timbre

Tailored Improvement of Brownfield Regeneration in Europe

Guideline for application of Tree Coring as an initial screening tool for typical pollutants in the subsurface

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Content

Prefatory note

This *Guideline for application of tree coring as an initial screening tool for typical pollutants in the subsurface* was developed within the project of **TIMBRE, Tailored Improvement of Brownfield Regeneration in Europe**, funded by the European Commission's Seventh Framework Programme (FP7)**.** The project aims at overcoming existing barriers to brownfield regeneration by developing and providing customised problem- and target-oriented packages of approaches, technologies and tools.

This guideline is an outcome of the work accomplished in working package 4; **Strategies and technologies for integrated site characterisation and remediation are investigated**. One of the tasks was to test the feasibility of vegetation sampling as a screening tool for typical pollutants in the subsurface.

Previous guidelines of tree coring as bio-indicators for subsurface pollution are available (Holm et al. 2011ab, Trapp et al. 2012, Vroblesky 2008). These guidelines report that tree coring is more or less useful for a variety of volatile organic compounds (VOC) such as BTEX (benzene, toluene, ethylbenzene, xylenes), methyl tert.-butyl ether (MTBE), trimethyl benzene and chlorinated solvents (PCE, TCE, DCE, VC). **This new guideline goes beyond the previous guidelines**, and the main novelty is that it also includes the application for screening of heavy metals, plus some new examples for BTEX. It is based on field applications at sites polluted with BTEX, chlorinated solvents and/or heavy metals. The guideline describes the method and the application of it including sampling, chemical analysis and data treatment. Finally a short overview of current literature obtained within the TIMBRE project and by others is given.

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1. Introduction

Using vegetation as bio-indicators to detect pollutants in the subsurface is termed "phytoscreening". One method of phytoscreening is tree coring where trees are used as bio-indicator.

1.1 Phytoscreening

Phytoscreening takes advantage of the plants' natural ability to absorb water, nutrients and prospective pollutants from the soil matrix and groundwater through their roots. From the roots, the pollutants may be translocated to other parts of the plant above the surface. These parts of the plant are collected and analysed in order to detect possible pollutants in the subsurface. Based on the results of the chemical analysis, it is possible to locate and map subsurface pollution. A variety of plants and plant parts are useful for phytoscreening (Lintern et al. 2013, Algreen et al. 2012, Stefanov et al. 2012, Limmer et al. 2011 among others).

1.2 Tree coring

The use of wood, extracted from the stem of trees, as a bio-indicator for subsurface pollution s is termed tree coring. Trees are to be preferred to smaller plants as their large root system can absorb chemicals from a larger area (the root system can be larger than the crown of the tree [Dobson and Moffat 1995]) and greater soil depths (pollution down to 19 m has been detected by trees [Sorek 2008]). Furthermore, the wooden parts of the tree are available all year in contrast to e. g. leaves.

Figure 1: A tree core sample with the bark removed. ***Photo by M. Algreen.***

The objective of this guideline is application of tree coring as a screening tool at VOC and/or heavy metal polluted sites, where a small core sample of wood from the stem of the tree is collected (see figure 1). Heavy metals will be bound into the wood structure; other compounds such as VOC will be stored in the wood for a shorter time due to their volatile properties. Therefore are sample preparation, the sampling procedure, the analytical method and data interpretation depended on the target compounds (organic or inorganic compounds), which will be described later in this guideline.

Tree coring is a semi-quantitative screening tool which can be applied initially for location and mapping of pollution. The method is well suited for large sites and to locate (single) hot spots. It can also be applied in dense forests, on swamps, and in urban areas. However, the results should be confirmed by quantitative screening methods such as groundwater sampling or soil sampling.

