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# The Development of a Novel and Potentially Nondestructive Pretreatment for the Radiocarbon Dating of Archaeological Artifacts

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THE DEVELOPMENT OF A NOVEL AND POTENTIALLY NONDESTRUCTIVE  
PRETREATMENT FOR THE RADIOCARBON DATING OF ARCHAEOLOGICAL  
ARTIFACTS

by

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Thesis

Submitted to the Department of Chemistry

Eastern Michigan University

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for the degree of

MASTER OF SCIENCE

in

Chemistry

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## **DEDICATION**

I would like to dedicate this thesis to my husband Rob for his continuous and much appreciated support.

## **ACKNOWLEDGEMENTS**

I would like to thank my research advisor, Dr. Ruth Ann Armitage for her guidance and assistance throughout this process. I would also like to thank my thesis committee members, Dr. Lindsay and Dr. Edwards, for their support. Also, I thank Jamie Brown-Sinha for her help with using the instruments.

## ABSTRACT

Because the traditional acid-alkali-acid method for pretreating archaeological artifacts prior to radiocarbon dating is destructive to most materials, the possibility of developing an alternative pretreatment is important to preserving culturally- and historically-significant artifacts. The alkali step usually involves a 1M or higher concentration NaOH wash to remove humic acid contamination. The purpose of this work is to replace the alkali step with an equally effective, yet less harsh treatment. Charcoal soaked in a humic acid standard was used to mimic a contaminated artifact. Residues from treatments with NaOH, phosphate buffer (pH 8), and deionized water were characterized using THM-GC-MS. Preliminary results show that both NaOH and phosphate buffer are at least equally effective. The phosphate treatment was also used on two archaeological artifacts that were prepared nondestructively by using the plasma chemical oxidation (PCO) pretreatment. The resulting radiocarbon dates from these were statistically indistinguishable from dates produced by the traditional treatment process coupled with standard destructive combustion.

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# CHAPTER 1

## INTRODUCTION

### 1.1 Introduction to accelerator mass spectrometry radiocarbon dating (AMS) and plasma chemical oxidation (PCO)

Willard F. Libby developed a method in the 1940s for determining the age of organic materials, called radiocarbon dating<sup>1</sup>. The technique is based on the fact that carbon occurs in nature as three isotopes,  $^{12}\text{C}$ ,  $^{13}\text{C}$ , and  $^{14}\text{C}$ . Carbon-14 is radioactive and decays with a half-life of about 5,730 years. When an organism dies, it stops taking in new carbon of any isotope and all the carbon-14 remaining in it continues to decay. All organisms are assumed to take in a certain amount of carbon-14 in their lifetimes. The ratio of carbon-14 to other isotopes present in organisms is known and remains constant until death. By measuring the amount of carbon-14 left in organic material, the time since its death can be determined.<sup>2</sup>

The conventional radiocarbon dating technique requires amounts of material in the range of grams. The carbon in the sample combines with oxygen in a combustion reaction to form carbon dioxide and then is typically reduced to form benzene. The radiation resulting from the  $\beta$ -decay of carbon-14 in the sample is measured either by gas proportional counting or liquid scintillation counting. The sensitivity of the technique is limited, and is only applicable to a maximum age of 40,000 to 50,000 years.<sup>2</sup>

Developments in the 1980s led to accelerator mass spectrometric (AMS) radiocarbon dating, a technique which requires only fractions of milligrams of material to produce a reliable date.<sup>3,4</sup> AMS dating uses tandem accelerators and mass spectrometry to count the numbers of different carbon isotopes in a sample. AMS dating has proven to

provide results as accurate as conventional techniques and with far less sample (micrograms for AMS, as opposed to grams for conventional  $^{14}\text{C}$  dating).

One problem with AMS dating is that because of the very small sample size, contamination by older or younger carbon can drastically affect the results.<sup>5</sup> Contaminating carbon comes from inorganic sources, such as carbonates and oxalates, and from organic sources, such as humic acids. Humic acids are large, complex organic molecules, which are ubiquitous in all soils around the world.<sup>6</sup> They result from the decay of organic material and represent one step in the humification process and soil diagenesis. Compounds that can be expected to make up humic acids include decay products of fatty acids, proteins, carbohydrates, and nucleic acids.<sup>6</sup> Research into the characterization of humic acids has determined that they contain a wide assortment of organic molecules including alkanes, alkenes, aromatics, hydroxyl- and methoxybenzoic acids, and fatty acids.<sup>7,8,9</sup> Lipids are one of the main components of humic acids because of their stability over time. Amino acids and carbohydrates are found less frequently as components due to their rapid degradation in the soil.<sup>7</sup>

Based on years of research, the International Humic Substances Society (IHSS) has developed a standard method for extracting humic acids from soil. The IHSS technique uses 1 M NaOH and 0.1 M KOH to dissolve and extract humic acid from soils.<sup>10</sup> This standard technique of using bases to dissolve humics is used in conjunction with other steps to remove all contaminating carbon from archaeological samples to be radiocarbon dated. In the first step of this acid-alkali-acid (AAA) treatment, inorganic carbon, typically present as carbonates, is removed by treating the sample with an acid wash. Next, the humic acids are dissolved with base. Finally, another acid wash

removes any carbon dioxide that has adsorbed to the now-basic sample from the atmosphere.<sup>11</sup> Each step of this process is destructive to the material being treated because of the high acidity and alkalinity of the washes used. Typically, 1M NaOH and 1M HCl solutions are used. The AAA pretreatment method has other problems as well. Studies have shown that the acid wash does not always sufficiently remove oxalate contamination, particularly in the case of rock painting samples.<sup>12</sup>

The Rowe group at Texas A&M University developed an alternative method of pretreating samples using a low temperature, low pressure oxygen plasma called plasma chemical oxidation (PCO). The technique was developed to radiocarbon date the very small samples available from prehistoric rock paintings.<sup>13</sup> The low temperature plasma selectively oxidizes organic carbon to CO<sub>2</sub>, which is then collected for AMS dating. Carbonates and oxalates do not decompose in the plasma and so do not affect the radiocarbon date.<sup>14,15</sup> In traditional radiocarbon dating, the sample is combusted to CO<sub>2</sub> at high temperatures. Inorganic carbon in the sample, such as, carbonates and oxalates are also combusted in this process, so the acid washes to remove them first are necessary. The oxygen plasma in the PCO technique will combust only organic carbon, rendering the acid wash unnecessary. It does not, however, distinguish between organic carbon from the sample and organic carbon from humic acids and other contaminants.<sup>14,15</sup> The alkali step, it is assumed, is still needed to ensure accurate radiocarbon dates.<sup>16,17</sup>

## 1.2 Introduction to thermally-assisted hydrolysis and methylation gas chromatography-mass spectrometry (THM-GC-MS)

Challinor<sup>18</sup> first developed thermally-assisted hydrolysis and methylation (THM) to analyze polyesters. Using tetramethylammonium hydroxide (TMAH), he methylated compounds of interest to identify them using pyrolysis GC-MS. Since then, TMAH has been used as a sample preparation technique to better identify waxes, resins, and fatty acids.<sup>19</sup> These studies have shown that this method is simple and fast; preparation involves simply adding a small volume of TMAH to the sample before injection.

