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Quantification of sulphur in *Picea abies* by LA-ICPMS

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Introduction

Sulphur profiles in Swiss peat bogs have been studied in our working group before. While the profile of a Duedingen peat bog core (580 m a.s.l., Swiss Plateau) correlates with the sulphur dioxide emission profile of the 20th century in Switzerland (Jeker 2001), the profile of an alpine peat bog in Mauntschas near St. Moritz (1900 m a.s.l., Engadin) shows a different behaviour (Schreier et al. 2002). These results are to be compared and consolidated with data provided by measurement of trees growing nearby. *Picea abies* (L.) Karst. is widely spread and represents 48% of all forest trees in Switzerland. Therefore, it was chosen as the tree type representative for all of the regions and altitudes mentioned. Six trees were chosen in both regions, and additional six specimens in the forest of Frieswil (740 m a.s.l., Swiss Plateau) for comparison with the anthropogenic Duedingen site, which is situated next to a highway.

In 1987, 88% (150'000 kt) of the total emissions of sulphur dioxide were anthropogenic (Parlar & Angerhöfer 1995). In Switzerland, these emissions have decreased since, due to several measures such as the replacement of coal by oil and the desulphurisation of mineral oil. But in other countries such as China, SO_2 emissions are still increasing, which has also been seen in trees near the heavily polluted city of Chengdu (Gaoming 1996). Due to the extent of these emissions, sulphur dioxide became an additional source of S for plants.

Sulphur metabolism in trees is complex and not completely deciphered. Sulphur is one of the six macronutrients for plants, although it is only 3% to 5% as abundant as nitrogen (Leustek & Saito 1999). It is found in the two amino acids cysteine and methionine, and in a variety of metabolites like the tripeptide glutathione, which plays an important role as an anti-oxidiser. Plants cover their demand for sulphur by reduction of inorganic sulphate. Recent studies showed that sulphur metabolism is linked to carbon and nitrogen metabolism (Kopriva & Rennenberg 2004).

Sulphur dioxide enters the needles via stomata without regulatory control. It is rapidly fixed into cysteine by the cysteine biosynthetic pathway (Noji et.al. 2000). This is an important step in the detoxification of the sulphite ion, leading to an increase of sulphate and glutathione contents. In wood, the uptake of gaseous sulphur compounds is reflected by higher sulphate content in case of sulphur dioxide, or by a larger amount of organic sulphur compounds in presence of hydrogen sulphide (Rennenberg 2004), and reduces the uptake via roots substantially but not completely (Giesemann et.al. 2000).

Some physiological aspects of sulphur in wood are not fully understood yet. Examples are the diminution of total sulphur content in compression wood compared to normal wood, as well as the huge increase observed in case of fungal attack in the heartwood of old trees. These results were already obtained at our laboratory by analysis of drill core pieces from Frieswil (Barrelet et.al. 2003).

Aim of the present study is the assessment of the spatial distribution of sulphur in tree rings and the investigation of the suitability of this archive for non-metals. It should be tested if LASER ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) is an adequate technique for quantification of sulphur in Picea abies (Norway spruce) drill cores. The main advantages of LA-ICP-MS are its high spatial resolution and high sensitivity and the minimized risk of chemical contamination and sulphur losses due to sample digestion.

As a quasi non destructive method, LA-ICP-MS is mainly used in research applications on solids such as archaeological, metal and glass samples, or other solids (Devos et.al. 2000, Senn-Bischofberger 2004). The hyphenated technique was also applied to plant materials such as bark (Liebergeld et.al. 1996), wood (Prohaska et.al. 1998) or leaves (Hoffmann et.al. 2000), e.g. to investigate the variation of metals in tree rings. However, little is known about its application in dendroecology for quantifying non metals. The present work presents development and optimisation of LA-ICP-MS for sulphur analysis in wood, as well as first results.

Experimental

Sampling

At three sampling sites in Switzerland Frieswil, Duedingen and St. Moritz, six trees were sampled using a 5 mm increment borer (Suunto). The sampling procedure was optimised with special regards to minimised contamination. The use of gloves was necessary and furthermore, the cleaning and lubricating oil used for the preservation of the borer had to be free of sulphur. Previously, sulphur contents in different lubricants were determined using ICP-OES. PhEur grade paraffin (Hänseler) was chosen because of lowest contamination risk.