1.2.1 Advantages of tree coring

- Fast (60-80 trees can be sampled by two persons in one day), easy (just a small hand drill is needed), and only little effort is needed to screen a large test site.
- Inexpensive.
- Only a tree borer and some storage material are needed in the field, which makes the method very mobile.
- Suitable at sites where application of conventional site characterization methods is limited/problematic due to the sensitivity of a site; certain site features (moor, rocks, forest), location of the site (inner cities, risk of pipes or cables) or due to former/current activities at the site (e. g. drilling is not possible on sites contaminated with explosives).
- Minimal impact on the site and the environment.
- The tree core sample represents a large volume of the subsurface. Root systems can be larger than the crown of the tree (Dobson and Moffat 1995).
- Due to the low costs, a high number of samples can be taken with a high density. This enhances the chance of detecting scattered hot spots. The method allows a fast overview of the spreading of the pollutants and can this way serve as initial screening method.
- Gives information about single compounds and ratios between parent and daughter products.
- Gives information about plant uptake, which can be useful for further planning of regeneration (the usefulness of phytoremediation).

1.2.1 Limitations of tree coring

- Limited by the fact that there have to be trees at the test site. A lack of trees may be due to toxic levels of chemicals in the subsurface. That's why the method can be limited by phytotoxic levels.
- Lower detection limit by the analytical methods (concentrations found in extracts from wood are typically low [ug/L]).
- The results vary with weather and season.
- The results vary with tree species and tree size.
- The results are affected by the soil properties (pH, redox etc.) and the groundwater level.
- The pollutants have to be available for the root systems and mobile within the tree. This may be limited by the chemical properties, the soil conditions (clay or other and impermeable soil layers) and the physical properties of the trees.
- The method is semi-quantitative (showing pollution levels) due to the fact that the uptake and accumulation of contaminants in the stem depend on the physico-chemical properties of the pollutants, the soil conditions and the tree species. However, correlations between concentrations measured in the groundwater and in the tree cores were found for some pollutants (Gopalakrishnan et al. 2007, Larsen et al. 2008).
- There can be false negatives; i. e. contaminations cannot be detected by tree core sampling even though the subsurface is contaminated.
- The method is "only" applicable for shallow pollutants (however pollution down to 19 m has been detected in trees (Sorek 2008), for TCE even down to > 30 m [Larsen and Trapp 2005, unpublished]).

2. Application of tree coring

This guideline describes application of tree coring as a screening tool at sites polluted with BTEX, chlorinated solvents and/or heavy metals.

2.1 Sampling

Tree cores are sampled using a Suunto increment borer – a tool which is normally used by foresters to examine wood quality and to measure growth rates.

Sampling of tree cores should be done by a standardized procedure, like the one presented in the following, as the procedure (such as sampling height, depth and direction) can affect the results.

2.1.1 The 6 steps of sampling

The sampling procedure can be divided into 6 steps (see figure 2):

1. **Select a suitable tree.** The tree should be healthy and mature (stem diameter approximately 10 cm or greater). The results can depend on the tree species; therefore, some species (often willow, aspen or other poplars; in order of priority) are better suited than others depended on the compound of interest.

2. **Screw the borer into the stem.** Place the sampling point 1 m above ground. Screw the borer 6 cm in to the stem (water is predominantly transported through the outermost rings). Align the drill vertically.

3. **Insert the core retractor.** The core retractor is inserted into the borer. Give it a small push to make sure the retractor has reached the bottom of the borer.

4. **Loosening the tree core.** Turn the borer one rotation back using the handle.

5. **Take the tree core sample.** The samples are retracted by pulling out the core retractor. The outer inches of a tree core, containing bark and phloem, have to be removed to prevent cross-contaminations from e. g. air pollution.

6. **Transfer to sample storage.** Plastic bags are used as storage medium when screening for heavy metals and GC analytical vials are used when screening for VOC. When screening for VOC, the transfer from tree to vial has to be rapid and the vials sealed immediately to avoid loss of the contaminates by volatilization.

Record by GPS the location of sampled trees for later mapping of analytical results. VOC store cold and proceed soon.

