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X-RAY DENSITOMETRY OF NORWAY SPRUCE SUBFOSSIL WOOD FROM THE AUSTRIAN ALPS

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ABSTRACT

The processing of subfossil wood poses some difficulties in densitometric research. Problems arise because of the physiochemical changes of wood occurring in the sedimentation environment. Subfossil wood modification can result from the uptake of mineral and organic substances into the wood tissue. It can also occur as the effect of microbiological degradation of wood. The goal of this study was to identify the appropriate method of subfossil wood preparation for the densitometric research. For this purpose the wood of Norway spruce from Lake Schwarzensee was subjected to extraction in de-ionized water, acetone and diluted acetic acid. The application of acetic acid did not significantly influence the density of the wood and acetone seemed to be too aggressive. The best result was obtained by rinsing the samples in cold de-ionized water. This extraction procedure allowed removal of unwanted water-soluble, organic and inorganic compounds from wood and simultaneously did not lead to the degradation of subfossil samples.

ZUSAMMENFASSUNG

Die radiodensitometrische Bearbeitung von sub-fossilem Holz wirft einige Fragen auf. Die Aufnahme von organischen und mineralischen Substanzen während der Wasserlagerung führt zu physikalisch-chemischen Veränderungen des Holzes. Mikrobieller Abbau ist die Ursache weiterer Modifikation des Holzes. Ziel der Untersuchung ist die Auswahl einer optimalen Vorbehandlung (Extraktion) des sub-fossilen Holzes. Für diesen Zweck das Fichtenholz vom Schwarzensee wurde mit deionisertem Wasser, Aceton und verdünnter Essigsäure extrahiert. Die verdünnte Essigsäure veränderte die Dichte des Holzes nur marginal. Jedoch gab es nach der Aceton-Extraktion Veränderungen der Dichte. Das beste Ergebnis wurde mit der Reinigung mit deionisertem Wasser erzielt. Hierbei war es möglich sowohl lößliche organische als auch lößliche anorganische Stoffe zu extrahieren ohne die Dichte des sub-fossilen Holzes zu verändern.

Keywords: tree-ring densitometry, wood density, sample extraction, Norway spruce, subfossil wood.

INTRODUCTION

The aim of our research was to establish the best method of subfossil wood preparation for densitometric research. During densitometric studies wood is usually subjected to the standard extraction processes by means of organic solvents (Schweingruber *et al.* 1978; Singleton *et al.* 2003). In the dendrochronological laboratory at BOKU University, the samples are ordinarily rinsed in acetone for 24 hours at 20°C. This procedure is followed by seven days water extraction at 60°C. However, this technique was developed for living trees. Therefore, their application could cause some problems as physio-chemical characteristics of subfossil wood differ considerably from the living ones.

Subfossil wood is of high interest because it provides the possibility of building multi-centennial dendrochronological time-scales. Nevertheless, only a small number of long densitometric chronologies have been published so far. Among them are three density chronologies from the Alps. These dendrochronological time-scales were constructed on the basis of archaeological and historical wood. The first of them is the Lauenen chronology from

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Switzerland, which reaches back to AD 982 (Schweingruber *et al.* 1988). The second one, also of Swiss origin, spans the period AD 755–2004 (Büntgen *et al.* 2006). Finally, the third of them from Tyrol in Austria encompasses AD 1053–2003 (Esper *et al.* 2007).

Outside of the Alps, a millennium-long densitometric chronology comes from the Columbia Icefield in Canada. This dendrochronological time-series extends from AD 950 to 1994 (Luckman *et al.* 1997; Luckman and Wilson 2005). From the subarctic region of Canada, a 1300-year-long densitometric chronology was developed (Wang *et al.* 2001). Relatively close to the Canadian chronologies, namely in Arctic Alaska territory, an MXD (maximum wood density) chronology reaching back to AD 1073–2002 was constructed (Anchukaitis *et al.* 2013). Another multi-centennial timescale, dated to AD 924–2005, derives from the Pyrenees Mountains (Büntgen *et al.* 2010). In turn, a densitometric chronology spanning the years AD 778–2006 from Siberian Polar Urals region was published (Briffa *et al.* 1995; Briffa *et al.* 2013). Yet another chronology, covering the period AD 481–2004, was described from northern Sweden. This chronology was developed from wood excavated from Lake Torneträsk (Briffa *et al.* 1990; Grudd 2008). Subfossil wood of lake origin was also used for the construction of multi-century chronology from Finland. This chronology ranges from AD 673 to 1788 (Helama *et al.* 2008, 2010).

