fuels.

**Compound specific :**

- Strongly hydrophobic contaminants with high LogK<sub>o/w</sub>, such as PCBs, are not as readily absorbed and translocated in trees, compared to dissolved contaminants –also known as solutes- such as HVOCs.
- Analysis of PAHs and n-alkanes by GC x GC / MS should avoid conifers due to their naturally high organic content which limits peak identification or can damage detector.
- Organic compounds with high octanol/water partitioning coefficients (LogK<sub>o/w</sub>) are not readily taken up by tree roots. This is the case for PCBs, which were measured in tree xylem by only one independent study. However, phytoscreening for PCBs in conifer needles and bark is possible.
- Bioavailability of chromium is limited and concentration remain in a narrow range, whether or not trees are exposed to chromium contamination.
- What about metals?

**Others :** In dendrochemistry, the plume's impact to the trees is posterior to the contaminant release, time for the plume to migrate from source. When tree is planted, which is often the case in urban settings, the earliest rings corresponding to nursery growth before planting at site must be discarded. In temperate areas, dormant season events are largely absent from the tree record.

**Field sampling :**

- Phytoscreening methods on volatiles are best used during the growing season when sap flowrates are greatest.
- Although field sampling may be difficult in winter, the effectiveness of dendrochemical analysis is usually independent of season. Phytoscreening results from separate sampling campaigns may not be directly comparable due to potential differences in sap uptake and contaminant concentration due to moisture availability, temperature, and other factors.

Note that the sampling of control trees is an important part of phytoforensic investigations. Duplicate or greater redundancy in field sampling tools should be available as equipment can fail or break

### D.III. Major Findings

- Dendrochemistry can distinguish between contamination events that were slow and progressive in (e. g. SG) from releases that were massive and abrupt (e. g. GE).
- Background noise : owing to the large amount of analytical data gained by PIT, it has been possible to determine, for example, that mercury concentrations at unpolluted sites does not exceed 5 ng/g, and that dendrochemical chlorine background is below 5 cts (at PIT's settings) for all tested tree species.
- On an environmental forensic standpoint, phytoscreening methods can identify, map, and locate the source of a contaminant plume. Where the site context allows (GE case), combined

phytoscreening and dendrochemical methods, can allow the forensic allocation of torts between two different polluters.

- Dendrochemistry allows calculation of the effective migration rate of the plume, when several trees can be sampled along same direction.

#### **As to tree physiology :**

- While it is generally recommended that sampled trees are chosen from same species and of similar age and size this is rarely possible unless the site is densely vegetated. Still, for contaminants such as HVOs in solution, PIT's campaigns have not revealed significant differences between tree types, angiosperms or gymnosperms.
- When PAHs are legitimately investigated at polluted sites, PIT has shown that PAHs (PL case), are not naturally present in angiosperms.
- Decaying trees should be avoided. Such decay may not be readily visible ; however phytoscreening samples where the moisture content is significantly below 40% should be used with caution since it may show limited representativeness of sap uptake as compared to other samples which commonly range between 40% and 60 % water content.

#### **Other potential applications at polluted sites.**

Recent development in phytoforensic methods include new extraction methods by freeze/centrifuge, which combined with ultra fast liquid chromatography, provides access, at sub-ppb level to other contaminants including explosives.

Developments in Laser Ablation ICPMS allow to search for several elements by line scanning methods, including the lightest ones, such as Boron or Fluorine, or heavy ones such as Uranium that are not available by EDXRF dendrochemistry.

#### **Official recognition of the methods and their Legal admissibility :**

As of today, phytoscreening methods have been used or accepted for site investigation by official organizations including academic institutions in Belgium, Canada, France, Germany, Switzerland, and USA. The methods have been used as a research tool in many additional countries. Note that many of these research laboratories have conditions that limit the use of their data in private legal cases.

Dendrochemistry data has today been admitted as legal evidence in Belgium, France, Germany, USA.

### **D.IV. Future improvements and research**

Four more publications are planned by PIT's team, on Scanchem©, on GE case, on Mercury and on PAHs, for which the data is already in place under PIT.

Laboratory Certification: PIT's research has been served by 5 main laboratories (University of Missouri S&T for organics and metals, University of Strathclyde for organics, Cebam Analyticals for Hg, University of Franche Comté for metals, Stockholm University for EDXRF dendrochemistry. It is apparent from bibliographic research and its 450 references that at least another hundred laboratories have been serving phytoscreening campaigns worldwide. However very few are certified under ISO 17025, even if they qualify as per US EPA criteria. If such ISO certification is not needed for preliminary phase I site assessment, as phytoscreening is used at a reconnaissance level, it is important in the future that such ISO certification is gained by more laboratories, and that national laboratory accreditation, such as COFRAC in France, becomes available, as a judicial requirement if relating to a litigation case.

