







SAFE PREPARATION AND DELIVERY OF GRAPHITE TARGETS FOR ^{14}C ANALYSIS: PROCEDURES OF BRAVHO LAB AT BOLOGNA UNIVERSITY

Laura Tassoni^{1*}  • Bernd Kromer²  • Ronny Friedrich³  • Lukas Wacker⁴  • Maurizio Cattani⁵ • Michael Friedrich⁶ • Dragana Paleček¹  • Enrico Pelloni¹ • Kehua Peng¹ • Michael Eric Thomas¹ • Sahra Talamo¹ 

¹Department of Chemistry “Giacomo Ciamician”, Alma Mater Studiorum, University of Bologna, 40126 Bologna, Italy

²Institute of Environmental Physics, Heidelberg University, 69120 Heidelberg, Germany

³Curt-Engelhorn-Centre Archaeometry, Mannheim, Germany

⁴Laboratory of Ion Beam Physics, ETH, CH-8093 Zurich, Switzerland

⁵Department of History and Culture, Alma Mater Studiorum, University of Bologna, 40124 Bologna, Italy

⁶University of Hohenheim, Hohenheim Gardens (772), 70599 Stuttgart, Germany

ABSTRACT. Nowadays, most radiocarbon (^{14}C) laboratories can reliably avoid and remove any possible sample contamination during the pretreatment of organic samples (e.g., bones, charcoal, or trees) thanks to a series of methods commonly used by the radiocarbon community. However, what about the final step, the storage of graphite? Rarely do the laboratories produce their graphite and ship it as pressed targets to accelerator mass spectrometry (AMS) facilities for measurement. Pressed graphite in aluminum targets are vulnerable to contamination, and during shipment or storage, exogenous carbon can be introduced again. Here we report a test on various archaeological sample materials from different environments and different periods (from the past three millennia to the Middle Paleolithic period). We transformed them into graphite, pressed the graphite into targets and sent them to two different AMS laboratories to be dated. We observe that packing details of the targets, extended shipment and storage time may lead to contamination which can be avoided by appropriate packaging in tight metal cans and sealed in vacuum bags. Close cooperation and coordination between our chemistry laboratory and the AMS facilities, high standards in contamination removal, and efficient measurement planning enabled us to obtain reliable ^{14}C ages within a short time.

KEYWORDS: AMS dating, contamination, pretreatment, radiocarbon.

INTRODUCTION

Nowadays, it is well recognized that sample contamination during preparation and radiocarbon (^{14}C) analysis must be avoided to arrive at high-precision and accurate data, especially when we are trying to solve human evolution disputes in the Paleolithic period. The process of avoiding contamination during the pretreatment of organic samples, such as bones, charcoal, or wood, has been widely discussed in several scientific papers (Higham 2011; Talamo et al. 2012, 2021; Brock et al. 2013; Bird et al. 2014; Cercatillo et al. 2021). The Bologna Radiocarbon laboratory devoted to Human Evolution (BRAVHO lab) deals with a variety of samples for ^{14}C dating. The challenge for our laboratory is the critical period of the samples involved in the pretreatment, the Middle to Upper Paleolithic (the period between 55,000 to 30,000 cal BP). Today we have reached a level of confidence where most of the laboratories can truly remove contamination during this first step. However, the graphitization process and the risk of contamination during storage/shipment of graphite have a minor relevance to the contamination topic. Some laboratories pretreat samples and send their extracted material to the AMS laboratory without contamination. On the other hand, it is uncommon that a chemistry lab proceeds with the graphitization and sends graphite to the AMS facility already pressed in targets. This step is the very last phase where there is a

*Corresponding author. Email: laura.tassoni@unibo.it

possibility of contamination, due to the potential introduction of exogenous CO₂ or organic vapor from the atmosphere into the graphite target during storage or shipment (Paul et al. 2016).