These densitometric records were often analyzed as a component of much larger dendrochronological networks because mutual matching of multi-century-long density chronologies allows assessment of large-scale climate signals and helps to develop temperature reconstruction over millennia (Briffa *et al.* 2002a, 2002b; Collins *et al.* 2002; Frank and Esper 2005; Schneider *et al.* 2015). The crucial issue in the case of densitometric research is however, the proper chemical pretreatment of wood for the construction of chronologies. In general, subfossil samples in the above-mentioned records were exposed to standard extraction procedures. However, in some instances the process of resin removal was most probably omitted.

The presented research focuses on the extraction of organic and inorganic components from subfossil wood of Norway spruce (*Picea abies* (L.) Karst.). Subfossil samples used in this study come from Lake Schwarzensee, an environment that helped conserve wood in relatively unchanged form. Deposition of wood in the water environment significantly limited biodegradation processes and provided conditions that allowed the preservation of stems in a subfossil state. Despite this fact, physico-chemical properties of wood changed noticeably over the time of submergence. The chemical profile of the lake was an important factor that influenced ongoing wood modifications. Schwarzensee is rich in organic matter and abounds with calcium carbonate from dissolved bedrock (Sommaruga *et al.* 1999; Dokulil 2005). Prolonged penetration of these mineral and organic compounds into the plant tissue may thus cause an increase of wood density. On the other hand, the activity of microorganisms contributes to wood decay, which led to density reduction of wood. Consequently, chemical composition and physical properties of the analyzed samples differ considerably from the modern ones (Buurman 1972; Fengel 1991; Passialis 1997). This generated problems with sample preparation during densitometric research.

THEORETICAL BACKGROUND

X-ray densitometry is a well-known method frequently applied in dendroclimatology and wood quality studies (Lenz *et al.* 1976; Rozenberg *et al.* 1999; Briffa *et al.* 2001). The standard pretreatment procedure in densitometry is sample extraction with organic solvents, like ethyl alcohol, benzene or acetone. Extraction is an important process because the wood tissue comprises not only lignin, cellulose and hemicelluloses which are building materials for cell walls, but it also contains other natural chemical components. These include resins, heartwood substances and other organic and inorganic extracellular compounds (Lewin and Goldstein 1991). They are non-structural and secondary elements of wood, and their presence leads to an overestimation of the wood density during densitometric research (Grabner *et al.* 2005a). Moreover, these constituents are characterized by their changeable content in wood and may be transferred between neighboring growth rings. As

a result, they are rather unevenly distributed along the radius of a tree (Harlow *et al.* 2006). Therefore, extractives must be removed. The problem in the application to subfossil wood arises because the usual techniques of wood pretreatment were developed for living trees (Polge 1970; Lenz *et al.* 1976; Schweingruber *et al.* 1978).

The state of subfossil wood preservation is determined mainly by the conditions of environment where deposition took place. The intensity of wood degradation on one hand, and the degree of external substances' accumulation on the other, depend on many factors. In the case of aquatic environment these are: water depth, chemistry, redox potential, pH, temperature, oxygen concentration, content of organic and inorganic matter and sedimentation time. Thus, the process of wood modification is rather site-specific and to a lesser extent depends on fossilization period (Guyette and Stambaugh 2003).

The waterlogged environment may allow wood preservation over many centuries or even thousands of years because it significantly slows down wood biological decay. The limiting factor for microorganism development is, in this case, a reduced oxygen abundance. Nevertheless, the process of wood decomposition is not completely inhibited here (Fengel 1991). The state of wood preservation depends mainly on the action of erosion and tunneling bacteria and, to some extent, on the soft rot fungi activity. All these microbes produce enzymes that break down the wood cell walls in the aquatic environment (Eriksson *et al.* 1990).

Erosion bacteria are the most common type of waterlogged wood degrading organisms. They attack whole wood tissue whereas soft rot fungi and tunnelling bacteria tend to focus on the outer layers of wood. This results from lower oxygen level within the inner zones of wood. Erosion bacteria operate efficiently under these conditions whereas soft rot fungi and tunnelling bacteria require higher oxygen concentration (Björdal *et al.* 1999).

Erosion bacteria destroy secondary cell-wall layers and deplete cellulose and hemicellulose content in wood. Wall material that survives these processes consists of residual modified lignin and middle lamellae compound. In turn, tunnelling bacteria can metabolize all cell wall components. However, their impact on lignin is not extensive. Tunnelling bacteria start attack from the S3 layer and proceed deeper into the cell wall, forming a tunnel within the S2 and S1 layers and within middle lamellae. On the other hand, soft rot fungi most intensively decompose the secondary cell wall, but the middle lamella is intact or lightly damaged. Soft rot considerably reduces carbohydrate content. Consequently, lignin concentration increases in residual wood. Moreover, the remaining lignin is modified only to a small degree (Blanchette 2000; Powell *et al.* 2001).