As fatty acids are a major component of humic acids, THM is well suited to the study of humic acid contamination. The alteration to methyl esters makes the lipids more volatile, which is necessary when using gas chromatography. This technique has been applied to the study of resins, lipids, waxes, carbohydrates, proteins, humic substances, and soil.<sup>20</sup>

At high temperatures and in the presence of the derivatizing agent tetramethylammonium hydroxide (TMAH), the carboxyl groups of fatty acids are converted to fatty acid methyl esters (FAMES).<sup>19,20</sup> Figure one shows the mechanism by which a compound, A-B, undergoes hydrolysis, forms tetraalkylammonium salts, and finally the alkyl derivative.<sup>18</sup> In a study by Lehtonen et al.<sup>21</sup> of different derivatizing agents, tetramethyl, tetraethyl, and tetrabutylammonium hydroxide (TMAH, TEAH, TBAH) were all investigated. The researchers found that for studying fatty acids by GC-MS, TMAH was most effective. The TEAH and TBAH did not alkylate compounds as extensively as TMAH. In a similar study, Drechsel et al.<sup>22</sup> used several different derivatizing agents on fatty acids and analyzed the products using pyrolysis GC-MS.

They found that using TMAH yielded 90% of the fatty acids that were expected to appear in the chromatogram.

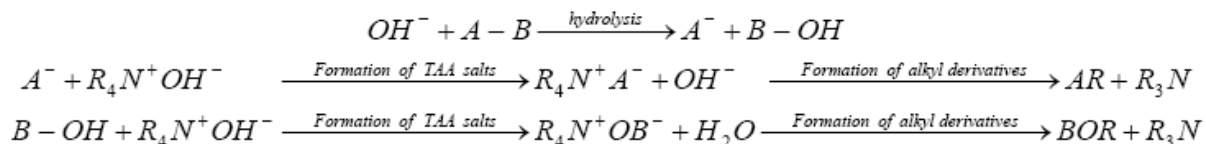


Figure 1. The mechanism for hydrolysis and methylation of compounds by tetraalkylammonium salts at high temperatures. (after reference 18)

### 1.3. Review of the literature

The ability to accurately date archaeological artifacts is essential to understanding ancient cultures. The development of accelerator mass spectrometry (AMS) for radiocarbon analysis allows for reliable dating of as little as 0.1 mg of carbon. With this technique, archaeologists can minimize the destruction of artifacts by using very small samples, as well as date small, residual materials like rock paintings and food residues.<sup>23</sup> Another problem, however, still remains. Any sample that is to be radiocarbon dated needs to be free of carbon unrelated in time to the artifact being dated. Dates obtained for contaminated samples may be older or younger, depending on the source of the contamination. For example, humic acids must be removed as they originate from decomposition of organic matter in soils; such soils may pre- or post-date the event to which the artifact represents.

Two main techniques exist for dealing with contamination. Compounds of interest may be isolated from the sample, as is commonly carried out for collagen from bones. Collagen is the proteinaceous fraction of bone that is less subject to diagenesis

than the carbon-bearing hydroxyapatite mineral fraction<sup>24</sup>. Dating of inorganic ceramic materials has been attempted by extracting from potsherds the residues of their use, typically hydrophobic lipids<sup>25</sup> and occasionally proteins<sup>26</sup> and directly AMS dating the compounds themselves. In both the cases of bone and ceramics, some portion of the original artifact or remains must be destroyed. Some studies have found the results of directly dating extracted lipids to be inconclusive. Stott et al.<sup>27</sup> found that palmitic acid (C<sub>16:0</sub>) and stearic acid (C<sub>18:0</sub>) yielded different dates, neither of which correlated well with the stylistic age of the ceramics from which they were extracted. Mori et al.<sup>28</sup> dated a pictograph in Western Libya by extracting amino acids using alkali and acid solvents. The resulting amino acids were separated into different fractions using HPLC. The fractions were dated using AMS and were found to have dates consistent with inference from archaeological data and with reconstruction of the region's climate.

The other option is to remove contaminating carbon from the sample. Charcoal is perhaps the most widely radiocarbon dated material; its porous structure makes it particularly susceptible to contamination from water-borne humic substances, as well as dissolved carbonates that surround the charcoal in the burial environment. Standard AAA pretreatments described in Section 1.1 were developed to remove these ubiquitous contaminants from large samples of charcoal, leaving behind the cellulosic lignin for dating. Such pretreatments have also been applied to rock paintings prior to applying PCO-AMS dating. For charcoal pigments, pretreatment is appropriate in most cases. However, the effect of the treatments on uncharacterized binders remains unknown.

Van Klinken et al.<sup>15</sup> reviewed the results obtained by both removing compounds to date and removing contamination and concluded that in most cases, the latter technique



is preferred. They concluded from reviewing various studies, that the traditional AAA cleaning method was most successful in removing humic acids from the sample. Because some of the isolated collagen in bone samples was complexed to humic substances, isolation of collagen was not ideal.

### *1.3.1. Previous studies on effective pretreatment methods*

Little work has actually been done on investigating the effectiveness of the AAA treatment, let alone any alternative pretreatments. Gillespie et al.<sup>29</sup> dated volcanic ash at a site in New Zealand. They found that using the traditional AAA treatment led to dates that were younger than expected. Because the carbon they dated was in very fine-grained volcanic sediments, the humic acids were particularly difficult to remove. They found that the only way they could achieve the expected maximum age of the sample was to pretreat the ash with a complex series of steps using a hot, 70% nitric acid solution as well as chlorate oxidation. Santos et al.<sup>30</sup> dated wood of known age after different pretreatments in order to determine the best method for cleaning a sample. They found that the first acid step and the base step of AAA followed by a wet oxidation with  $K_2Cr_2O_7$  to replace the final acid step gave the expected dates. Using the AAA treatment alone resulted in dates that were younger than expected, implying that the traditional pretreatment does not sufficiently remove contamination. In another test, the researchers used their new technique with wet oxidation, but left out the alkali wash step. This treatment resulted in younger than expected dates. They concluded from this, that the alkali step is necessary to remove younger carbon from humic acid contamination. Cook et al.<sup>31</sup> found that time rather than NaOH concentration determined the amount of humic

acid contamination removed from peat samples that were to be radiocarbon dated, though the destructiveness of the treatment was not an issue with the large amounts of material used in this study. Turnbull et al.<sup>32</sup> found that the radiocarbon dates of AAA-treated textiles and those obtained on CO<sub>2</sub> generated selectively from treating amino acids with ninhydrin were comparable, yet destructive and only useful for samples containing amino acids.

