INTERPRETING RADIOCARBON DATES FROM THE PALEOLITHIC LAYERS OF THEOPETRA CAVE IN THESSALY, GREECE

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ABSTRACT. Theopetra Cave is a unique prehistoric site for Greece, as the Middle and Upper Paleolithic, Mesolithic, and Neolithic periods are present here, bridging the Pleistocene with the Holocene. During the more than 20 yr of excavation campaigns, charcoal samples from hearths suitable for ^{14}C dating were collected from all anthropogenic layers, including the Paleolithic ones. Most of the samples were initially dated using the ABA chemical pretreatment protocol in the Laboratory of Archaeometry of NCSR Demokritos, Greece, and the Radiocarbon Dating and Cosmogenic Isotopes Laboratory of the Weizmann Institute of Science, Israel. The ^{14}C results, which were not always consistent versus depth, showed that the earliest limit of human presence is ~50,000 yr BP, thus reaching the age limits of the ^{14}C dating method. However, 10 TL-dated burnt flint specimens unearthed from the lower part of the Middle Paleolithic sequence of the cave gave ages ranging between ~110 and 135 kyr ago. These results are in disagreement with the ^{14}C dates, as they support a much later date for these layers. In order to clarify the situation further, charcoal samples originating from hearths were conventionally dated in the Laboratory of Archaeometry of NCSR Demokritos using the ABA pretreatment. Additionally, hand-picked charcoal fragments also underwent ^{14}C dating by AMS in the Oxford Radiocarbon Accelerator Unit using the acid-base wet oxidation (ABOX-SC) pretreatment protocol. The ^{14}C dates from the cave's Paleolithic layers obtained by both pretreatment protocols suggest a probable charcoal diagenesis affecting the ^{14}C results of these very old samples. However, the dates obtained with ABOX-SC pretreatment are considered more reliable and in the younger stratigraphic part produced consistent results with the TL dating.

INTRODUCTION

The long-known Theopetra Cave (21°40′46″E, 39°40′51″N) is a unique prehistoric site for Greece (Figure 1), as the Middle and Upper Paleolithic, Mesolithic, and Neolithic periods are present here, bridging the Pleistocene with the Holocene (Kyparissi-Apostolika 1998, 1999; Facorellis and Maniatis 2000; Facorellis et al. 2001).

Figure 2 shows the established 2 × 2 m square excavation grids that cover the greater part of the cave. The deposits reach their thickest at ~6.40 m in the central area of the cave, while at the periphery they reach a thickness of only 3–3.50 m. The Pleistocene deposits are quite different from the Holocene ones (maximum thickness of 4.5 and 1.5 m, respectively). The Pleistocene sediments entered the cave partly through karstic aquifers and partly through the entrance of the cave. In particular, the sequence of Pleistocene stratigraphy is a thick sandwich of water-lain sediments with intervening deposits of numerous superimposed ashy hearths (Karkanas 1999, 2001). The upper part of the Paleolithic sequence, although represented over the whole excavated area (layers II10-II12), preserve very few recognizable Upper Paleolithic lithic artifacts. The lower part of the Middle Paleolithic sequence (I–II9) is represented by the most clearly stratified lithic assemblages, beginning with the Quina phase in the lower part of the sequence, Levallois in the middle part (Figure 3), and Levallois with an Upper Paleolithic component in the upper part (Panagopoulou 1999, 2000).

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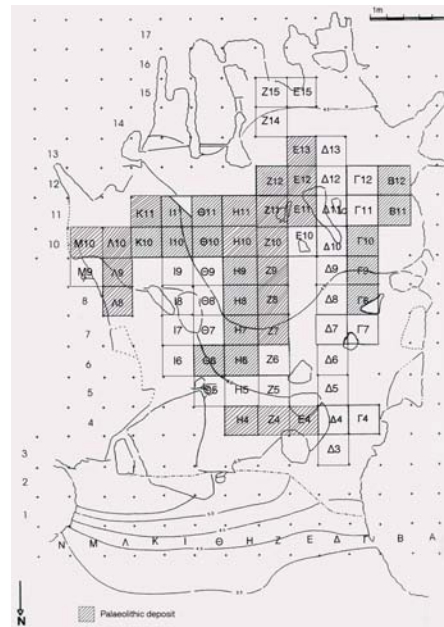Figure 1 Map of Greece showing the location of Theopetra Cave.