Analytical costs range between 70 € and 150 € per phytoscreening sample, depending on sought contaminant. Dendrochemical analysis is at 1500 € per core. These costs include preparation, shipment, quality check and analytical report.

Statistics : where PIT has generated considerable amount of analytical data, complex statistical methods such as PCA have not been applied at each site. Nor have the full range of sites

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and tree species been compared, except for VOCs (Limmer & Bruken, in press). However, all data can be made available for such overall studies .

On site HVOC phytoscreening analysis using portable GC/MS (Inficon Hapsite Smart) with Tenax concentrators, along with Automated Mass-Spectral Deconvolution and Identification System (MADIS) has been tested by Matt Limmer and Joel Burken of the University of Missouri S & T. When the portable GC data proved to be reliable in this case when compared to conventional phytoscreening results, the benefits of swift portable GC uses remain offsetted by the sampling and analytical times of around ½ hour per sample, when using trees that have been pre-fitted at last one day before with In-Planta port, or the equilibrium needed time by headspace of one day.

PIT was :

- designed to primarily address soil and ground water pollution, and not to research on direct atmospheric contamination
- focused on tree xylem, not root, bark, needle or leaf tissue.
- to deal with contaminants sought under mandatory site assessment protocols (chlorinated solvents such as HVOCs, 8 metals, fossil fuels, PCBs)

Further research plans, backed up by international fundamental research and analytical developments can now be considered, including :

- The use of bark, needles and leaves for phytoscreening of atmospheric gaseous contaminants,
- The use of needles for dendrochemistry of metals and organic contaminants of low water solubility,
- The application of phytoforensic methods to emerging pollutants, including explosives.
- The use of simple extraction methods, such as freeze-centrifugation to hasten and reduce costs of sample analyses, and to further test analytical methods of high resolution capacity, such as UfLGC and GC x GC / ToFMS, on the broadest possible spectrum of contaminants.
- Test the dendrochemical methods on ringless trees for well-documented sites, and test line scanning ICPMS on more elemental tracers.

When dendrochemical research based on isotopes is ongoing in different universities worldwide to document past climatic events, such isotopic research may help phytoforensic scientists further document contamination sources. Alternatively, elemental dendrochemistry may complement research on past climates, as pioneered by Poussart (2006) on ringless trees.

## E. List of references

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## **E.II. as per PIT**

An Excel file for the 460 references gathered by PIT during this research program is provided on the web site.

## F. PIT's Glossary

Hereby Glossary is provided for readers' assistance only. Not all terms used in PIT's methodical guides are incorporated. This glossary is largely inspired by PIT's team's work, especially by the *Dictionary of environmental forensics* as edited by Gil Oudijk, second edition, © Triassic Technology, Inc. 2010

absorption, *n* – [HYDROGEOLOGY] assimilation of FLUIDS into INTERSTICES. The PROCESS by which IONS or MOLECULES present in one PHASE tend to penetrate into and concentrate in the interior of a SOLID or a LIQUID<sup>13</sup>.

accretion, *n* – the accumulation of seasonally built tissues and rings in trees.

accumulator The plant uptakes an amount of the contaminant to which it is exposed.

admissible, *adj* – [LAW] a term used to describe EVIDENCE that may be considered by a JURY or JUDGE in CIVIL and CRIMINAL cases<sup>19</sup>.

aerial : Part surrounded by atmosphere. As seen from above. aerial photograph, *n* – [GEOGRAPHY] a photograph of a portion of the earth's surface, often from an airplane or other aircraft.

Age/size (trend) : reflects that trees' ring width declines as they get older.

age-dating investigation, *n* -- [AGE DATING] estimating the TIME frame of a CONTAMINANT RELEASE to the ENVIRONMENT.

aerobic : occurring only in the presence of oxygen

Age dating : Scientific methods and processes by which origin of materials or past events can be dated. Such methods can be absolute or relative. They include dendrochronology, radiometric /isotopic dating as for absolute ages, or can be relative when to state prior or after, and to include geological fundamentals such as superposition principle.

aliquot, *n* – [CHEMISTRY] a measured portion of a SAMPLE taken for ANALYSIS. One or more aliquots make up a sample<sup>11</sup>. *Also see sample and specimen.*

allocation, *n* – [LAW] an assignment or allotment<sup>15</sup>; the percentage allocated or assigned to a specific RESPONSIBLE PARTY. Allocation studies are often a part of, and the basis for many, forensic investigations.

Anaerobic : an environment or a process occurring in absence of oxygen.

Angiosperm : plant that produce flowers. Over 235,000 species, further classified as monocotyledons or as eudicotyledons, where seeds' leaves are either unique or doubled.

anion, *n* – [CHEMISTRY] a negatively CHARGED ION<sup>4</sup>. *Also see cation and ion.*

anisotropic, *adj* – [PHYSICS] denoting a MEDIUM in which certain PHYSICAL PARAMETERS are different in different directions<sup>4</sup>. *Also see isotropic, homogeneous and heterogeneous*

anomaly, *n* – [SCIENTIFIC METHOD] 1. a departure from the expected or normal. 2. the difference between an observed *value* and the corresponding computed value<sup>24</sup>.

