ANNEX A

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TRACE METAL DETERMINATION IN TREE RINGS: FROM SAMPLING TO ANALYSIS

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Abstract. This study is aimed to present the necessary steps for tree-rings sampling and trace metal analysis. The most common and reasonable methods were described for: selecting site, research object, sampling, tree-rings dating, sample preparation, trace metal analysis and statistical analysis. This scientific paper is guidance for precise field sampling, following all necessary steps, for easier work and accurate results.

Keywords: Tree-ring, sampling, dendrochronology, increment borer, trace metal.

Introduction

Forest ecosystems, especially those of central Europe have been exposed to pollution for decades. Especially coniferous forest stands appeared to be affected. Forest showed signs of discolouration as well as sporting necrotic alterations to the leaves, impaired root-growth were observed, also changes to the stem, such as a decreasing growth-rate and an increase in damage caused by insects or fungi (Rademacher 2003).

Main factors that cause decline in coniferous forests: SO_2, trace metal and fluor (Pukacki 2000). To assess the role of trace metals in forest decline often require comparison of laboratory studies with field observation (Narasimha 2004).

Though trees can be contaminated through the atmosphere the main uptake pathway for elements to enter into a tree is from soil via the roots (Padilla 2002).

SO_2 increase acidity of soil. Particularly in acidic soil more mobile trace metals such as zinc (Zn), manganese (Mn) and cadmium (Cd) were increasingly taken up by plant and animal organisms of the forest ecosystems and also led to increasing surface and ground water pollution. Less mobile trace metals such as lead (Pb) and, partly, copper (Cu), have been accumulating for a long time in the organic soil layer, particularly in the top soil and, even in regions with decreasing atmospheric input, there is a danger in the case of their release (Rademacher 2003).

There are a lot of evidences about how forests ecosystem is damaged by pollution and dendrochronology appears as a useful tool for such researches.

Dendrochronology, or tree-ring analysis, shows tree-rings variation in response to the environment (Alexander 1999); indicates tree-ring growth and tree-ring dating in order to piece together a timeline of past events (Firestone 2003); helps to address both the extent and causes of impacts of regional scale environmental pollution on the productivity and function of forest ecosystems (McLaughlin 2002); shows the spatial extent of contamination (e.g. downwind from a smelter) if trees are sampled over a certain area; reveals the timing or chronology of the contamination event (Gilberd 1997).

The aim of this study was to make literature-based analysis and develop methodology about trace metal determination in tree-rings.

Selection of site and research object

The search and selection of suitable regions, sites, species, and trees are fundamentally important in dendrochronological studies.

Since dendrochronology is practically the only discipline that can provide the long-term historical dimension in pollution research on forested ecosystems, the range of addressed problems is very wide: *Local*, *Re-*

gional/national* and *Continental/hemispheric* (Cook 1990).

Conifers are best to choose for dendroindication, because they are sensitive to the effects of environmental contamination than deciduous trees, and improved environmental condition indicators (Stravinskiene 2003). Radial growth rate of Scots pine (*Pinus sylvestris L.*) is sensitive to environmental conditions changes (Stravinskiene 2008).

Before going to field it is important to estimate the scale and coordinates of area that will be investigated. This can be done by the usual methods: cartographic methods, GIS (Geographic Information Systems) or simply with map, compass and tape measure (Trapp 2007).

The amount of data needed depends strongly on the precision desired (Braker 2002). However, the more points sampled the greater the accuracy of deposition monitoring and the greater the statistical power to detect changes (Ware 2001).

Stravinskiene (2008) in her research was investigating how does Scots pine radial growth indicates unfavorable environmental conditions in Vilnius and Kaunas cities in comparison to relatively clean environment. From 330 to 456 trees were sampled to represent area of about 40 thousand ha in Vilnius and about 3.7 thousand ha in Kaunas respectively (Stravinskiene 2008).

Trying to establish baseline values of 12 trace metals and to assess differences in metal concentrations related to automobile traffic, Kirchner (2008) selected two field collection sites with similar ecologic attributes, one representing to a heavily traveled highway, and the other isolated from any local source of auto emissions. At each site two pine trees with similar features were selected, and two increment cores collected from each tree (Kirchner 2008).

According to Cook (1990) taking samples it has to be not less than 16 – 20 samples per specie and site. But there is no single method how to decide how much samples would be needed (Cook 1990).

The next step of dendrochronological research is how the chosen number of trees should be selected from the selected territory.

There are several methods that could be used:

Random sampling. The most common design in vegetation science is simple random sampling (Wilson 2005). Most common is stratified random sampling. For a given sampling intensity, stratification often yields more precise estimates of the forest parameters than does a simple random sample of the same size (Husch 2002).

Fixed-area plot sampling. In fixed are plot samplings we assume that the plot area is representatively in the remainder of the area of interest. Usually statistics about the stand are reported on the standard units of measure. For example we measure one tree in ¼ are (= 100 m^2) plot we assume that there are four trees just like one we measured per are (Larsen 1999).