Alon et al.<sup>33</sup> used Raman spectroscopy to determine the effectiveness of the AAA method on charcoal. They found that by using Raman, they could examine very small amounts of sample nondestructively to see if contamination had been removed. They did this by comparing the relative amount of humic substances on the cleaned charcoal to the amount in modern charcoal. Tatzber et al.<sup>51</sup> compared humic acid extraction yields from soil using NaOH, Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, and Na<sub>2</sub>CO<sub>3</sub>. They found that NaOH extracted less humic acids than Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> or Na<sub>2</sub>CO<sub>3</sub>. Nakamura et al.<sup>34</sup> dated pottery fragments with AMS and used the traditional AAA pretreatment. They used a fairly low concentration, 0.2 M NaOH, for the humic removal step because they had a very limited sample size. The dates were in the expected range, based upon the style of pottery, in spite of the low NaOH concentration. In dating cave paintings, Valladas<sup>35</sup> also used lower concentrations of base with successful results. She dated cave paintings using AAA pretreatments. One sample was treated with strong acid and base, and another with weaker acid and base. She found that each sample resulted in comparable dates, demonstrating that lower concentrations of acid and base may clean samples effectively.

### *1.3.2. Plasmas applied to art and archaeological materials*

Plasmas have been frequently used to clean objects including archaeological artifacts. Daniels<sup>36</sup> used a plasma to remove tarnish from the silver in daguerreotypes, which are old photographs with the image exposed on a polished silver surface. The plasma was able to remove tarnish without removing any of the silver and without destroying the image. Patscheider et al.<sup>37</sup> used a low pressure hydrogen plasma to remove chlorides from iron artifacts. Rutledge et al.<sup>38</sup> used a low pressure oxygen plasma to remove smoke damage from an ink drawing and from a painting. They found that the plasma cleaned the works without visibly damaging the surfaces. Saettone et al.<sup>39</sup> used argon plasma sputtering to clean artifacts from the Royal Tomb of Sipan. They found that the artifacts were satisfactorily cleaned, but that oversputtering could remove more of the artifact than was intended. The PCO method developed by the Rowe group has been used extensively to selectively remove organic carbon from rock paintings to be radiocarbon dated as described in Section 1.1.

### *1.3.3. GC-MS for analysis of art and archaeological materials*

GC-MS is the single most widely used method for characterizing the organic fractions of art and archaeological materials. Craig et al.<sup>40</sup> used solvent extraction and catalytic hydrolysis (pyrolysis with high pressure hydrogen gas) to extract bound lipids from the interiors of archaeological ceramics. The resulting fractions were identified with GC-MS. Fezzey et al.<sup>41</sup> used pyrolysis- and THM-GC-MS to identify the source of a black coating covering rock paintings in Little Lost River Cave in Idaho. They compared the coating to humic acid, cooking residue, and amberat from pack rats. They concluded that the coating was humic acid, and therefore had a geologic origin. Scalarone et al.<sup>42</sup> used THM-GC-MS to analyze the results of aging on copal and sandarac, two natural resins often found in archaeological materials. With this method, they were able to identify diterpenoid acids in the resins.

Andreotti et al.<sup>43</sup> identified lipids from oils, waxes, and resins in paintings with GC-MS. To identify specific lipids, they used the chromatographic profiles as well as characteristic marker compounds. Cappitelli<sup>44</sup> used THM-GC-MS to identify the components of binding media in 20<sup>th</sup> century art. She identified several oils by determining the palmitic to stearic acid ratio. Buonasera et al.<sup>45</sup> extracted lipids from burned cooking rocks from an archaeological site in Texas. They then used GC-MS to identify several long chain fatty acids, such as C<sub>14</sub>, C<sub>16</sub>, and C<sub>18</sub>. Spades and Russ<sup>46</sup> quantified the lipids in samples of Pecos River Genre rock paintings, which were the first to be dated using the Rowe group's PCO-AMS method. They found no more lipids in the paint samples than was observed in samples of unpainted substrate from the same site, indicating that if any binder does remain in these paints, it does not consist of lipids.

There are many different techniques used to pretreat artifacts, but exactly how well they work remains unclear. The best approach may be to pretreat using methods that are known to be less destructive than NaOH and HCl washes, such as PCO and lower pH alkali solutions.

#### **1.4 Significance of project**

This project was significant because it is important to preserve the structural integrity of historically and culturally significant artifacts. Earlier projects have produced alternative techniques to the acid steps in the traditional cleaning method. Only a replacement for the alkali step was needed to make the plasma-chemical oxidation process, which is inherently less destructive, more reliable.

#### **1.5 Objectives**

The objectives of this project were to evaluate the effectiveness of the traditional alkali wash in removing humic substances from artifacts and to develop a new, less destructive, but equally effective wash. Any new treatment that removes contaminants as well as the standard AAA treatment, yet preserves the integrity of the material being treated, is a significant step in developing a non- or at least minimally-destructive radiocarbon dating process.

## CHAPTER 2

### EXPERIMENTAL

#### 2.1 Instrumentation

The instrument used for this project was the Varian 3800 gas chromatograph and Saturn 2200 ion trap mass spectrometer. The Varian ChromatoProbe was used for sample introduction to the GC. The Chromatoprobe utilizes a programmable temperature 1079 injector. The experimental parameters for the GC-MS are listed in Tables 1 and 2.

Table 1. Instrumentation parameters for GC

<b>Stationary Phase</b>	5% phenyl PDMS
<b>Column Dimensions</b>	30 m long, 0.25 mm id, 0.25 mm film thickness
<b>Column Flow Rate</b>	40 psi for 1.10 min, 9.3 psi for 47 min
<b>Carrier Gas</b>	99.999% He
<b>Temperature program for oven</b>	40°C for 5 min, 250°C at 6.5°C/min for 10 min
<b>Temperature program for Chromatoprobe</b>	40°C for 0.10 min, 84°C at 200°C/min for 1.00 min, 300°C at 200°C/min for 10 min, no split

Table 2. Instrumentation parameters for MS

<b>Ionization</b>	Electron Impact
<b>Ion Range</b>	35-650 m/z
<b>Solvent Delay</b>	8 min
<b>Pressure</b>	<40 $\mu$ Torr
<b>Trap Temperature</b>	150°C
<b>Manifold Temperature</b>	35°C
<b>Transfer Line Temperature</b>	260°C

The specialized, high temperature conditions of expensive pyrolysis equipment can be mimicked using the Chromatoprobe injector. In pyrolysis, the temperature of the injector increases very quickly at a rate of 20,000°C per minute. With the Chromatoprobe, the injector heats up at a fairly quick rate of 200°C per minute.<sup>47</sup> Using the Chromatoprobe, solid samples can be introduced to the GC in a similar way to the injector of a pyrolysis instrument. The sample is placed into a small glass vial, which is placed into the Chromatoprobe injector. The probe is then injected into the Varian 1079 injection port. The temperature increases, the sample volatilizes, and the gas is swept into the GC.