Figure 2 Plan of the cave showing the excavation grid and topographic contours at 0.5-m intervals.

Figure 3 Middle Paleolithic tools

During the more than 20 yr of excavation campaigns under the direction of archaeologist Dr Nina Kyparissi-Apostolika, evidence of human activity, such as charcoal samples from hearths suitable for ^{14}C dating, were collected from all anthropogenic layers, including the Paleolithic ones. Initially, the ^{14}C results published by Facorellis et al. (2001) showed that the earliest limit of human presence probably exceeds 50,000 yr BP, thus reaching the age limits of the ^{14}C dating method (Facorellis and Maniatis 1999). Nevertheless, the lowest combustion layers II2 and II4 gave ^{14}C ages in the range 36–46 kyr BP. However, later on, 10 burnt flint specimens unearthed in layers II2 and II4, corresponding to the lower part of the Middle Paleolithic sequence of Theopetra Cave, were dated by the thermoluminescence (TL) method and gave ages ranging between ~110 and 140 kyr ago. Moreover, 1 burnt flint from layer II11 gave a TL age of 57 ± 6 kyr ago (Valladas et al. 2007). The positions of the TL-dated samples are shown in Figure 4. These results, which support a much later date for the

Middle Paleolithic layers, are in disagreement with the ^{14}C dates, and question the assignment of the upper part of the sequence to the Upper Paleolithic that nevertheless was based on very few artifacts.
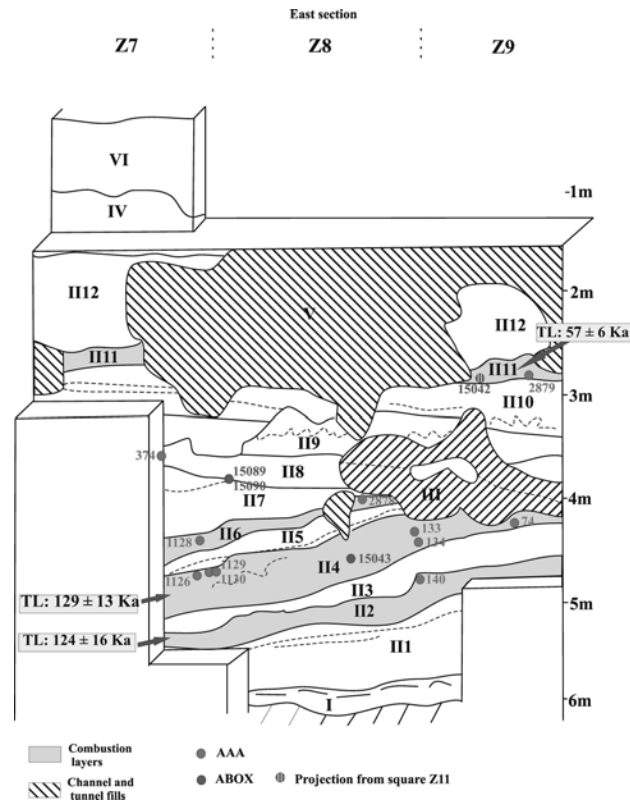

Figure 4 Stratigraphic profile Z7–Z9 showing the positions of the samples and the TL ages.

The depositional sequence of Theopetra Cave is complex with frequently appearing filled channels and underground tunnels as well as labyrinthine large burrows. Resolving the stratigraphy during excavations was very difficult, but all the above features and the thick combustion layers are distinct in appearance and can be easily followed in the excavated profiles. Therefore, in order to clarify the reason for the discrepancies with the ^{14}C ages we wanted first to eliminate the possibility of post-depositional movement of younger charcoal down the sequence by ^{14}C dating more samples, and then to find out if the charcoal was diagenetically modified. In addition, by using anthracological analysis we wanted to see if the possible diagenetic alteration affected the charcoal integrity.