At the BRAVHO lab, we acquired the AGE3 graphitization system, and our effort was devoted to test the graphitization, storage time, and commercial shipment of graphite. In this paper, we report on a test with various archaeological samples which were transformed into graphite and pressed into targets at the BRAVHO lab and sent to two different AMS laboratories to be ¹⁴C dated. The data obtained would be used to investigate our graphitization protocol and how shipment/storage could affect the final results of samples from different periods. The two AMS labs chosen for this experiment are the Curt-Engelhorn-Centre Archaeometry, CEZA, Mannheim, Germany (lab code MAMS) and the Laboratory of Ion Beam Physics, ETH, Zurich, Switzerland (lab code ETH) with which we have long-standing collaboration. The experiment confirms that avoiding significant contamination on pressed graphite targets is not an easy task, but our results and observations can also be useful to other chemical laboratories.

MATERIALS AND METHODS

Twenty-one samples were chosen for the test (Table 1). The sample selection considered different materials from various chronological periods to investigate every variability of (old/young) contamination during the experiment. We decided to analyze 11 collagen samples, 3 archaeological charcoal samples, and 7 cellulose samples. The collagen samples include one bone from an Italian site from the 15th to 17th century AD and the other samples are different pieces of the BRAVHO lab background bone (Austrian cave bear). The three charcoal samples are from two periods: Bronze Age and Middle-Upper Paleolithic. Different pieces of BRAVHO lab background wood (Wintersdorf gravel pit, Rhine valley) are used as cellulose samples and they were pretreated via different methods.

All bone samples and backgrounds were extracted using Method 2, as explained in Talamo et al. (2021). The extraction of cellulose from wood was performed using the BABAB method described in Němec et al. (2010) and Cercatillo et al. (2021). For charcoal, we studied several pretreatment methods (Brock and Higham 2009; Southon and Magana 2010; Haesaerts et al. 2013; Santos and Ormsby 2013; Bird et al. 2014) and tested two of them for the pretreatment. The charcoal sample from the Paleolithic period (charcoal A) was split into two parts: one was pretreated using the ABA method, and the other with the ABOx method. The other two samples from the Bronze Age (charcoal B and C) were pretreated using the ABA protocol. To guarantee that no contamination was introduced during this pretreatment, we used our BLK (blank) wood (Wintersdorf) as a background for charcoal, being pretreated by ABA and ABOx methods without the bleaching step at the end. The result of this study led us to define our ABA and ABOx pretreatment methodology for charcoal. Specially, we slightly modified the Bird et al. (1999) protocol by changing the molarity of HCl and by reducing the reaction time in the oxidation step.

ABA METHOD

The samples are crushed to powder or smaller charcoal grains of 20–50 mg. The BRAVHO lab method starts with using 2M HCl at 60°C in a heater block for 1 hr to remove contamination by carbonates; thereafter, we proceed with 1M NaOH at 60°C for 30 min to remove humic acid. This process is repeated until the solution remains clear, with at least one rinse with

Table 1 All results of our first graphitization test. Each sample is linked to the pretreatment method (UF = ultrafiltration), days of storage of unpressed graphite (plus 11 days storage at ETH, 28 days at MAMS) and all results measured at ETH and MAMS labs. In the final columns, the dates are obtained from aluminum capsules graphitized and measured at ETH and MAMS labs. The dates obtained from targets in glass vials broken during shipment are indicated with an asterisk. All dates are reported as uncalibrated values.

