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STABLE CARBON ISOTOPE ANALYSIS OF SUBFOSSIL WOOD FROM AUSTRIAN ALPS

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Abstract: The presented studies were carried out in order to check the usefulness of subfossil wood for stable isotope analysis. The aim of research was also to define the optimal method of subfossil samples preparation. Subfossil samples used during the presented studies are a part of the multi-century dendrochronological scale. This chronology originates in an area situated around a small mountain lake — Schwarzersee, in Austria.
The obtained results of stable carbon isotope measurements confirmed that the method of α-cellulose extraction by the application of acidic sodium chlorite and sodium hydroxide solutions removes resins and other mobile compounds from wood. Therefore, in the case of the analysed samples, the additional chemical process of extractives removing was found to be unnecessary. Studied wood samples contained an adequate proportion of α-cellulose similar to the values characteristic for the contemporary trees. This proved an adequate wood preservation which is essential for the conduction of isotopic research.

Keywords: stable carbon isotopes, subfossil wood, α-cellulose, extractives.

1. INTRODUCTION

The goal of this research was to test the usefulness of subfossil wood for the stable carbon isotope measurements. The study was conducted on the basis of multi-centennial dendrochronological scale which encompasses the period 1526 BC – 2008 AD. This chronology is planned to be used for the reconstruction of climatic conditions in the area of northern Alps. The article presents the first stage of this research aimed at the formulation of the most appropriate way of subfossil sample preparation.

Hitherto, stable carbon isotopic studies based on millennial long dendrochronological scales have been published rather sporadically. An application of living trees rarely allowed obtaining of multi-century carbon isotope scales. An example of a millennial long record based on living trees could be the stable carbon chronology of bristlecone pine (*Pinus longaeva* Bailey) from White Mountains in California. This chronology reaches back to 1085 AD (Epstein *et al.*, 1990 and Bale *et al.*, 2011). Another one is 1171-year long chronology established for juniper (*Juniperus turkestanica* Kom. and *Juniperus excelsa* M. Bieb.) trees in Karakorum Mountains in northern Pakistan (Treydte *et al.*, 2009). Sometimes for chronology construction the dead trees were used. Such situation took place in the case of fir (*Abies alba* Mill.)

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chronology which ranges from 1004 AD to1980 AD and originates in Black Forest in Germany (Lipp *et al.*, 1991 and Edwards *et al.*, 2000). Similarly, 1011-year chronology from Forfjord in north-western Norway was based on living and deadwood Scots pine (*Pinus sylvestris* L.) trees (Young *et al.*, 2012). Nevertheless, to extend the time range of chronologies most effectively the best way is to take advantage of the subfossil wood. Stable carbon isotope measurement of subfossil wood was performed during the 8230 BC – 905 AD bog oak (*Quercus robur* L. and *Quercus petraea* Liebl.) chronology construction in southern Germany region (Mayr *et al.*, 2003). From Germany was also derived subfossil pine (*Pinus sylvestris* L.) chronology which embraces the period 11 919 cal BP – 9900 cal BP (Becker *et al.*, 1991 and Friedrich *et al.*, 1999). Moreover, subfossil pine (*Pinus sylvestris* L.) wood was applied for 7000 BP – 3500 BP chronology development in the area of Kola Peninsula in Russia (Boettger *et al.*, 2003). In turn, historical, archaeological and subfossil wood samples served to the Irish oak (*Quercus robur* L. and *Quercus petraea* Liebl.) chronology set up. By means of these samples an exceptionally long time-scale series spanning the period of 5140 BC – 1810 AD was formed (McCormac *et al.*, 1994).

Unfortunately, an application of subfossil wood during the stable isotope research entails a problem concerning the wood preservation state. It results from the fact that with the increasing age of wood the decomposition of its organic compounds progresses (Schleser *et al.*, 1999 and van Bergen and Poole, 2002). Because sufficient content of lignin or cellulose is indispensable factor necessary to the conduction of isotopic research therefore, the first question asked in presented article was about the quantity of these elements in the analysed subfossil wood.