## 2.2 Materials

Humic acid standard was purchased from Alfa Aesar (Ward Hill, MA). Sodium hydroxide pellets along with 18M $\Omega$ , Barnstead Nanopure deionized water to were used to make a 1M solution. Na<sub>2</sub>HPO<sub>4</sub> and NaH<sub>2</sub>PO<sub>4</sub> were dissolved in deionized water to create

a solution with a pH of 8. Charcoal made from hardwood and unbleached, undyed, and unsized linen and cotton (TestFabrics, Inc., provided by K. Jakes, Ohio State University) were prepared to model contaminated archaeological materials. Charcoal was chosen because it is one of the most commonly found materials in archaeological sites that can be radiocarbon dated. Linen and cotton were also chosen as fabrics which may be found as artifacts. One concern with charcoal is that because both charcoal and humic acids result from the decay and diagenesis of organic materials, they have similar chemical compositions. Finding compounds that are present in humic acids, but not in charcoal can be difficult for this reason. Linen and cotton were chosen because the composition is expected to be significantly different from humic acids.

In addition to the synthetic samples that were created to evaluate the efficacy of the new treatment, two artifacts excavated from Little Lost River Cave, an archaeological site in Idaho, were studied as well. The cave, with a Smithsonian designation of 10BT1, was first “discovered” by Albert Whiting in 1954.<sup>48</sup> Significant evidence of human utilization of the site include both rock paintings covering the walls of the cave as well as hearths excavated by Gruhn.<sup>49</sup> During the course of the excavations in 1990, several unique artifacts were found. These include a ring of knotted juniper bark (Figure 2) with associated antelope hair and a reed artifact, also knotted (Figure 3). These artifacts were submitted to our laboratory for nondestructive radiocarbon dating using the PCO-AMS method. Small fragments that broke off these artifacts were used in this study to evaluate the efficacy of the new treatments. Instead of comparing the composition of the artifact fragments to standard humic acids, the composition of soil obtained from 10BT1 was studied.



### **2.3 Preparation of materials**

A standard humic acid solution was made by dissolving solid humics in deionized water. Fragments of modern, hardwood charcoal were soaked overnight in this saturated solution of humic acid standard. Several threads of linen and cotton, removed with tweezers, were also soaked overnight. All three were then dried in an oven at about 100°C for two to three hours. When dry, the charcoal fragments were ground up into a uniform powder with a porcelain mortar and pestle. Small fragments of juniper and reed were removed from the artifacts with tweezers.

A solution of 1M HCl was made by diluting 12M concentrated HCl. 6.3 mL were diluted to 75 mL with deionized water. A solution of 1M NaOH was made by dissolving 1 g of NaOH pellets in 25 mL of deionized water.



Figure 2. Juniper bark ring from Little Lost River Cave, with centimeter scale, before plasma-chemical oxidation.



Figure 3. Knotted reed artifact, with millimeter scale, before plasma-chemical oxidation.

### *2.3.1 Traditional pretreatment*

Armitage<sup>50</sup> and Pace et al.<sup>17</sup> modified the standard AAA-pretreatment to better fit with the capabilities of the PCO method, simplifying the treatment to sonication in 1M NaOH only. Samples were placed in 1 mL centrifuge tubes and filled with approximately 1 mL of 1 M NaOH solution. The tubes were placed in a 60°C water bath and sonicated for one hour. The vials were then centrifuged to separate the samples from the liquid. The liquid was poured off and saved in a clean centrifuge vial. The samples were then rinsed and filtered with deionized water. The samples were subjected to repeated treatments until the solution became clear, which indicates that the sample is free of humates.

### *2.3.2 New pretreatment*

Phosphate buffer with a pH of 8 was chosen as an alternative to 1M NaOH because it is basic, but less so than NaOH. Tatzber et al.<sup>51</sup> used FTIR to analyze the results of extracting humic acids from soil with different bases. They used 1M NaOH, 0.1M Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and 1M Na<sub>2</sub>CO<sub>3</sub>. They found that the NaOH extraction had lower yields than the extractions from the other two bases. Valladas<sup>35</sup> also used sodium pyrophosphate as a potentially less harsh alternative to NaOH. She found that resulting radiocarbon dates from two samples treated with each base were comparable. These studies show that phosphate compounds may be a viable alternative to NaOH.

The phosphate buffer was made by dissolving 8.88 g of Na<sub>2</sub>HPO<sub>4</sub> and 0.55 g of NaH<sub>2</sub>PO<sub>4</sub> in deionized water up to 40 mL. The solution was then adjusted to pH 8 using HCl and NaOH and checked with a pH meter. Samples were placed in 1 mL centrifuge

tubes and filled with the phosphate buffer solution. The tubes were placed in a 60°C water bath and sonicated for one hour. The vials were then centrifuged to separate the samples from the solution. The solution was poured off and saved in a clean centrifuge vial. The samples were then rinsed and filtered with distilled water. The samples were subjected to repeated treatments until the solution became clear, indicating that the sample was clean.

#### **2.4 Thermally-assisted hydrolysis and methylation conditions**

Thermally-assisted hydrolysis and methylation (THM) is a technique used to volatilize compounds for gas chromatography. Tetramethylammonium hydroxide (TMAH) converts the carboxyl groups on fatty acids to methyl esters. The esters then hydrolyze at high temperature. Tetramethylammonium salts are formed and the methylated derivatives are thermally dissociated.<sup>20</sup> Small, uniform pieces of each sample, after pretreatments, were put into a sample vial for the Chromatoprobe to which 0.5 µL of TMAH was then added.

#### **2.5 Identification of compounds**

Compounds were identified using the NIST 98 database. To consider a compound a good match, forward and reverse matches were at least 500, and in most cases much greater. A less important parameter to consider was the probability. This determines how likely the peak is to be the compound matched compared to other possible matches. To determine the presence or absence of a marker compound, selected ion chromatograms were used. With this technique, the ions specific to a compound were

searched for within the larger chromatogram. If peaks for the compound were found at similar retention times, the compound was determined to be present.

## CHAPTER 3

### RESULTS AND DISCUSSION

To determine whether or not humic substances were removed from samples during the cleaning processes, marker compounds were identified. For charcoal, chromatograms for the humic acid standard, the clean sample, and the sample soaked in humic acids were overlaid to compare the peaks. Compounds corresponding to peaks that appeared in the humic acid standard but not in the samples prior to contamination acted as markers for humic substances. If these compounds did not appear in the cleaned samples, successful removal of contamination was concluded. To determine whether or not a compound disappeared, a selected ion chromatogram was created to search for the ions representative of that compound. A similar strategy was used for the reed and juniper artifacts. Instead of comparing the artifacts to the humic acid standard, however, they were compared to soil from the archaeological site at which they were found.

#### 3.1 Charcoal

Nonanedioic acid ( $C_9H_{16}O_4$ ), dimethyl ester was found at a retention time of 25.964 minutes in the chromatogram for charcoal soaked in humic acids. The compound also appears definitively in the humic acid standard, but not in the charcoal alone. Long chain fatty acids are expected to be found in humic acids and are, therefore, good markers for humic substances.<sup>52</sup> A few other compounds were found exclusively in the humics and humic-soaked charcoal, but were not expected as markers for humic substances. Figure 4 shows the selected ion chromatograms for nonanedioic acid ( $m/z$  55, 83, 111, 152, 185). The absence of a peak confirms that the compound does not exist in charcoal

alone. The humic-soaked charcoal has a larger peak than the humic acid alone because all samples were taken qualitatively. More charcoal was sampled than humic acid standard.