Figure 2: The tree coring procedure using a SUUNTO borer. ***Photos by M. Algreen.***

2.1.2 Orientation of sampling

Due to variations of chemical concentration inside trees, replicates should be sampled from different directions of the tree to minimize the risk of false negatives (Holm et al. 2011ab, Holm & Rotard 2011).

2.1.3 Timing of sampling

The concentrations measured in the trees will vary with changes in the weather and groundwater level. Sampling after rainfall should be avoided due to dilution effects. Sampling at a site should be done within a short time frame (to ensure similar weather conditions and similar groundwater levels). This way the best conditions to compare the concentrations measured in the trees throughout the site and to assess a relative pollution level are obtained.

Screening of VOC is preferred to be done in autumn (Wittlingerova et al. 2013), while screening of heavy metals can be done all year. The sampling of BTEX on hot summer days is not advised (Algreen et al. 2011).

2.1.4 Effects on the trees

The extraction of tree cores leaves holes in the stems behind (see figure 2 and 3). The trees respond to these wounds with compartmentalization. Compartmentalization includes the process of forming boundaries to isolate the injured tissues and hereby resist pathogens (Shigo 1984). This means that tree coring should not increase the frequency of tree death, even when excessive drilling is done (Weber and Mattheck 2006).

Figure 3: Stem of a willow tree one year after sampling. The two sampling perforations have almost healed. *Photo by M. Algreen.*

To minimize the impact of tree coring, some precaution may be taken. Cleaning the borer between coring different trees will avoid the transfer of pathogens. A sharp borer should be used, because it will avoid ripping of the wood tissue which would result in greater cambial damage. Additionally, holding the borer slightly upwards can avoid water and dirt entering into the hole (Norton 1998). It is also possible to attempt to plug the holes in order to prevent infections. However, the results show that plugs tend to increase the infection rate (Norton 1998). Therefore, plugging is not recommended.

2.2 Precautions when sampling

Following precautions should be taken into account when applying tree core sampling as a screening tool.

2.2.1 Volatile organic compounds

Sampling for VOC, two (at least) individual samples from opposite site, are sampled and transferred into analytical vials. Sampling will preferably be done in autumn (Wittlingerova et al. 2013) i.e. after a long period of water uptake (and uptake of chemical compounds) and when the volatilization is lower compared to the summer period because of the lower temperatures.

2.2.2 Heavy metals

When sampling for heavy metal three (at least) tree cores from opposite directions are collected and mixed into one and later divided into two (at least) samples. Sampling can be done all year because these compounds are bound into the wood structure. The uptake of heavy metal in trees depends on the tree species. Willow was found to be best suited. Samples from reference trees of the same species as the ones at the polluted area have to be sampled out site the polluted for later comparison. Typical background concentrations of heavy metals in wood are given in Algreen et al. (2013).

2.2.3 Quality control sampling

Additional sampling are needed during a sampling campaign to ensure the quality of the screening approach.

Table 1: Overview of additional sampling to ensure the quality.

Type	*Excursion blank*[1]	*Site blank*[1]	*Air blank*[1]	*Background*[2]
What and how	Analytical vials prepared as described below (section 3.1). Internal standard is added before leaving to the site and the vials are closed immediately. Place the vials in the storage freezer during the sampling campaign.	Analytical vials prepared as descript below (section 3.1). Internal standard is added at the site when starting the sampling and by the end of sampling. Close vials immediately after addition of internal standard and store in a freezer.	Analytical vials prepared as descript below (section 3.1). Leave the vials open during sampling to catch some air, add internal standard and close the vial. Place the vials in storage freezer during the sampling campaign.	Samples from trees located outside the test site are sampled and stored as described for heavy metals (section 3.2). If possible use same tree species as at the test site.
Object	The samples show whether the level of the internal standard (and thus also sampled compounds) changes during the transport[3].	The samples show if the level of the internal standard changes during sampling[3].	To confirm that the contamination originates from the subsurface and not from the air.	To measure the background level of heavy metals inside the trees as basis for a statistical comparison.