The second question was related to the necessity of extractives isolation. The sample extraction is a standard procedure performed during the stable isotopes measurements. The preparation process is carried out by means of different organic solvents (Cullen and Macfarlane, 2005). This chemical pre-treatment allows to remove resins, heartwood substances and other organic and inorganic ingredients from wood (Borella *et al.*, 1998 and Boettger *et al.*, 2007). The isolation procedure is commonly used because the presence of extractives disturbs isotope measurement as these compounds are enriched or depleted in ^{13}C in comparison to cellulose. Moreover, these constituents are characterised by changeable contents in wood and may be transferred between neighbouring growth rings (Harlow *et al.*, 2006). Nevertheless, the extraction process is sometimes omitted because chemical substances applied for α-cellulose separation frequently remove also resins and other mobile elements from wood, to the degree sufficient for the conduction of isotopic research. Therefore, it is recommended to check the need for extraction in the case of samples of different species, ages and localities (Rinne *et al.*, 2005).

In order to find the answers for above mentioned problems the isotopic measurements of the selected subfossil wood samples were carried out. The aim of presented research was not, however, to study the variability of stable carbon isotope within the whole range of chronology period because this analysis will be the next stage of the project to be conducted. The main purpose was rather to test the usefulness of subfossil woods from Schwarzersee for isotope measurements, to establish if analysed samples contain appropriate amount of α-cellulose and to validate the simplified sample preparation method.

2. THEORETICAL BACKGROUND

Stable carbon isotopes can be measured in the whole wood material containing lignin and cellulose as well as separately in lignin or in cellulose only. The choice between these methods is often difficult. The problem arises because both cell wall ingredients have a specific isotopic ratio (McCarroll and Loader, 2004). This is caused by the fact that the processes that lead to their formation are characterised by a different degree of fractionation. Consequently cellulose is much more enriched in ^{13}C in comparison to lignin (Leavitt and Long, 1982). Additionally, lignin and cellulose may contain the climatic information from different periods of growing season because these compounds are deposited in cell walls at a various rate and time (Loader *et al.*, 2003).

Some analyses indicate that the whole wood samples have a stronger climatic signal than isolated lignin or cellulose. The reason of the whole wood advantage is uneven distribution of photosynthates between these particular cell walls elements (Schleser *et al.*, 1999 and Savard *et al.*, 2012). The other studies suggest, however, that the best effects are obtained by application of lignin or cellulose separately. This is caused by various lignin and cellulose content which occurs within one plant within the growth rings formed in the same year and in the following years, as well as by the changes in lignin and cellulose proportion between particular trees (Helle and Schleser, 2004a). Nevertheless, in the case of subfossil woods the requirement of lignin or cellulose extraction is undisputed because in subfossil samples the mutual quantitative ratio of these cell-wall constituents is disturbed (Loader *et al.*, 2003).

The amount of lignin and cellulose in subfossil wood differ in time and depends on the sample history and conditions of wood deposition (Loader *et al.*, 2003). Therefore, for isotopic research it is essential to select one of these compounds. The common choice of cellulose results from its chemical properties, the consistency of structure and a single biosynthetic pathway. Cellulose is an immobile substance, and its partial decay does not change its isotopic composition significantly. It also possesses a stronger climatic signal in comparison with lignin. In turn, lignin biosynthesis relies on secondary cell

metabolites derived from complex processes. Moreover, lignin is transformed while its decomposition progresses and this leads to considerable fluctuations in its isotopic content (Schleser *et al.*, 1999 and Harlow *et al.*, 2006). On the other hand, a disadvantageous factor for conducting the isotope research on cellulose is the faster rate of its decay which results in the depletion in this organic compound in subfossil wood (Loader *et al.*, 2003 and Sass-Klaassen *et al.*, 2005).

