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$\delta^{13}C$ IN TREE RINGS α-CELLULOSE. A METHODOLOGY OF MEASUREMENTS AND INTERPRETATION: STATE OF THE ART

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Abstract. The development of a tree is dependent upon the climatic and environmental conditions under which it grows. Formed growth rings represent integration of these variables during each growing season. More parameters other than ring-width are required to produce satisfactory reconstruction of past environmental changes. Stable isotope variations within the growth rings may provide other parameters. The variation of $\delta^{13}C$ indices in the latewood α-cellulose is highly correlated with combined July and August environmental variables.

Investigations of isotopic carbon composition in annual tree rings from Upper Silesia and ecologically clean Augustów Wilderness region were recently undertaken in the ^{14}C Laboratory in Gliwice. In that time a series of radiocarbon concentration measurements in single tree rings and $\delta^{13}C$ in the entire organic matter were carried out. Such evaluated values of $\delta^{13}C$ are used to correct radiocarbon concentration. However they cannot be used to reconstruct climatic and environmental conditions.

1. INTRODUCTION

The development of a tree is dependent upon the climatic and environmental conditions under which it grows. Formed growth rings represent integration of these variables during their growing season. Because of that, doing an assessment of the nature of changes in the rings width, reconstruction of some aspects of past environmental changes is feasible by traditional dendrochnological techniques. These techniques give convincing results if the growth is limited by a single factor, e.g. humidity. Such method often fails if a more complex set of conditions exists. Then, parameters other than ring width are required to perform satisfactory reconstruction. These parameters can be changes of stable isotopic composition in the subsequent rings (Loader and Switsur, 1997).

Processes controlling isotopic fractionation in trees are not fully explained, yet. However, one of the widely used models proposed to explain changes in isotopic sequences of the tree rings is the model described by Francey and Ferquhar (1982). According to this model, during absorption of atmospheric CO_2 through stomata, the initial isotopic composition is modified by fractionating processes. The leaf CO_2 is then fractionated by an enzyme that works as a part of the assimilation process and

$$\delta^{13}C_{cellulose} = \delta^{13}C_{atmosphere} - a(1 - P_i/P_a) - b(P_i/P_a) \qquad (1)$$

where: a – $\delta^{13}C$ changes (of about 4.4%) during diffusion through stomata, b-change (of about 27%) caused by an enzyme working as a part of the assimilation process, P_i – internal partial pressure of CO_2 in a leaf, P_a – partial pressure of CO_2 in the atmosphere.

The sizes of stomata change with changes of factors shaping the environment (e.g. light, humidity, temperature), and with efforts made by the plant to effectively control functioning of the organism.

As it was already mentioned, analyses of stable isotopic ratios in organic matters provide a great deal of valuable information to paleoclimatology. In dendroclimatology the analyses of stable isotopic ratios in samples of the wholewood give less readable signal than some analyses in samples of α-cellulose being one of the components of a tree (Loader *et al.*, 1996). Investigations showed that isotopic composition of a tree changes significantly from year to year with changes of ratios of various tree components. It was also noticed that tree components accumulate in tree rings with different rate after the initial development of wall cells. Because of accuracy and time integration α-cellulose constitutes a material used for isotopic analyses utilised in paleoclimatic investigations. In these analyses the latewood is considered because $\delta^{13}C$ in the earlywood refers to climatic changes that revealed in the year proceeding rings growth, see **Fig. 1** (Robertson *et al.*, 1997).