METHODS

The initial group of ^{14}C dates (Facorellis et al. 2001) were derived from 7 charcoal samples (DEM-374, -74, -133, -134, -140, -247 and -613) originating from hearths in the Paleolithic layers. They were dated in the Laboratory of Archaeometry of NCSR Demokritos in Athens, Greece. The removal of any carbon compounds of non-archaeological origin was performed using the acid-base-acid (ABA) chemical pretreatment protocol. This consisted of samples soaking in 4% HCl solution in a water bath at 80 °C for 1 hr to remove soil carbonates and soluble organic compounds, followed

by extraction of the humic acids with 4% NaOH solution for 12 hr. The samples were then re-acidified with 4% HCl in a water bath for 30 min, washed with deionized water to pH 6, oven-dried at 105 °C, and finally ground to fine powder. Samples were then converted to CO_2 using a de Vries-type continuous combustion system (de Vries and Barendsen 1953) and after several purification stages were conventionally measured inside cylindrical gas proportional counters (Facorellis 1996; Maniatis et al. 2010). Another 3 Paleolithic charcoal samples, which also underwent ABA chemical pretreatment, were dated using liquid scintillation counting in the Radiocarbon Dating and Cosmogenic Isotopes Laboratory of the Weizmann Institute of Science in Rehovot, Israel (Karkanas et al. 1999; Karkanas 2001). The samples originate from the neighboring grid squares Z7–Z11 (Figures 5 and 6) and Θ10/10 (Figure 7) sorted by depth. The positions of the samples are shown in Figures 4 and 8, respectively.
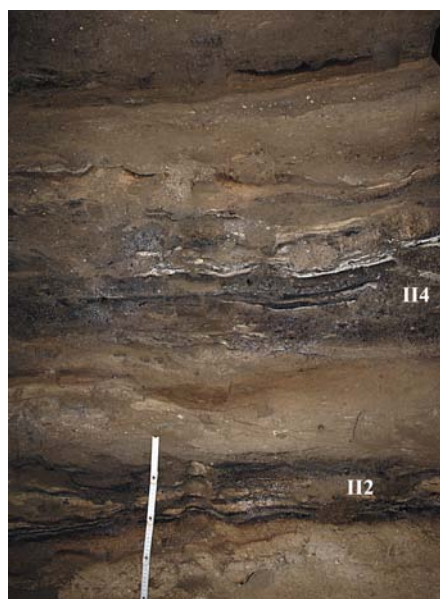