The degree of cellulose destruction is connected with the age of wood and even more strongly associated with the place of wood origin (Eriksson *et al.*, 1990 and Passialis, 1997). Hence wood of equal age but of different genesis could be characterised by various lignin and cellulose proportion. The best state of preservation usually characterises the wood from the localities where the process of microbiological decomposition had been substantially slowed down or entirely inhibited. Such delay in wood biodegradation could result from decreased oxygen availability which occurs in the case of submerged or buried wood, or might be an effect of very low humidity of environment, or a consequence of extremely high or low temperature influence (Fengel, 1991 and Kim and Singh, 2000).

Subfossil samples used in the presented research originate from the Schwarzersee lake. Accordingly, an aquatic environment secured a good state of wood preservation. Limited oxygen access slowed down the development of aerobic bacteria and fungi that, under usual open-air conditions, lead to fast wood decomposition. However, the degradation of the material was not completely The wood composition was changed due to its penetration and accumulation of mineral substances and organic matter dissolved in the lake water. Wood destruction was caused also by the activity of anaerobic microorganisms. This fact has a crucial significance since it has been proven that the stable isotope ratios could be disturbed in cellulose derived from strongly altered subfossil wood (Savard *et al.*, 2012). For this reason during presented research only samples characterised by good preservation state were used. These samples had no macroscopically visible indications of changes and no damages of anatomical structure of wood.

The second problem concerning appropriate sampling procedure and involving also subfossil woods results from the fact that for stable carbon isotope analysis the whole ring, earlywood or latewood could be selected. This choice has an important practical meaning owing to the difference in carbon isotope composition that exists between particular growth ring zones. Observed variation in carbon isotope ratio depends largely on amount of reserve substances which have been used for wood formation. Stored substances are significantly enriched in ^{13}C during their production processes. Moreover, if these compounds come from previous year, they have the isotopic record from the earlier vegetation period. Therefore, the larger amount of reserves is utilised, the higher is the ^{13}C wood enrichment that results from biochemical factors, unrelated to the current climatic conditions. The amount of nutrient reserves varies during the year and demonstrates the highest level in the spring. Because of that, for isotopic studies the application of latewood exclusively is often recommended. This is frequently practiced in spite of the fact that the general isotopic pattern of particular ring zones is very similar (Jäggi *et al.*, 2002 and Helle and Schleser, 2004b). On the other hand, at marginal sites with short growing season as material for conifer ring formation serve predominantly the photosynthates from the current year. It is caused by high turnover rates and small reserve pools at tree-line locations. Therefore, in this case the separation of latewood is not required or could be even disadvantageous factor because using the whole ring may provide some analytical benefits and consequently may improve climate calibrations (Kress *et al.*, 2009).

During Schwarzersee chronology construction the sampling was done almost at the timberline. Hence for measurements the material from whole growth ring was selected. This decision was also motivated by small size of growth rings and difficulties with an accurate early and latewood separation.

3. MATERIAL AND METHOD

The studied wood is a part of a multi-century dendrochronological scale which comes from small area situated around a mountain lake — Schwarzersee (47°31'N, 13°49'E, 1450 m a.s.l.). This lake lies in the region of the Dachstein Mountains which belong to the Limestone Alps (**Fig. 1**). Subfossil wood used for chronology construction was collected in the form of stem disks from the trunks deposited on the bottom of Schwarzersee. These trunks originated from the trees growing on the nearby mountain slopes. Also the present-day wood was sampled from the trees growing around the lake (Grabner *et al.*, 2006). The whole chronology material contains the wood from spruce (*Picea abies* (L.) Karst.) and larch (*Larix*

Fig. 1. *Map of Austria showing the location of Schwarzersee.*

decidua Mill.) trees. However, because of differences in isotopic composition and resin content that occur between various tree species for the analysis solely the spruce wood was applied.