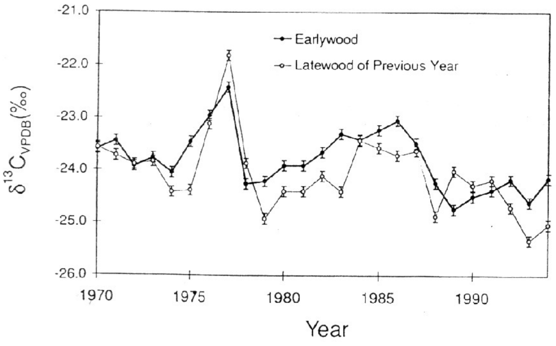


***Fig. 1.** Relationship between $\delta^{13}C$ of the earlywood and $\delta^{13}C$ of the latewood of the previous year (after Robertson et al., 1997).*

2. α-CELLULOSE EXTRACTION

The laboratory station set for wood preparation in order to extract α-cellulose is based on the Green method. This technique relies on sodium chloride oxidation. For preparation of α-cellulose from wood strokes an ultrasonic bath and small Soxhlet thimbles are employed. This allows a rapid processing of wood samples into α-cellulose and provides a material of sufficient homogenity required for palaeoclimatic reconstruction (Loader *et al.,* 1996).

The diagram of α-cellulose extraction from wood samples can be briefly presented as follows (Robertson and Waterhouse, 1996): wood after mechanical processing is subject to sodium chloride and acetic acid; to the obtained holocellulose NaOH is added then, and finally α-cellulose is obtained.

$$\text{wood} \xrightarrow[CH_3COOH]{NaClO_2} \text{holocellulose} \xrightarrow{NaOH} \alpha\text{-cellulose}$$

In practice, the following should be done:

1. Mechanical wood processing:
   The latewood of every ring is cut into very small pieces (strokes) with a hammer, chisel or scalpel, and then it is weighted.
2. Resin extraction:
   a) The sample is put into a 1000 ml beaker and 50 ml of toluene is added. The beaker is placed in an ultrasonic bath for 30 min. The toluene is removed and the process is repeated until resin removal;
   b) The Soxhlet apparatus is cleaned (**Fig.2**);
   c) At the bottom of the Soxhlet apparatus there are subsequently placed: 2.5 cm of glass wool, the sample, 2.5 cm of glass wool. Next, 400 ml of 95% ethanol is poured into a flask that is heated then;
   d) The Soxhlet apparatus is put together and the cooling water valve is open;
   e) Heating is turned on to allow ethanol evaporation;
   f) After 24 hours the heating is turned off and the sample is left to cool;
   g) 95% ethanol solution is removed;
   h) The sample is dried;
   i) If the sample is not clean enough a 500 ml beaker is filled up with 400 ml of 95% ethanol and the extraction is repeated;
   j) After 24 hours the heating is turned off and the sample is left to cool;
   k) The sample is taken off, put into a 1000 ml beaker and dried in a drier with a low heating level;
   l) 95% ethanol is removed;
   m) The Soxhlet apparatus is cleaned.
3. Rinsing of the sample in order to be cleaned after extraction:
   a) A beaker is placed on a hot plate and about 500 ml of distilled water is added; the beaker is covered with transparent glass and the sample is left to boil for 6 hours. The distilled water is added if necessary;
   b) The heating is finished and distilled water is removed. If the procedure cannot be continued the sample should be dried and saved (Kalin *et al.*, 1995).
4. Holocellulose extraction:
   a) The sample is put into a marked borosillicate Soxhlet thimble (**Fig. 3**);
   b) The thimbles are placed in a beaker filled with distilled water, sodium chloride and acetic acid, in amounts as it is presented in **Table 1**;
   c) The beaker is covered with glass and placed in an ultrasonic bath (the temperature should be about 70°C);
   d) Three times, every hour, acetic acid and sodium chloride are added;

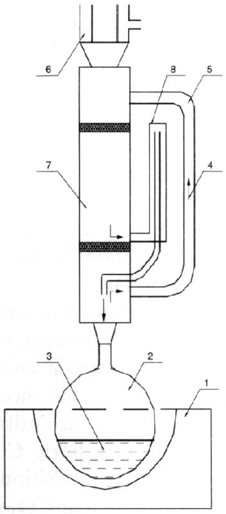


***Fig. 2.** Soxhlet apparatus: 1 – heater, 2 – flask, 3 – solvent, 4 – direction of solvent flow, 5 – arm leading to condenser, 6 – vapour condenser, 7 – place for sample, 8 – siphon.*