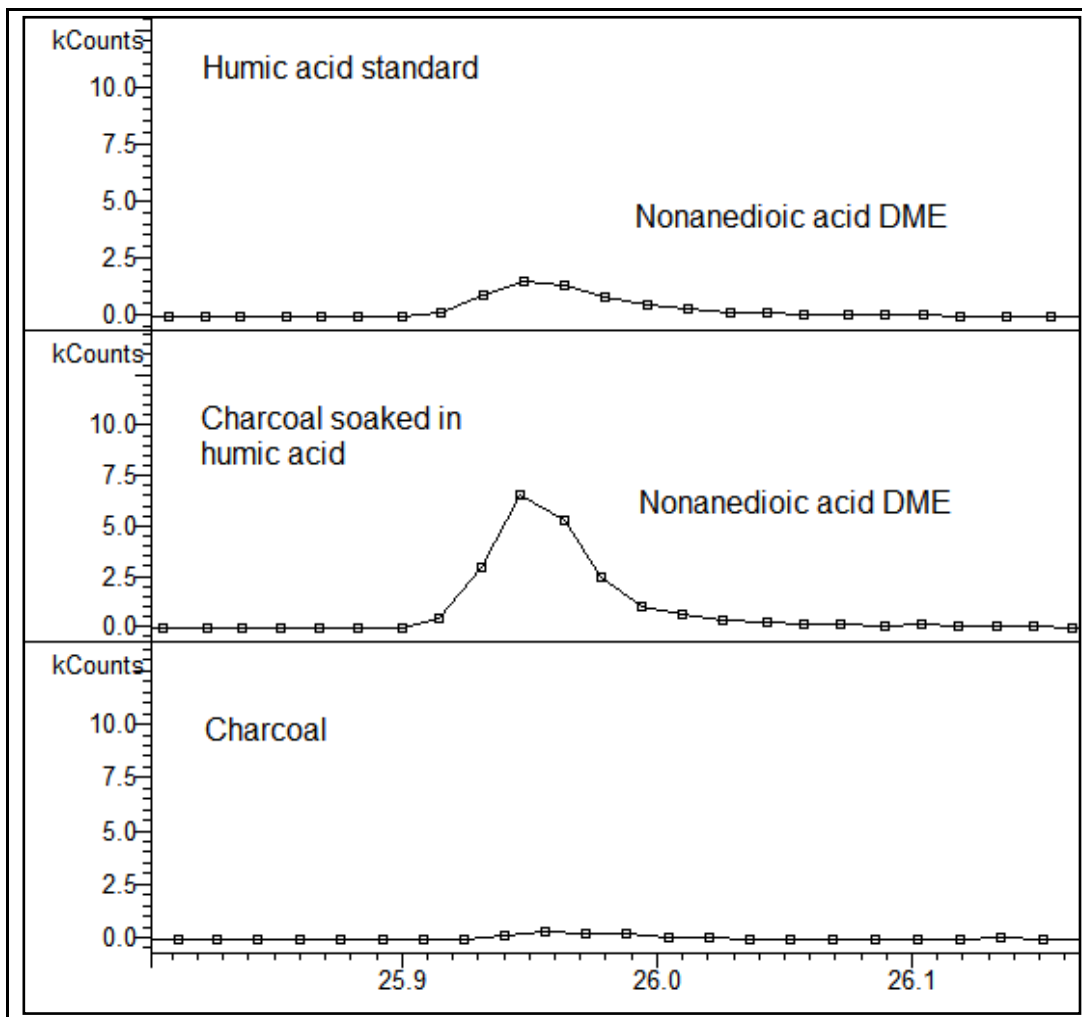


Figure 4. Selected ion chromatograms of charcoal samples

The charcoal soaked in humic acid standard was cleaned with NaOH. After eight treatments, the liquid became clear. A piece of charcoal after each of the eight washes was analyzed by THM-GC-MS. After the sixth treatment, the peak for nonanedioic acid disappeared. Another sample of the charcoal soaked in humics was treated with the

phosphate buffer seven times until the liquid became clear. Figure 5 shows that the peak for nonanedioic acid disappeared after the third treatment. These results suggest that, not only does the phosphate buffer successfully remove humic acid, it works in fewer steps than NaOH. The phosphate treatment required three washes to remove the marker compound, while the NaOH treatment needed six washes.