The samples of various age representing six periods at more or less regular intervals within the chronology range were chosen. In order to ensure the appropriate amount of measuring material, 10 sequential samples, composed from four consecutive rings (in equal proportion), were taken from 10 pieces of wood. Each of these sequential samples was divided into two equal parts. As a result these parts constituted the subsamples composed of the same rings. Altogether 20 subsamples were prepared in this way. The age of particular growth rings was determined during previous dendrochronological research and encompassed the time period from the year 790 BC to the year 1956 AD. Selected samples were precisely weighed and cut with a knife into very small pieces, approximately 0.5 millimetres thick. The aim of this fragmentation was the maximisation of an active surface that is in contact with chemical reagents.

Thereafter, one subsample from every piece of wood was chosen for the processing in Soxhlet extractor according to the protocol described by Sheppard and Thompson (2000). The second subsample was used as reference material. As a result, the treated and untreated wood had equal age and they originated from the same rings. The applied extraction procedure was threefold. In the first stage the samples were treated with toluene and 99.8% ethanol solution (1:1 ratio) for the period of 4 hours. In the second step the samples were processed in 99.8% ethanol, also for 4 hours. Finally, the samples were washed in deionised water for 1 hour. After the removal from the extractor the woods were rinsed several times in boiling deionised water and then dried in the temperature of 70°C for 2 hours. Then the samples were weighed again.

Subsequently, 10 samples prepared in this way and the remaining 10 not extracted samples were placed in separate tubes. The procedure of α-cellulose isolation was conducted in accordance with the modified Green (1963) protocol and with the application of ultrasonic bath technique (Pawełczyk *et al.*, 2004 and Pawełczyk and Pazdur, 2004). Ultrasonic treatment promotes a rapid and more complete penetration of the reagents into the wood tissue and assist greatly in the disaggregation of the individual cellulose fibres (Loader *et al.*, 1997). This method allows obtaining α-cellulose of the quality that meets the requirements of isotopic studies (Cullen and MacFarlane, 2005). The procedure of α-cellulose separation is described in the **Table 1**.

For the stable carbon isotope measurements three subsamples from each sample of α-cellulose were prepared. They were precisely weighted (*ca.* 60 μg) and placed in tin capsules. Thereafter samples were combusted "online" at 1020°C using the elemental analyser (EuroVector) in Gliwice Mass Spectrometry Laboratory. Isotopic composition of α-cellulose was measured by the application of IsoPrime EA-CF-IRMS continuous flow isotope ratio mass spectrometer. During measurements the mass spectrometer was directly connected to the elemental analyser. The precision of these methods was 0.1‰.

Obtained carbon isotope measurements were calibrated to VPDB standard and were expressed in the isotope delta (δ) notation. Following tradition, $\delta^{13}C$ were presented in units of part per thousand and communicated in per mil shown as ‰ (Brand and Cuplen, 2012).

4. RESULTS AND DISCUSSION

The first stage of the presented research was the procedure of resin extraction from a solid wood material by means of organic solvents. This study was aimed at checking the necessity of extractives isolation. For this purpose one part of each sample was subjected to the pretreatment in the Soxhlet apparatus. All samples, pretreated and no pre-treated in the Soxhlet extractor, were subjected to the cellulose extraction procedure next. Measurements of $\delta^{13}C$ (**Table 2**, **Fig. 2**) obtained for the samples processed and not processed in the Soxhlet apparatus were compared and then checked if there was no significant difference between them.

Values of $\delta^{13}C$ of wood samples processed in the Soxhlet apparatus were very similar to values of unprocessed samples, and no directional systematic changes between them were observed. The results of measurements received for the samples subjected to the resin extraction were sometimes higher and sometimes lower when compared to the unextracted ones. Such situation was noticed in the whole analysed period. The mean values of $\delta^{13}C$ calculated separately for prepared and unprepared samples were equal to –21.462‰ and –21.421‰ respectively.

