

# Sample Selection, Characterization and Choice of Treatment for Accurate Radiocarbon Analysis—Insights From the ETH Laboratory

**Journal Article****Author(s):**

Hajdas, Irka; Guidobaldi, Giulia; Haghypour, Negar; Wyss, Karin

**Publication date:**

2024

**Permanent link:**

<https://doi.org/10.3929/ethz-b-000662476>


**Rights / license:**

[Creative Commons Attribution 4.0 International](#)

**Originally published in:**

Radiocarbon, <https://doi.org/10.1017/RDC.2024.12>

## SAMPLE SELECTION, CHARACTERIZATION AND CHOICE OF TREATMENT FOR ACCURATE RADIOCARBON ANALYSIS—INSIGHTS FROM THE ETH LABORATORY

Irka Hajdas<sup>1\*</sup>  • Giulia Guidobaldi<sup>1</sup> • Negar Haghpor<sup>1,2</sup> • Karin Wyss<sup>1,3</sup>

<sup>1</sup>Laboratory of Ion Beam Physics, ETHZ, Otto-Stern-Weg 5, 8093 Zurich, Switzerland

<sup>2</sup>Earth Sciences Department, ETHZ Zurich, 8092 Zurich, Switzerland

<sup>3</sup>Berner Fachhochschule BFH, Hochschule der Künste Bern HKB, 3027 Bern, Switzerland

**ABSTRACT.** Accurate radiocarbon ( $^{14}\text{C}$ ) analysis depends on a successful carbon separation relevant to the studied object. The process of  $^{14}\text{C}$  dating involves the following steps: characterization and sample choice, sample treatment, measurements, and evaluation of the results. Here, we provide an overview of conventional approaches to macromolecular samples and address specific issues such as detecting and removing contamination with roots, dolomite, and conservation products. We discuss the application of elemental analysis (%N, %C) in the preparation of bones and the infrared analysis in monitoring the contamination of samples. Our observations provide the basis for the discussions of the existing results and for planning the future sampling.

**KEYWORDS:** contamination, FTIR, radiocarbon AMS dating, sample treatment, sieving.

### INTRODUCTION

All laboratories strive to achieve the highest precision and accuracy when performing radiocarbon ( $^{14}\text{C}$ ) analysis. Although these two terms are often exchanged to express the desire for the best chronological estimates, they are not synonymous. Modern measurement techniques, certificates, and intercomparison studies provide quality assurance. However, the most precise ages can also be inaccurate (Geyh 2008).

The accuracy of radiocarbon ages is dependent on various factors. First is the source of carbon built into the sample at the time of its formation, i.e.,  $^{14}\text{C}$  age or the isotopic signal of the reservoir. Other factors are the stage of preservation or degradation of the sample and contamination with allochthonous carbon, which might be related or amplified by the degradation (van Klinken and Hedges 1998; van Klinken 1999). Last is the selection of the original sample and its purification before  $^{14}\text{C}$  analysis.

The wide range of materials and applications of radiocarbon analysis requires using protocols developed for different types of material (Hajdas 2008; Wood 2015; Hajdas et al. 2021a). In general, all laboratories follow standard procedures of ABA, cellulose separation, Longin method or Ultra Filtration for bones, but modifications of protocols are standard practice (Brock et al. 2007; Hajdas et al. 2007, 2009; Brock et al. 2010a, 2010b, 2013, 2018; Rubinetti et al. 2020; Pawelczyk et al. 2022). New opportunities for the separation of carbon suitable for radiocarbon dating arrived with the development of compound-specific radiocarbon analysis (CSRA) (Eglinton et al. 1996; Ingalls and Pearson 2005). The range of applications of CSRA expanded the field of RA, especially in studies of sedimentary records (for example Blattmann et al. 2020; McNichol and Lindauer 2022), and archeology, including dating pottery (Casanova et al. 2022) and bones (Deviese et al. 2018). Our overview concentrates on the

\*Corresponding author. Email: [hajdas@phys.ethz.ch](mailto:hajdas@phys.ethz.ch)

preparation of macromolecular type of samples and conventional pretreatment (van Klinken and Hedges 1998).

In addition to the sample treatment (purification), material selection is essential. The assignment occurs during the fieldwork. Later, the refined choice of material or separation of the suitable fraction is performed in the laboratory after a visual investigation using binoculars. Depending on the type of material, the selection of datable carbon by wet or dry sieving, separation of macro and micro remains, drilling, and cutting suitable pieces is chosen.

Binocular observation is most effective and paramount to the understanding of the sample. For example, a mixture of anthracite and charcoal has been observed in the samples of rock varnish, which allowed us to scrutinize and question the validity of the radiocarbon dating of rock varnish (Beck et al. 1998). Often, synthetic materials such as textiles can be easily identified. The most common problem detected using the microscope is contamination by roots (in situ) or anthropogenic contaminants such as dust, hair, and fiber. The latter, is somewhat random and difficult to deal with because there is no guarantee that all contaminants can be ever picked out of the samples. More common is contamination with roots observed in soils, peat, sediments, wood, and charcoal. Sieving removes roots from sediments and peat (Hajdas et al. 2021b); however, the infested wood and charcoal used for radiocarbon analysis can be contaminated even if visible roots are removed from the sample. Sieving is also used when macro and micro remains are selected for radiocarbon analysis. Separation of terrestrial macrofossils assures that the material is free of reservoir effect (hard water effect) (Hajdas et al. 1993, 1998, 2021a).

Another material that requires sieving and selecting suitable carbon fraction (grain size) is lime mortar (Lindroos et al. 2007). An alternative method is the cryo-breaking and ultrasonic separation (Nawrocka et al. 2005; Marzaioli et al. 2014; Michalska et al. 2017).