Experiment protocol	BRAVHO target results										Results for samples graphitized by the AMS labs					
	ETH (11 days)					MAMS (28 days)					ETH			MAMS		
	Sample type	Pretr. method	Storage days	ETH code	¹⁴ C BP	F ¹⁴ C	MAMS code	¹⁴ C BP	F ¹⁴ C	ETH code	¹⁴ C BP	F ¹⁴ C	MAMS code	¹⁴ C BP	F ¹⁴ C	
10th-17th century AD collagen	BLK	UF	14	116908.5	44864±170	0.0038	54470.1	*43445±300	0.0045	122349.1	45978±198	0.0033	54499.1.1	44521±335	0.0039	
	collagen		14	116908.3	45337±175	0.0035	54471.1	*44379±299	0.0040	122346.1	46460±194	0.0031	54500.1.1	44068±315	0.0041	
			14	116908.4	45905±180	0.0033	54472.1	45566±349	0.0034	122350.1	46059±191	0.0032				
			13	116908.6	44625±165	0.0039	54476.1	43122±346	0.0047							
			13	116908.7	45298±170	0.0036	54477.1	44407±329	0.0040							
			13	116908.8	45565±176	0.0034	54478.1	43506±311	0.0044							
			12	116908.10	44993±172	0.0037	54479.1	*42646±303	0.0049							
			10	116908.11	44101±160	0.0041	54482.1	44622±340	0.0039							
			9	116908.12	44862±169	0.0038	54485.1	*42650±299	0.0049							
			7	116908.9	44523±163	0.0039	54491.1	45633±367	0.0034							
15th-17th century AD collagen		9	120785.1	373±14	0.9547	54490.1	370±22	0.9550	122347.1	364±21	0.9557	54497.1.1	387±21	0.9529		
7th BLK cellulose	BLK	ABA	14	114055.3	44911±171	0.0037	54473.1	43166±289	0.0046	122364.1	48952±227	0.0023	54512.1.1	47893±412	0.0026	
	cellulose	ABA	14	114055.4	44693±166	0.0038	54474.1	44750±369	0.0038							
		ABOX	14	114055.5	46649±192	0.0030	54475.1	41205±727	0.0059	122354.1	49863±242	0.0020	54502.1.1	45822±367	0.0033	
		ABOX	10	120784.2	45060±175	0.0037	54483.1	*46125±385	0.0032	122362.1	50501±250	0.0019	54510.1.1	47028±404	0.0029	
		BABA-B	9	114054.7	47485±197	0.0027	54486.1	46791±430	0.0030	122363.1	52077±277	0.0015	54511.1.1	47159±411	0.0028	
Charcoal A	ABOX	9	120784.3	46078±184	0.0032	54488.1	*43672±334	0.0044	122354.1	49863±242	0.0020	54502.1.1	45822±367	0.0033		
	ABOX	7	120784.1	46357±190	0.0031	54494.1	46919±414	0.0029	122362.1	50501±250	0.0019	54510.1.1	47028±404	0.0029		
	ABA	12	120788.1	31265±172	0.0204	54480.1	30958±371	0.0212	122356.1	31095±130	0.0208	54504.1.1	30109±227	0.0236		
	ABOX	12	120789.1	32952±209	0.0165	54481.1	*32569±449	0.0173	122361.1	32490±151	0.0175	54509.1.1	32548±295	0.0174		
Charcoal B	ABA	9	120790.1	3391±17	0.6557	54487.1	3396±27	0.6552	122357.1	3420±23	0.6533	54505.1.1	3441±25	0.6516		
	Charcoal C	ABA	7	120791.1	3616±17	0.6375	54492.1	3638±27	0.6358	122358.1	3570±23	0.6412	54506.1.1	3593±26	0.6394	

Ultrapure water between each step. This procedure can be repeated up to 3 times. The chemical process ends with a second 2M HCl step to remove any absorbed atmospheric CO₂. At the end of each step, samples are centrifugated for 2 min at 2800 rpm to take out the liquid part easily and then samples are neutralized with three rinses of Ultrapure water. Finally, the glass tubes with samples are covered with aluminum foil with some holes to allow vapor exit and are dried in the oven at 70°C for 1–2 days.

ABOx Method

We took between 100–120 mg of charcoal in this process. The acid-base steps are the same as those explained for ABA methods. The oxidation step is carried out using 0.1M K₂Cr₂O₇ in 2M H₂SO₄ for 6–8 hr in a heater block at 60°C. The reaction time depends on the sample quality, for this reason, it is important to check the progression of the chemical reaction every hour. After each step, the samples are neutralized with three rinses of Ultrapure water and they are finally dried in the oven for 1–2 days at 70°C covered with aluminum foil with holes to allow vapor exit.

After the chemical pretreatment, a graphitization protocol was designed (Table 1), including samples being graphitized on different days and stored unpressed at different times to investigate various contamination sources. Each pretreated sample was prepared in two aluminum capsules (one for each AMS lab involved) for a total of 44 caps. These were graphitized in our lab and in addition, Oxalic Acid II and Phthalic Acid were included in the graphite magazine preparation. Finally, 5 empty aluminum capsules were delivered to each AMS lab to investigate any possible contamination from our capsule cleaning procedure. In order to evaluate our graphitization system and shipment protocol, we asked each AMS laboratory to graphitize our samples with their own system and date them. In this way, we could establish if our graphitization system provides similar values to those graphitized at ETH-CEZA AMS labs.