Accordingly, in an aqueous environment, hemicellulose disintegration is most advanced, followed by cellulose and finally lignin decay. However, alteration in lignin structure and weak lignin destruction frequently occur. Moreover, preferential degradation of the S2 layer and the presence of a relatively unchanged middle lamella are typical features of waterlogged wood (Kim and Singh 2000). Additionally, because cell-wall integrity is disturbed by the cellulose depolymerization, the remaining cell walls are porous and marked by very low strength (Blanchette 2000).

Microbiological decomposition of wood enhances the accumulation of salts and other organic and inorganic ingredients in subfossil wood. Furthermore, it allows better wood penetration by solutions and chemical compounds. Biochemical disintegration enlarges the active surface area of wood and thus facilitates the precipitation of minerals. During mineral deposition the role of crystallization centers is fulfilled by free chemical bonds that exist within cellular walls. They form at an early stage of the wood biodegradation process (Buurman 1972). Therefore, waterlogged wood is often characterized by an increased concentration of inorganic elements. However, this relative enrichment could also be attributed to the original mineral content that had not been removed during microbiological decay and only to some extent can be related to the infiltration of external substances into the wood tissue (Passialis 1997).

Because the preservation state of subfossil wood depends on many diverse factors, the optimal pretreatment method for densitometric research should be determined separately for each specific material and site. During the selection of reagents

for this study, the chemical composition of water from Lake Schwarzensee as well as the degree of subfossil wood destruction were taken into account. On this basis it was decided to test hot and cold de-ionized water, acetone and acetic acid during the extraction process. Acetone was used in order to dispose of the substances removable by organic solvents. De-ionized water removes water-soluble components from wood, whereas acetic acid was aimed at calcium carbonate dissolution. Calcium carbonate was expected to be found in the wood because this mineral is abundant in Lake Schwarzensee.

MATERIALS AND METHODS

The studied wood is a part of a multi-century dendrochronological time-scale that ranges from 1526 BC to AD 2008 and comes from a small area situated around a mountain lake Schwarzensee (47°31′N, 13°49′E, 1450 m a.s.l.) (Grabner *et al.* 2006). Schwarzensee is located in the region of the Dachstein Mountains, within the northern Limestone Alps in Austria (Figure 1). On the bottom of this small lake, numerous trunks were deposited over hundreds of years. They originated from the trees that had grown on the surrounding hills. These trees were felled by the impact of snow or wind, then slid down the steep slopes and finally sank into the lake waters. Besides the subfossil trees, the Schwarzensee chronology consists of the wood collected from living trees. This recent wood was sampled from the trees growing around the lake (Grabner *et al.* 2006).

Figure 1. Map of Austria showing the location of Lake Schwarzensee.

The wood of various age was designated for the purposes of analysis (Table 1). In the first stage of research, ten samples of Norway spruce (*Picea abies* (L.) Karst.) were chosen. Their age had been determined during previous dendrochronological studies and was in the range of AD 631 to 2003. Thin strips were cut out from this wood using a double-bladed circular saw (Schweingruber *et al.* 1978). These strips were *ca.* 1.2-mm thick and their tangential width was about 10 mm. In turn, their size in radial direction depended on sample distance from pith to bark.

The samples were then subjected to an acclimatization process in order to achieve uniform moisture content of the wood. The moisture of cell walls should be kept at a constant level because its variability could falsify the density results (Echols 1973; Lenz *et al.* 1976). The conditioning was performed over a 48-hour period in a room with constant temperature of 20°C and relative air humidity of 65%. The described treatment should ensure stable moisture of the fibers at a 12% level, which is required for densitometric research (Schweingruber *et al.* 1978). However, different age and various states of preservation could possibly influence the equilibrium water content of wood (García Esteban *et al.* 2006). Therefore, in spite of these efforts, the final humidity could slightly differ between analyzed samples.

Before any extraction, X-ray photos of the samples were taken. This enabled further comparison of wood density changes that occurred as the result of successive reagent application. For the X-ray procedure, the wood strips were placed on films together with the calibration wedges. During analyses, AGFA D3 SC NIF STRUCTURIX Industrial X-ray films were used. The exposure was performed from a distance of 2.5 m, during a 25-minute period, with the accelerating potential difference of 24 kV and intensity of 10 mA, with application of Seifert ISO-DEBYEFLEX 3003 apparatus (Grabner *et al.* 2005a, 2005b).