Radiocarbon dating of bone, tooth and antler requires the separation and purification of collagen (Weiner and Bar-Yosef 1990; Yizhaq et al. 2005). A degree of preservation determines the success of radiocarbon analysis (van Klinken 1999). Brock et al. (2010b) tested an assessment based on elemental analysis of %C and %N content, or C/N ratio of the original bone, and found that bones with %N < 0.76 are less promising. Another technique used by radiocarbon laboratories employs the infrared analysis to detect characteristic absorption lines of the bone to assess the preservation of collagen (D'Elia et al. 2007; Lebon et al. 2016; Cersoy et al. 2017; France et al. 2020; Leskovar et al. 2022).

More sophisticated methods of sample screening and preselection can be supported by the Fourier transform infrared (FTIR), thermal gravimetric analysis (TGA), scanning electron microscope (SEM), Raman, direct temperature-resolved mass spectrometry (DT-MS), Py-GC-MS, and other techniques. Identifying specific components using FTIR spectroscopy can be utilized in studies of bones, paintings, and charcoal (Alon et al. 2002). The infrared light absorbed (or transmitted) depends on the studied material's molecular composition. Molecules with different types of vibration modes absorb characteristic wavelengths. The specific regions and peaks of absorption/transmission minima allow the identification of molecules/material, which is often supported by the existing databases of FTIR spectra (<https://centers.weizmann.ac.il/kimmel-arch/infrared-spectra-library>). In the preparation of radiocarbon samples, the detection of synthetic and conservation materials is critical. Most synthetic polymers, which are long-chain carbon molecules, are made of fossil carbon; therefore, the dead carbon

contamination is significant. Often, such contamination requires modification of the standard treatment as well as additional control of the clean sample before combustion and AMS  $^{14}\text{C}$  analysis (Yizhaq et al. 2005).

The use of FTIR in radiocarbon laboratories is not limited to the detection of synthetic contaminants added during the conservation and preservation process, including the use of pesticides (Tiilikkala et al. 2010). As mentioned above, FTIR is useful in screening for well-preserved bones and in the characterization of mortars (Paama et al. 1998; Al Sekhaneh et al. 2020; Calandra et al. 2022). Moreover, the FTIR analysis of sediments can provide information about the carbonate content and, most importantly, indicate the presence of dolomite and other minerals. The standard treatment of acid-base-acid is insufficient to remove the dolomitic component. The effect of contamination with carbon-free dolomite is amplified by the fact that glacial and fluvial sediments have a very low organic %C, and dolomite is free of  $^{14}\text{C}$ . Also, organic carbon in soil and sediments might have old components trapped by minerals, such as clay (Scharpenseel and Becker-Heidmann 1992), which requires a different approach such as a stepped-combustion or Ramped pyrolysis/oxidation (McGeehin et al. 2001; Wang et al. 2016; Hemingway et al. 2019).

This paper presents an overview of the most common methods used to characterize and select material suitable for radiocarbon dating. Establishing and monitoring treatment efficiency is the key to accurate radiocarbon dating.

## METHODS

The spectrum of sample material submitted and processed at the ETH radiocarbon laboratory is wide. Thus, the overview of methods used to select and purify material for radiocarbon dating is based on our observations gained during a couple of decades. Table 1 shows methods applied in a pre-screening process followed by sample preparation (Table 1 Supplementary Material) before the AMS analysis.

### Microscope/Binocular (Magnification 10 $\times$ –50 $\times$ )

A visual investigation and documentation of suspicious contaminants is the first step before selection. Some samples, such as macrofossils, foraminifera, and sieved fractions of mortar, are selected and identified by the researchers before submission. The microscope is indispensable for determining macrofossils (Hajdas et al. 1993, 1998) or foraminifera shells (Broecker et al. 1990) but also for removing contaminants such as exogenous fiber, roots, or remains of insects and hair.

### Sieving

Sieves, mesh 125  $\mu\text{m}$  (also 150  $\mu\text{m}$  can be applied) are used to separate fine (roots-free) fractions from samples of sedimentary deposits (peat, soil, sediments). At ETH laboratory, we apply 100 mm diameter stainless steel sieves (Retsch) and a collection pan with a funnel to collect water and a fine fraction (Figure 1).

It is essential to work on fresh or stored in a freezer (wet) material; samples should not be dried, crushed, or milled before the sieving and are soaked in MiliQ water to soften and disintegrate the bulk.

Table 1 Sample types, pre-screening methods and preparation methods. Details of pretreatment methods are summarized in Table 1 of the Supplementary Material.

Sample type	Potential Contamination	Prescreening Methods	Material selected/ observation	Prescreening Methods	Material chosen/ observation	Pretreatment method	Check after treatment
Peat/soil/gyttja/ Sediments	Roots, aquatic plants dolomite	Microscope	First assessment				
		Ssieving	Top sieve >125 µm Fine fraction <125µm	Microscope  FTIR	identifiable macro remains (Terrestrial/Aquatic) No dolomite observed	ABA  ABA	
					Dolomite present	Strong and long ABA 1 M HCl ABA Cellulose ABA/cellulose	FTIR  Microscope
Wood/charcoal Wood Wood/canvas/ textiles	Roots Worms Conservation	Microscope Microscope Microscope	Remove roots Intact wood	  FTIR (multiple points)	  Absorption lines of sample material  Conservation/additional absorption lines Conservation observed	  ABA Cellulose ABA/cellulose  Solvents/ABA	  FTIR  FTIR
Bone, tooth, antler	Conservation	FTIR  %C, %N %C, %N	Whole bone, tooth, antler  %N>1 C/N <sub>at</sub> from combustion		  Gelatin	  UF of gelatin	  FTIR
Paint	Carbon bearing pigments	Pigment analysis	binder  Lead White	FTIR  FTIR	  Carbonate	Acid  Solvent/ thermal decom- position 4 consecutive fractions of CO <sub>2</sub> collected	FTIR  FTIR after solvent
Mortar	Geogenic carbon (limestone, dolomite)	Characterization of mortars, sieving	45-63 µm  45-63 µm & Lime lumps	Dissolution speed	  Recrystallization	  Thermal decomposition	



Figure 1 Setup for wet sieving of sediment and peat samples. The fine fraction ( $<125\ \mu\text{m}$  or  $150\ \mu\text{m}$ ) is collected in a glass beaker. The larger fraction (top sieve) can be investigated under binoculars.