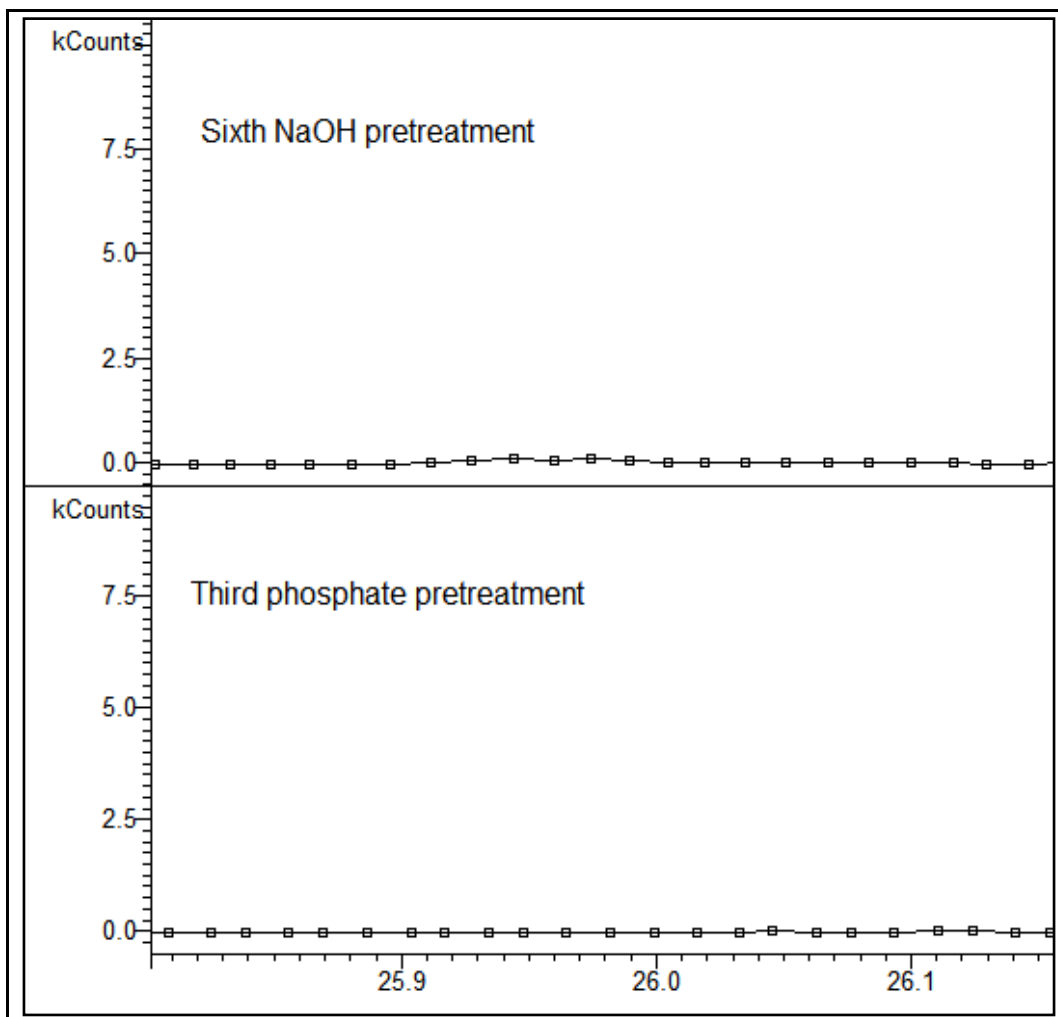


Figure 5. Selected ion chromatograms of charcoal after NaOH and phosphate pretreatments



The humic-soaked charcoal was also treated with deionized water as a control. After nine washes the liquid became clear, indicating a clean sample. Only the chromatogram for the ninth treatment showed that the nonanedioic acid disappeared. Water may eventually remove humic acid contamination, but because it takes nine treatments to do so, it is not very efficient. Each cycle of treatment takes at least two hours.

### **3.2 Juniper**

Fragments of a juniper bark wreath from an archaeological site were treated with both NaOH and phosphate buffer. To find marker compounds for humic acids in the contaminated juniper, chromatograms for the 10BT1 soil and juniper treated with the AAA method were compared. No suitable compounds were found to act as markers and to confirm the efficacy of the treatments. The chromatogram for clean juniper was very complex and many of the peaks were the same as the peaks in the soil.

### **3.3 Reed**

A fragment of reed from the knotted artifact was treated with the traditional AAA method and with the phosphate buffer. A small piece was analyzed before any treatment and one piece was analyzed after AAA and phosphate treatments. The methyl ester of decanoic acid was found in the untreated reed and not in the AAA treated reed. This compound was used as a marker for humic acids. Figure 6 shows the selected ion chromatogram for the ions characteristic of decanoic acid ( $C_{10}H_{20}O_2$ ) ( $m/z$  43, 74, 87,

143). The compound appears in the reed artifact prior to cleaning. The compound no longer appears in the AAA-cleaned fragment, or in the phosphate-treated sample. In the AAA-treated chromatogram, the background was very high and the peak that may correspond to the marker compound does not rise above it. This confirms that phosphate buffer removes humic acids as well as the alkali step of AAA.

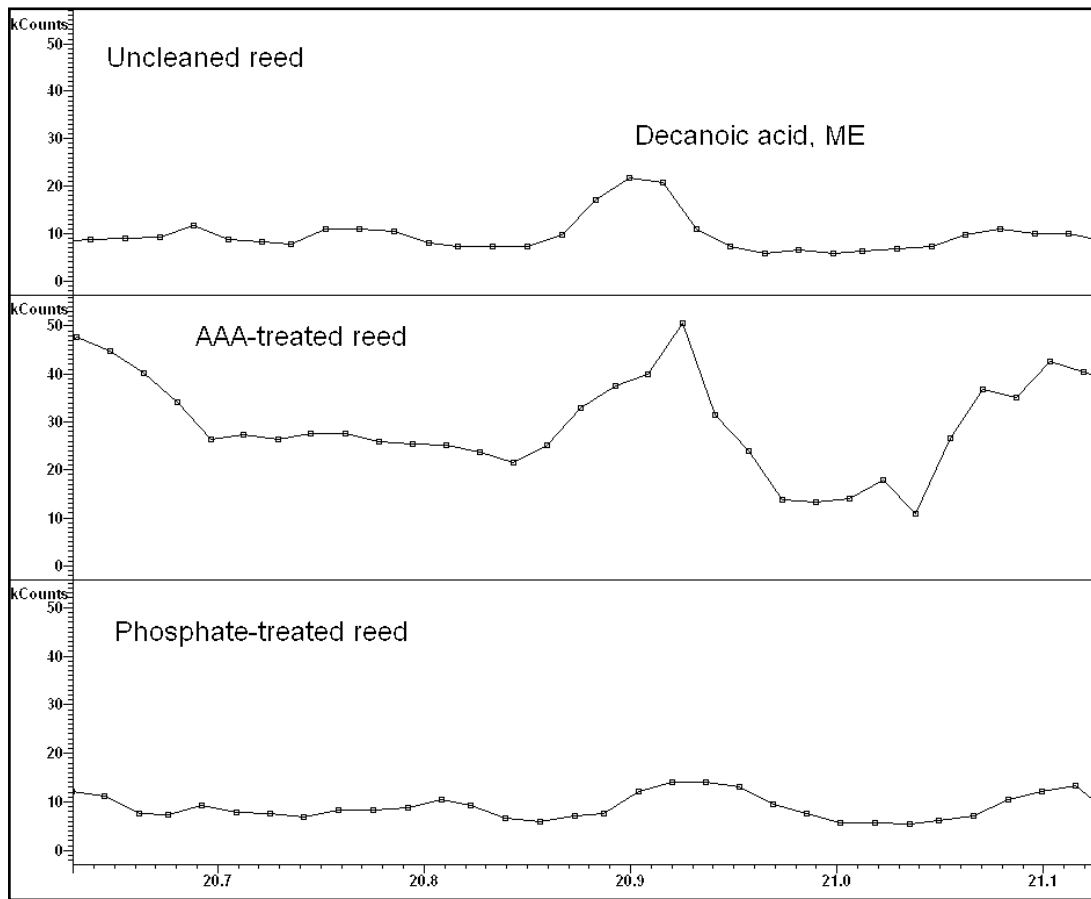


Figure 6. Selected ion chromatograms for decanoic acid, methyl ester in reed.

### **3.4 Cotton and linen**

Samples of modern cotton and linen were each contaminated with humic acids, cleaned with NaOH and phosphate buffer and then analyzed. The results of cleaning were inconclusive. Marker compounds were difficult to find in the humic acid-soaked fabrics and not in the clean fabrics, possibly because the plant-based structure of cotton and linen is too similar to humic acids.

### **3.5 Appearance of artifacts after pretreatments**

After each treatment of the humic-soaked charcoal with NaOH, the amount of charcoal decreased. Mass was not measured, but the loss was significant enough to detect visually. After treating with phosphate buffer, significantly more remained. The appearance of the charcoal did not change, only the amount.

The amounts of cotton and linen also decreased significantly after treatment with NaOH. They also appeared mushy afterwards. After phosphate treatments, most of the fabric remained intact.