The ¹⁴C graphitization starts with the sample combustion and elemental analysis performed by the Elementar vario ISOTOPE select (below called EA). The AGE3 (Automated Graphitization Equipment, IonPlusAG, Switzerland) (Wacker et al. 2010) is coupled to the EA and during the graphitization, it converts CO₂ to graphite. The Ionplus AGE3 system contains seven reaction vessels, and the samples were processed in batches of seven at a time. Before each set of measurements, a series of initial EA runs (typically two to five) were performed without a sample to ensure the background levels of carbon and nitrogen were acceptable (0% C and <0.2% N). One phthalic acid sample (2.5–3 mg) was measured before each set of seven samples as a check and to purge the system.

After the reaction, the glass tube with the unpressed graphite was closed with aluminum foil (sterilized at 300°C for 1 hr) and covered with parafilm; then, the tubes were stored in a glass jar closed by its cap (Figure 1a). Finally, we coordinated with each AMS facility to define when samples should arrive in their labs. Immediately before shipment, all graphite samples were pressed inside a sample holder (target) using a pneumatic press (Figure 1b). Three targets for each magazine were closed in an Eppendorf[®] tube, the other targets were shipped using glass vials with snap-cap (Figure 1c).

The samples were placed into two boxes and shipped on the same day. The Zurich AMS lab measured the samples 6 days after their arrival; hence, pressed graphite stayed in targets for 11 days. Unfortunately, some glass vials were broken during the transport to the Mannheim AMS



Figure 1 (a) AGE glass tubes closed with Al foil and parafilm, then stored in a glass jar; (b) targets preparation; (c) targets are closed in glass vials for the shipment.

lab. These were measured nonetheless to test any possible contamination. All samples delivered to MAMS were measured in the AMS 28 days after they arrived at the Mannheim facility, due to delays during Christmas vacation. This variant gave evidence of a longer period of storage in targets of pressed graphite and could help us to define a maximum time for target conservation.

RESULTS AND DISCUSSION

The results for targets graphitized and pressed at BRAVHO laboratory, and samples graphitized and pressed at AMS facilities are shown in Table 1. All dates are reported as uncalibrated dates expressed in BP and only charcoal samples and Italian bone of the 15th to 17th century AD are background corrected. During the shipment to the MAMS AMS facility, some glass vials were broken; the dates obtained from those targets are identified with an asterisk and these are not included in the statistic. Before going deeper into the analysis of the results we report our pretreated collagen and cellulose background ranges for two years obtained before this test: the collagen blanks mean values are $45,731 \pm 163$ (std. dev. $\sigma = 1857$; ETH) and $46,223 \pm 364$ ($\sigma = 1004$; MAMS). For cellulose, we obtained at the ETH AMS lab $48,793 \pm 214$ ($\sigma = 1738$) and $47,206 \pm 434$ at the MAMS lab ($\sigma = 1311$).

BRAVHO Targets vs. Al Capsules

Starting from collagen extracted from bone of the 15th to 17th century AD, the BRAVHO target values (ETH 120785, MAMS 54490) do not differ from those graphitized by ETH and MAMS labs. Concerning the charcoal dates graphitized at BRAVHO lab compared to AMS

labs graphite, results agreed for the paleolithic samples, pretreated using ABA (ETH 120788, MAMS 54480) and ABOx (ETH 120789, MAMS 54481), and for those from the Bronze Age (ETH 120790-91 and MAMS 54487, 54492). The values testify to the success of the graphitization procedure in our laboratory for modern and archaeological samples.

For the background collagen, the comparison between BRAVHO targets measured at ETH (filled black squares in Figure 2a) and those graphitized at ETH (half-filled red squares) shows some differences from the archaeological samples. The mean age (M) of collagen graphitized at BRAVHO is $45,007 \pm 170$ compared to $46,166 \pm 194$ for samples graphitized at ETH lab. The mean age of the BRAVHO targets is slightly lower and this trend is clearly shown in Figure 2a. The results reported from MAMS ($M = 44,476 \pm 340$; half-filled red dots) are identical to the BRAVHO targets results ($M = 44,295 \pm 325$; open black dots).