Anomaly (dendrochemical) : Dendrochemical anomalies associated to soil or groundwater contamination (Balouet et al. 2009) depart from background, in annual minima, maxima and average over at last one ring. Typically, such anomalies are followed by "recovery" after a few rings, where concentrations remain slightly higher than earlier background, even when contamination still present by tree. Such partial "recovery" as shown by dendrochemical record is to be attributed to some compensation mechanism within rhizosphere, such as microroot renewal and turnover (Wang et al, 2012). Dendrochemical anomalies can be monoelemental, such as is the case with chlorine in

chlorinated solvents, or multielemental, such as would be the case for leaded gasoline, where it is characterized by CL, S and Pb.

apoplast : cell walls continuum in plants, as opposed to symplast (constituted by cytoplasm).

attenuation, *n* – [REMEDIACTION TECHNOLOGY] general term that relates to the reduction in MAGNITUDE, intensity, or CONCENTRATION of a SUBSTANCE once it has been released to the environment<sup>29</sup>. Also *enhanced natural attenuation, natural attenuation and monitored natural attenuation*.

auger, *n* – [DRILLING TECHNOLOGY] a tool for drilling into UNCONSOLIDATED earth materials consisting of a spiral blade wound around a central stem or shaft that can be hollow<sup>6</sup>.

background, *n* – [CHEMISTRY] the CONCENTRATION of a SUBSTANCE in an ENVIRONMENTAL MEDIA (AIR, WATER, SOIL, etc.) that occurs NATURALLY or is not the result of human activities<sup>22</sup>.

background, *n* – [TOXICOLOGY] in exposure ASSESSMENT, the concentration of a substance in a defined control area, during a fixed period of TIME before, during, or after a data-gathering operation. Also see *ambient and natural*.

bark : or Phloem. Protective layer to trees trunk, branches and roots outside of vascular cambium. To include living phloem and phelloderm.

binding: to tie together... (rather than fixation)

borer : a tool used for boring. In hereby applications to include Pressler borer or auger bits/spiral drills as well as sampling hammer (increment borer).

cambium : a layer of meristematic plant tissue, between the inner bark and wood, that produces new inner bark and wood cells, causing the stem or trunk to grow in diameter and forming the annual ring in trees.

cation, *n* – [CHEMISTRY] an ION that moves, or would move toward a CATHODE; thus nearly always synonymous with positive ion such as K or Ca. Also see *anion and ion*.

chlorinated : chemical compound to contain chloride or chlorine. HVOCs are volatile chlorinated hydrocarbons. Also called halogenated.

control sample, *n* – [ENVIRONMENTAL INVESTIGATION] a SAMPLE normally collected outside of the subject area to assess BACKGROUND conditions. laboratory control sample, *n* – [CHEMISTRY] an ALIQUOT of the SAMPLE MATRIX, FREE from the ANALYTES of interest, spiked with verified known amounts of analytes, or a material containing known and verified amounts of analytes.

correlation, *n* – [STATISTICS] 1. a mutual relation or degree of relationship between two or more things<sup>6</sup>. 2. interdependence of variable qualities.

correlation coefficient, *n* – [STATISTICS] a STATISTIC used to measure the strength of a relationship between two VARIABLES. Also see *regression analysis*.

cycle, *n* – [PHYSICS] a periodic, repetitive fluctuation in time series data from either a constant mean or trend line<sup>15</sup>. Also see *fluctuation and oscillation*.

Daubert challenge, *n* – [LAW] an attempt to prevent or QUASH an EXPERT'S TESTIMONY based PRECEDENCE of the *Daubert v. Merrell Dow Pharmaceuticals, Inc 509 U.S. 579 [1993]* case. In this case, the Supreme Court effectively made TRIAL JUDGES the gatekeepers of scientific expert testimony on the basis of four criteria: 1. whether the theory used by the expert can be and has been tested. 2. whether the theory or technique has been subjected to peer review. 3. the known or potential rate of error of the method used. 4. the degree of the methods or conclusion's acceptance within the relevant scientific community.

decay : breakdown in components' parts. See also phyto and rhizo degradation. Here also includes decomposition of wood tissues by bacteria / molds.