Table 1. *The procedure of α-cellulose isolation (intermediate products of α-cellulose extraction according to Sensuła* et al., *2011).*

Preparation stage	Chemicals for 1 g of wood	Products	Substances removed
1	sodium chlorite — 2.5 g × 7 acetic acid 80% — 1.7 ml × 7 distilled water — 175 ml	holocellulose	lignin
2a	sodium hydroxide 10% — 75 ml	α-cellulose	hemicellulose: mannose, arabinose, galactose, xylose, rhamnose
2b	sodium hydroxide 17% — 67 ml		

Table 2. *Results of stable carbon isotope measurements and percentage cellulose content in wood. Sample signatures A, B or C are related to different radii of the same stem disk distinguished during dendrochronological measurements.*

Sample	Years	Soxhlet extraction	$\delta^{13}C$ (‰)	Wood weight (mg)	Cellulose weight (mg)	Cellulose content (%)
73B	790–787 BC	yes	–23.26	56.76	7.11	12.53
73B	790–787 BC	no	–23.44	56.82	3.98	7.00
101A	790–787 BC	yes	–22.89	60.67	16.21	26.72
101A	790–787 BC	no	–22.90	60.58	16.83	27.78
104B	243–240 BC	yes	–21.60	88.01	15.43	17.53
104B	243–240 BC	no	–21.77	88.28	22.96	26.01
12C	148–151 AD	yes	–23.21	101.11	41.07	40.62
12C	148–151 AD	no	–23.08	101.04	35.85	35.48
28A	1056–1059 AD	yes	–20.32	101.25	25.57	25.25
28A	1056–1059 AD	no	–20.38	101.49	27.34	26.94
43A	1056–1059 AD	yes	–17.63	102.00	20.42	20.02
43A	1056–1059 AD	no	–17.49	102.30	36.33	35.51
117B	1606–1609 AD	yes	–24.46	92.58	31.02	33.51
117B	1606–1609 AD	no	–24.46	91.62	31.19	34.04
121A	1606–1609 AD	yes	–18.73	52.80	15.50	29.36
121A	1606–1609 AD	no	–18.34	52.43	14.79	28.21
159A	1953–1956 AD	yes	–21.47	57.67	21.68	37.59
159A	1953–1956 AD	no	–21.38	58.87	22.67	38.51
187A	1953–1956 AD	yes	–21.06	102.59	40.33	39.31
187A	1953–1956 AD	no	–20.98	102.81	37.70	36.67

With the aim of precise comparison of results obtained for these two groups of samples some statistics were applied. At first, the absolute difference between-mean values (Δx) was calculated according to the following equation:

$$\Delta x = |x_i - x_j| \tag{4.1}$$

where:
x_i, x_j are mean values measured respectively for sample pre-treated and not pre-treated in Soxhlet extractor.

Next, the uncertainties of measurement values were counted and expressed as standard deviations ($u(x_i)$, $u(x_j)$). The uncertainty of Δx was calculated according to:

$$u(\Delta x) = \sqrt{u(x_i)^2 + u(x_j)^2} \tag{4.2}$$

The expanded uncertainty $U(\Delta x)$ was obtained by multiplying $u(\Delta x)$ by a coverage factor (k) equal to 2. It providing a level of confidence of approximately 95%.

$$U(\Delta x) = 2 \times u(\Delta x) \tag{4.3}$$

If:

$$\Delta x \leq U(\Delta x) \tag{4.4}$$

Results are presented in the **Table 3**. On the basis of expanded uncertainty calculations it can be concluded that there is no significant difference between the measurements obtained for pairs of pre-treated and not pre-treated samples.