The mean age of cellulose samples graphitized at ETH is $50,348 \pm 249$ identical to the best background of this lab whereas for the cellulose graphitized by BRAVHO lab, the mean age is $45,891 \pm 182$. The age difference is twice the standard deviation of the measurements (BRAVHO=1037; ETH=1316) so it is significant.

A meaningful source of contamination from the aluminum caps can be excluded because they were analyzed by both laboratories. Therefore, the marginally younger dates detected for BLK collagen and cellulose could be due to two main factors: variable storage times of graphite and unsafe target packaging.

Storage of Graphite

All our BRAVHO target results are represented in Figure 2b organized following the time of storage (expressed in days) as already outlined in Table 1. Neither BLK collagen nor BLK cellulose show any significant variability due to the increase in storage time because most of the values are within the same range. On the other hand, we note that the BRAVHO targets measured at the AMS in Mannheim gave younger dates than those of ETH and younger than samples graphitized in the AMS labs. This result was expected because a long time has passed between the graphitization and the measurement by the MAMS AMS: 11–14 days of storage plus 28 days in the target before the analysis in the AMS. However, it seems not to be a problem of different storage times of graphite because the BLK samples appear to be uniformly contaminated.

Safe Target Packing: Multiple Shipment Tests

The second factor is represented by some targets which arrived at Mannheim in broken vials. Their younger results are quite evident (Figure 2a,b; open black dots/squares with crosses) and demonstrate possible contamination that could affect the targets during the shipment. Based on this first graphitization test, we have decided to study this issue in detail. We wanted to investigate and minimize the contamination of our backgrounds (especially BLK cellulose). We made some shipment tests with phthalic acid ($C_8H_6O_4$) graphitized in our laboratory. These experiments involved changes in packing the targets and the way of shipment: each target was wrapped in aluminum foil, previously baked at $300^\circ C$, and put inside a metal box (Figure 3a). In the final tests, we decided to seal all metal boxes in a vacuum bag (Figure 3b). Using this method, older results were obtained for the blanks compared to our first graphite test and shipment without the vacuum bag (Figure 4a).

This packing procedure was finally verified with our background wood, which was graphitized, pressed within three days, shipped by personal transport within a day and immediately