Figure 5 Stratigraphic profile of grid square Z8

Figure 6 Stratigraphic profile of grid square Z9

Figure 7 Stratigraphic profile of grid square Θ10

To clarify the situation further, 9 charcoal samples originating from hearths found in the layers II6, II4, and II2 of the profiles of grid squares Z7 (Figure 4; DEM-1128, -1126, -1129, and -1130) and Θ10 (Figure 8; DEM-1122, -1119, -1123, -1120 and -1121) were conventionally ^{14}C dated in the Laboratory of Archaeometry of NCSR Demokritos using the aforementioned procedure. Additionally, another 12 wood charcoal fragments were handpicked from stratigraphically secure parts of layers II2 to II11 of the profiles of grid squares Z8, Z9, and Z11 with special attention to include combustion features associated with the TL-dated samples. These charcoal samples, previously analyzed for botanical identification of the plant species (mostly *Quercus* sp. and *Prunus* sp.), were sent for accelerator mass spectrometry (AMS) dating to the Oxford Radiocarbon Accelerator Unit, where they underwent the acid-base-wet oxidation (ABOX-SC) pretreatment protocol. This method removes contaminants more efficiently than a standard ABA pretreatment and has been shown to yield significantly older dates for some samples (Bird et al. 1999, 2003; Turney et al. 2001; Santos et al. 2003; Higham et al. 2009a,b; Brock and Higham 2009). The applied pretreatment described by Brock et al. (2010) consists of the following steps. The charcoal samples were roughly crushed before being treated with 6M HCl (room temperature, 1 hr), followed by 1M NaOH (room temperature, 30 min). The base may be replaced with fresh solution 1 or more times during this period if it becomes particularly dark colored. The samples were washed 3 times with ultrapure water after both treatments and then treated with 0.1M potassium dichromate in 2M sulfuric acid in a sealed tube at 60 °C for ~20 hr. The remaining material (described as "oxidation resistant elemental carbon" or OREC by Bird et al. [1999]) was then rinsed 3 times with ultrapure water at 35 °C for 5 min and the fine material discarded. The samples were then freeze-dried before ~20 mg was precombusted at 630 °C for 2 hr in the presence of copper oxide (~200 mg, 4 × 0.5 mm wire) and quartz wool in a sealed evacuated quartz glass tube. Prior to combustion, the copper oxide and quartz wool were heated in an evacuated sealed tube at 850 °C for 8 hr. The remaining OREC sample was then combusted and graphitized as normal for dating.
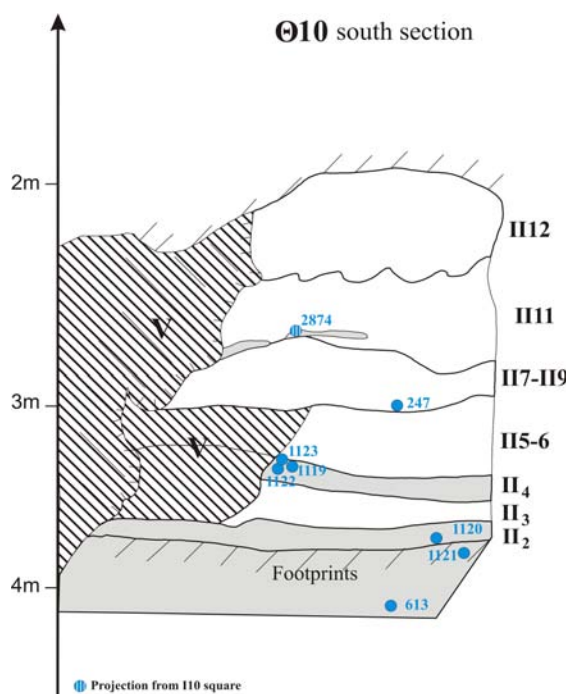

Figure 8 Stratigraphic profile Θ10 showing the positions of the samples.

RESULTS AND DISCUSSION

Table 1 presents the ^{14}C dating results produced by all 3 laboratories. Only 3 of the 12 ABOX-SC pretreated samples (OxA-15042, -15089, and -15043) produced a ^{14}C date as most of them failed in this pretreatment procedure due to low yields, 8 of them during the wet oxidation stage, which is very harsh, and 1 during the precombustion stage. These samples appeared to have been very fragile and were quickly reduced in size. In one case, the same sample was dated twice (OxA-15089: 50,100 ± 1300 yr BP and OxA-15090: 43,400 ± 1500 yr BP). Although it was split in 2 and identically treated as part of the Oxford laboratory's autoduplicate program, undertaken in order to examine routine sample reproducibility, there is poor agreement between the 2 determinations (beyond 2 standard deviations). We argue that the older date (OxA-15089) is more reliable and free of carbon contaminants since the OxA-15090 sample was very small, and hence more susceptible to contaminants, and smaller than the normal AMS standards. Interestingly, the internal structure of the charcoal did not show indications of chemical alteration such as humification, hence its species identification. The δ^{13}C values of these samples are as follows: OxA-15042: δ^{13}C = –21.0‰; OxA-15043: δ^{13}C = –24.0‰; OxA-15089: δ^{13}C = –23.2‰; and OxA-15090: δ^{13}C = –23.6‰.

Table 1 Summary of ^{14}C dating results from the Paleolithic layers of Theopetra Cave. The dates of charcoal samples from the neighboring grid squares Z7–Z11 and Θ10/10 are sorted by depth, reporting also the pretreatment protocol used. The positions of the samples are shown in Figures 4 and 8, respectively. (*Dates extend out of calibration curve range, **Previously published dates).