Determination of tree characteristics

Trees of different sizes were selected in order to assess ageing effects. Four drill cores per tree were taken at breast height in each direction. Additionally, two drill cores were taken 5 cm under the southern and western drill holes. Since in Picea abies there is no difference in colour between sapwood and heartwood (mature wood), the extent of the humid sapwood had to be measured immediately after sampling. The samples were pressed into weighted and previously cleaned plastic rails and dried in an oven at 40°C until weight constancy was reached. Water content of the drill cores was finally determined by difference weighing. The density and tree ring width of the two supplementary, parallel drill cores was measured at WSL by radiodensitometry on a DENDRO-2003 Densitometer (Walesch Electronic GmbH).

Instrumentation

An EMPA modified Nd:YAG LASER Perkin Elmer/Sciex sampler 320 (Wanner et.al. 1999, Bleiner et.al. 2003) was coupled to an ELEMENT 2 high resolution magnetic sector field ICP-MS (ThermoFinnigan). The LASER was operated at a wavelength of 266 nm in Q-switch mode using a Q-switch delay of 300 ns. The formed craters show diameters of about 100 µm. The ablated particles were transported by 5.0 grade helium at an average flow of 1.5 l/min. An additional argon gas flow was added after sampling cell to keep the plasma stable. The ICP was operated under hot plasma conditions using RF power 1250 W, 1.3 l/min Ar sample gas, 15 l/min cool gas and 0.8 l/min auxiliary gas.

Sample preparation for LA-ICP-MS

For LA-ICP-MS, drill core pieces of 3 cm length were cut using a scalpel and inserted into plastic rail pieces of corresponding size. After counting of the tree rings, a mark was applied onto the rail every 5 year rings. Two samples in rails were placed onto a small aluminium holder in a cavity of the sampling cell designed by the EMPA.

Standardisation for LA-ICP-MS

A precondition for quantification using LA-ICP-MS is the availability of suitable solid standards such as commercial reference materials. For sulphur in wood however, no appropriate calibration standards procurable. Self-made doped cellulose powders had been used by other authors (Hoffmann et.al. 1996). Also in this study standards had been produced in the laboratory. Two different base materials were tested:

- cellulose powder (20 microns, Aldrich) with 5 % activated charcoal (p.A., Merck),
- homogenised wood powder characterised at EMPA.

Both standards were prepared as follows: 2 g portions of powder were put into six Teflon vials and doped with 0, 40, 200, 500, 1000 and 2000 µl of a solution containing 200 µg/ml sulphur (1:5 dilution of S ICP standard solution, 1000 µg/ml S in water, Alfa Aesar). 650 µl of a 100 ppm Scandium solution (1:5 dilution of Sc ICP standard solution, 1000 µg/ml Sc in 5 % HNO_3, Alfa Aesar) was added as an internal standard. Six millilitres of ultrapure water were then poured into the vials while mixing thoroughly with a plastic stick until a homogeneous paste was obtained. The paste was dried in an oven at 80°C until weight constancy. Subsequent to the drying, the content of the vials was crushed in an agate mortar. The homogeneity of the obtained powder and the appropriateness for calibration was verified by analysis of two aliquots with ICP-OES. Afterwards, 300 mg of powder were transformed into pellets.

Results and Discussion

For each sampled tree, sapwood width, circumference and wood water content were determined. Knowledge of the sapwood extent is important due to the physiological processes of heartwood formation. Some authors describe soluble mineral compounds

containing P, K and S which migrate to the sapwood through the rays (Bamber 1976, Ziegler 1968). While sapwood contains living parenchyma cells, heartwood is inactive and has no water-conducting functions anymore.
Furthermore, density and cambial age of two drill cores per tree were measured by radiodensitometry. The averages of six trees at each site are presented in the table below, as well as the individual characteristics of the drill cores D1W and D5W, which were investigated by LA-ICP-MS so far. Further studies will be presented soon.

Table 1: Characteristics of the sampled trees.