Subsequently, a paired-samples t-test was conducted to evaluate the impact of resin extraction on $\delta^{13}C$ values. This coefficient allowed studying the differences in means between prepared and unprepared samples. Anoth-

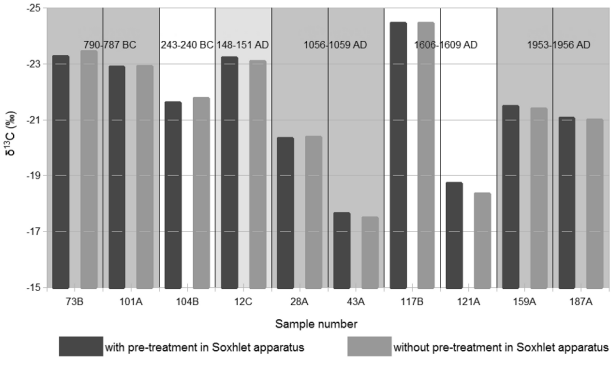

Fig. 2. *Comparison of $\delta^{13}C$ values obtained for the samples subjected to an extraction in Soxhlet's apparatus with unprepared woods.*

Table 3. *Comparison between $\delta^{13}C$ for samples with pre-treatment and without it. In all cases it can be observed that Δx < U(Δx).*

Sample	Δx	U(Δx)	Comments
101A	0.010	0.402	no significant difference
73B	0.175	0.318	no significant difference
104B	0.129	0.323	no significant difference
12C	0.173	0.324	no significant difference
28A	0.061	0.230	no significant difference
43A	0.143	0.256	no significant difference
117B	0.002	0.287	no significant difference
121A	0.385	0.637	no significant difference
159A	0.085	0.150	no significant difference

er statistical method applied for checking the variations between these two groups of samples was the analysis of variance (ANOVA). Both t-test and analysis of variance procedures are carried out by means of testing the null hypothesis and the alternative hypothesis. The null hypothesis asserts that there is no difference between the population groups, and that any observed variation is due to chance alone.

For t-test statistics if t Stat < –t Critical two-tail or t Stat > t Critical two-tail, the null hypothesis can be rejected. This is not the case, because –2.262 < –0.781 < 2.262 (**Table 4**). Therefore, the null hypothesis is not rejected. It means that observed difference between the sample means (–21.462‰ and –21.421‰) is not convincing enough to say that $\delta^{13}C$ of pre-treated and not pre-treated samples differ significantly.

In turn, ANOVA calculated F statistics was smaller than F crit (0.002 < 4.414), and p value was greater than accepted threshold (0.967 > 0.05). Therefore, it is failed to reject the null hypothesis. Obtained results point that there is a 96.7% chance that differences don't occur between studied groups (**Table 5**). Analysis of variance confirmed that no significant divergences exist between the mean values $\delta^{13}C$ of pre-treated and not pre-treated samples. Moreover, it was observed that variance was larger within analysed groups than between these groups of measurements. This influenced also the values of repeatability coefficient. The obtained value of repeatability was negative because the F ratio was less than unity. The repeatability calculated for the $\delta^{13}C$ amounted to –99.6.

As an effect of the conducted research, it can be assumed that analysed wood samples do not require the process of resin extraction by organic solvents because no significant differences occur between prepared and unprepared wood. This most probably result from small amount of extractives contained in analysed wood. An important factor which influences extractives preservation in the case of studied samples could be their origin from the Schwarzenegger lake. This could play a decisive role because waterlogged wood is characterised by an abnormally low resin content. Resinous substances are degraded in water environment. Decomposition is caused by the action of microorganisms and is a consequence of chemical dissolution by water (Kim, 1990 and Pan *et al.*, 1990).

The same conclusions were obtained also on the basis of the previous growth ring density measurements, performed for the spruce wood from the Schwarzersee lake. In the case of X-ray densitometry, similarly as during the analysis of stable isotopes, the wood is commonly extracted with the application of alcohol or acetone solvents (Schweingruber *et al.*, 1978). Such sample pre-treatment is used for the disposal of all organic substances that are not the constituents of wood cell walls. These extractives should be removed because their presence leads to an overestimation of the wood density (Grabner *et al.*, 2005). However, conducted densitometric research demonstrated that wood preparation procedure is needless due to insignificant content of resins included in the studied This suggests that small amount of extractives is a distinctive feature of analysed wood.