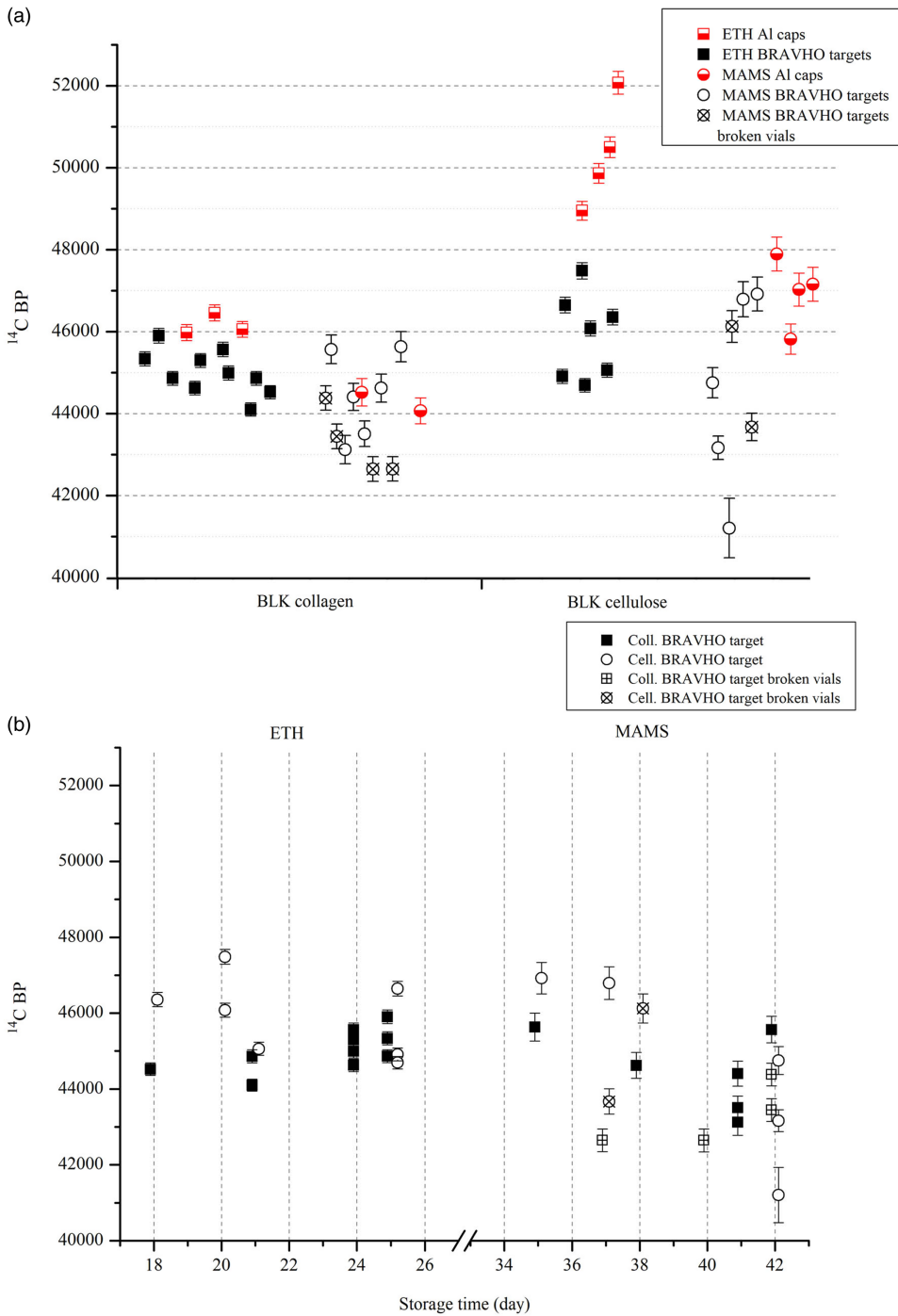


Figure 2 ^{14}C age vs. storage days for BLK collagen and cellulose: (a) comparison between the BRAVHO targets result and samples graphitized at ETH and MAMS AMS labs; (b) distribution of the BRAVHO targets results through days of storage.

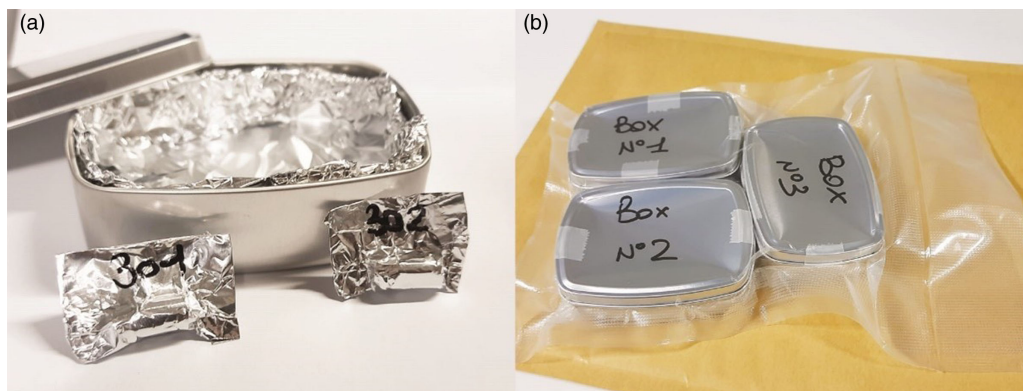


Figure 3 The shipment procedure at the BRAVHO lab: (a) targets wrapped in aluminum foil and closed in a metal box; (b) the metal boxes are sealed in a vacuum bag and shipped to the AMS facilities.

analyzed by AMS labs (July and August 2022). In Table 2 and Figure 4b all values of our background cellulose are reported. Our improvement in shipment packaging allows us to obtain older dates for all samples and fix our main contamination problem related to the shipment procedure.