Stainless steel sieves (100 mm Retsch), mesh  $45\ \mu\text{m}$  and  $63\ \mu\text{m}$ , and a collection pan are placed on the Retsch dry-sieve shaker to sieve mortar. Before sieving, the sample is investigated, and if present, any lime lumps are collected from the bulk. Small sample fragments are then crushed, and if present, stones (aggregates of mortar) are removed, and the powder is sieved. The process is repeated to collect at least 100 mg of powder  $45\text{--}63\ \mu\text{m}$ . The smaller and larger fractions are also collected and archived.

### Elemental Analysis

The carbon content of sedimentary deposits (TOC) varies greatly; therefore, %C analysis is performed on clean fractions before combustion and the AMS analysis. A few milligrams (5–10 mg) of pure material are weighed and packed in the aluminium (Al)<sup>1</sup> or tin (Sn) cups for analysis with an Elemental Analyzer (EA). The measured %C is used to calculate the mass of the sample material, which contains 1 mg of C. For example, 10 mg of material with a C content of 10% needs to be combusted for the graphite target to contain 1 mg of C. Samples with poor %C (less than 1%) are planned for analysis with gas ion source (GIS) (Ruff et al. 2010; Haghypour et al. 2019) and the equivalent of ca. 100  $\mu\text{g}$  of carbon is packed in Al cups.

Nitrogen and carbon content of original bones (%N and %C) can be measured in an EA. A small portion of the cleaned original bone (5–10 mg) is weighed and packed in Al cups for EA

<sup>1</sup>Al cups are used at the ETH laboratory due to simplicity of cups treatment (pre-cooking at  $500^\circ\text{C}$ ) and low blank values

analysis. The values of %N, %C, and C/N ratio are saved into the database. Currently, at the ETH laboratory, the preparation of samples with very low %N (<1%) is stopped.

The C/N<sub>at</sub> ratio of gelatin is obtained during combustion and graphitization (Nemec et al. 2010). This value is stored in the database, and the yield (mass gelatin/mass bone sample) indicates the gelatin's purity/quality.

The C/N<sub>at</sub> ratio can also be used to identify the type of material (Hajdas et al. 2014) and purity (Boudin et al. 2013).

### **Fourier Transform Infrared Spectroscopy**

Characterization of sample material and detection of potential contamination is possible with the help of FTIR spectroscopy. No preparation is required for analysis using the ATR modus (for example, PerkinElmer Spotlight 200i used at the ETH laboratory), but multiple subsamples should be analyzed (heterogeneity of contamination). The transmission/absorption spectra are compared with the spectra of specific materials. Cross-check of clean samples is performed before combustion. Moreover, the FTIR can be applied to screen/assess the preservation of bones.

### **Sample Pretreatment**

Different types of samples are treated differently after the characterization of the sample and selection of suitable fractions. For example, the methods described below are routinely applied at the ETH laboratory. The pretreatment details used at the ETH laboratory are summarized in Table 1 of the Supplementary Material.

#### *Acid-Base-Acid (ABA)*

The sequence of washes in acid and base is applied before the combustion of organic samples. The ABA procedure can be modified to adjust to sample contamination or preservation degree. Some steps, such as base, can be omitted (for example, when dating TOC of soils), or a stronger solution and longer treatment time is applied, for example, when dolomite is present and acid wash is extended to multiple days. Modifications are also applied in the case of a base step, which is destructive for wool and silk, therefore, such samples are subject to a short base step and performed at room temperature.

Poorly preserved charcoal can dissolve in the base, and only humic acid can be collected for radiocarbon analysis; however, one must be aware that it can be of mixed carbon sources. Also, peat and sediment fine fraction treatment can be modified to separate and date humin and humic fractions.

#### *Solvents*

A standard sequence of hexane, acetone and ethanol is applied when FTIR analysis indicates the presence of complex carbon molecules (oil, fat, waxes, conservation material). The Soxhlet apparatus is often applied to wash the sample in a clean solvent (Hajdas et al. 2004; Hajdas 2008). However, glass vials are used when chloroform treatment is necessary (Liccioli et al. 2017; Kessler et al. 2022) because samples float and might discharge via the siphon of the Soxhlet apparatus. The glass vials with samples and solvents are placed on a shaker for a few hours in the heated block (60°C). The solvent is replaced, and the wash continues for one working day. The sample is left to dry overnight and checked with FTIR the next day. If

required, the cleaning is repeated. It is worth noting that the use of glass vials and shaker tables is more sustainable and requires a lower quantity of solvents but more of pipetting out the liquid solvents, which must be done under the fume hood.

#### *Ultra-Filtration of Gelatin*

The treatment of bones and antlers requires the separation and purification of gelatin (Table 2). The main modification of the procedure (Hajdas et al. 2009) is a return to a dissolution of fragments of bones without crushing them (Hajdas et al. 2007). The modification was introduced following studies of Fewlass et al. (2017, 2019), who showed that demineralization of larger pieces of bones improves collagen recovery and allows radiocarbon analysis to be performed on much smaller samples of bone.

#### *Sequential Dissolution Mortar*

Mortar powder (45–63  $\mu\text{m}$ ; ca. 100 mg) is dissolved in condensed phosphoric acid. The  $\text{CO}_2$  is collected and closed in a glass tube in four intervals, each 3 seconds long. Depending on the amount of  $\text{CO}_2$ , the sample is sealed in a glass tube for graphitization (>200  $\mu\text{g}$ ) or for GIS (Hajdas et al. 2020a, 2020b).

#### *Special Samples*

Occasionally, unique samples such as paint (Hendriks et al. 2018), lime lumps (Lindroos et al. 2018), cremated bones (Lanting et al. 2001; Major et al. 2019), iron (Hüls et al. 2004), wine (Quarta et al. 2022) and other liquid samples can be subjects of radiocarbon analysis. Methods used to prepare such samples are summarized in Table 1 Supplementary material.