1: Not relevant when screening for heavy metals
2: Not relevant when screening for volatile compounds
3: Changes can be due to volatilization or degradation of the chemicals during the campaign.

2.2.4 Sample contamination

Contamination of tree core samples can occur by transfer of wood from a previous sample to the next, due to remnant wood stocked inside the borer. Therefore, make sure during sampling to empty the borer between samples. Further cleaning of the borer and extractor is not needed for chlorinated solvents (Vroblesky 2008). Sampling for heavy metal analysis, the extractor could be wiped off to avoid wood particle which could cause a carryover of heavy metals.

Concentrations in wood are typically much lower than concentrations in soil. Therefore, it is important that the sampling tool and equipment have no contact with soil (especially not contaminated soil).

Contamination may also happen in the laboratory. Likely sources of heavy metals in the laboratory are acids with background content, non-carefully cleaned glass ware, air (VOC) and dust (heavy metals). We recommend determining the method blank repeatedly.

3. Chemical analysis

The sample preparation and chemical analysis will depend of the compounds of interest, divided into volatile organic compounds and heavy metals.

3.1 Volatile organic compounds

Before sampling, analytical vials (20 ml) are prepared with 4 ml water (volumes depend on the specific analytical method). During sampling, 0.5 ml of internal standard is added together with the tree core. Type and volume of the internal standard depend on the specific analytical method and the target compound. The internal standard should have similar chemical properties as the target compound, and it will be used for calculating concentrations and as indicator to detect losses of the compounds due to leaking vials or degradation. The samples need to be cooled until the chemical analysis takes place to avoid the volatilization and degradation of the compounds. Sample preservation is possible by 1 mL of 0.01M HNO_3 (Larsen et al. 2008).

Samples can – for example - be analysed by HS-GC/MS (Headspace Gas Chromatography / Mass Spectrometry) using a system of Agilent 7980 gas chromatograph equipped with a Agilent 5975C electron impact (70 eV) triple-axis mass-selective detector.

Samples analysed for chlorinated solvents, BTEX (and MTBE) are analysed according to the following procedure. Initially incubated on a rotary shaker at 250 rpm and 70 °C for 5 min. This shall support that pollutants sufficiently equilibrate between tree core, water and air. 2 ml headspace is injected in pulsed splitless mode at 85°C with the injection pulse pressure at 20 psi until 0.2 min and the purge flow 100 ml/min to the split vent at 0.2 min. Chromatographic separation is achieved on a 30 m x 0.25 mm I.D x 1.00 µm film thickness ZB-5 capillary column (Phenomenex). The initial column temperature is set to 60 °C, and then ramp by 15 °C/min to 75°C, 3°C/min to 90 °C for 2 min, 25 °C/min to 170 °C for 5 min, 50 °C/min to 300 °C. The final temperature is held for 4 min and the total run time is 22.8 min with helium (1.0 ml/min) as carrier gas. The mass-selective detector temperatures are 230 °C for the EI source and 150 °C for the quadropole with the transfer line held at 250 °C. The spectra are monitored in selected ion monitoring (SIM) mode.

3.2 Heavy metals

The tree cores are stored in plastic bags at room temperature until sample preparation. Sample preparation is needed in order to breakdown the wood structure and release the heavy metals, see figure 4. It is done as follows. The tree cores are dried at 75-85°C until they reach a constant weight (approximately 24 h). 0.5-0.8 g (at least) of the dried tree core is weighed and given into a 50 ml volumetric flask followed by 10 ml 65 % HNO_3. The flasks are placed on a sand bath for 2 hours at 70-80 °C and then remove and cool at room temperature for 10 min, afterwards, add 2.5 ml of 30 % H_2O_2 and place back the flasks on the sand bath until the gas reaction is completed. The procedure is repeated with additional 2.5 ml of 30 % H_2O_2. Add Mili-Q water to obtain a total volume of 50 ml. Shake for 1 min, and transfer approximately 5 ml of the sample to a centrifuge glass, shake and empty (discard). Transfer the rest of the sample to the same centrifuge glass and centrifuge for 10 min with 2500 rpm. Transfer 7 ml supernatant to the test tubes and the samples are ready for analysis. Only use glass and acid wash equipment to avoid contamination. Other methods like autoclave or microway can also be used for break-down of wood (Algreen et al. 2012).