After the first irradiation step, the same samples were prepared in hot de-ionized water, acetone and acetic acid. The rinsing with hot de-ionized water was conducted for 24 hours at a temperature of

Table 1. Characteristics of the samples from Lake Schwarzensee used during densitometric research.

Sample	First year [AD]	Last year [AD]	Number of rings	Mean ring [mm]	Max ring [mm]	Standard deviation	Auto-correlation	Mean sensitivity
13A	1480	1617	138	0.69	2.08	0.446	0.963	0.140
15A	1303	1399	97	1.23	2.74	0.456	0.862	0.169
18B	1461	1643	183	0.63	1.68	0.346	0.957	0.139
21A	926	1143	218	0.90	1.94	0.246	0.719	0.156
21B	962	1124	163	0.81	1.31	0.192	0.711	0.148
26A	1015	1262	248	1.07	1.69	0.222	0.640	0.145
26B	1014	1175	162	1.06	1.91	0.341	0.822	0.166
27A	1797	1876	80	1.38	2.82	0.572	0.852	0.176
34A	809	1080	272	1.07	2.67	0.422	0.853	0.171
43A	1036	1370	335	0.69	1.36	0.216	0.773	0.174
45A	852	1144	293	0.67	1.63	0.252	0.813	0.191
46A	1003	1264	262	0.72	1.56	0.183	0.642	0.166
52A	984	1272	289	0.77	1.96	0.206	0.673	0.169
53B	845	1101	257	0.99	2.52	0.434	0.869	0.164
57B	450	867	418	0.73	1.69	0.394	0.934	0.170
60A	1002	1267	266	0.76	1.80	0.207	0.681	0.178
61A	838	1120	283	0.81	2.82	0.527	0.933	0.198
110A	903	1019	117	1.02	2.04	0.317	0.794	0.138
117A	1547	1673	127	0.86	2.21	0.426	0.897	0.176
136A	1498	1569	72	1.49	2.64	0.496	0.820	0.146
143B	905	1196	292	0.31	1.16	0.180	0.827	0.238
194A	1729	2003	275	0.71	2.05	0.353	0.872	0.191
197A	1776	2003	228	0.85	1.99	0.318	0.784	0.203
199A	1797	2003	207	1.25	2.31	0.283	0.570	0.160

60°C. This method should be sufficient to remove all relevant water-soluble substances (Grabner *et al.* 2005b; Adamopoulos and Voulgaridis 2012). Then the samples were pretreated in acetone for 24 hours at 20°C, according to the previously developed protocol (Gierlinger *et al.* 2002; Grabner *et al.* 2005a, 2005b) and finally rinsed in 20% acetic acid for one day. After the application of successive reagents, the samples were subjected to the acclimatization process. Each step of the preparation and acclimatization was followed by X-raying. Thus, the X-raying was repeated four times for each of the samples.

In the second stage of research, fourteen samples of Norway spruce were selected. The age of the wood ranged from AD 813 to 1873. During the second experiment, these samples were subjected to 24 hours of soaking in de-ionized water at room temperature. The X-ray films were made for the acclimatized wood, firstly without preparation and then for the same wood strips extracted in de-ionized water and conditioned.

The irradiated films obtained during both steps of this research were developed and then digitized by the means of an X-ray dendrodensitometer that was constructed at BOKU University (Grabner *et al.* 2005b). This device consists of a specially designed scanner with a motor and a line camera on a light microscope (Stemi 2000, C. Zeiss, Jena, Germany). Tree-ring transparency (gray level) was obtained by this microdensitometer at 5 μm intervals along the radial direction of the stem. The scanned measurement track amounted to about 5 mm of wood tangential width. Thereby, the density measurement performed after every single step of sample preparation process followed almost the same measurement path.

The specific gravity of the wood was derived by using the relationship that exists between the optical density of the radiograph and the density of the wood for a given thickness of the specimen and the settings of the X-ray apparatus as well as the parameters of the X-ray film (Polge 1966; Vaganov *et al.* 2006). To calibrate wood density recorded by the means of X-ray radiation on the

X-ray sensitive films, the standard optical references in the form of cellulose acetate wedges were used. The density of samples was calculated by computer software compiled at BOKU University. This software identified the boundary between earlywood and latewood as the position of half way between the maximum and minimum values within an annual density profile, according to the definition established by Lenz (Lenz *et al.* 1976; Schweingruber 2007).