Depending on C content, purified samples are graphitized (Nemec et al. 2010) or analyzed as gas samples (Ruff et al. 2010) at MICADAS (Synal et al. 2007).

## RESULTS AND DISCUSSION

Effects of the applied screening and sample treatment are illustrated by the example of various materials analyzed and sometimes re-analyzed after additional treatment. Evaluation of results for samples measured with GIS (minimal C mass) considers constant mass contamination (Welte et al. 2018; Haghypour et al. 2019). The results reports include: Radiocarbon ages and  $F^{14}\text{C}$  (Stuiver and Polach 1977; Reimer et al. 2004),  $\delta^{13}\text{C}$  measured during AMS analysis,  $\text{C}/\text{N}_{\text{at}}$ , (%C and %N from combustion prior graphitization), C mass of targets analyzed by AMS and, if available Yield=mass after treatment/mass start. The yield value provides information about sample conditions but sometimes cannot be provided (wet sample, small sample, missing notes).

In addition, for collagen samples, we might provide IRMS analysis  $\text{C}/\text{N}_{\text{at}}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$

### Roots

Roots present in peat (Figure 2a) or sediment samples can be removed. Sieving was successfully applied in dating the sedimentary deposits in the Italian valley Val Ferret southeast of the Mont Blanc Massif (45°56'35"N 7°05'26"E) to clarify the controversial chronology of the 1717 avalanche (Hajdas et al. 2021b). However, contamination of wood samples or charcoal is often impossible to remove. Roots are growing deep into the sample (Figure 2b), and a web of roots is hidden inside the pieces of charcoal or wood.



Table 2 Contamination (wavenumber  $\text{cm}^{-1}$  in bold Italics) detected with the help of FTIR and the results of radiocarbon dating after additional treatment.

Material	Pretreatment	$F^{14}\text{C}$	$\pm 1\sigma$	$\delta^{13}\text{C}$ (‰)	Absorption lines (wavenumber $\text{cm}^{-1}$ )
Sediments and soils from regions with dolomite rocks/or catchment area					
<b>Dolomite</b>		<b>0.000</b>		<b>~0</b>	<b><i>1436, 888, 730</i></b>
BulkSed1	ABA°60, 1 hr	0.040	0.001	-0.9	3626, 3400, 1634, <b><i>1436</i></b> , 1164, 983, 909, <b><i>888, 730</i></b>
	ABA°60, 1 hr; 1M HCl°60, 72 hrs	0.271	0.001	-29.1	3626, 3400, 1634, 1164, 983, 796, 776
BulkSed2	ABA°60, 1 hr	0.092	0.001	-8.8	3626, 3400, 1634, <b><i>1436</i></b> , 1164, 983, 909, <b><i>888, 730</i></b>
	ABA°60, 1 hr; 1M HCl°60, 72 hrs	0.226	0.001	-27.1	3626, 3400, 1634, 1164, 983, 796, 776
Objects of cultural heritage					
Flax	No conservation	na	na	na	3335, 2918, 1643, 1426, 1315, 1202, 1157, 1103, 1028, 897, 661
Canvas	Solvents: H-A-Eth- ABA°60, 1 hr	0.961	0.0026	-21.1	3332, 2921, <b><i>2854, 1457, 1376</i></b> , 1315, 1203, 1158, 1105, 1029, 897, 661
	Solvents: H-A-Eth-Chloroform, ABA°60, 1 hr	na	na	na	3332, 2895, <b><i>1697</i></b> , 1428, <b><i>1335, 1251</i></b> , 1160, 1102, 1029, 897, 663
	Solvents: H-A-Eth-Chloroform, ABA°60, 1 hr	0.973	0.0027	-24.9	3336, 2897, 1428, 1314, 1203, 1161, 1104, 1054, 1029, 897, 662
Whole wood (Pine)	No conservation	na	na	na	3340, 1738, 1640, 1508, 1264, 1025
PEG (Polyethylenglycol)		<b>~0</b>			<b><i>2888, 1467, 1367, 1342, 1147, 1107, 1058, 963, 842</i></b>
Wood with PEG	No treatment	0.2690	0.0010	-29.4	3400, <b><i>2884</i></b> , 2741, 2163, 1590, 1508, 1455, <b><i>1466</i></b> , 1455, 1414, <b><i>1359, 1341</i></b> , 1279, 1241, <b><i>1145, 1100, 1060, 961</i></b> , 947, 886, <b><i>842</i></b> , 567
	Solvents: H-A-Eth-Chloroform, ABA°60, 1 hr	0.5630	0.0017	-24.8	3400, <b><i>2884, 1466, 1360, 1340</i></b> , 1279, 1240, <b><i>1147, 1104, 1060, 947, 842</i></b>
	Solvents: H-A-Eth-Chloroform, ABA°60, 1 hr; mechanical separation	<b>0.6140</b>	<b>0.0018</b>	<b>-24.6</b>	3400, <b><i>2884, 1466, 1360, 1340</i></b> , 1279, 1240, <b><i>1147, 1104, 1060, 947, 842</i></b>

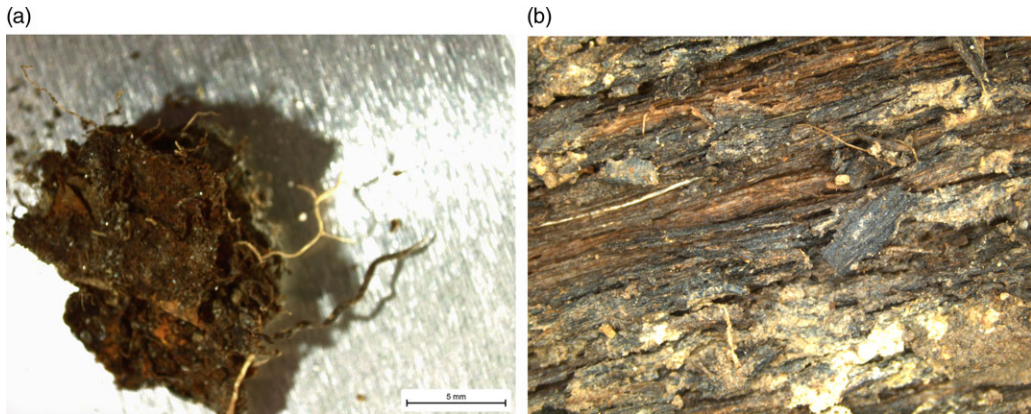


Figure 2 (a) Sample of peat VF-28 (Val Ferret) (Hajdas et al. 2021b) was sieved to remove the visible roots (b) roots observed in the wood submitted to the laboratory could not be removed.