Lab code	Grid square	Strat. unit-layer	Depth (m)	Age (yr BP)	Calibrated age (cal yr) (1σ, 2σ)	Pretreatment protocol	Reference
OxA-15042	Z11	II11	2.70	45,750 ± 750	50,000*–48,480 50,000*–47,250	ABOX-SC	
RT-2879**	Z9	II11	2.90	25,625 ± 500	30,970–29,870 31,130–29,530	ABA	Karkanas et al. 1999
DEM-374**	Z7	II8	3.62–3.77	32,672 ± 1503	39,260–35,500 41,270–34,810	ABA	Facorellis et al. 2001
OxA-15090	Z8	II8-7	3.70–3.80	43,400 ± 1500	48,320–45,440 49,810–44,890	ABOX-SC	
OxA-15089	Z8	II8-7	3.70–3.80	50,100 ± 1300 [a]		ABOX-SC	
RT-2878**	Z8	II6	3.95	36,550 ± 420	41,900–41,270 42,250–40,950	ABA	Karkanas et al. 1999
DEM-74**	Z9	II4	4.28	38,079 ± 1942	44,490–42,280 47,370–39,400	ABA	Facorellis et al. 2001
DEM-1128	Z7	II6	4.42	>51,000	>50,000	ABA	
DEM-133**	Z8-Z9	II4	4.39–4.49	39,274 ± 4771	48,100–41,220 50,000*–38,940	ABA	Facorellis et al. 2001
OxA-15043	Z8	II4	4.50–4.70	48,000 ± 1100 [a]		ABOX-SC	
DEM-134**	Z8-Z9	II4	4.55	36,827 ± 845	42,410–41,150 43,170–40,370	ABA	Facorellis et al. 2001
DEM-1126	Z7	II4	4.56–4.69	50,180 ± 1500		ABA	
DEM-1129	Z7	II4	4.64–4.70	>50,000	>50,000	ABA	
DEM-1130	Z7	II4	4.64–4.70	>50,000	>50,000	ABA	
DEM-140**	Z8	II2	4.80	39,414 ± 3914	47,520–41,420 50,000*–39,740	ABA	Facorellis et al. 2001
RT-2874**	I10	II11	2.52	25,820 ± 270	30,880–30,400 31,110–30,210	ABA	Karkanas et al. 1999
DEM-247**	10	II7-9	3.03	33,085 ± 1573	40,010–36,400 41,690–35,010	ABA	Facorellis et al. 2001
DEM-1122	10	II4	3.31–3.34	40,030 ± 750	44,570–43,400 45,210–42,900	ABA	

Table 1 Summary of ^{14}C dating results from the Paleolithic layers of Theopetra Cave. The dates of charcoal samples from the neighboring grid squares Z7–Z11 and Θ10/10 are sorted by depth, reporting also the pretreatment protocol used. The positions of the samples are shown in Figures 4 and 8, respectively. (*Dates extend out of calibration curve range, **Previously published dates). *(Continued)*

Lab code	Grid square	Strat. unit-layer	Depth (m)	Age (yr BP)	Calibrated age (cal yr) (1σ, 2σ)	Pretreatment protocol	Reference
DEM-1119	10	II4	3.28–3.34	45,803 ± 1200	50,000*–48,080 50,000*–46,760	ABA	
DEM-1123	10	II4	3.35	44,369 ± 1539	49,020–46,280 50,000*–45,670	ABA	
DEM-1120	10	II2	3.52–3.68	43,294 ± 1000	47,600–45,460 49,180–45,020	ABA	
DEM-1121	10	II2	3.57–3.66	43,420 ± 2000	48,660–45,350 50,000*–44,710	ABA	
DEM-613**	10	II2	4.07–4.17	46,327 ± 1590	50,000*–48,110 50,000*–46,700	ABA	Facorellis et al. 2001

^{a}Date out of calibration range.

