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Bone Degradation under Differing Environments

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This senior honors thesis is dedicated to everyone who did not let me quit. I am so thankful to those who pushed me and stayed positive when I found it difficult to do so.

This thesis is also dedicated to Xanax – my best friend throughout this process (kidding, mostly).

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Abstract

Degradation of organic materials is a very important aspect of study for archaeologists and especially the rate at which it happens. No other organic remnants are studied as widely in this respect as bones. They are able to tell us so much about the human population at the time including health and diet. For this experiment I wanted to test the diagenetic process of faunal bones in a neutral and controlled environment and from there also test them in basic and acidic environments which could be subject to water or heat. From this I hoped to gain an understanding of the exchange of trace elements between bone and their surrounding environment. This is important to study because it can help tell us if the chemical makeup of bones is the same pre and post deposition. If it differs then those differentiations need to be accounted for when measuring the organic composition of bone to look at aspects such as diet.

Table of Contents

List of Tables

Background

The idea for this experiment arose due to the issue of our limited knowledge on how bones degrade and how their chemical makeup is altered post-deposition. A great deal of research has already been done on how bones are preserved when exposed to different geochemical processes (Berna et al., 2004; Hedges, 2002; Hedges and Millard, 1995; Karkanas et al., 2000; Karkanas, 2010). Karkanas explains that open-air sites are not widely studied when it comes to bone degradation because of how quickly dissolution happens (2010: 66). Simply put, this means that a great deal of the research done on bone diagenesis has a focus on the bones found in caves. However, they are done extensively, done well, and still provide insight into the process. My experiment will differ, in that, those experiments were under the conditions of soils containing multiple elemental factors and the intriguing concept here is what the individual elements do in isolation with the bone in order to create the end result. If this experiment shows significant data, then this could provide context to many archaeological data aspects. One highly interesting factor of bone recovery is studying elements such as Strontium and Calcium in the bone to note trends in diet for certain groups of people or animals. My experiment could help tell us if certain trace elements are key in altering the organic levels of elements such as Strontium and Calcium or if the bones remain mostly similar to their chemical make-up post-deposition. If the elements due incite change, how significant is it and how can it be read into a wider context?

Bone is chemically made up of carbonated hydroxyl apatite [(Ca, Na, Mg)5(HPO4, PO4, CO3)3(OH, CO3)] (Berna, et al., 2004). When introduced to a soil-based environment, bone is altered depending upon the elements in play and this can vary from one geographic location to the next (Hedges and Millard, 1995: 155). There are a few primary conditions that induce post-depositional change in bones. “Kinetic factors”, such as water flow for example, influence the

rate of reactions (Hedges and Millard, 1995: 155; Karkanas, 2000: 916; Karkanas, 2010: 64). It is already known that chemical reactions in soil, such as acidic environments, are some of the most destructive agents when it comes to organic material (Berna et al., 2004; 872; Hedges and Millard, 1995; Karkanas, 2000: 916; Karkanas, 2010: 66; Keely et al., 1977: 19). Finally, the levels of microbial activity are also a large factor when considering the diagenetic change of bones (Collins et al., 2002; Hedges, 2002; Karkanas, 2010: 63).

Looking into the stability of bone as it is exposed to varying levels of pH, the conclusions are that bones are stable in samples with a pH level higher than 8.1, then bone undergoes re-crystallization and is replaced by more stable forms between pH levels of 8.1 and 7.4, and any pH level lower than 7 will dissolve rapidly (Berna et al 2004: 879, 878; Hedges and Millard, 1995; Karkanas, 2010: 66). Keeping that last note in mind, it is important to remember that when looking for spatial distribution of minerals, the fact that most minerals dissolve completely makes it difficult to find definitive signs of chemical reactions (Karkanas 2010:64). Therefore, it is important to remember that an absence of bones can be just as telling as a presence of bones.

While Berna says that bones are stable when their pH is higher than 8.1, he also says that "even when bones do not dissolve, their mineral phases change (2004: 867). Therefore, looking at a bone from a basic context can prove just as interesting as a bone from an acidic context, despite the outward appearance of stability. Here, we are going to look at bones in a neutral environment, and acidic environment and a basic environment. These three extremes will hopefully give us a glimpse at what elements within the same vein can do and how it may complicate the preservation of bone specifically. Post-depositional change is an evident occurrence, but the question remains as to what individual elements do to the bones and under what conditions.