To evaluate possible differences in wood density profiles resulting from the application of different extraction methods statistical tests were applied. For this purpose, an analysis of variance (one-way ANOVA) was performed to compare the means of unprepared as well as hot water-, acetone- and acetic acid-prepared samples. ANOVA was calculated separately for mean, minimum and maximum ring density, earlywood and latewood density both before preparation and after extraction in successive reagents. Moreover, a paired t-test was employed to determine if the means for unprepared and cold water-prepared samples are equal. In both tests the differences were considered significant at the 5% probability level. The SPSS 21.0.0 software package was used for statistical studies. Altogether, the density of 11,692 growth rings was analyzed in this way.

Because Lake Schwarzensee is rich in dissolved organic and inorganic matter (Sommaruga *et al.* 1999; Dokulil 2005) an increased accumulation of minerals was expected in wood. To verify this assumption mineralogical analysis was additionally conducted. The mineral content of 6 selected samples was established on the basis of X-ray diffraction technique with the application of X-ray powder diffractometer Philips PW 3710. Diffraction patterns were analyzed using the X'PERT software. The operating conditions during X-ray measurements were: Co Kα1 radiation graphite, X-ray lamp voltage 45 kV and intensity 30 mA, impulse counting: 3, 9, 10 seconds.

RESULTS AND DISCUSSION

During the first stage of research, the values of densities determined for prepared samples were sometimes higher and sometimes lower than the samples that were not pretreated, which was the case for the whole analyzed period (Supplementary Material Table 1). Nevertheless, the average measurements of mean density were equal to 344.32 kg/m^3 for unprepared samples, 338.91 kg/m^3 for water processed samples, 325.05 kg/m^3 for acetone extracted samples and 318.99 kg/m^3 for acetic acid treated wood. In total, the mean density of rings decreased by 1.57% after water preparation, by 4.09% after acetone extraction and by 1.86% after acetic acid pretreatment (in each case the percentage decrease was calculated in relation to the preceding preparation stage).

The results of statistical analyses was ambiguous. As an effect of analysis of variance (ANOVA), it was established that, out of 10 samples subjected to extraction in hot de-ionized water, mean ring density of four samples differed in comparison to unprepared samples, four samples differed from hot-water prepared samples after the acetone preparation, and nine samples differed from acetone-prepared samples after acetic acid application. Similar results were obtained for maximum ring density – in this case it was 3, 3 and 10 samples, respectively. For the minimum ring density, 4, 3 and 9 samples, respectively, differed between the successive stages of wood extraction. Mean earlywood density of 3, 5 and 8 samples, respectively, and mean latewood density of 4, 3 and 10 samples, respectively, were different from the samples obtained during the previous stage of wood extraction. Therefore, attempts to select the most appropriate method of subfossil wood preparation on the basis of statistical approach appear unhelpful in the case of subfossil wood from Schwarzensee Lake because it wasn't possible to determine unequivocally if all of the samples demonstrate differences in the density values.

With the aim of more precise testing of the changes resulting from extraction procedures, the density graphs were examined (Figure 2). During the research, the density profiles spanning the period from AD 631 to 2003 were analyzed visually, and special attention was paid to the variation in wood density resulting from the application of particular reagents (Figure 3). Overall, the hot water pretreatment in well-preserved parts of samples led to a lowering of the density because of removal