The conventional ^{14}C ages were calibrated with the IntCal09 calibration curve (Reimer et al. 2009) using OxCal v 4.1.7 software (Bronk Ramsey 2009) (Table 1). Figures 9 and 10 present the probability distribution plots of the calibrated dates, wherever this was possible, from the grid squares Z7–Z11 and Θ10/I10, respectively, sorted by depth. Some dates appear truncated at 50,000 cal yr BP as they extend beyond the range of the calibration curve. The dates shown in gray and black correspond to those produced using the ABA and ABOX-SC chemical pretreatment protocols, respectively. Figure 11 presents bar plots of the uncalibrated ^{14}C ages from grid squares Z7–Z11 (see Figures 4, 5, and 6) and Θ10/I10 (see Figures 7 and 8) versus depth. Similarly, the gray and black bars correspond to ages produced using the ABA and ABOX-SC chemical pretreatment protocol, respectively. The closed and open bars represent 1 and 2 standard deviations, respectively. The samples (DEM-1128, -1129, and -1130) shown with dashed bars gave background measurements. In several instances, there is no consistency of the obtained ABA ages originating from the same square versus depth (Figure 11). In Table 1 and Figure 11, one can see that the ages of the samples from grid squares Z7–Z11 dated at the Oxford AMS facility produced by the ABOX-SC pretreatment protocol are consistently older in relation to their depth and closer to the limit of the ^{14}C dating method than those produced by the ABA method, and so they may be considered as more reliable.

According to the TL date (57 ± 6 ka) of layer II11 (Figure 4) all charcoal samples originating from layers II11 and below should have produced results beyond the ^{14}C dating method limit, but instead, as mentioned above, only 3 samples gave background measurement. However, the age of the ABOX sample OxA-15042 that originates from this layer (11) overlaps within 2 standard deviations with the aforementioned TL date. The fact that the ABA pretreated samples produce younger ages than the ABOX-SC pretreated ones is evidence that they are contaminated with more recent carbon. This contamination can be very important as it is for instance in the case of the samples RT-2879 and OxA-15042, both originating from layer II11 (Figure 4), producing an age difference of ~20,000 yr (RT-2879: 25,625 ± 500 and OxA-15042: 45,750 ± 750 yr BP).

Although 3 of the charcoal samples from the lower layers II2 and II4 that TL dated to ~110–140 kyr ago failed in the ABOX-SC pretreatment procedure, 1 sample from layer II4 gave an age of 48,000 ± 1000 yr BP (OxA-15043). We note that the sample is located 1.8 m below sample OxA-15042 coming from layer II11 and dated to 45,750 ± 750 yr BP (Figure 4). The paleoenvironmental data of Theopetra Cave based on charcoal and phytolith analysis (Ntinou and Kyparissi-Apostolika
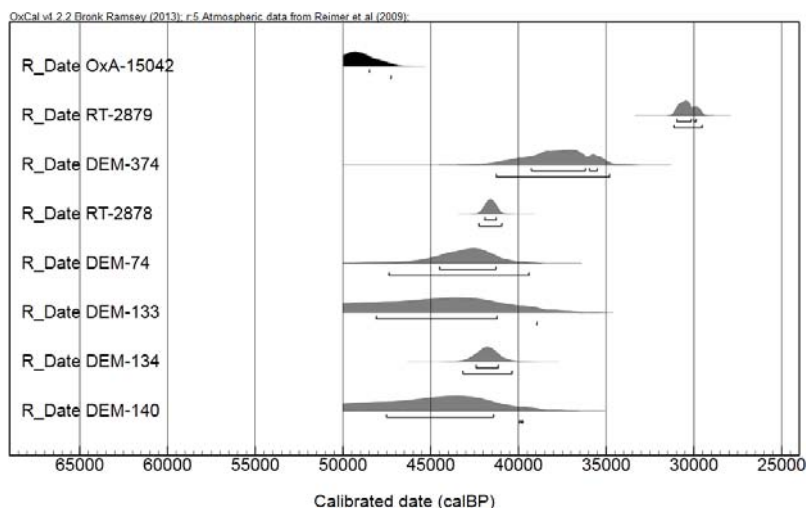
Figure 9 Probability distribution plot of the calibrated dates from the grid squares Z7–Z11 sorted by depth (see Figures 4, 5, and 6). The dates shown in gray and black correspond to those produced using the ABA and ABOX-SC chemical pretreatment protocols, respectively. Dates truncated at 50,000 cal yr BP extend beyond the range of the calibration curve.
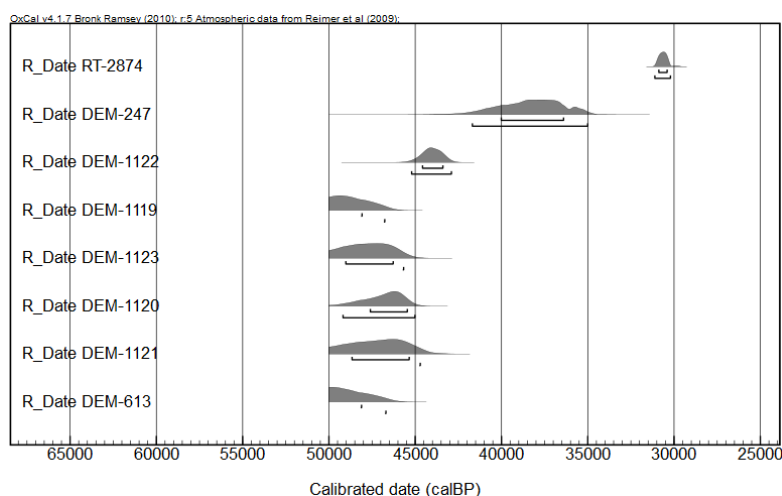