On the other hand, the method of α-cellulose separation by means of acidic sodium chlorite and sodium hydroxide solutions was proven be able to remove resins and other mobile compounds from wood, to the degree sufficient for the conduction of isotopic research (Rinne *et al.*, 2005). Because during chemical pre-treatment of subfossil samples from Schwarzersee the same chemical substances were applied therefore, it could be stated that it is not necessary to use any organic reagents for additional sample preparation. The extractives if occur could be easily removed during α-cellulose isolation process.

In spite of the lack of any significant differences between subsamples originated from one tree the $\delta^{13}C$ values obtained for the whole analysed material were marked by a wide range of variability observed between particular trees. Therefore, the analysis of variance was also performed for $\delta^{13}C$ values of equally-dated samples. For this purpose all $\delta^{13}C$ values received for particular time periods were averaged together. Analysis demon-

Table 4. *Results of paired two-sample t-test conducted for samples with pre-treatment in Soxhlet extractor and without it.*

	$\delta^{13}C$ for samples with pre-teatment	$\delta^{13}C$ for samples without pre-treatment
Mean	–21.462	–21.421
Variance	4.550	4.951
Observations	10	10
Hypothesized Mean Difference	0	
df	9	
t-Stat	–0.781	
P(T ≤ t) one-tail	0.227	
T critical one-tail	1.833	
P(T ≤ t) two-tail	0.455	
T critical two-tail	2.262	

Table 5. *Analysis of variance (ANOVA output from Microsoft Excel) conducted for samples with pre-treatment in Soxhlet extractor and without it.*

Groups	Count	Sum	Average	Variance
$\delta^{13}C$ for pre-treated samples	10	–214.623	–21.462	4.550
$\delta^{13}C$ for not pre-treated samples	10	–214.214	–21.421	4.951

Source of variations	SS	df	MS	F	P value	F_{Crit}
Among groups	0.008	1	0.008	0.002	0.967	4.414
Within group	85.508	18	4.750			
Total	85.517	19				

SS — sum of squares; df — degrees of freedom; MS — mean square; F — F ratio statistic; F_{Crit} — F ratio statistic for significance at 5% level of confidence.

strated that variance differs considerably between samples of various ages. The largest divergence existed for samples representing the period 1606–1609 AD and the smallest for samples representing the period 1953–1956 AD (**Table 6**). Additionally, $\delta^{13}C$ values of equally-dated samples were compared on the basis of expanded uncertainty calculations. The obtained results allow concluding that between samples belonging to equally-dated wood significant variation occurs (**Table 7**).

Presented measurements indicate also that couples of equally-dated samples differ widely from each other (**Fig. 2**). Observed variations between two trees of the same age could result, among other things, from decomposition of subfossil wood. In spite of the fact that some authors exclude the possibility of carbon fractionation during partial decay of subfossil samples (Loader *et al.*, 2003), there are also some evidences that this process may occur during aqueous bacterial degradation of wood. It involves especially the oxygen isotopes (Sass-Klaassen *et al.*, 2005 and Savard *et al.*, 2012). Nevertheless, the appearance of changes in isotopic composition is characteristic of highly altered wood. Usually, when the wood anatomical structure is intact or even slightly altered, the isotopic signal of α-cellulose can be considered reliable (Schleser *et al.*, 1999, van Bergen and Poole, 2002 and Savard *et al.*, 2012). As previously mentioned, for the presented research only samples characterised by good preservation state were selected. Therefore observed variations in $\delta^{13}C$ values should be rather attributed to metabolic specimen-specific phenomena. However, for further research an important issue is to choose well preserved samples without any visible signs of wood structure disintegration.