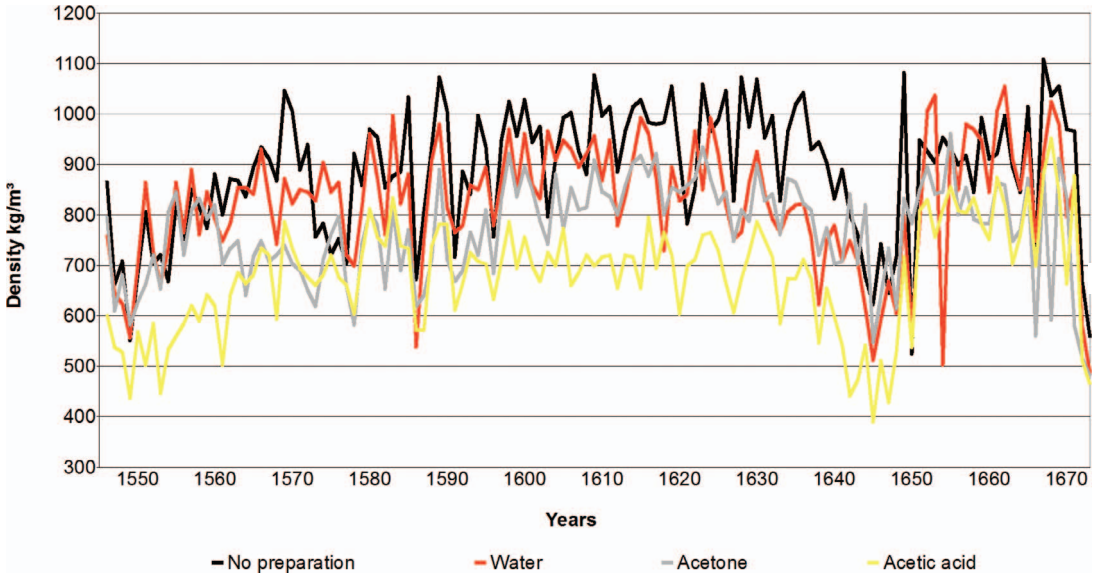

Figure 2. Example graph demonstrating maximum density values obtained for sample 117A before extraction, as well as after the application of consecutive reagents.

of water-soluble mineral and organic compounds from wood. In damaged parts of samples, however, it contributed to an increase of density, probably because of the changes in the hygroscopic behavior of wood, which are frequently associated with the wood aging process (García Esteban *et al.* 2006). On the other hand, acetone extraction distinctly diminished wood density and seemed to be very aggressive chemical treatment. However, detailed analysis of radiographs suggests that the amount of resins and heartwood substances in the subfossil wood was very low. This can be assessed because the presence of wood extractives manifests itself in light spots on X-ray films, which were not visible in the subfossil wood.

This fact is in accord with the data obtained during stable isotope analyses carried out for the same spruce wood from Lake Schwarzensee. In the case of stable isotope measurements, as with X-ray densitometry, the wood is commonly extracted by the means of organic solvents (Cullen and Macfarlane 2005). Such sample preparation is used for the removal of all organic substances that are not the constituents of wood cell walls. The extractives should be removed because their presence influences the bulk isotope composition as these compounds are enriched or depleted in ^{13}C in comparison with wood structural compounds (Borella *et al.* 1998; Boettger *et al.* 2007). However, the stable isotope research demonstrated that the wood extraction procedure was unnecessary because of the insignificant content of resins in the Lake Schwarzensee wood. Furthermore, we concluded that no differences exist between the samples without any chemical pretreatment and the specimens subjected to the preparation in benzene and ethyl alcohol. This is not surprising because an important feature of waterlogged wood is abnormally low resin content. Resin removal in sedimentation environment is caused by microbiological decay. Moreover, extractives could also, to some extent, dissolve in water during prolonged submergence (Kim 1990; Pan *et al.* 1990; Martínez-Iñigo *et al.* 1999; Björdal and Nilsson 2002).

Additional information was gained from previous mineralogical research. The results of these studies indicated that, although Schwarzensee samples predominantly contained expected calcite, the overall mineral content of the wood was less than one percent of weight (Table 2). This concentration is in the range of the values found for living trees (Wagenführ and Scheiber 1985; Hedges 1990; Surmiński 2007). Therefore, the quantity of minerals in subfossil wood from Schwarzensee was low and