Figure 10 Probability distribution plot of the calibrated dates from grid squares Θ10 and I10 sorted by depth produced using the ABA chemical pretreatment protocol (see Figures 7 and 8). Dates truncated at 50,000 cal yr BP extend beyond the range of the calibration curve.

2008; Tsartsidou 2013) show that layers II2–II7 are associated with warm interglacial conditions followed by harsh cold climatic conditions in layers II8–II12 also characterized by intensive freeze-thaw activity (Karkanas 2001). Layer II11 represents slightly milder conditions probably associated with an interstadial. Therefore, given the stratigraphic position and paleoenvironmental data it is highly unlikely that layer II4 could be of the same age as layer II11, as the ^{14}C age of sample OxA-15043 suggests. In this line of evidence, the TL dates of layers II2–II4 are more consistent with the paleoenvironmental analysis than the age of sample OxA-15043.
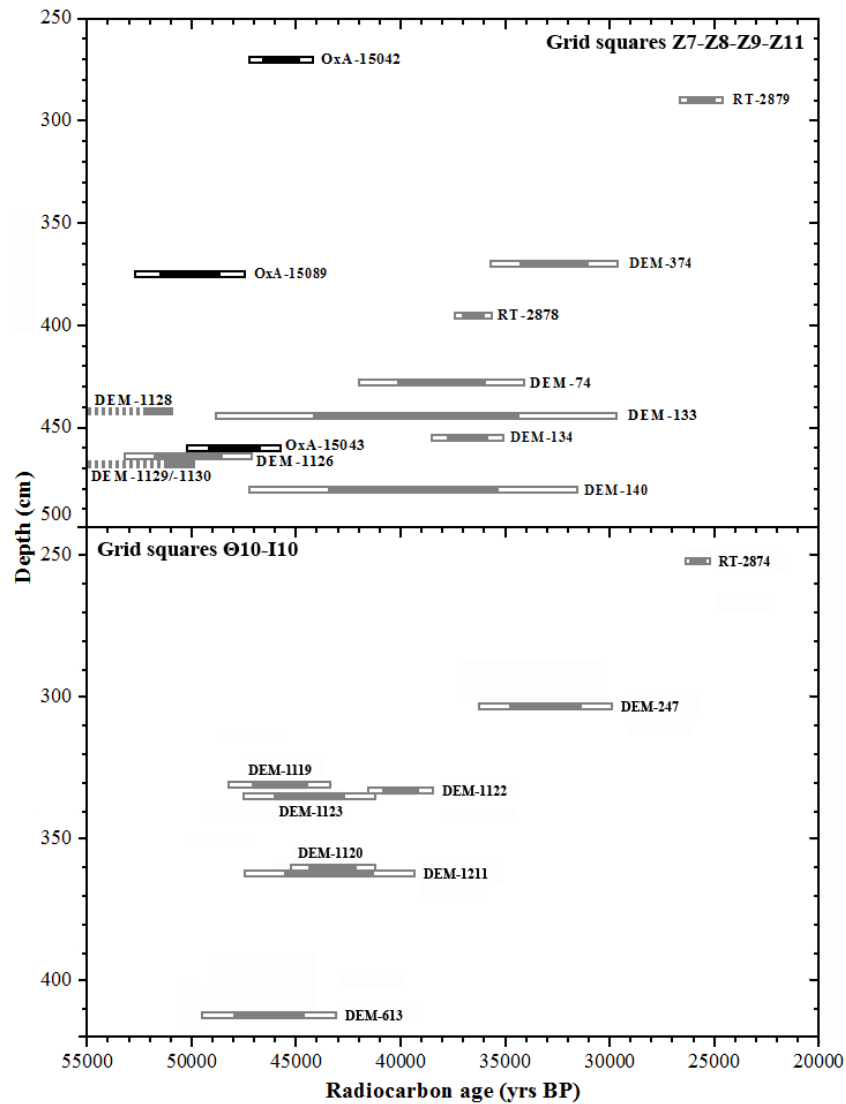
Figure 11 Bar plot of the uncalibrated ^{14}C ages from grid squares Z7–Z11 (see Figures 4, 5, and 6) and Θ10-I10 (see Figures 7 and 8) versus depth. The gray and black bars correspond to ages produced using the ABA and ABOX-SC pretreatment, respectively. The closed and open bars represent 1 and 2 standard deviations, respectively. The samples shown with dashed bars gave background measurements.