  - e) The thimbles are taken out and the solution is removed;
  - f) Holocelullose is rinsed, first with hot distilled water (about 50 ml) and then with cold distilled water (about 50 ml);
  - g) Its colour is checked – holocellulose should be quite white. In case of tinge the cleaning should be repeated.
5. α-cellulose extraction.
  - a) Single thimbles are put into a clean beaker and then 10% solution of NaOH is added to each of them. The beaker is placed in an ultrasonic bath for 45 min (the temperature should be about 70°C);
  - b) The thimbles are taken off, the solution of NaOH is removed and the sample is rinsed with cold distilled water (about 50 ml);
  - c) The thimbles are put into the beaker with 17% solution of NaOH and they are subject to ultrasonic for 45 min in the room temperature;
  - d) α-cellulose is rinsed, first with 17% solution of NaOH (about 20 ml), next with big amount of distilled water, then with 1% solution of hydrochloric acid (about 20 ml) and finally with big amount of cold distilled water (the solution should be neutral).
6. α-cellulose is dried (Loader *et al.,* 1996).

Table 1. *Amounts of reagents used in the extraction of α-cellulose from small sample (after Loader et al., 1996).*

Reagent	Amount of reagent
Wood shavings	1 g
Distilled water	175 ml
Sodium chlorite	2.5 g (per addition)
Acetic acid [80%]	1.7 ml (per addition)
10% sodium hydroxide	75 ml
17% sodium hydroxide	67 ml

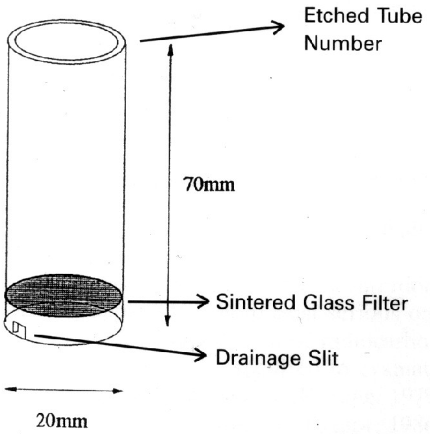
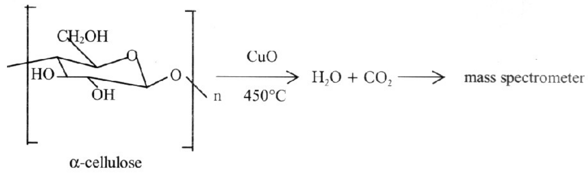


Fig. 3. *The Soxhlet thimble used in the extraction of α-cellulose (after Loader et al., 1996).*

3. PREPARATION OF CO_2 FOR $\delta^{13}C$ MEASUREMENTS

CO_2 for $\delta^{13}C$ measurements with mass spectrometer is prepared from the dried α-cellulose (Robertson and Waterhouse, 1998).

A glass tube, in which 3 mg of α-cellulose and 400 mg of preheated CuO are placed, is closed in the vacuum. Next, it is put into a metal thimble and heated in muffle furnace for 18 hours in the temperature of 450°C. Obtained CO_2 is analysed with the aid of mass spectrometer.



Results are expressed in per milles as a deviation from the PDB standards:

$$\delta^{13}C = \frac{\delta^{13}C_{(\mathrm{sample})} - \delta^{13}C_{(\mathrm{standard})}}{\delta^{13}C_{(\mathrm{standard})}} \cdot 1000\,‰. \qquad (2)$$

4. INVESTIGATIONS OF $\delta^{13}C$ IN TREE RINGS FROM BABINGLEY AND SANDRINGHAM

Changes of $\delta^{13}C$ in α-cellulose coming from the latewood are highly correlated with environmental changes in July-August (**Figs. 4** and **5**). The relation between changes of rings width and environmental changes is not so clear (Robertson *et al.,* 1997).