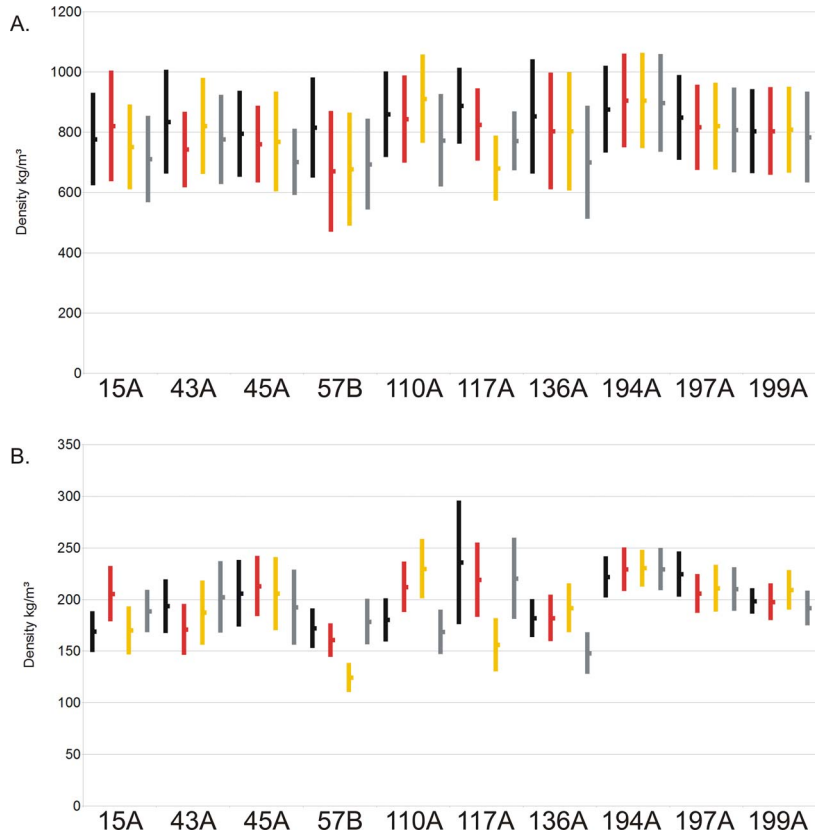

Figure 3. The values of maximum (a) and minimum (b) ring density averaged for particular samples before preparation (black) and after the application of hot de-ionized water (red), acetone (yellow) and diluted acetic acid (gray). Middle point of each line represents the mean value and length of the line refers to standard deviation range.

its removal was not a major improvement for densitometric research. On the other hand, acetic acid application reduced mean wood density by over one percent of wood mass. This demonstrates that not only were mineral substances dissolved by acetic acid, but cell wall components were also removed during extraction process.

The above-mentioned factors point to cell-wall decomposition as a predominant reason for reduction of wood density observed during the first stage of our experiments. The highest density reduction was associated with acetone extraction. Most likely this reagent induced the destruction of cell walls and the release of their decomposition products from subfossil wood. Presumably, the pretreatment with acetic acid solution produced a similar result. In summary, the analysis of measurement data, radiographs, density profiles and

Table 2. The results of mineralogical analyzes of subfossil wood from Lake Schwarzensee. Mineral composition of the samples was established on the basis of X-ray diffraction technique by means of an X-ray powder diffractometer, Philips PW 3710.

Sample	Dating	Mineral content [%]	Organic content [%]	Calcite [%]	Anhydrite [%]	Other
41B	1475–963 BP	0.75	99.25	*ca.* 90	*ca.* 9	quartz, goethite, bassanite
101A	838–638 BP	0.76	99.24	*ca.* 91–92	*ca.* 8	quartz
44C	578–267 BP	0.96	99.04	*ca.* 90	*ca.* 8	quartz, bassanite
20B	377–517 AD	0.83	99.17	*ca.* 90	*ca.* 10	quartz
34B	820–982 AD	0.86	99.14	*ca.* 87	*ca.* 12	quartz, goethite
83B	1622–1748 AD	0.47	99.53	*ca.* 91–92	*ca.* 8	

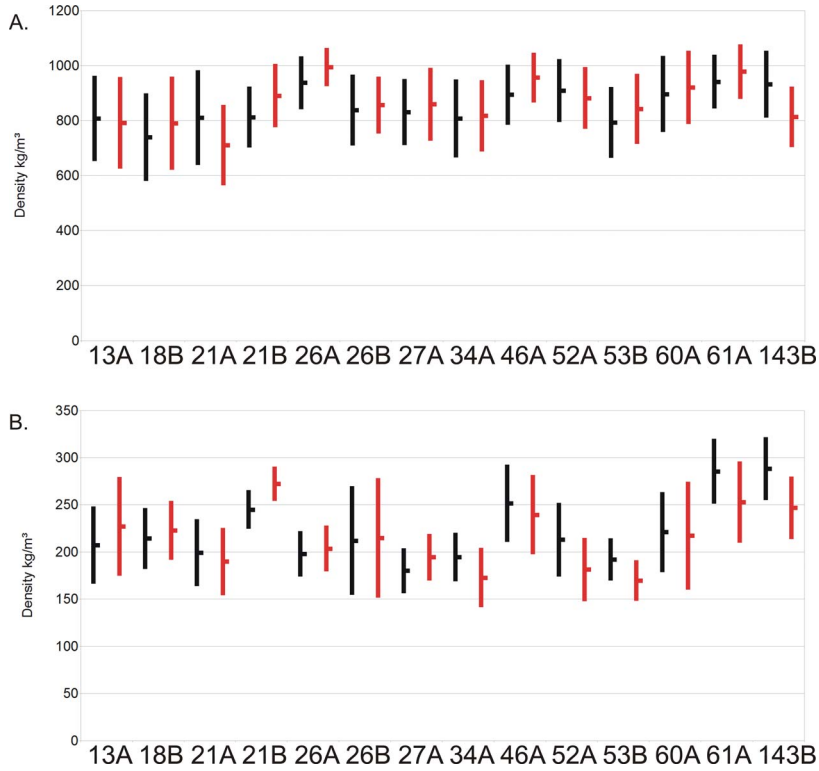

Figure 4. The values of maximum (a) and minimum (b) ring density averaged for particular samples before preparation (black) and after the application of cold de-ionized water (red). Middle point of each line represents the mean value and length of the line refers to standard deviation range.

mineralogical results indicates that the pretreatment in organic solvents was not suitable and the application of acetic acid was not desirable for the studied wood. It also suggests that subfossil wood from Schwarzensee is characterized by small amounts of extractives and minerals.