Consequently, the aforementioned analytical data raise questions about these very old charcoal samples' integrity and homogeneity, suggesting possible contamination with exogeneous carbon through diagenesis (Alon et al. 2002; Cohen-Ofri et al. 2006, 2007; Ascough et al. 2011). It is well established by sediment micromorphological analysis that the Pleistocene sediments underwent an intense diagenetic mechanical and chemical alteration related to the cave's hydrological regime. More specifically, the amount of phosphates increased during cold episodes in the cave also affected the underlying deposits. These phosphatization episodes have strongly cemented the sediments, which are characterized by the presence of the phosphate minerals leucophosphite, taranakite, and

variscite, which are known to form in low-pH environments and are probably caused by the accumulation of large amounts of guano in the cave (Karkanas 1999, 2001), which eventually could have been a possible source of the charcoal's organic contaminants. Nevertheless, it appears that the ABOX-SC pretreatment protocol was able to eliminate most of this contamination and produce ages that are more reliable particularly in the stratigraphically younger layers.

CONCLUSIONS

Twenty-three charcoal samples originating from hearths excavated in the Pleistocene layers of Theopetra Cave were ^{14}C dated by 3 different ^{14}C laboratories (Laboratory of Archaeometry of NCSR Demokritos in Athens, Greece; Radiocarbon Dating and Cosmogenic Isotopes Laboratory of the Weizmann Institute of Science in Rehovot, Israel; Oxford Radiocarbon Accelerator Unit, UK) using the ABA and the ABOX-SC pretreatment protocols. Comparison between the combined ^{14}C dates with the much older TL dates of 11 burnt flint specimens from the lower part of the Middle Paleolithic sequence indicates that most of the charcoal samples have been contaminated by a progressively increasing versus depth unidentified amount of exogeneous carbon thus yielding more recent dates. This is most probably attributed to the severe chemical alteration of the cave's Paleolithic layers that caused charcoal diagenesis affecting the ^{14}C results of these very old samples. Diagenesis seems not to have had any visible effect in the microscopic structure of charcoal. However, the ^{14}C dates obtained with the ABOX-SC protocol are more reliable and in the younger stratigraphic part of the cave are more consistent with the TL results.

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