Charcoal Pretreatment at the BRAVHO Lab

Together with the first BRAVHO graphitization test, our ABA and ABOx methods were tested to pretreat charcoal. This section will only consider the results obtained by the charcoal and BLK cellulose in aluminum caps graphitized by the two AMS laboratories. For charcoal A, we obtained two dates for each AMS lab. For the ABOx method, the ETH value is $32,490 \pm 151$ BP (ETH 122361.1) and $32,548 \pm 295$ BP (MAMS 54509.1.1) from MAMS lab: these two dates agree fully. The same cannot be said for samples pretreated with the ABA method because these were $31,095 \pm 130$ BP (ETH 122356.1) and $30,109 \pm 227$ BP (MAMS 54504.1.1). However, both from ETH and MAMS we obtained older dates with ABOx pretreatment compared to ABA, as was already demonstrated by Haesaerts et al. (2013). As an additional test, new charcoal samples were collected during fieldwork in Portugal (<https://site.unibo.it/resolution-erc/en>); the ABOx method was used to extract carbon. As a potential background, we received an age of $46,499 \pm 139$ BP (ETH 127956.1.1) that proves the usability of the method.

CONCLUSION

As it is well-known in the radiocarbon community, removing potential contaminants in multiple steps requires careful laboratory procedures. Another consideration emerges when pretreated samples are also graphitized in the chemistry laboratory. For this reason, our effort was devoted to maintaining a high standard in avoiding contamination during the production of graphite and shipment of targets to an AMS facility. The first graphite test at BRAVHO lab using the combination of the Elementar Analyser and Automated Graphitization Equipment gave reliable results in the measurement of different types of sample materials. Furthermore, we also tested the accuracy of our charcoal pretreatment methods on samples from different archaeological periods. The graphitization test confirms that avoiding contamination on graphite targets is an arduous goal. It can be minimized by wrapping targets with aluminum