Systematic sampling. Systematic sampling involves laying out the grid of points over the forest area, from a randomly selected starting point, with spacing such that the number of grid points equals the sample size required. The advantage of systematic sampling is that is very straightforward to apply in field. This sampling technique is appropriate to simple random sampling, but not to fixed-are sampling. The main disadvantage of systematic sampling is that it is appropriate only for forest with relatively little variations, when simple random sampling is most appropriate (West 2003).

Best sampling time is the vegetation period, from late April to October. From early November, concentrations decline (Trapp 2007). However, springtime is recommended for sampling, when plants are most physiologically active (Hill 2002).

Methods of sampling

Samples of tree-rings can be taken by different methods:

- Planing (Baltrėnaitė 2007, Husch 2004);
- Severing with arched or simple chisels (Baltrėnaitė, Butkus 2005, Braker 2007);
- Using increment borers (Haglöf 2009, Edwards 2005),

Tree sections and cores are the basic material for sampling of tree-rings. On the one hand tree sections are better for identifying locally missing rings, however these types of sampling are lethal for the tree. Cores give less information but are less destructive for the trees (Bräker 2002). The increment borer (Fig. 1) is known to be used as instrument for fast and trustworthy data when evaluating tree age, pollution control, wood density, increment and decay examination (Haglöf 2009).

Figure 1 Haglöf increment borers (Haglöf 2009)

Sampling with an increment borer is comparatively non-destructive, environmentally harmless and usually simple to implement (Smith 1996).

Commonly, for analysis of tree rings it's used a 4, 5 or 12 mm diameter corer. The advantage of the smaller diameter corers is that they are more easily inserted into trees, than the 12 mm diameter corer. Moreover, the advantage of the larger corer is that more samples are obtained, and this can be critical if trace metals are at a low concentration or the time step for the analysis is small.

The major weakness to the larger corer is that it is nearly impossible to insert into hardwood species (Baes 1985). Additionally, the 12 mm core diameter borers are mostly used for measuring fibre length of wood and when making quantitative analysis where larger samples are necessary (Haglöf 2009).

Tree cores can be taken in different heights, it depends what metals are investigated. Because metals can be translocated in plants very different. Once the ions have been absorbed through the roots or leaves and have been transported to the xylem vessels, there is the possibility of movement throughout the whole plant. The rate and extent of movement within plants depends on the metal concerned, the plant organ and the age of the plant. They can be classified Mn, Zn, Cd, B, Mo and Se as elements which were readily translocated to the plant tops; Ni, Co and Cu were intermediate, and Cr, Pb and Hg were translocated to the least extent (Adriano 2001). So, the height of sampling point can vary from the bottom of tree to 1.5 m. The most usable and comfortable way of taking sample is from 1 m to 1.5 m.

Measurement of tree rings

Measurement of ring widths can be carried out using any system that is sufficiently accurate. Much early work used the traditional travelling microscope of the physic laboratory. These are very slow to use as each measurement requires the reading of a vernier scale (Cook 1990).

Many computer programs are available for developing and analyzing tree-ring data. One of the most widely used programs is WinDENDRO (Grissino – Mayer 2009).

In Lithuania most common way of wood samples measuring is with measuring table LINTAB. LINTAB is a digital positioning table for tree-ring analysis. It is ergonomically easy to operate, robust and splash-water proved. LINTAB could be used together with the TSAP software (Time Series Analysis and Presentation). The accuracy of the measurements is ± 0.01 mm (Walesch Electronic 2009).

Preparation of samples

The next step before metal analysis in tree-rings is a wood sample preparation. It depends on further analysis applies, but traditionally involve cracking and grinding. In accordance with Greece Forest Soil Laboratory (2004) wood chips were cracked with a mill to 1 mm wood fragments. Approximately 2 g of dry wood fragments were used for the analysis. The wood fragments were diluted with the acid digestion method, with acid solution consisting of 500 ml HNO_3, 100ml H_2SO_4 and 50 ml $HClO_4$ 60 % solution. The wood samples were placed in 30 ml Kjeldhal tubes on a hot plate with 120-1300C and 6 ml of acid solution added. Cu, Fe, Zn, Mn, and Pb concentration was estimated with the use of Atomic absorption, directly to the 50 ml filtered solution (Pantera, Papadopoulos 2004).

According to Canada Trent university samples were placed in 50 ml borosilicate glass tubes, dried at 70°C for 48 h, weighed and then dry-ashed at 400°C in a muffle furnace for 6 h. Solutions were analyzed for Mg, K, Ca, Mn, P, Fe, Cu, Ni, Zn, Sr, Cd and Pb using ICP-MS (Watmough, Hutchinson 1995).

Depending on type of analysis performed, the acid and amount of it used could vary. For instance, it's 5.0 ml of acid solution used because that is the minimum amount necessary for inductively coupled plasma (ICP) analysis. The same amount can be necessary for flame atomic absorption techniques (AA). With graphite furnace AA techniques 1.0 ml or less would suffice. For atomic absorption, nitric acid (HNO3) and perchloric acid (HClO4) are the best acids (Baes 1985).