Expectations

With the knowledge of how pH levels affect bone, I especially wanted to emphasize that change by setting up an experiment that showcased both extremes. Naturally I wanted to maintain a control but also choose elements I was sure would place my bone in a very basic environment on one hand, and a very acidic environment on the other. My expectation, of course, being that the bone within the higher pH range would maintain its stability (Berna et al., 2004: 880; Karkanas, 2010: 66) while the bone on the lower end of the spectrum would quickly begin to show signs of change and degradation rather quickly (Berna et al., 2004; Karkanas, 2010: 66; Keeley et al., 1977: 19).

Water was added to three of my samples because of the numerous articles discussing how water flow could speed up the rate of chemical change (Hedges and Millard, 1995: 155; Karkanas, 2000: 916; Karkanas, 2010: 64). For this reason, I especially expect to see interesting changes in solutions which involve both the aqueous environment and the acidic element. Finally, three of the samples were kept under a heat lamp. A great deal of the literature noted how stabilizing colder temperatures could be for bone (Karkanas et al., 2010: 925) so I wanted to see if I could create a less stable environment by turning up the heat. The trace elements I plan on paying specific attention to are Strontium, Calcium, and Phosphorous. The first two are important in terms of measuring diet balances between meat and vegetables in individuals. Phosphorous is important because it usually indicates human activity and human activity is typically the very reason that archaeologists become interested in a site (Holliday and Gartner, 2007: 301). Phosphorous is widely noted in several of the articles as an important element to study, but one that is tricky to interpret. So, I worry that phosphorous may provide confusing or irrelevant data (Holliday and Gartner, 2007).

Methods

X-Ray Fluorescent Analyzer

The handheld X-ray fluorescent analyzer is being used to test these samples because it is a quick and non-destructive technique for determining the quantity of most elements between Magnesium (Mg) and Uranium (U) in a sample. It is an especially useful device when working with materials that touch base with NAGPRA. The way it works is an x-ray beam is emitted from the analyzer and connects with the object being tested, interacting with and displacing electrons within the atoms (Bruker 2013). Displacement occurs only when the x-ray being emitted has a higher energy than the energy that binds the electrons to the atom (Bruker 2013). Based upon the unique spacing between orbital shells in an atom, one can tell the specific element (Bruker 2013). Once an electron is bumped out of place another electron must move from a higher orbit to fill the empty space left behind or else the atom is left unstable: this is fluorescence (Bruker 2013). As electrons move from higher orbits to fill a vacancy closer to the nucleus of an atom, they lose a unique amount of energy which is dependent on the element because of the characteristic distance between electron shells (Bruker 2013). With this measurement, the instrument can calculate the quantity of the elements present.

Bone Processing

The proposed experiment was to be conducted over the course of three months and shots were to be taken using the x-ray fluorescent analyzer on four separate occasions. Due to some scheduling complications with the holidays, the three month timespan was maintained but only three shots were able to be taken. The time span was chosen because it was a manageable length but still allowed time for changes to happen to the deer bones being tested. The experiment involves the isolated manipulation of 9 sections of a metatarsal from a white tailed deer. The first

metatarsal (Bone 1) was cut, using a saw, into sections 1-8 from the proximal to distal end. The very distal end (Section 9) was left out due to its highly porous nature. Bone 2 was cut into 7 sections, also leaving out the very distal end (Section 8) due to its porous nature. The bones were always handled with gloves since the experiment involves seeing what these bones may have picked up from their post-depositional environments and I did not want them picking up anything else from direct handling.

Establishing Controls

To measure the controls of all organic materials involved in the experiment, the x-ray fluorescent analyzer was used to take an initial shot of every material involved. Each of the 9 sections of bone being used in the experiment (Bone 1, Sections a-h and Bone 2, Section a) was marked and shots were taken on the flattest part of the bone in order to achieve the most accurate read. Shots were taken using both the blue and green filters so that we might view a wider spectrum of change. The pure quartz sand was divided evenly into the 9 beakers which the bones would be buried in and a subsample of sand from each beaker was taken to be analyzed and ensure the sand's consistency. Quartz sand was chosen for the soil base because it provides a very neutral environment in which we could more easily detect change (Karkanas et al., 2000: 924; Karkanas 2010: 67). Then, our basic element, shell (aka calcium carbonate), was crushed as finely as possible using a mortar and pestle. Calcium carbonate was chosen to be tested because the literature agrees that it provides stability for bones (Berna et al., 2004: 880; Karkanas, 2010: 66). Its pure form was also tested using the XRF analyzer. Finally, a sample of aluminum sulfate, our acidic element, was taken and also tested in isolation using the XRF. Ideally, to replicate an acidic environment, we would have used a form of phosphorous. Phosphorous provides a strong indicator for the presence of human activity and is, therefore, and element of great interest to

archaeologists. As far as cave sites go, phosphorous can be found in bat guano and when groundwater is added to the mix to interact with that acidic bat guano, we see a great deal of degradation in bones. However, Holliday and Gartner's intensive look at Phosphorous indicates that it is a difficult element to test and it takes a significant amount to detect change (2007). For this reason, aluminum sulfate was chosen as an alternative. Referencing the knowledge of a paleoethnobotanist, Dr. Gary Crites, and a few soils specialists over in the UT gardens, Sue Hamilton and Dr. Hugh Savoy, I learned that aluminum sulfate would provide the acidic environment desired and in the shortest amount of time possible.