The samples can – for example – get analyzed by ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometry) on a system of Varian Vista MPX Axial View with a Varian SPS3 autosampler, Varian Axial Quartz Torch 2.3 mm injector, white/white (ID 1.02 mm) sample tubing, a cinebar spray chamber and conical nebulizer type. The operating conditions for the use of this analytical method are the following: Plasma power 1.0 kW, nebulizer pressure 220 kPa, plasma flow rate 15 L/min., auxiliary flow rate 1.5 L/min., replicate read time 30 s, instrument stabilization delay 15 s, sample uptake delay 30 s, pump rate 15 rpm, rinse time 30 s. Background correction is fitted. Three replicate measurements are done of each sample; the average of these is reported. 1 mg/L Yttrium used as internal standard was introduced online by a Y-piece with orange/orange pump tubing (ID 0.89 mm) to the sample stream. Alternative methods are AAS or ICP-MS.

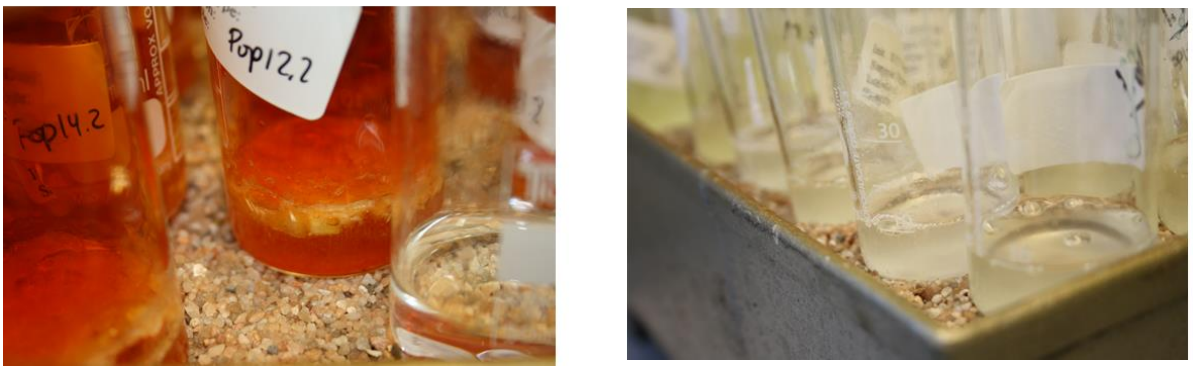

Figure 4: a) Decay of the tree cores in HNO_3 on a sand bath.

b) After the addition of H_2O_2 and end reaction. *Photos by M. Algreen.*

3.3 Detection limits

Concentrations in wood are relative low and this requires methods with sufficient precsion and quite low detections limits. The DL (detection limit [or qualification limit]) and QL (quantification limit) are dynamic and will depend on the analytical method including sample preparation, instruments status at the time of analysis and the variance of the standard curves. Therefore, DL and QL should be reported in conjunction to reported analytical results. Measurements below the DL or QL can be adjusted to ½ of DL and QL.

4. Data interpretation

The interpretation of results depends on the type of compound.

4.1 Volatile organic compounds

VOC compounds do, with few exceptions, not naturally occur in the environment. Hence, the detection of the compounds in the tree cores indicates a pollution of the subsurface (if sample contamination via air can be excluded by quality control sampling, section 2.2.3). It is important to mention that false negatives can occur when screening for organic compounds, i. e. the pollutants could not be detected in the tree core samples even though the subsurface is contaminated.