Methods used for trace metal analysis

Most mature, effective spectrometric techniques which can be used for trace metal evaluation includes:

- Flame Atomic Absorption Spectrometry (FAAS),
- Graphite Furnace Atomic Absorption Spectrometry (GFAAS),
- Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, or ICP),
- Inductively Coupled Plasma Mass Spectrometry (ICP-MS).

Flame AA would be appropriate only for analysis of Na, Mg, possibly Al, K, Ca, Mn, Sr, and Ba. ICP will typically give results for roughly 30 elements including

Ag, Al, As, B, Ba, Be, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hf, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Se, Si, Sr, Ti, V, Zn, and Zr. However, rarely are Ag, As, Co, Cr, Ga, Mo, Sb, Se, V, and Zr, detected in wood by ICP. Graphite furnace techniques are probably the best for any given single element, but its major disadvantages are the time necessary to set up and calibrate the tube, single element analysis only, and the short life of the graphite tubes (Baes 1985).

The atomic spectrometry techniques atomize or ionize samples at very high temperatures, and then analyze the constituent atomic parts by mass, or by interaction with electromagnetic radiation (both absorption and emission). The nature of these techniques means that, unless the technique is coupled with a chromatographic process, there is no speciation or chemical state data produced from these techniques, only total elemental content. However, these techniques, especially ICP-MS, provide access to the lowest limit of detection (LODs) currently available in routine analytical chemistry. They are particularly useful since, theoretically, they provide analysis of the total amount of element present in the sample, regardless of the matrix or the environment of the target element (Brown, Milton 2005).

According to all given information it's shown that ICP-MS is one of most important techniques for assessing environmental pollution also detecting trace metal concentration.

Statistical analysis

ANOVA (Analysis of variance – one way) – analysis of variance is a statistical technique for analyzing data that tests for a difference between two or more means by comparing the variances "within" groups and variances "between" groups (Merron 2003). The reason for doing an ANOVA is to see if there is any difference between groups on some variable. A one way repeated measures ANOVA is used when you have a single group on which you have measured something a few times (Monash University 2009).

Main decades of trace metals and interacting terms on total decade ring width can be statistically analyzed using ANOVA.

Conclusion

1. The first steps in trace metal analysis in tree-rings, careful selection of location, sampling method and investigation object, is important for further study of data accuracy and reliability. Usually at least 16 – 20 samples are needed. Springtime is recommended (from late April) for sampling, when plants are most physiologically active. Weather conditions should be estimated during sampling: temperature should be not less than minus 5oC.

2. Among tree-ring sampling methods – planing, sampling with arched or common chisels or increment borer, the increment borer is believed to be more practical and less harmful for trees. The one of 12 mm in diameter provides more mass for analysis of trace metals.

3. Various ways of chemical sample preparation are used for trace metal analysis, however first of all it's required cracking or burning of wood sample. Commonly 1-5 mm wood fragments are cracked. GFAAS and ICP–MS methods are most used; as a consequence it can detect extremely small trace metal concentrations.

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Santrauka

Didėjančios užterštumo problemos reikalauja kruopštesnių tyrimų ir naujų būdų teršalų koncentracijoms, sklaidai nustatyti. Vienas iš taršos bioindikatorių – medžiai. Jautriausi iš jų – paprastoji pušis (*Pynus sylvestris*). Remiantis dendrochronologijos mokslu galima ne tik nustatyti medžio biologines savybes, bet ir nustatyti sunkiųjų metalų koncentracijas. Šio tyrimo tikslas yra pateikti būtinus žingsnis medžio rievių mėginių ėmimui ir sunkiųjų metalų analizei. Šiame straipsnyje buvo aptarti dažniausiai naudojami ir pagrįsti metodai: vietos parinkimui, tyrimo objekto parinkimui, mėginių ėmimui, medžio rievių skaičiavimui, bandinių paruošimui, sunkiųjų metalų analizei ir statistinei analizei. Vietos parinkimas priklauso nuo jos dydžio ir siekiamų rezultatų. Paprastai reikalingas mėginių kiekis yra bent 16 - 20. Pavasaris (nuo balandžio pabaigos) geriausias metas mėginių ėmimui, kai augalai yra fiziologiškai aktyvūs. Oro sąlygos turėtų būti įvertintos imant mėginius: temperatūra turi būti ne mažesnė kaip minus 5^{o}C. Mėginių paėmimo būdai: nukertant medį, atskyrimas kaltai ir ėmimas su Preslerio grąžtu, darantis mažiausią žalą medžiui. Tinkamiausias diametras – 12 mm. Geriausi būdai sunkiųjų metalų koncentracijų nustatymui yra naudojant grafitinį liepsnos atominį

absorbcinį spektrometrą (GFAAS) arba indukuotos plazmos masių spektrometru (ICP-MS).