- degreaser, *n* – [INDUSTRIAL TECHNOLOGY] a substance which is used to remove GREASE, especially from tools and mechanical parts. Often composed of some type of ORGANIC SOLVENT. *Also see chlorinated solvents and dense, non-aqueous phase liquids (DNAPLs).*
- dendrochronology, *n* – [DENDROLOGY] the use of TREE-RING analysis to estimate the AGE of a TREE and local ENVIRONMENTAL conditions in the past<sup>12</sup>. These METHODS can also be used to estimate the ages of past POLLUTION events. *Also see dendroecology and dendrology.* Dendrochronology : the science of dating past events and environmental changes by comparative study of aged wood and their growth rings;
- dendroecology, *n* – [DENDROLOGY] the SCIENCE that uses TREE RINGS to study factors that affect the EARTH'S ecosystems. *Example:* analyzing the effects of AIR POLLUTION on tree growth by studying changes in ring widths over TIME<sup>12</sup>. *Also see dendrochronology and dendrology.*
- dendrochemistry : The chemistry of trees' tissues as analyzed for their chemical content.
- diffusion : Spontaneous process by which molecules move from most contaminated to less contaminated adjacent tissues or media.
- directional (PS). Relating to direction in space. Also called cardinal when referring to North, East, West and South.
- early wood : wood tissues formed early in the growing season, where cells are commonly larger, and cell walls thinner as compared to late wood.
- element, *n* – [CHEMISTRY] any SUBSTANCE identified by a specific number of PROTONS in its NUCLEUS (known as the ATOMIC NUMBER) that cannot be separated into smaller COMPONENT substances except by nuclear DISINTEGRATION (RADIOACTIVITY)<sup>17</sup>.
- Elemental (mono, bi-, multi-, + elemental majors)
- environmental forensics, *n* – 1. the use of EXPERTISE to settle ENVIRONMENTAL disputes<sup>58</sup>. 2. the use of environmental expertise to settle disputes (note the difference between definitions 1 and 2). 3. the systematic and scientific evaluation of physical, chemical and historical information for the purpose of developing defensible scientific and legal conclusions regarding the source or age of a contaminant release into the environment<sup>59</sup>.
- enzyme : any protein or conjugated proteins, acting as catalyst or in digestion processes, as capable of producing changes in organic substances.
- false ring : a layer of wood tissues that represent less than full annual tree ring growth. False rings are commonly associated with cold spells or significant environmental events that interrupt normal growth.
- fiber, *n* – [BIOLOGY] a fragment or piece of plant tissue that retains a recognizable cellular structure and is large enough to be retained after wet sieving on a 100-mesh sieve (openings 0.15 mm).
- fiber, *n* – [MINERALOGY] elongate crystalline structure ranging in length from a few millimeters to several feet and in diameter from one micron to 0.05 inch.
- fingerprinting : Initially dealing with the ridges of outer skin surfaces such as humans' fingers and palms, their leftover marking traces as used in criminal cases, the term fingerprinting is here used in a chemical approach to precisely identify the chemical signature, including its co-tracers, as used in environmental forensics to identify original source, and its impacts after migration or bio-attenuation.
- fingerprint, *n* – [ENVIRONMENTAL INVESTIGATION] 1. correlating a PATTERN or SIGNATURE to a known standard; used to compare known and unknown CONTAMINANTS or groups of contaminants. 2. something that identifies as a trait, trace or characteristic revealing origin and/or responsibility<sup>15</sup>.
- fossil fuel, *n* -- [PETROLEUM CHEMISTRY] a nonrenewable ENERGY source, such as OIL, GAS, or COAL, that derives from the ORGANIC remains of past life. Fossil fuels consist primarily of HYDROCARBONS.