Both the reed and juniper amounts decreased significantly after NaOH treatments. The amounts decreased only somewhat after phosphate treatments. The appearance of each artifact after NaOH changed dramatically. The fragments lost structure and had a mushy appearance. After phosphate treatments, the appearance of the reed and juniper remained largely unchanged. The surface of each piece appeared lighter in color, but retained their structure. Figures 7 and 8 show a piece of juniper before and after phosphate treatments.

### 3.6 Radiocarbon dates

The long-term application of this pretreatment process is to clean samples for nondestructive radiocarbon dating. While THM-GC-MS can be used to show that markers for contamination have decreased significantly to below what is easily detected by the method, its effectiveness can only truly be shown by comparing the results of radiocarbon analysis on a material subjected to the standard AAA treatment to those for the same material subjected to the new process combined with PCO-AMS. The two



Figure 7. Fragment of juniper before any pretreatment.



Figure 8. Same juniper fragment as in Figure 7, after phosphate treatment.

artifacts from Little Lost River Cave were an opportunity to compare AAA-combustion-AMS to nondestructive PCO-AMS both with and without the phosphate treatment.

A piece of the juniper bark ring was treated with the traditional AAA treatment and sent to the Lawrence Livermore National Laboratory, where it was combusted at high temperature to produce  $\text{CO}_2$ , converted to graphite and dated by AMS. Another fragment of the juniper bark was treated with the phosphate treatment developed herein (pictured in Figure 8) and subjected to PCO; unfortunately, only 20  $\mu\text{g}$  of carbon was obtained from the fragment, which was not sufficient for a reliable radiocarbon date even by AMS. Additionally, the whole artifact was subjected to “nondestructive” sample preparation using PCO. An initial 5-minute 40 W oxygen plasma provided sufficient material for a measurement of the carbon isotopic fractionation. The  $\text{CO}_2$  resulting from two subsequent oxygen plasmas was submitted for AMS analysis.

The results of the PCO-AMS dating of the juniper bark ring are summarized in Table 3. The three radiocarbon dates are statistically indistinguishable at the 95% confidence level, and yield a pooled mean age of  $4500 \pm 20$  radiocarbon years before present (BP).

A fragment from the knotted reed artifact was also subjected to AAA treatment and submitted for combustion and AMS dating at the Livermore laboratory. Another fragment was treated with phosphate buffer and subjected to PCO to obtain CO<sub>2</sub> gas for radiocarbon analysis. Again, the entire artifact was subjected to two oxygen plasmas for “nondestructive” dating as well. The radiocarbon results for the reed artifact and the pretreated subsamples are shown in Table 4.

Table 3. Summary of radiocarbon analysis results for juniper ring artifact from Little Lost River Cave.

<b>CAMS #</b>	<b>Sample name</b>	<b><sup>14</sup>C age, years BP</b>	<b>μg C</b>
127362	juniper bark, AAA treated	4460 ± 30	n/a
134005	juniper bark, PCO fraction 2	4550 ± 40	230
134006	juniper bark, PCO fraction 3	4520 ± 50	170
n/a	juniper bark, PO <sub>4</sub> treated	n/a	20

Table 4. Summary of radiocarbon analysis results for knotted reed artifact from Little Lost River Cave.

CAMS #	Sample name	$\delta^{13}\text{C}$	$^{14}\text{C}$ age, years BP	$\mu\text{g C}$
134008	Reed, AAA pretreated	-29.73	1180 $\pm$ 80	n/a
132755	reed, PCO fraction 1	-29.73	1615 $\pm$ 40	210
n/a	Reed, PCO fraction 2	n/a	n/a	100
134007	reed, PO <sub>4</sub> treated	-29.73	1100 $\pm$ 110	50

The radiocarbon results for the reed artifact show that the AAA and phosphate treatments yielded statistically indistinguishable results at the 95% confidence level. The “nondestructive” date for the surface of the artifact, however, was significantly older, indicating that surface contamination may be a significant problem with applying PCO without any chemical cleanup step. Unfortunately, the second PCO fraction from the reed failed to produce any ion current in the AMS (T. Guilderson, personal communication) and thus did not yield a radiocarbon date. The conclusion that surface contamination is problematic is borne out by the fact that the juniper bark ring dates after a 5-min cleaning plasma were statistically indistinguishable. Combining the phosphate treatment with the PCO sample preparation appears to be the ideal approach, and will be investigated more fully in the future.

## CHAPTER 4

### CONCLUSIONS

#### 4.1 General conclusions

Three different pretreatments for removing humic acids from various materials were proven to work in this study. The traditional 1M NaOH and phosphate buffer successfully removed marker compounds from charcoal and reed. Deionized water also removed the marker from charcoal.

Phosphate buffer removed humic acids most efficiently. It took only three washes of charcoal and one wash of reed to see the marker compound disappear. NaOH required six washes and water required nine. Because the washing procedure is so lengthy, the difference is significant.

The most convincing evidence that phosphate buffer removed contamination from the artifacts as well as did the NaOH treatment are the radiocarbon data. The two pieces of reed subjected to each different treatment and then dated, yielded statistically indistinguishable dates. Furthermore, the reed treated only with PCO gave an older than expected date, showing the significance of some kind of alkali wash.

Finally, the significance of the results lies in the appearance of the materials after treatments. The NaOH treatments dissolved away much of the charcoal, reed, juniper, cotton, and linen. The phosphate buffer left each material largely intact, but with surfaces that appear cleaner. This indicates that phosphate is far less destructive to artifacts than NaOH.



## **4.2 Future analyses**

The results of these experiments give an initial indication that the alkali step of the AAA pretreatment of artifacts may be replaced with a less harsh base. Before a conclusive conclusion is reached, the results shared here should be reproduced. The removal of marker compounds from samples by phosphate buffer was evidenced qualitatively only. The compound was present and then it was not. Quantifying these results would help to give more conclusive evidence that the phosphate buffer is removing humic acids. Also, the liquids that were saved from each treatment step could be analyzed to find the humic acid markers.

## REFERENCES

1. Taylor, R.E.; Aitken, M.J. *Chronometric Dating in Archaeology*; Plenum Press: New York, 1997; p. 65-96.
2. *Handbook of Rock Art Research*; Whitley, David S.; Ed.; Altamira Press: Walnut Creek, 2001; p. 139-166.
3. Schwarcz, H.P. *Acc. Chem. Res.* **2002**, *35*, 637-643.
4. Elmore, D.; Phillips, F.M. *Science* **1987**, *236*, 543-550.
5. Pettitt, P.; Bahn, P. *Antiquity* **2003**, *77*, 134-142.
6. Stevenson, F.J. *Humus Chemistry. Genesis, Composition, Reactions*; Wiley: New York; p. 1-40.
7. Allard, B.; *Geoderma* **2006**, *130*, 77-96.
8. Li, L.; Jia, W.; Sheng, G.; Fu, J.; Huang, W. *Appl. Geochem.* **2006**, *21*, 1455-1468.
9. Chefetz, B.; Salloum, M.; Deshmukh, A.P.; Hatcher, P.G. *Soil. Sci. Soc. Am. J.* **2002**, *66*, 1159-1171.
10. Swift, R.S. *Methods of Soil Analysis: Part 3 – Chemical Methods*; Soil Science Society of America: Madison, 1996; p. 1011-1070.
11. Hatte, C.; Morvan, J.; Noury, C.; Paterne, M. *Radiocarbon* **2001**, *43*, 177-182.
12. Hedges, R.; Ramsey, C.B.; Van Klinken, G.J.; Pettit, P.B.; Nielsen-Marsh, C.; Etchegoyen, A.; Fernandez Niello, J.O.; Boschin, M.T.; Llamazares, A.M. *Radiocarbon*, **1998**, *40*, 35-44.
13. Russ, J.; Hyman, M.; Shafer, H.J.; Rowe, M.W. *Nature*, **1990**, *348*, 710-711.
14. Stott, A.; Berstan, R.; Evershed, R. *Anal. Chem.* **2003**, *75*, 5037-5045.