### Dolomite

Contamination with calcium carbonates  $\text{CaCO}_3$  (limestone) is removed from samples (any material) in the acid step of ABA treatment. Most samples are sufficiently treated in this step, and radiocarbon analysis provides accurate ages. However, sediment or soil samples from the regions with dolomite require stronger treatment because dolomite  $\text{CaMg}(\text{CO}_3)_2$  reacts slowly with HCl. Table 2 shows  $F^{14}\text{C}$  measured on bulk sediment treated with standard ABA, which resulted in ages outside of the expected range. The FTIR investigation of the remaining clean sample has shown that the dolomitic component is still present in the sample, showing absorption lines  $1436, 888, 730 \text{ cm}^{-1}$  (Table 2).

Additional, extensive treatment lasting for a few days, with stronger HCl acid (1 M instead of 0.5 M) removed dolomite, and higher  $F^{14}\text{C}$  values of the sample were measured. Higher  $F^{14}\text{C}$  indicates the removal of contaminants such as dolomite and has values of  $F^{14}\text{C}$  close to 0. The effect is also visible as a change in  $\delta^{13}\text{C}$  of the sample from  $\sim 0$  toward more negative values (Table 2).

### Conservation Materials

Treatment of samples from heritage objects often depends on the contamination type. Table 2 shows examples of results obtained for a variety of samples. The radiocarbon age of the canvas, dated too old for the expected age, was treated with an additional solvent. The change in FTIR spectra of the clean sample was confirmed by radiocarbon analysis returning a higher  $F^{14}\text{C}$  of the clean canvas, i.e., the contamination with fossil carbon was removed.

Results of radiocarbon analysis of wood treated with polyethylene glycol (PEG) is an example of difficulties in removing PEG. The FTIR absorption spectra of PEG-treated wood show strong absorption peaks of PEG ( $2888, 1467, 1367, 1342, 1147, 1107, 1058, 963, 842 \text{ cm}^{-1}$ ). These peaks are still in the spectra even after additional solvent treatment with ethyl acetate. The measured  $F^{14}\text{C}$  increased from  $0.269 \pm 0.001$  to 0.614 after treatment with solvents, but it is still inaccurate due to the detected PEG. The contamination appears to be heterogenous, and mechanical scraping affected the measured  $F^{14}\text{C}$  (Table 2).

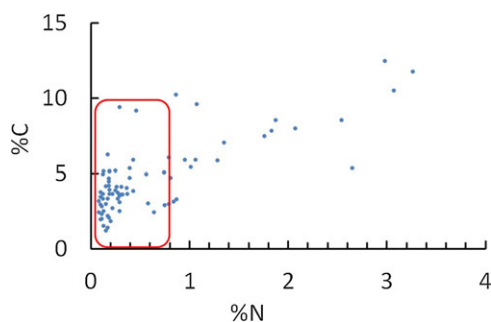


Figure 3 Results of elemental analysis %N and %C of 80 bones which gave no gelatin.

### Collagen Preservation

Prescreening of bones for collagen preservation was only introduced at the ETH laboratory after the Elemental Analyzer was installed in 2012. However, at that time, the practice of checking the %C, %N analysis of the original bone was yet to be part of the standard protocol. Nevertheless, the data collected for over 1000 bones prepared in the ETH laboratory during the last nine years (2013–2022) indicate a wide range of values: %N between 0.05 and 7 and %C values of 0.15 to 28.

The majority of successfully dated bones ( $N=880$ ) had  $\%N > 1$ . From 150 bones with  $\%N < 1$ , 80 bones failed, most ( $N=57$ ) of which had  $\%N < 0.76$  i.e., below limit proposed by (Brock et al. 2010b). It is worth noting that some bones with  $\%N > 1$  did not provide gelatin (Figure 3) showing limitations of this prescreening method thus a combination of methods can be of help (van Klinken 1999; Brock et al. 2010b). The poor preservation results in a very low yield but the low yield can also be due to sample handling, especially of fine grain samples. The quality of UF-purified gelatin is well illustrated by the  $C/N_{at}$  values of the graphitized sample (AGE system; Nemec et al. 2010) and the yield. Most samples with  $C/N_{at}$  between 3.1–3.4 show higher yield, while samples with  $C/N_{at}$  outside 3.0–3.5 range had very low yield (Figure 4). Evaluation of the success of sample decontamination remains difficult (van Klinken 1999) however the observed abnormal  $C/N_{at}$  values require check and repeated analysis. Observations from the ETH laboratory suggest that often  $C/N_{at}$  values closer to 3.2–3.3, which is the range for modern bones (Ambrose 1990), result in a better agreement with expected ages.