- forensic, *adj* – [LAW] relating to or dealing with the application of some type of expertise, such as SCIENTIFIC KNOWLEDGE, to LEGAL problems and/or dispute resolution. *Also see environmental forensics and forensics.*
- forensics, *n* – [LAW] pertaining to, connected with, or used in COURTS of LAW; suitable or analogous to pleadings in court; belonging to, used in, or suitable to courts of judicature or to public discussion and debate; the art or practice of formal debate and argument; or of used in connection with courts of law, especially in relation to crime detection; the use of expertise to resolve disputes. *Also see forensic and environmental forensics.*
- fluorescence, *n* – [PHYSICS] LIGHT emission by ORGANIC OR INORGANIC MATTER when excited by ENERGY such as ULTRAVIOLET light or X-Ray.
- ground water, *n* — [HYDROGEOLOGY] that part of the subsurface WATER that is in the saturated zone<sup>4</sup>. Ground water is also considered to be at or greater than atmospheric pressure. + GW direction
- gymnosperm : Seed-bearing plants whose ovules are not enclosed in an ovary. This group of plants includes the conifers, the cycads, the ginkgo and the gnetophytes.
- heartwood, *n* – [HYDROLOGY] the inner, mostly dark layer of XYLEM which, in the growing tree, has ceased to contain living cells<sup>12</sup>. Commonly denser than the sapwood. *Also see sapwood.*
- HSB : Heartwood to sapwood boundary.
- humidity, *n* – [PHYSICS] the actual quantity or mass of water vapor present in a given volume of air, generally expressed in grams per cubic foot or in grams per cubic meter<sup>6</sup>.
- hydrogeology, *n* – [GEOLOGY] the study of the interrelationship of GEOLOGIC materials and PROCESSES with WATER, especially GROUND WATER. Hydrogeology tends to be more qualitative, whereas GEOHYDROLOGY is more quantitative. *Also see hydrology and geohydrology.*
- Hyperaccumulator : The plant uptakes a greater amount of the contaminant. (It is not based on speed of uptake, just a larger quantity.)
- impervious (layer) : *adj* – [HYDROLOGY] pertaining to a LAYER of NATURAL and/or MAN-MADE material of sufficient thickness, DENSITY and COMPOSITION so as to have a maximum PERMEABILITY for WATER of  $10^{-7}$  centimeters per second at the maximum anticipated HYDROSTATIC PRESSURE<sup>18</sup>. *Also see permeability.*
- increment : distance or time between separating repeated procedures.
- increment borer, *n* – [DENDROLOGY] an AUGER-like instrument with a hollow shaft that is screwed into the trunk of a TREE, and from which an increment core (or tree core) is extracted using an extractor (a long spoon inserted into the shaft that pulls out the tree core)<sup>12</sup>.
- Infection barrier : physiological process by which trees naturally protect infections from extending to other sane tissues.
- Inter-ring : comparisons between two or more consecutive annual growth rings.
- Intraring : comparisons within an annual growth ring, as opposed to interring.
- landfill, *n* — [WASTE DISPOSAL] a place, location, tract of LAND, area, or premises used for the disposal of SOLID WASTES as defined by state solid waste regulations. The term is synonymous with the term solid waste disposal site and may also be known as a garbage DUMP, trash dump, or similar term<sup>7</sup>. *Also see dump.*
- latewood, *n* – [DENDROLOGY] dense and often dark WOOD produced in the TREE RING during the later part of the GROWING SEASON characterized by small, thick-walled cells in GYMNOSPERMS, or a zone with few small vessels in ANGIOSPERMS<sup>12</sup>.

limit of detection : Concentration above or below which the chemical compounds can or cannot be identified by given analytical method.

limit of quantification : Concentration above or below which chemical compounds can or cannot be quantified. quantitation limit, *n* — [CHEMISTRY] the lowest result that would be considered quantitative, normally regarding the analytical results from a LABORATORY. *Also see detection limit.*

line-scanning : process by which identical analytical procedures are repeated along one physical axis.

meristem : plant tissue capable to divide indefinitely.

metals : A chemical element in a specific portion of the Mendeleev periodic table. PIT investigations focused on so-called heavy-metals that are frequently implicated as pollutants : As, Cd, Cr, Cu, Hg, Ni, Pb, Zn. Where other metals such as Fe or P are also available in PIT's dendrochemical investigations.

micro-extraction : extraction from small samples of minute amounts of material or chemicals by physical or chemical means.

migration (GW, plume and including migration rate) : process by which groundwater or contaminants migrate from one location to another one.

mineralization, *n* — [GEOLOGY] the PROCESS of depositing MINERALS or NATURALLY occurring INORGANIC CHEMICALS. This may be a normal BIOLOGICAL process which takes place during the life of an ORGANISM such as the the formation of bone tissue or egg shells. Alternatively, it may a process which begins after death and burial within sediments by the total replacement of the ORGANIC material with various minerals known as fossilization. Frequently this involves either CALCITE or QUARTZ, but many other minerals such as PYRITE may be involved. Mineralization is also used to describe the hydrothermal deposition of economically important METALS in the formation of orebodies<sup>4</sup>.

missing ring, *n* — [DENDROCHRONOLOGY] a TREE RING which in a sample CORE is absent due to the failure of CAMBIAL activity. Missing rings can be located by CROSS DATING<sup>12</sup>. *Also see false rings.*

octanol-water partitioning coefficient ( $K_{ow}$ ), *n* — [CHEMISTRY] a coefficient representing the ratio of solubility of a compound in octanol to its solubility in water. As  $K_{ow}$  increases the water solubility of the chemical decreases. The greater the value of  $K_{ow}$ , the more the chemical will be adsorbed to soil.  $K_{ow}$  values are often used in fate and transport calculations. *Also see organic carbon partitioning coefficient and partitioning coefficient.*

organic chemical, *n* — [CHEMISTRY] chemical compounds of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides, metallic carbonates, and ammonium carbonate. *Also see volatile organic chemical.*

partitioning, *n* — [CHEMISTRY] the act of movement of CONTAMINANTS from one soil residence phase to another.

parts per billion (ppb), *n* — [CHEMISTRY] a common unit of measure of concentration. Preferred terms are micrograms per kilogram ( $\mu\text{g}/\text{kg}$ ) for solid phases or micrograms per liter ( $\mu\text{g}/\text{l}$ ) for aqueous phases or molar concentrations such as microequivalents per liter.