15. Van Klinken, G.; Hedges, R. *Radiocarbon* **1998**, *40*, 51-57.
16. Armitage, R.A.; Brady, J.E.; Cobb, A.; Southon, J.R.; Rowe, M.W. *Am. Antiq.* **2001**, *66*, 471-480.
17. Pace, M.F.N.; Hyman, M.; Rowe, M.W.; Southon, J.R. *American Indian Rock Art* **2000**, *24*, 95-102.
18. Challinor, J.M. *J. Anal. Appl. Pyr.* **1989**, *16*, 323-333.
19. Asperger, A.; Engewald, W.; Fabian, G. *J. Anal. Appl. Pyr.* **2001**, *61*, 91-109.
20. Challinor, J.M. *J. Anal. Appl. Pyr.* **2001**, *61*, 3-34.
21. Lehtonen, T.; Peuravuori, J.; Pihlaja, K. *J. Anal. Appl. Pyr.* **2003**, *68-69*, 315-329.
22. Drechsel, D.; Dettmer, K.; Engewald, W. *Chromatographia* **2003**, *57*, S-283-S-289.
23. Linick, T.; Damon, P.; Donahue, D.; Jull A. *Quaternary International* **1989**, *1*, 1-6.
24. Jorkov, M.L.; Heinemeier, J.; Lynnerup, N. *J. Arch. Sci.*, **2007**, *34*, 1824-1829.
25. Evershed, R.P.; Dudd, S.N.; Copely, M.S.; Berstan, R.; Stott, A.W.; Mottram, H.; Buckley, S.A.; Crossman, Z. *Acc. Chem. Res.* **2002**, *35*, 660-668.
26. Evershed, R.P. *J. Arch. Sci.* **1996**, *23*, 429-436.
27. Stott, A.; Berstan, R.; Evershed, P.; Hedges, R.; Ramsey, C.; Humm, M. *Radiocarbon* **2001**, *43*, 191-197.
28. Mori, F.; Ponti, R.; Messina, A.; Flieger, M.; Havlicek, V.; Sinibaldi, M. *Journal of Cultural Heritage* **2006**, *7*, 344-349.
29. Gillespie, R.; Hammond, A.P.; Goh, K.M.; Tonkin, P.J.; Lowe, D.C.; Sparks, R.J.; Wallace, G. *Radiocarbon* **1992**, *34*, 21-27.

30. Santos, G.M.; Bird, M.I.; Pillans, B.; Fifield, L.K.; Alloway, B.V.; Chappell, J.; Hausladen, P.A.; Arneth, A. *Radiocarbon* **2001**, *43*, 239-248.
31. Cook, G.T.; Dugmore, A.J.; Shore, J.S. *Radiocarbon* **1998**, *40*, 21-27.
32. Turnbull, J.; Sparks, R.; Prior, C. *Nucl. Instrum. Methods Phys. Res., Sect. B* **2000**, *172*, 469-472.
33. Alon, D.; Mintz, G.; Cohen, I.; Weiner, S.; Boaretto, E. *Radiocarbon* **2002**, *44*, 1-11.
34. Nakamura, T.; Taniguchi, Y.; Tusji, S.; Oda, H. *Radiocarbon* **2001**, *43*, 1129-1138.
35. Valladas, H. *Meas. Sci. Technol.*, **2003**, *14*, 1487-1492.
36. Daniels, V. *Studies in Conservation* **1981**, *26*, 45-49.
37. Patscheider, J.; Veprek, S. *Studies in Conservation* **1986**, *31*, 29-37.
38. Rutledge, S.K.; Banks, B.A.; Forkapa, M.; Stueber, T.; Sechkar, E.; Malinowski, K. *Journal of the American Institute of Conservation* **200**, *39*, 65-74.
39. Saettone, E.A.; da Matta, J.A.; Alva, W.; Chubaci, J.F.; Fantini, M.C.; Galvao, R.M.; Kiyohara, P.; Tabacniks, M.H. *J. Phys. D.: Appl. Phys.* **2003**, *36*, 842-848.
40. Craig, O.E.; Love, G.D.; Isaksson, S.; Taylor, G.; Snape, C.E. *J. Anal. Appl. Pyrolysis* **2004**, *71*, 613-634.
41. Fezzey, S.; Armitage, R.A. *J. Anal. Appl. Pyrolysis* **2006**, *77*, 102-110.
42. Scalarone, D.; Lazzari, M.; Chiantore, O. *J. Anal. Appl. Pyrolysis* **2003**, *68-69*, 115-136.
43. Andreotti, A.; Bonaduce, I.; Colombini, M.P.; Gautier, G.; Modugno, F.; Ribechini, E. *Anal. Chem.* **2006**, *78*, 4490-4500.

44. Cappitelli, F.; Koussiaki, F. *J. Anal. Appl. Pyr.* **2006**, *75*, 200-204.
45. Buonasera, T. *J. Arch. Sci.* **2005**, *32*, 957-965.
46. Spades, S.; Russ, J. *Archaeometry* **2005**, *47*, 115-126.
47. Pitthard, V.; Finch, P.; Bayerova, T. *J. Sep. Sci.* **2004**, *27*, 200-208
48. Fichter, E.; Hopkins, M.; Isotoff, A.; Liljeblad, S.; Lyman, R.A.; Strawn, M.; Taylor, E. Idaho State College, Pocatello, ID., *Exploratory Excavations in Little Lost River Cave; No.1 A Progress Report*. Unpublished report, **1954**.
49. Gruhn, R.; Bryan, A. *Report on a Test Excavation at Little Lost River Cave No. 1 (10BT1) in 1990*; Report on Cultural Resource Permit ID-I-27727; Bureau of Land Management: Idaho Falls, ID, October, **1990**.
50. Armitage, R. A. Ph.D. dissertation, Chemistry, Texas A&M University, 1998.
51. Tatzber, M.; Stemmer, M.; Spiegel, H.; Katzlberger, C.; Haberhauer, G.; Mentler, A.; Gerzabek, M.H. *J. Plant Nutr. Soil Sci.* **2007**, *170*, 522-529.
52. Saiz-Jimenez, C.; Hermosin, B. *J. Anal. Appl. Pyro.* **1999**, *49*, 349-357.