Therefore, in the second stage of research, 14 samples of spruce wood were prepared in cold de-ionized water only (Figure 4). The results show that the mean ring density before water treatment was very similar to the mean density measured for the samples after water treatment. The average measurements of mean ring density were equal to 381.70 kg/m^3 for untreated samples and 383.19 kg/m^3 for those that were extracted with cold water. Thus, the mean density of rings increased by 0.39% after cold water preparation (Supplementary Material Table 2). In turn, calculation of paired t-test frequently demonstrated the difference between the results received for untreated samples and the data obtained for the same samples when extracted with cold de-ionized water. For mean ring density, differences were recorded for 9 samples, 10 samples for maximum density, 12 samples for minimum density, 8 samples for the mean earlywood density, and 10 samples for mean latewood density.

The rinsing in cold de-ionized water enabled the extraction of water-soluble organic and mineral compounds from wood and simultaneously did not lead to the degradation of subfossil material. Moreover, de-ionized water had an additional, beneficial impact. Soaking in water was observed to straighten and smooth the tracheid structure in the crumpled rings in the wood (Figure 5). The occurrence of crushed zones could be the result of the load acting perpendicularly to the main axis of the stem in the sedimentation environment, or it might be an effect of cell-wall collapse during the drying of wood that

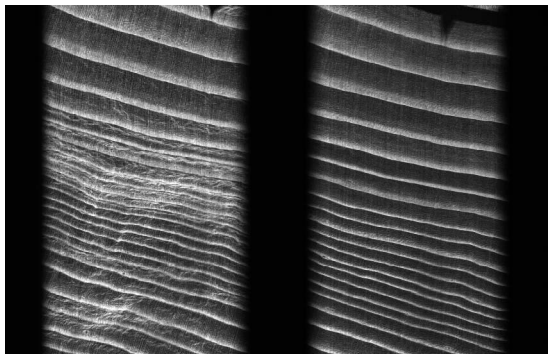

Figure 5. X-ray film presenting the creased zone of sample 117A before preparation and the same place smoothed after soaking in cold de-ionized water.

followed the excavation of trunks from the lake. Therefore, de-ionized water pretreatment also helps avoid the bias in density profiles, which could occur within these local zones of subfossil wood.

Summing up, cold de-ionized water seems to be the best reagent to use with the wood we tested. However, our results are rather species- and site-specific and relate to spruce subfossil wood from Schwarzensee Lake. Nevertheless, the results of this study coincide to some degree with findings of other publications. The necessity of subfossil wood pretreatment for the purpose of densitometric measurements had been previously discussed during the studies carried out on Scots pine (*Pinus sylvestris* L.) wood retrieved from Lake Herajärvi (Helama *et al.* 2008, 2010). Initially, these studies found subfossil wood does not require any chemical pretreatment before densitometric research (Helama *et al.* 2008). However, after more detailed examination, it was determined that the use of acetone extraction provided more precise results (Helama *et al.* 2010). In turn, some analysis of living trees demonstrated that because of the negligible overall content of extractives in the wood of Norway spruce, the procedure for removing these non-structural compounds is unnecessary and could be omitted during densitometric research (Jaakkola *et al.* 2005, 2006). These analyses performed for recent wood of Norway spruce, together with the fact that some fraction of resinous substance is always removed during the sedimentation process, confirm the conclusion obtained during presented research. The extractives removal prior to X-ray density measurements seems to be superfluous in the case of subfossil wood from Schwarzensee Lake.

CONCLUSIONS

On the basis of the presented research, rinsing in cold de-ionized water was the best extraction technique for subfossil wood. Furthermore, this treatment did not destroy altered cell walls. Therefore, it was decided to use the above-described preparation in cold de-ionized water in order to remove water-soluble mineral and organic compounds and because the pretreatment with de-ionized water resulted in restoring rings with crushed cells back to their original appearance. This method of extraction appears to be the best solution in the case of subfossil wood from Lake Schwarzensee.

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Supplementary Material is available at *http://www.treeringsociety.org/TRBTRR/TRBTRR.htm*

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