parts per million (ppm), *n* — [CHEMISTRY] a common unit of measure of concentration. Preferred terms are milligrams per kilogram ( $\text{mg}/\text{kg}$ ) for solid phases or milligrams per liter ( $\text{mg}/\text{L}$ ) for aqueous phases or molar concentrations such as milliequivalents per liter.

parts per trillion (ppt), *n* — [CHEMISTRY] a common unit of measure of concentration. Preferred terms are nanograms per kilogram ( $\text{ng}/\text{kg}$ ) for solid phases or micrograms per liter ( $\text{ng}/\text{L}$ ) for aqueous phases or molar concentrations such as nanoequivalents per liter.

phase I environmental site assessment (ESA), *n* — [ENVIRONMENTAL INVESTIGATION] an INVESTIGATION to identify, to the extent feasible, recognized environmental conditions in connection to a property. Phase I assessments normally do not include any environmental sampling.

phase II environmental site assessment (ESA), *n* – [ENVIRONMENTAL INVESTIGATION] an INVESTIGATION conducted to evaluate the recognized environmental conditions identified in a Phase I ESA. Typically, the purpose of a Phase II ESA is to: develop sufficient information from which an environmental professional can render a professional opinion on whether or not hazardous substances have been releases at a particular property, thereby satisfying the innocent purchaser defense under CERCLA. In addition, the Phase II ESA may be developed to provide sufficient information to meet the business objectives of the client and assist in making a business decision.

phloem, *n* – [DENDROLOGY] food conducting tissue in vascular plants. *Also see cambium and xylem.*

phytohydraulic : Use of deep-rooted plants (usually trees) to contain, sequester, degrade or volatilize ground water contaminants that come into contact with their roots.

phytoextraction : Also known as phytoaccumulation. Plants take up or hyperaccumulate contaminants through their roots and store them in the tissues of the stem or leaves. The contaminants are not necessarily degraded but are removed from the underground environment.

The uptake of contaminants by plant roots and translocation into the above ground portion of the plants, where it is generally removed by harvesting the plants. This is usually applied to soil or water that has been contaminated with metal.

phytoextraction, *n* – [TREATMENT TECHNOLOGY] the use of plants or algae to remove contaminants from soil, sediments or water. The contaminants are uptaken by the plants and becomes part of the biomass.

phytoforensics : the use of plants to adress disputes.

phytostabilization : Immobilization of a contaminant through absorption and accumulation by roots and absorption onto roots, or precipitation within the root zone of the plants.

phytovolatilization : Plants take up volatile compounds through their roots, and transpire the same compounds, or their metabolites, through the leaves, some through the bark, thereby releasing them into the atmosphere. The uptake and transpiration of a contaminant by a plant, with the release of the contaminant or a modified form of the contaminant to the atmosphere from the plant.

phytodegradation : Contaminants are taken up into the plant tissues where they are metabolized, or biotransformed. Where the transformation takes place depends on the type of plant, and can occur in roots, stem or leaves.

The breakdown of contaminants taken up by plants through metabolic processes within the plant, or the breakdown of contaminants external to the plant through the effect of compounds (such as enzymes) produced by the plants.

phytostabilization : Immobilization of a contaminant through absorption and accumulation by roots and absorption onto roots, or precipitation within the root zone of the plants.

physiology : the study the functions of living organisms and their parts.

phytoscreening : the use of plants to document extent, source and chemical composition of a contamination.

pith : The biological center of a tree containing remnants of cells produced by the primary growing tip.

plume, *n* – [HYDROGEOLOGY] a relatively concentrated mass of emitted CHEMICAL CONTAMINANTS spreading in the ENVIRONMENT. In SURFACE WATER, the EFFLUENT added to a receiving stream near a point source. For example, when a heated-water discharge is added to a stream, the heated water does not mix immediately with the stream water. The mass of hot water remains detectable for some distance downstream. In ground water, the LEACHATE leaking down-gradient from a site of buried waste material.

pore, *n* — [HYDROGEOLOGY] synonymous with INTERSTICE or INTERSTITIAL.

porous : referring to trees : ring porous trees such as oaks, have large and more numerous vessels in earlywood. In diffuse porous trees vessels such as beech are same size in early and latewood. In non-porous trees , pores are not visible, as is the case in pines or some tropical trees.

precision, *n* — the degree of agreement of repeated measurements of the same parameter expressed quantitatively as the standard deviation computed from the results of a series of controlled determinations.

Profile : graphic showing physical or chemical paramaters as they evolve with distance or time.