4.2 Heavy metals

Heavy metals are ubiquitous in the environment. Therefore, the measured concentrations in the tree cores have to be compared with references samples (see section 2.2.2). A statistical comparison of the concentrations allows for the identification of increased concentration levels. Such a comparison consists of testing the normal distribution of data by e.g. a Kolmogorov–Smirnov test at $\alpha = 5$ %. If a normal distribution cannot be rejected, parametrical statistical tests can be applied, such as a two-tailed t-test for differences in mean with an error probability of 0.05 ($\alpha = 5$ %). Else, non-parametrical tests for differences in mean, such as the Mann-Whitney-U-test, are an alternative.

5. Applications and additional literature

Some recent literature regarding plant uptake in trees and tree coring is presented for select compounds, together with outcomes from site investigations at polluted sites during the TIMBRE project.

5.1 Volatile organic compounds

Two types of VOC have been of interest: BTEX and chlorinated solvents.

5.1.1 BTEX

Most current literature concerning the uptake of BTEX compounds in trees is about phytoremediation. Trees have demonstrated to be useful in the remediation of BTEX compounds mainly by stimulating bio-degradation in the groundwater and soil (Barac et al. 2011, Weishaar et al. 2009, Zhang and Bouwer 1997, Morgan et al. 1993). This is caused by the impact of the trees on the groundwater table; fluctuations in the groundwater occur when the trees take up water. This will induce a falling water table and a higher oxygen level in the vadose zone resulting in better conditions for aerobic bio-degradation in the subsurface (Larsen et al. 2001). However, the presence of trees will also draw BTEX compounds from the groundwater to the bioactive area (root zone or soil area close to the roots), where they can be taken up (if not degraded) and later detected in the wood. This is due to the fact that the groundwater will recharge contaminants at times when the trees do not transpire (nightly hours) (Weishaar et al. 2009).

Due to the bio-degradation of BTEX, it is expected that the use of tree coring is useful only at high groundwater table. Tree coring has been applied above a lens of petroleum compounds 8m below the surface. Results showed relatively low concentrations in wood, generally below 100 ng/g, compared to concentrations of 1,100, 2,400 and 860 µg/L (benzene, toluene and xylene, respectively) in the groundwater (Sorek et al. 2008). Similarly, only few trees growing at the mega-site Zeiss with free-phase benzene in 8 m depth hat measurable benzene concentrations in wood (Algreen et al. 2011). In conclusion, tree coring may be useful for BTEX, but the requirements to the analytical method are more demanding. A greenhouse study confirms detectable concentrations in tree, but also that a lower groundwater level may cause lower BTEX concentrations in trees. This may be due to the increased aerobic degradation of BTEX in the rhizosphere and bulk soil (Wilson et al. 2013).

Within the TIMBRE project the feasibility of the method as a screening tool for BTEXs was tested at a former military air field in Poland. Tree core samples from pine, birch, willow and asp were collected together with sampling by other semi-quantitative and quantitate screening methods. Some outcomes are published:

- Kalisza M, Algreen M, Stalder M, Krupaneka J, Martac E, Trapp S, Bartke S. (2014). Application of a step-wise multiple screening methods for efficient site characterization. In progress.
- Martac E, Trapp S, Clausen L, Algreen M, Stalder M, Krupanek J, Kalisz M, Fatin-Rouge N. (2014) Comparative study of DP-based site investigation approaches and potential in situ remediation techniques: model-assisted evaluation of advantages and uncertainties. TIMBRE project, FP7 - ENV-2010.3.1.5-2, contract no: 265364. In Progress. Will be available at: http://www.timbre-project.eu/.
- Algreen M, Broholm M, Trapp S, Kalisz M, Krupanek J, Stalder M, Martac E, (2014a). Conference abstract (Poster), Ninth International Conference Remediation of Chlorinated and Recalcitrant Compounds. Monterey, California. Available at: http://www.battelle.org/docs /chlorinated-conference/chlorinated-conference-abstracts.pdf?sfvrsn=2

From the site investigation in Poland was concluded that BTEX can be detected in some tree species; willow and aspen are to be preferred, compared to birches (not recommended at all) and pines. Pines can be used as bio-indicators for toluene if the preferred species do not grow. Results obtained by tree coring show some discrepancies when compared with other methods; however tree coring was a useful initially screening tool for location of hot spots and single hot spots. Discrepancies between methods can, of course, not only be contributed to one method (Kalisza et al. 2014), and method comparison is generally statistically challenging (Wahyudi et al. 2012). Further case studies can be found in Vroblesky (2008).