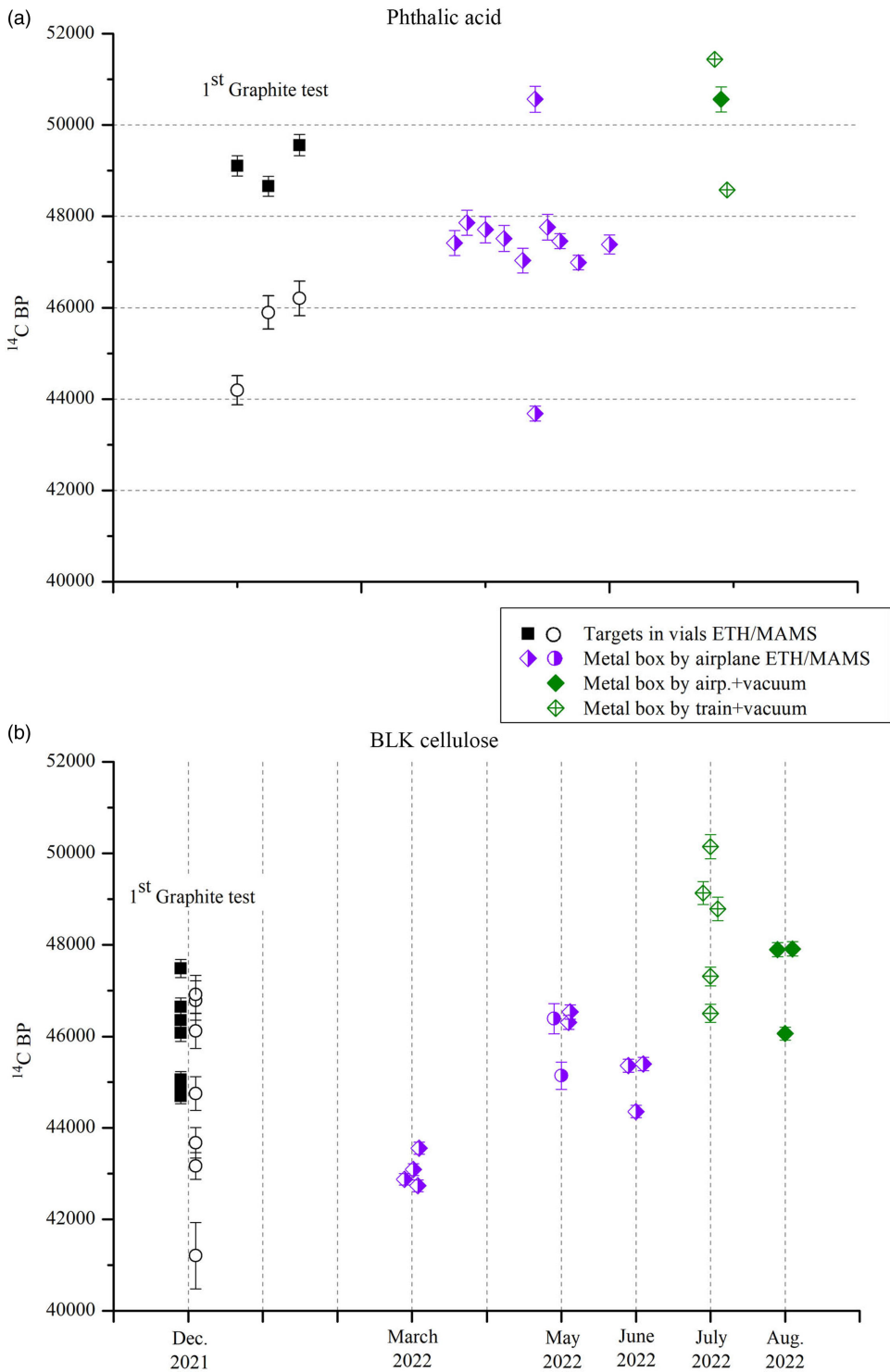


Figure 4 (a) Different shipment tests using phthalic acid graphitized at BRAVHO lab and (b) variation of BLK cellulose results through time using different shipment methods (data listed in Table 2).

Table 2 BLK cellulose ¹⁴C-ages obtained from ETH and MAMS AMS measurements over 6 different shipment/measurement dates, as shown in Figure 4.

BLK cellulose results ¹⁴ C BP through time						
Dec 2021	F ¹⁴ C	March 2022	F ¹⁴ C	May 2022	F ¹⁴ C	F ¹⁴ C
MAMS 54486.1	46791±430	ETH 114054.10	42876±125	ETH 114054.11.1	0.0048	46309±153
ETH 114054.7	47485±197	ETH 114054.8	43555±131	ETH 114054.11.2	0.0044	46535±155
MAMS 54474.1	44750±369	ETH 114054.9	43088±128	MAMS 56500.1	0.0047	45140±300
ETH 114055.4	44693±166	ETH 114055.6	42733±128	MAMS 56501.1	0.0049	46390±330
MAMS 54473.1	43166±289					
ETH 114055.3	44911±171					
MAMS 54494.1	46919±414					
ETH 120784.1	46357±190					
MAMS 54475.1	41205±727					
ETH 114055.5	46649±192					
MAMS 54488.1	43672±334					
MAMS 54483.1	46125±385					
ETH 120784.3	46078±184					
ETH 120784.2	45060±175					
June 2022	F ¹⁴ C	July 2022	F ¹⁴ C	August 2022	F ¹⁴ C	F ¹⁴ C
ETH 125873.1	45401±144	ETH 114054.16	48786±258	ETH 114054.15.2	0.0023	46065±143
ETH 125873.2	44355±135	ETH 114054.17	49135±249	ETH 114054.15.3	0.0022	47913±157
ETH 125873.3	45362±141	ETH 114054.18	50145±264	ETH 114054.15.4	0.0019	47897±153
		ETH 114054.14	47315±205		0.0028	
		ETH 114054.15.1	46506±197		0.0031	

foil and storing them in a metal box. Furthermore, shipping under vacuum in plastic bags is very useful to avoid the introduction of exogenous carbon in an extended interval of shipment.

As a direct consequence, the final step of graphitization should be carried out in the pretreatment laboratory close to the time of shipment to the AMS lab. The vulnerability to further contamination of targets can be delayed by valid packaging techniques. To minimize possible contamination during the waiting period of targets at the AMS laboratory, tight coordination is crucial between the chemistry laboratory and the AMS facility. These efforts, combined with high standards of contamination removal and efficient measurement planning, enabled us to obtain reliable results.

ACKNOWLEDGMENTS

This project is funded by the European Research Council under the European Union's Horizon 2020 Research and Innovation Program (grant agreement No. 803147 RESOLUTION, awarded to Sahra Talamo).

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