Proxy-recorder : equipment or living organism that record changes in physical or chemical parameters (similar to black box in aircraft)

pyrogenic, *adj* – [CHEMISTRY] COMPOUNDS derived from the COMBUSTION of HYDROCARBONS, such as POLYCYCLIC AROMATIC HYDROCARBONS (PAHS). *Also see petrogenic.*

quality control (QC), *n* — [ENVIRONMENTAL INVESTIGATION] the overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of users. The aim is to provide quality that is satisfactory, adequate, dependable, and economical. Normally regarding sampling procedure or laboratory analytical procedures.

Reference material (analysis) : reference sample, *n* — [CHEMISTRY] a MATRIX whose ANALYTES of interest are of known or accepted CONCENTRATION or property.

regression analysis, *n* -- [STATISTICS] STATISTICAL method developed to investigate the interdependence or relationship between two or more measurable variates. The most common form of regression analysis is linear regression. *Also see correlation coefficient.*

regression line, *n* – [STATISTICS] a 'best fit' line through a series of points on a graph showing the form of the relationship between two sets of data.

repeatability : or test-retest reliability is the variation in measurements taken on the same sample by same instrument and under same conditions.

rhizodegradation : This takes place in the soil or ground water immediately surrounding the plant roots. Exudates from plants stimulate rhizosphere bacteria to enhance biodegradation of soil contaminants. The breakdown of a contaminant in soil through microbial activity that is enhanced by the presence of the root zone.

rhizosphere, *n* – [AGRONOMY] SOIL and MICROBES adjacent to and influencing plant roots.

ring : In temperate zone trees, the layer of new wood added to the outside of the woody stem, roots, and branches.

rot : partial or complete breakdown of wood structure caused by microorganisms, primarily wood decay fungi.

sample, *n* — [ENVIRONMENTAL INVESTIGATION] 1. a portion of material taken from a larger quantity for the purpose of estimating properties or composition of the larger quantity. 2. one or more items or portions collected from a lot or population. 3. a portion of material which is collected for testing or for record purposes.

sampling process, *n* — [ENVIRONMENTAL INVESTIGATION] the method and procedure of collecting PHYSICAL SAMPLES from a defined POPULATION.

sap, *n* – [DENDROLOGY] the fluid part of a plant, a watery solution that circulates through a plant's vascular system<sup>12</sup>.

sapstream : sap flowing in trees' tissues.

sapwood, *n* – [DENDROLOGY] the outer layers of XYLEM which, in the growing tree, conducts water and contains living cells with stored food reserves.

saturate, *v* – [HYDROLOGY] to fill with moisture or liquid, to soak thoroughly. Saturated zone refers to underground zones below watertable. See also unsaturated zone and vadose.

Scanchem©: Excel / VBA software and template developed by PIT to computerize dendrochemical data.

sediment, *n* -- [GEOLOGY] an assemblage of individual mineral grains that were deposited by some geologic agent such as water, wind, ice or gravity.

solvent, *n* — [CHEMISTRY] a CHEMICAL COMPOUND that is capable of dissolving another SUBSTANCE and, potentially, a HAZARDOUS substance, used in a number of manufacturing/industrial processes including but not limited to the manufacture of paints and coatings for industrial and household purposes, equipment clean-up, and surface degreasing in metal fabricating industries.

solubility, *n* – [CHEMISTRY] the amount of MASS of a COMPOUND that will dissolve in a unit VOLUME of SOLUTION. Aqueous Solubility is the maximum concentration of a chemical that will dissolve in pure water at a reference TEMPERATURE. The solubility of a certain chemical can also be dependent of the pressure and the presence of other chemicals in the solution.

solute phase, *n* — [CHEMISTRY] a condition of CONTAMINANT residence in which contaminants are dissolved in ground water in either the saturated or the vadose zone.

source, *n* — [HYDROGEOLOGY] the location at which CONTAMINATION has entered the NATURAL ENVIRONMENT.

spectrometry : the analysis of wavelengths or other electromagnetic radiation to identify chemical compounds.

smelter : Industrial plant where ores are smelted.

symplast : see apoplast.

tolerant : The plant is able to grow with the contaminant but does not necessarily uptake the contaminant.

tracer, *n* – [HYDROGEOLOGY] easily detectable material which may be added in small quantities to flowing SURFACE WATER or GROUND WATER to depict the path lines or to serve in the measurement of characteristics of flow, such as velocity, transit times, age, dilution, etc.

translocation, *n* [DENDROLOGY] the movement of elements or chemical compounds within SAP from one plant part to another.

transpiration, *n* – [HYDROLOGY] the PROCESS by which WATER VAPOR is lost to the ATMOSPHERE from living plants. The term can also be applied to the quantity of water thus dissipated. *Also see evapotranspiration.*

uptake : absorbing and incorporating.

vadose zone, *n* — [AGRONOMY] the HYDROGEOLOGICAL region extending from the SOIL SURFACE to the top of the principle WATER TABLE; commonly referred to as the “unsaturated zone” or “zone of aeration”. These alternative names are inadequate as they do not take into account locally saturated regions above the principle water table (for example, perched water zones).

weathering, *n* — [GEOLOGY] the process of DISINTEGRATION and DECOMPOSITION as a consequence of exposure to the ATMOSPHERE, to CHEMICAL action, and to the action of frost, WATER, and HEAT. The ALTERATION of ORGANIC COMPOUNDS through EVAPORATION, DISSOLUTION and BIODEGRADATION. *Also see biodegradation, dissolution, erosion, evaporation and resistant.*

xylem : A type of plant cellular tissue in roots, stems, and leaves. In stems, branches, and roots, mature xylem is wood.