5.1.2 Chlorinated solvents

The use of trees a bio-indicator for chlorinated solvents has been widely applied (Holm et al. 2011ab, Limmer et al. 2011, Larsen et al. 2008, Sorek et al. 2008, Gopalakrishnan et al. 2007, Vroblesky et al. 1999, among others). The literature demonstrates that tree cores are useful to detect different chlorinated compounds in groundwater and soil matrix. Even correlations between concentrations in wood and groundwater were observed (Gopalakrishnan et al. 2007), but they are not always linear or constant (Larsen et al. 2008). Concentrations varied with factors like tree species and sampling height. In the last couple of years, investigations have been brought up to a higher level and further options for the use of tree coring for chlorinated solvents have evolved. Limmer et al. (2013) and Holm & Rotard (2011) investigated if the concentration linked to the orientation of sampling could be related to the subsurface contamination gradient. Holm and Rotard found that concentration clearly depended on the sampling direction, but that the variations in concentrations measured around the tree could not provide secure information on the flow direction of the plume in the groundwater. Weather conditions were found to have a greater influence than the variations inside the tree. On the other hand, Limmer et al. showed that trees had contaminant centroids at the side of the stem closest to the plume center and that there was a relation

between the contaminant gradients in trees and in the groundwater. The conflicting findings of these two studies can be due to different steep gradients at the test sites and due to the tree species. Limmer et al. studied trees which exhibited sectoriality, which is not common for all tree species. Some tree species have winding xylem pores which complicate the use of the method to provide compass-like information (Holm and Rotard 2011). However, it can be learned from the studies that there is a large variation in concentrations for tree cores sampled in different directions of the stem. To avoid false negative results, at least two replicates should be collected from different sides of each tree. Other approaches to clarify the plume direction aimed ratios between parent and metabolites product. The ratios of c-DCE/TCE increased with increasing distance from the source (Limmer et al. 2011, Larsen et al. 2008).

When dealing with chlorinated solvents, tree coring can also help to get information about the timing of the spill event by analyzing the rings and the Cl^- content in the tree rings. This method is called “dendrochronology”. It was investigated by Balouet and co-workers among others and there are several papers published on the topic (Burken et al. 2011, Balouet et al. 2009, 2007). Dendrochronology allows tracking down the history of contamination releases, which may be of great value if a site had changing owners and the responsibilities have to be placed. A short-coming of the method is that chloride in the environment can have plenty of sources.

Because the method of tree coring already has shown to be useful, one focus within the project of TIMBRE was to implement the method in practice and to compare tree coring with the commercially applied method of soil air sampling. The method has been applied at several Danish sites polluted with PCE and/or TCE. Some outcomes are published:

- Algreen M, Stalder M, Riis CE, Petersen J, Kalisz M, Krupanek J,Trapp S, Broholm M. (2014b). Comparison of tree coring and soil gas sampling for screening of contaminated sites. Conference Abstract (oral presentation). 11th International Phytotechnologies Conference Heraklion, Crete, Greece.
- Region Zealand. (2012). Træprøver afslører forurening i Ølsemagle. Press statement. Avilable at: http://www.regionsjaelland.dk/nyheder/pressemeddelelser/Sider/Tr%C3%A6pr%C3%B8v er-afsl%C3%B8rer-forurening-i-%C3%98lsemagle.aspx. Danish.
- Graae P. (2011). Træer fortæller om forurenet grundvand. Televison feature. Available at: http://www.tv2east.dk/artikler/traeer-fortaeller-om-forurenet-grundvand . Danish.
- Holgersen S. (2011). Forurenet jord og vand måles i træprøver, Hurtig og billig screening kan nok snart erstatte dyre jordprøver. Grønt Miljø 1/ Januar. Available at: http://www.grontmiljo.dk/numre/2012/gm112.pdf. Danish.