### SUMMARY AND CONCLUSIONS

The wide range of sample material submitted to research and service  $^{14}C$  laboratories requires the application of different protocols. Instrumental support is the key to monitoring visible and invisible contamination. The presented examples highlight some of the most frequent obstacles to accurate radiocarbon ages. Contamination with roots might be challenging to observe, and it is quite possible that samples of bulk measured in the past were contaminated by roots. Conservation can also be invisible; if undocumented, contamination with old carbon is inevitable. The FTIR spectra provide only qualitative information about the possible contamination, but the results obtained on clean material are satisfactory. Our application of elemental analysis (%N, %C) to assess the preservation of bone collagen is possible because of the available equipment. However, using FTIR is an alternative method that can help save the

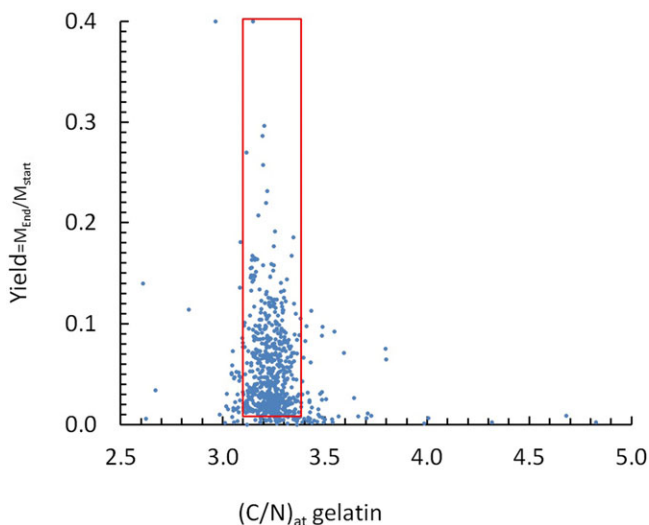


Figure 4 Correlation between the yield of gelatin and  $C/N_{at}$  ratio of the purified gelatin (%N and %C values from combustion prior to graphitization). The rectangle marks the samples with acceptable yield of  $>0.1\%$  and  $C/N_{at}$  in the range 3.1–3.4.

time required to weigh the samples. Finally, all the observations and characterization results are crucial in evaluating the final results. Heterogeneous pieces of mortar and poorly preserved bones need careful evaluation of parameters such as  $C/N_{at}$  or the preparation yield. In conclusion, a holistic assessment of so-called “outliers” can help improve the accuracy of radiocarbon analysis.

## ACKNOWLEDGMENTS

Many thanks to all the colleagues who worked in the preparation laboratory during the last 20 years: Sandra Isteri, Carole Biechele, Mantana Maurer and Maria Belen Röttig for their dedicated work. Caroline Welte, Hans-Arno Synal, Lukas Wacker, and Urs Ramsperger for their support of the AMS analysis.

## SUPPLEMENTARY MATERIAL

To view supplementary material for this article, please visit <https://doi.org/10.1017/RDC.2024.12>

## REFERENCES

- Al Sekhaneh W, Shiyab A, Arinat M, Gharaibeh N. 2020. Use of FTIR and thermogravimetric analysis of ancient mortar from The Church of the Cross in Gerasa (Jordan) for conservation purposes. *Mediterranean Archaeology and Archaeometry* 20:159–174.
- Alon D, Mintz G, Cohen I, Weiner S, Boaretto E. 2002. The use of Raman spectroscopy to monitor the removal of humic substances from charcoal: quality control for  $^{14}\text{C}$  dating of charcoal. *Radiocarbon* 44:1–11.
- Ambrose SH. 1990. Preparation and characterization of bone and tooth collagen for isotopic analysis. *Journal of Archaeological Science* 17:431–451.
- Beck W, Donahue DJ, Jull AJT, Burr S, Broecker WS, Bonani G, Hajdas I, Malotki E. 1998.