## **G. List of acronyms**

ADEME : French Agency for Environment and Energy Management.  
Bgs : Below Ground Surface  
BTEX : Benzene, Toluene, Ethylene, Xylen.  
DCM : Dichloromethane  
C-DCE : Cis-dichlorethane.  
CF : Chloroform  
CNRS : French National Center for Scientific Research  
CT : Carbon tetrachloride.  
Cts : counts  
CT CVAFS : Combustion Trap / Cold Vapor Atomic Fluorescence Spectroscopy.  
DNAPL : Dense Non-Aqueous Phase Liquid  
ECD : Electron Capture Detection  
EDXRF : Energy Dispersive XRay Fluorescence.  
EPA : US Environment Protection Agency  
FID : Flame Ionization Detection.  
GC : Gas Chromatography.  
HCs : Hydrocarbons  
HVOC(s) : Halogenated Volatile Organic Compound(s)  
ICP : Inductively coupled Plasma  
MS : Mass Spectrometry.  
MSE : Mean Square Error  
PAH : Polynuclear Aromatic Hydrocarbon.  
PCBs : Polychlorobiphenyls.  
PCE : Tetrachlorethylene.  
PIT : Pollution Investigation by Trees (Ademe's programme)  
PPB : parts per billion  
PPM : parts per million  
PPT : parts per trillion  
SPME : Solid Phase MicroExtraction.  
TCA : Trichloroethane.  
TCE : trichloroethene.  
THC : total hydroxarbon.  
TWCMA : Time weighted Centerd Moving Average.  
UfLGC : Ultra fast Liquid Chromatography  
UXO : Unexploded ordinance.  
VC : Vinyl Chloride  
VAC : Volatile Aromatic Compounds.

## H. List of web sites

<http://dendroremediation.univ-fcomte.fr/>

<https://www1.clermont.inra.fr/piaf-www1/Telechargements/telecharger.phphem.>

<http://www.clu-in.org/products/phyto/>

Maps generated by PIT were primarily prepared using the following tools:

- Google Earth: <http://earth.google.com/>
- Geoportail: <http://www.geoportail.gouv.fr/>
- Surfer 10: <http://www.goldensoftware.com/products/surfer> (maps generated by Surfer 10 include modélisation (see the PL, PO and SG cases))
- QGIS: also allows for modélisation (see La and MEU cases)
- AutoCAD: <http://www.autodesk.com/products/autodesk-autocad/overview> (VE case)
- PowerPoint: (<http://office.microsoft.com/fr-fr/powerpoint/>) (used frequently by PIT, especially when generating dated core pictures of EDXRF, and is coupled with “paint” to extract .png files)
- Excel: (<http://office.microsoft.com/fr-fr/excel/>) (used in many applications by PIT, such as to establish correlations and regression models (see Scanchem©))
- Matlab: <http://www.mathtools.net/MATLAB/> (used for principal component analysis (PCA); see the OB and PL cases)

## I. Annexes

Published papers under the hospices of PIT.

- [PIT Annex 1] : Limmer, M.A., Balouet, J.-C., Karg, F., Vrobley, D.A., Burken, J.G., 2011. Phytoscreening for Chlorinated Solvents Using Rapid in Vitro SPME Sampling: Application to Urban Plume in Verl, Germany. *Environ. Sci. Technol.* 45, 8276–8282. doi:10.1021/es201704v
- [PIT Annex 2] : Balouet, J.C., Burken, J.G., Karg, F., Vrobley, D., Smith, K.T., Grudd, H., Rindby, A., Beaujard, F., Chalot, M., 2012. Dendrochemistry of Multiple Releases of Chlorinated Solvents at a Former Industrial Site. *Environ. Sci. Technol.* 46, 9541–9547. doi:10.1021/es300318v
- [PIT Annex 3] : Smith, K.T., Balouet, J.C., Shortle, W.C., Chalot, M., Beaujard, F., Grudd, H., Vrobley, D.A., Burken, J.G., 2014. Dendrochemical patterns of calcium, zinc, and potassium related to internal factors detected by energy dispersive X-ray fluorescence (EDXRF). *Chemosphere* 95, 58–62. doi:10.1016/j.chemosphere.2013.08.017