Better correlation between $\delta^{13}C$ and temperature changes was proved for materials coming from dry sites. For investigations carried out by Robertson and his group two places were chosen: Babingley Osier Carr and Sandringham Park. Presented results refer mainly to Sandringham because it is a dry site and thus the results are better correlated with temperature changes.

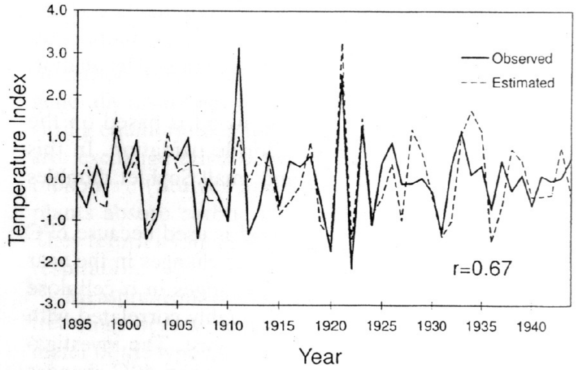


Fig. 4. *Temperature indices of July and August observed and estimated on the basis of $\delta^{13}C$ for Sandringham for 1895-1944 (after Robertson et al., 1997).*

The relation between $\delta^{13}C$ obtained for Babingley and Sandringham are depicted on **Fig. 6**.

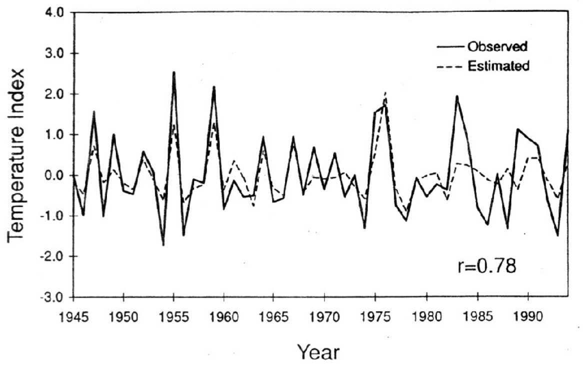


Fig. 5. *Temperature indices of July and August observed and estimated on the basis of $\delta^{13}C$ for Sandringham for 1945-1994 (after Robertson et al., 1997).*

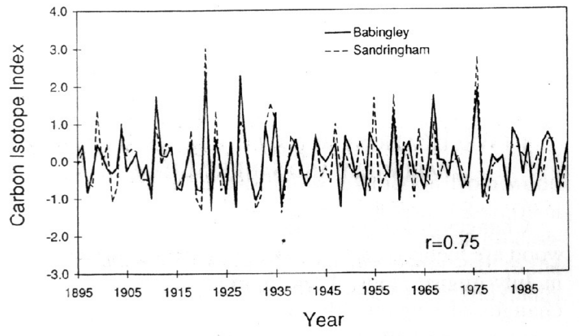


Fig. 6. *Relationship between $\delta^{13}C$ indices for Babingley and Sandringham (Robertson et al., 1997).*

5. SUMMARY

Basing on previous considerations it can be proved that there exists a strong relation between $\delta^{13}C$ changes and climatic changes, mainly temperature changes. The material suitable for $\delta^{13}C$ measurements is α-cellulose extracted from wood because it gives more readable signal than that, which can be obtained by analysis of the wholewood.

α-cellulose extraction from wood is based on the Green method of sodium chloride oxidation. In this process an ultrasonic bath and small Soxhlet thimbles are used.

For the analysis the latewood is used because $\delta^{13}C$ in the earlywood refers to climatic changes in the year proceeding rings growth. $\delta^{13}C$ changes in α-cellulose coming from the latewood are highly correlated with environmental changes in July-August. The investigations proved better correlation between $\delta^{13}C$ changes and environmental changes for wood samples acquired from trees growing in dry places.

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