The second problem considered during the presented research was related to the cellulose content in the analysed subfossil wood. Obtained results proved that analysed samples contain a proper amount of α-cellulose which allowed the conduction of isotopic research. Received values of α-cellulose proportion varied from 20.20% to 40.62% for prevailing number of samples (**Table 2**, **Fig. 3**). Only one specimen that is 73B demonstrated much smaller quantity of α-cellulose which was equal to 12.53% and 7.00% respectively for prepared and unprepared wood. Although any structural change in the anatomy was not macroscopically observed, the low extraction efficiency was undoubtedly connected with advanced age of this wood and with its decomposition through natural diagenetic processes. Such low values of α-cellulose content are characteristic of highly degraded wood that resided on lake floors for a prolonged time (Savard *et al.*, 2012). Nonetheless, it could be assumed that the rest of the analysed samples was in pretty good condition.

Taking into account previously mentioned values it could also be stated that α-cellulose content differs considerably among analysed samples. It results, to the largest extent, from the applied technique of α-cellulose isolation and is a consequence of material loss which occurs during rinsing and pipetting procedures. This conclusion could be proved by similar efficiency of α-cellulose extraction which is usually obtained for contemporary wood in Gliwice Laboratory. Simultaneously, stable carbon isotope ratios received during presented research do not vary from the values characteristic for the living trees. For analysed samples $\delta^{13}C$ remained within the range from –17.49‰ to –24.46‰. Therefore, it could be accepted that these measurement data demonstrate the usefulness of the wood from Schwarzersee for isotopic research.

Table 6. *ANOVA output from Microsoft Excel for $\delta^{13}C$ values of equally-dated samples.*

Groups	Count	Sum	Average	Variance
$\delta^{13}C$ for 790–787 BC	2	–46.240	–23.120	0.104
$\delta^{13}C$ for 1056–1059 AD	2	–37.908	–18.954	3.881
$\delta^{13}C$ for 1606–1609 AD	2	–42.995	–21.498	17.555
$\delta^{13}C$ for 1953–1956 AD	2	–42.445	–21.222	0.082

Table 7. *Comparison between $\delta^{13}C$ for equally-dated samples. In all cases it can be observed. $\Delta x > U(\Delta x)$.*

Period	Δx	$U(\Delta x)$	Comments
790–787 BC	0.507	0.209	significant difference
1056–1059 AD	2.704	0.058	significant difference
1606–1609 AD	6.041	0.270	significant difference
1953–1956 AD	0.401	0.089	significant difference

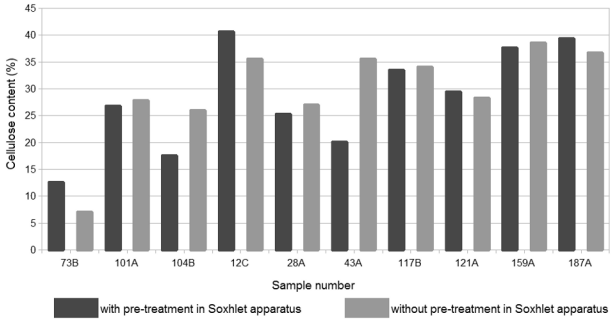

Fig. 3. *Comparison of α-cellulose content between analysed samples.*

5. CONCLUSION

This article presents the effects of stable carbon isotope measurements carried out with application of subfossil wood from Schwarzersee lake. The obtained results helped to define the optimal method of sample preparation which will be used during next stages of research. The planned measurements will encompass multi-century period and will be performed for whole range of chronology from Schwarzersee. Therefore the formulation of a suitable method for wood preparation is crucial for the quality of further research as well as for practical reasons, because the proposed exclusion of resin extraction procedure will allow a significant decrease of costs and a reduction in time of the planned research.

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