	Measured circumference [cm]	**Determined cambial age [y]**	**Measured sapwood width [cm]**	**Sapwood zone [y]**	**Determined wood water content [%]**
Duedingen 580 m a.s.l	167.5 +/- 26.3	95.7 +/- 3.4	5.9 +/-1.3	31 +/- 8	46 +/- 7
D1W	128	101	4.3	43	39.8
D5W	165	99	7.5	34	44.5
Frieswil 740 m a.s.l	208.2 +/- 39.4	141.8 +/- 6	5.2 +/-1.4	29 +/- 4	41.1 +/- 8
St. Moritz 1900 m a.s.l.	150.2 +/- 21.9	155.2 +/- 19.5	5.4 +/-1.4	62 +/- 16	60.5 +/- 8.3

After instrument optimisation and mass calibration using multi-element solutions, the LASER had been coupled to the ICP-MS using a self-designed adapter. The LASER parameters such as aperture, pulse energy and repetition rate were optimised and resulted in the final settings of 30 mJ for pulse output energy and 10 Hz for pulse repetition rate.
Before the development of the method for transient signal acquisition, the sulphur signals were optimised to maximum intensities on all three stable sulphur isotopes ^{32}S (95.02% relative abundance), ^{33}S (0.75%) and ^{34}S (4.21%). The transport gas flow rate through the cell had major influence and was finally set to 1.5 l/min. Validation was performed using wood and cellulose standards as well as selected samples.
A major problem was the high sulphur background level, which is of important concern considering the low concentration in wood. Consequent elimination of plasticine as fixation material for samples and sample holder finally led to lower background. Additionally, the use of 5.0 instead of 4.6 quality helium as transport gas lowered the background as well. Another positive effect resulted from careful cleaning of the sample cell before each sample change.
After careful method development and validation first measurements were performed on two drill cores from Duedingen, labelled D1W and D5W. These drill cores originate from two forest trees of different age, both located in a distance of 500 and 450 m to the highway A12, respectively. The peat bog is situated about 1 km away. Tree D1 has a cambial age of 101 years, while tree D5 has an age of 96 years. Details can be found in table 1. Both drill cores were sampled from western direction.

Test spots every 5 years as well as in the cambial zone and bark were selected in this preliminary study. All data were normalised using ^{13}C as internal standard. Figure 1 presents the obtained profiles for ^{32}S for the drill cores DW1 and DW5. The data present averages of multiple determinations.

From 1915 to 1970, the sulphur profile of D1W varies slightly between 0.1 and 0.2, while the profile of D5W ranges on a marginal higher level between 0.15 and 0.25. From 1970 on a significant sulphur increase was observed in both trees, with maxima in the particular tree rings corresponding to 1985. A similar peak was also found for the neighbouring Duedingen peat bog which reaches back to the year 1920 (Jeker 2001). The peak corresponds also to the SO2 emissions in Switzerland acquired by the Swiss Agency for the Environment, Forests and Landscape. The main sulphur source in the rural Duedingen area is likely to be mainly traffic (diesel fuel and tyre abrasion). This hypothesis is supported by the fact that the A12 highway in this area has been inaugurated in parts between 1971 and 1973. Even if the mobility of sulphur in wood cannot be neglected (Bamber 1976, Ziegler 1968), the anthropogenic origin of the peak described above is very likely. After the maximum, a significant signal decrease was observed, presumably due to the sulphur reduction in fuel.

The rise of the concentrations after 1995 is likely to be of physiological origin, since the decay of xylem cells, which begins shortly after the cambium, is accompanied by the decay of proteins. This leads to the presence of a ring-like zone with higher amino acid concentration in the tree (Sandermann et.al. 1967). Furthermore, there is a bidirectional exchange between phloem and xylem during long-distance transport along the stem, which has been described for sulphate, glutathione and cysteine (Gessler et.al. 2003). Thus, the observation that sulphur content is rising again in the years preceding the cambium is not surprising.

Due to the excellent spatial resolution of LA-ICP-MS, the cambial zone could be sampled separately, revealing higher sulphur content compared to xylem. This is consistent to protein contents of the cambium reported elsewhere, which range between 0.42 - 0.56 %, and fall shortly afterwards to 0.2 – 0.3 % (Schmid-Vogt et.al. 1977). From a previous study using microwave digestions of 5-year drill core segments determined by ICP-OES, the sulphur concentrations of Norway spruces from Frieswil are known to range between 20 and 80 mg/kg (Barrelet et.al. 2003). Bark showed higher concentrations of about 200 mg/kg sulphur. LA-ICP-MS results gave higher sulphur concentrations in bark, too. A similar pattern was found for total sulphur in the presented D1W and D5W investigations.