5.2 Heavy metals

To our knowledge no studies have tested the use of tree coring to detected elevated heavy metals concentration in soil. Therefore the method was applied at several polluted sites in Europe. Mainly willow and poplar trees were sampled. Results are published in:

- Algreen M, Trapp S, Rein A. (2013). Phytoscreening and phytoextraction of heavy metals at Danish polluted sites using willow and poplar trees. Environmental Science and Pollution Research. Open access at Springerlink.com. DOI 10.1007/s11356-013-2085-z.
- Algreen M, Rein A, Legind CN, Amundsen CE, Karlson UG, Trapp S. (2012). Test of tree core sampling for screening of toxic elements in soils from a Norwegian site. International Journal of Phytoremediation. 14(4): 305-319
- Martac E, Trapp S, Clausen L, Algreen M, Stalder M, Krupanek J, Kalisz M, Fatin-Rouge N. (2014). Comparative study of DP-based site investigation approaches and potential in situ remediation techniques: model-assisted evaluation of advantages and uncertainties. TIMBRE project, FP7 - ENV-2010.3.1.5-2, contract no: 265364. In Progress. Will be available at: http://www.timbre-project.eu/.
- Martac E, Bica I, Gaitanaru D, Gogu R, Krupanek J, Kalisz M, Algreen M, Trapp S, Fatin-Rouge N, Stalder M, Bartke S. (2013). Public briefing on Timbre project's test sites' knowledge status (database and site model) as a result of integrated site investigation techniques (D4.1 Brief Version). Available at: http://www.timbre-project.eu/.

Hereof can it be concluded that tree coring of heavy metals depends on the tree species. The use of willows indicated feasibility of tree coring for some heavy metals (Cd, Cu, Ni and Zn) at strongly polluted sites. Differences were less clear for poplar, only for Ni were the differences between test site and reference site significant. However, the range of "natural" background concentrations was relatively narrow and varied only a little with species, sampling time, and other conditions. Once the natural background concentrations of heavy metals in wood are established, results from heavy metals phytoscreening can be interpreted with more certainty. Yet, little data on heavy metals in wood is available, while more literature regarding the uptake of heavy metals into smaller plants and other plant parts such as leaves and roots can be found (McLaughlin et al. 2011, Zacchini et al. 2011, Djingova et al. 2004, Reimann et al. 2001 among others).

6. Summary

Tree coring has several advantages: it is fast, simple, inexpensive and suitable for most areas. Using tree coring as initial screening method followed by quantitative methods could make the site characterization more efficient (dense sampling grid; better basis for a conceptual model). When applying tree coring as a site characterization tool, it is important to emphasize that the sampling should be done by a standardized procedure like the one presented in this guideline as the procedure can affect the results.

Phytoscreening by tree coring for typical pollutants in the subsurface was tested during the project of TIMBRE. Test sites were contaminated with BTEX, chlorinated and/or heavy metals. It can be concluded that:

- **BTEX** can be detected in some tree species; willows and aspen are to be preferred compared to birches (not recommended at all) and pines. Pines can be useful as bio-indicators for toluene. Some discrepancies occur when compared with other methods, but tree coring was a good method to locate hot spots and erratic pollution. GW table had an influence on the BTEX concentration in wood.

- Trees are useful and reliable indicators for **chlorinated solvents**. Thus, tree coring is quite useful at sites polluted with e.g. PCE and TCE. Especially at large sites or at sites where the source is unknown, due to the dense sampling grid and low-cost.

- The method is useful as screening tool for **heavy metals** (Cd, Cu, Ni and Zn) at strongly polluted sites, but it will depend on the tree species. Willow is to be preferred compared to poplars.

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