- Ambiguities in direct dating of rock surfaces using radiocarbon measurements. *Science* 280: 2132–2135.
- Blattmann TM, Montluçon DB, Haghypour N, Ishikawa NF, Eglinton TI. 2020. Liquid chromatographic isolation of individual amino acids extracted from sediments for radiocarbon analysis. *Frontiers in Marine Science* 7:174.
- Boudin M, Boeckx P, Vandenabeele P, van Strydonck M. 2013. Improved radiocarbon dating for contaminated archaeological bone collagen, silk, wool and hair samples via cross-flow nanofiltrated amino acids. *Rapid Communications in Mass Spectrometry* 27: 2039–2050.
- Brock F, Dee M, Hughes A, Snoeck C, Staff R, Ramsey CB. 2018. Testing the effectiveness of protocols for removal of common conservation treatments for radiocarbon dating. *Radiocarbon* 60:35–50.
- Brock F, Geoghegan V, Thomas B, Jurkschat K, Higham TFG. 2013. Analysis of bone “collagen” extraction products for radiocarbon dating. *Radiocarbon* 55:445–463.
- Brock F, Higham T, Ditchfield P, Ramsey CB. 2010a. Current pretreatment methods for AMS radiocarbon dating at the Oxford Radiocarbon Accelerator Unit (ORAU). *Radiocarbon* 52: 103–112.
- Brock F, Higham T, Ramsey CB. 2010b. Pre-screening techniques for identification of samples suitable for radiocarbon dating of poorly preserved bones. *Journal of Archaeological Science* 37: 855–865.
- Brock F, Ramsey CB, Higham T. 2007. Quality assurance of ultrafiltered bone dating. *Radiocarbon* 49:187–192.
- Broecker WS, Klas M, Clark E, Trumbore S, Bonani G, Wolfli W, Ivy S. 1990. Accelerator mass-spectrometric radiocarbon measurements on foraminifera shells from deep-sea cores. *Radiocarbon* 32:119–133.
- Calandra S, Cantisani E, Salvadori B, Barone S, Liccioli L, Fedi M, Garzonio CA. 2022. Evaluation of ATR-FTIR spectroscopy for distinguish anthropogenic and geogenic calcite. *Journal of Physics: Conference Series* 2204:012048.
- Casanova E, Knowles TDJ, Bayliss A, Walton-Doyle C, Barclay A, Evershed RP. 2022. Compound-specific radiocarbon dating of lipid residues in pottery vessels: a new approach for detecting the exploitation of marine resources. *Journal of Archaeological Science* 137:105528.
- Cersoy S, Zazzo A, Rofes J, Tresset A, Zirah S, Gauthier C, Kaltnecker E, Thil F, Tisnerat-Laborde N. 2017. Radiocarbon dating minute amounts of bone (3–60 mg) with ECHoMICADAS. *Scientific reports* 7:1–8.
- D’Elia M, Gianfrate G, Quarta G, Giotta L, Giancane G, Calcagnile L. 2007. Evaluation of possible contamination sources in the  $^{14}\text{C}$  analysis of bone samples by FTIR spectroscopy. *Radiocarbon* 49:201–210.
- Deviese T, Comeskey D, McCullagh J, Bronk Ramsey C, Higham T. 2018. New protocol for compound-specific radiocarbon analysis of archaeological bones. *Rapid Communications in Mass Spectrometry* 32:373–379.
- Eglinton TI, Aluwihare LI, Bauer JE, Druffel ER, McNichol AP. 1996. Gas chromatographic isolation of individual compounds from complex matrices for radiocarbon dating. *Analytical Chemistry* 68:904–912.
- Fewlass H, Talamo S, Tuna T, Fagault Y, Kromer B, Hoffmann H, Pangrazzi C, Hublin J-J, Bard E. 2017. Size matters: radiocarbon dates of < 200 µg ancient collagen samples with AixMICADAS and its gas ion source. *Radiocarbon* 60:425–439.
- Fewlass H, Tuna T, Fagault Y, Hublin JJ, Kromer B, Bard E, Talamo S. 2019. Pretreatment and gaseous radiocarbon dating of 40–100 mg archaeological bone. *Scientific Reports* 9:1–11.
- France CAM, Sugiyama N, Aguayo E. 2020. Establishing a preservation index for bone, dentin, and enamel bioapatite mineral using ATR-FTIR. *Journal of Archaeological Science: Reports* 33:102551.
- Geyh MA. 2008. The handling of numerical ages and their random uncertainties. *E&G Quaternary Science Journal* 57:239–252.
- Haghypour N, Ausin B, Usman MO, Ishikawa N, Wacker L, Welte C, Ueda K, Eglinton TI. 2019. Compound-specific radiocarbon analysis by elemental analyzer-accelerator mass spectrometry: precision and limitations. *Analytical Chemistry* 91:2042–2049.
- Hajdas I. 2008. The Radiocarbon dating method and its applications in Quaternary studies. *Quaternary Science Journal – Eiszeitalter und Gegenwart* 57:2–24.
- Hajdas I, Ascough P, Garnett MH, Fallon SJ, Pearson CL, Quarta G, Spalding KL, Yamaguchi H, Yoneda M. 2021a. Radiocarbon dating. *Nature Reviews Methods Primers* 1:62.
- Hajdas I, Bonani G, Furrer H, Mader A, Schoch W. 2007. Radiocarbon chronology of the mammoth site at Niederweningen, Switzerland: Results from dating bones, teeth, wood, and peat. *Quaternary International* 164–65:98–105.
- Hajdas I, Bonani G, Thut H, Leone G, Pfenninger R, Maden C. 2004. A report on sample preparation at the ETH/PSI AMS facility in Zurich. *Nuclear Instruments & Methods in Physics Research Section B-Beam Interactions With Materials and Atoms* 223:267–271.
- Hajdas I, Bonani G, Zolitschka B, Brauer A, Negendank J. 1998. C-14 ages of terrestrial macrofossils from Lago Grande di Monticchio (Italy). *Radiocarbon* 40:803–807.
- Hajdas I, Cristi C, Bonani G, Maurer M. 2014. Textiles and radiocarbon dating. *Radiocarbon* 56:637–643.