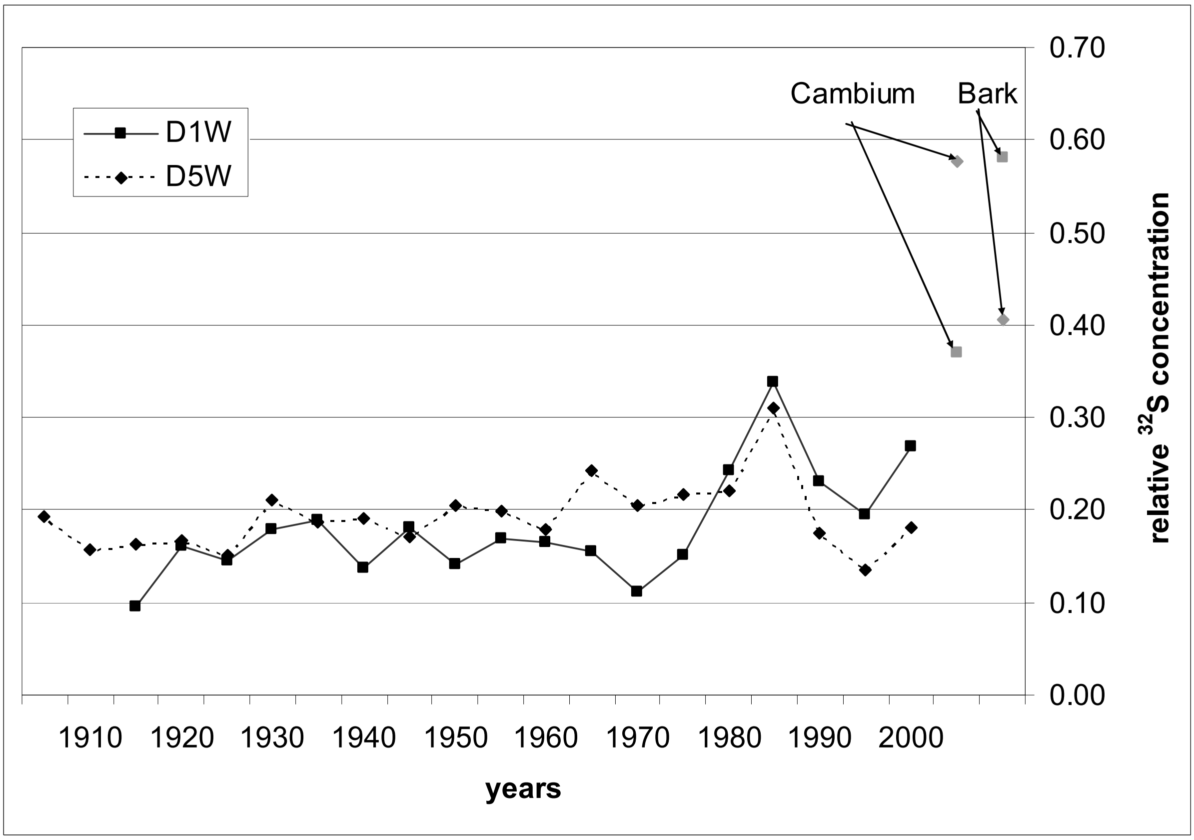

Figure 1: Relative total sulphur concentrations in the drill cores D1W and D5W

Outlook

Further steps are the optimization of an appropriate calibration strategy to get a reliable quantification. Since ICP-MS enables to perform simultaneous multi-element determination, it is planned to investigate additional information about metal distribution in spruce wood. A closer look at selected metal concentrations such as Al, K, Ca, Cr, Mn, Co, Ni, Cu, Zn, Rb, Sr, Cd, Ba and Pb might give further information about possible correlations of sulphur with these metals. Metal doped cellulose or wood pellets will be used for calibration.

A reliable validation of the LA-ICP-MS multi element method is planned by comparison to an independent digestion based procedure, where digested 5-years drill core segments will be quantified by inductively coupled plasma optical emission spectrometry (ICP-OES) as well as ICP-MS.

Conclusion

The two investigated trees gave similar sulphur profiles, which show several analogies with the profile found in the Duedingen peat bog. This leads to the assumption that Picea abies is an appropriate environmental archive for sulphur.

The excellent spatial resolution of LA-ICP-MS allows the determination of sulphur in each tree ring, whereas digestion-based procedures usually require 5 to 10 year segments for adequate determination. For this reason, only LA-ICP-MS enables to distinguish the cambial zone from bark. Even discrete analysis of earlywood and latewood would be possible by high spatial resolution of LASER Ablation with maximum spot sizes of about 100 µm. Therefore LA-ICP-MS is a suitable technique to determine sulphur in tree rings. The trees from St. Moritz and Frieswil will be investigated next.

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