- Hajdas I, Ivy SD, Beer J, Bonani G, Imboden D, Lotter AF, Sturm M, Suter M. 1993. AMS radiocarbon dating and varve chronology of Lake Soppensee – 6000 to 12000 C-14 Years BP. *Climate Dynamics* 9:107–116.
- Hajdas I, Maurer M, Röttig MB. 2020a. <sup>14</sup>C dating of mortar from ruins of an early medieval church Hohenrätien GR, Switzerland. *Geochronometria* 47:118–123.
- Hajdas I, Maurer M, Röttig MB. 2020b. Development of <sup>14</sup>C dating of mortars at ETH Zurich. *Radiocarbon* 62:591–600.
- Hajdas I, Michczynski A, Bonani G, Wacker L, Furrer H. 2009. Dating bones near the limit of the radiocarbon dating method: study case mammoth from Niederweningen, Zh Switzerland. *Radiocarbon* 51:675–680.
- Hajdas I, Sojc U, Ivy-Ochs S, Akçar N, Deline P. 2021b. Radiocarbon dating for the reconstruction of the 1717 CE Triolet Rock Avalanche in the Mont Blanc Massif, Italy. *Frontiers in Earth Science* 8.
- Hemingway JD, Rothman DH, Grant KE, Rosengard SZ, Eglinton TI, Derry LA, Galy VV. 2019. Mineral protection regulates long-term global preservation of natural organic carbon. *Nature* 570:228–231.
- Hendriks L, Hajdas I, Ferreira ESB, Scherrer NC, Zumbuhl S, Kuffner M, Wacker L, Synal HA, Gunther D. 2018. Combined C-14 analysis of canvas and organic binder for dating a painting. *Radiocarbon* 60:207–218.
- Hüls CM, Grootes PM, Nadeau M-J, Bruhn F, Hasselberg P, Erlenkeuser H. 2004. AMS radiocarbon dating of iron artefacts. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 223–224:709–715.
- Ingalls AE, Pearson A. 2005. Compound-specific radiocarbon analysis. *Oceanography* 18:18–31.
- Kessler NV, Hodgins GL, Butler BM, Kartha PS, Welch PD, Brennan TK. 2022. Tree-ring-radiocarbon dating paraffin-conserved charcoal at the Mississippian Center of Kincaid, Illinois, USA. *Radiocarbon* 65:173–199.
- Lanting JN, Aerts-Bijma AT, van der Plicht J. 2001. Dating of cremated bones. *Radiocarbon* 43:249–254.
- Lebon M, Reiche I, Gallet X, Bellot-Gurlet L, Zazzo A. 2016. Rapid quantification of bone collagen content by ATR-FTIR spectroscopy. *Radiocarbon* 58:131–145.
- Leskovar T, Zupanič Pajnič I, Jerman I, Črešnar M. 2022. ATR-FTIR spectroscopy as a pre-screening technique for the PMI assessment and DNA preservation in human skeletal remains – a review. *Quaternary International*.
- Liccioli L, Fedi M, Carraresi L, Mando PA. 2017. Characterization of the chloroform-based pretreatment method for C-14 dating of restored wooden samples. *Radiocarbon* 59:757–764.
- Lindroos A, Heinemeier J, Ringbom A, Brasken M, Sveinbjornsdottir A. 2007. Mortar dating using AMS C-14 and sequential dissolution: examples from medieval, non-hydraulic lime mortars from the Aland Islands, SW Finland. *Radiocarbon* 49:47–67.
- Lindroos A, Ringbom Å, Heinemeier J, Hodgins G, Sonck-Koota P, Sjöberg P, Lancaster L, Kaisti R, Brock F, Ranta H, Caroselli M, Lugli S. 2018. Radiocarbon dating historical mortars: lime lumps and/or binder carbonate? *Radiocarbon* 60:875–899.
- Major I, Dani J, Kiss V, Melis E, Patay R, Szabó G, Hubay K, Túri M, Futó I, Huszánk R, Jull AJT, Molnár M. 2019. Adoption and Evaluation of a sample pretreatment protocol for radiocarbon dating of cremated bones at HEKAL. *Radiocarbon* 61:159–171.
- Marzaioli F, Lubritto C, Nonni S, Passariello I, Capano M, Ottaviano L, Terrasi F. 2014. Characterisation of a new protocol for mortar dating: <sup>14</sup>C evidences. *Open Journal of Archaeometry* 2.
- McGeehin J, Burr GS, Jull AJT, Reines D, Gosse J, Davis PT, Muhs D, Southon JR. 2001. Stepped-combustion C-14 dating of sediment: A comparison with established techniques. *Radiocarbon* 43:255–261.
- McNichol AP, Lindauer S. 2022. Radiocarbon in the marine environment: an overview. *Radiocarbon* 64:673–674.
- Michalska D, Czernik J, Goslar T. 2017. Methodological aspect of mortars dating (Poznań, Poland, MODIS). *Radiocarbon* 59:1891–1906.
- Nawrocka D, Michniewicz J, Pawlyta J, Pazdur A. 2005. Application of radiocarbon method for dating of lime mortars. *Geochronometria* 24:109–115.
- Nemec M, Wacker L, Gaggeler H. 2010. Optimization of the graphitization process at Age-1. *Radiocarbon* 52:1380–1393.
- Paama L, Pitkänen I, Rönkkömäki H, Perämäki P. 1998. Thermal and infrared spectroscopic characterization of historical mortars. *Thermochimica Acta* 320:127–133.
- Pawelczyk F, Hajdas I, Sadykov T, Blochin J, Caspari G. 2022. Comparing analysis of pretreatment methods of wood and bone materials for the chronology of peripheral burials at Tunnug 1, Tuva Republic, Russia. *Radiocarbon* 64:171–186.
- Quarta G, Hajdas I, Molnár M, Varga T, Calcagnile L, D’Elia M, Molnar A, Dias JF, Jull AJT. 2022. The IAEA forensics program: results of the AMS <sup>14</sup>C intercomparison exercise on contemporary wines and coffees. *Radiocarbon* 64:1513–1524.
- Reimer PJ, Brown TA, Reimer RW. 2004. Discussion: reporting and calibration of post-bomb C-14 data. *Radiocarbon* 46:1299–1304.
- Rubinetti S, Hajdas I, Tarico C, Alessio S, Isella LP, Giustetto R, Boano R. 2020. An atypical Medieval burial at the Monte Dei Cappuccini

- Monastery in Torino (Italy): a case study with high-precision radiocarbon dating. *Radiocarbon* 62:485–495.
- Ruff M, Fahrni S, Gaggeler HW, Hajdas I, Suter M, Synal HA, Szidat S, Wacker L. 2010. On-line radiocarbon measurements of small samples using elemental analyzer and MICADAS gas ion source. *Radiocarbon* 52:1645–1656.
- Scharpenseel H, Becker-Heidmann P. 1992. Twenty-five years of radiocarbon dating soils: paradigm of erring and learning. *Radiocarbon* 34:541–549.
- Stuiver M, Polach HA. 1977. Reporting of C-14 data: discussion. *Radiocarbon* 19:355–363.
- Synal HA, Stocker M, Suter M. 2007. MICADAS: a new compact radiocarbon AMS system. *Nuclear Instruments & Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 259:7–13.
- Tiilikkala K, Fagernäs L, Tiilikkala J. 2010. History and use of wood pyrolysis liquids as biocide and plant protection product.
- van Klinken GJ. 1999. Bone collagen quality indicators for palaeodietary and radiocarbon measurements. *Journal of Archaeological Science* 26:687–695.
- van Klinken GJ, Hedges REM. 1998. Chemistry strategies for organic C-14 samples. *Radiocarbon* 40:51–56.
- Wang S-L, Burr GS, Wang P-L, Lin L-H, Nguyen V. 2016. Tracing the sources of carbon in clay minerals: an example from western Taiwan. *Quaternary Geochronology* 34:24–32.
- Weiner S, Bar-Yosef O. 1990. States of preservation of bones from prehistoric sites in the Near East: a survey. *Journal of Archaeological Science* 17:187–196.
- Welte C, Hendriks L, Wacker L, Haghypour N, Eglinton TI, Günther D, Synal H-A. 2018. Towards the limits: analysis of microscale <sup>14</sup>C samples using EA-AMS. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* 437:66–74.
- Wood R. 2015. From revolution to convention: the past, present and future of radiocarbon dating. *Journal of Archaeological Science* 56:61–72.
- Yizhaq M, Mintz G, Cohen I, Khalaily H, Weiner S, Boaretto E. 2005. Quality controlled radiocarbon dating of bones and charcoal from the early Pre-Pottery Neolithic B (PPNB) of Motza (Israel). *Radiocarbon* 47:193–206.