Before mixing any materials together, the pure quartz sand was tested in its pH and temperature (Table 1).

Beaker	**pH**	**Temperature (Celsius)**
1	7.57	23.8
2	7.47	23.1
3	7.51	23.3
4	7.65	23.3
5	7.68	23.4
6	7.61	23.8
7	7.50	23.7
8	7.56	23.6
9	7.42	23.7

Table 1: Initial sand testing of pH and temperature

Set-Up

Finally, on the 12th of October, 2014 the beakers had their individual elements added and the bones were exposed to their respective conditions. Beakers 1, 2, and 3 were from the control group. This means they only contained quartz sand and bone. Beaker 1 had nothing else done to it, beaker 2 had 80ml of deionized water added to it to fully saturate the sand, and beaker 3 had heat applied to it with a thermal heat lamp used for reptiles. Beakers 4, 5, and 6 followed the same setup, but the quartz sand also had 2.08g. (2.06g. for beaker 6) of calcium carbonate (CaCO3) mixed in with it to create a more basic environment. The amount of shell was chosen simply because that evenly divided what we had on hand into the three samples that required it. The pH and temperature of beakers 4, 5, and 6 were then taken with the added shell (Table 2). To beakers 7, 8, and 9, 54.43g. of aluminum sulfate $Al_2(SO_4)_3$ were added to the quartz sand in

Beaker	pH	Temperature (Celsius)
4	8.03	24.1
5	7.75	23.9
6	8.24	23.8

Table 2: Initial sand and shell combination testing of pH and temperature

accordance with suggestions from a Clemson University article called "Changing the pH of Your Soil" on how to increase acidity to a certain level (Kluepfel and Lippert, 1999). Unfortunately,

the results were a little more extreme than we had initially planned, but the overall experiment is extreme in its showing how elements under isolation affect bone degradation. No bone would be exposed to such a controlled environment out in the real world environments. The pH and temperature was taken from beakers 7, 8, and 9 as well (Table 3).

Beaker	pH	Temperature (Celsius)
7	3.49	24.3
8	3.57	26.0
9	3.26	25.0

Table 3: Initial sand and aluminum sulfate combination testing of pH and temperature

In the end, the bones were buried as such (Table 4):

Beaker	Bone Segment	Conditions
1	1A	Control
2	1B	Control with 80ml DI water
3	1C	Control with heat
4	1D	2.08g. of CaCO3
5	1E	2.08g. of CaCO3 with 80ml DI water
6	1F	2.08g. of CaCO3 with heat
7	1H	54.43g. of $Al_2(SO_4)_3$
8	1G	54.43g. of $Al_2(SO_4)_3$ with 80ml DI water
9	2A	54.43g. of $Al_2(SO_4)_3$ with heat

*Note: Bone segments G and H were swapped from chronological order due to the mistake of picking them up in the wrong order. This new order was maintained for the duration of the rest of the experiment.

Table 4: Set-up of experiment upon burial

Beakers 2, 5, and 8, to which 80ml of deionized water was added, each had their moisture levels read using a moisture meter that provided a digital number on a scale from 1 to 10 with the possibility of no reading. Beaker 2 produced a moisture reading of 3.0, beaker 5 read at 3.6, and beaker 8 did not give a reading. The attempted moisture readings were taken both midway in the beakers and all the way at the bottom of the beakers. The moisture of each of these beakers was tested two days later after giving the water time to settle. Beaker 2 gave a reading of 4.8, beaker 5 provided a reading of 5.8, and unfortunately, beaker 8 once again did not provide a reading.

First Reading Post-Deposition

All of the beakers were covered with tin foil to keep outside contaminants from entering the samples and then they were left to sit until November 21, 2014. One at a time the bones were removed from their beakers. The dry samples could easily have the sand brushed off of them. The samples (2, 5, and 8) which were submerged in water, were removed and rinsed with deionized water and then left to dry. They had to dry out in order for the XRF analyzer to read them properly. It is interesting to note here that the three bones which had been buried in water had turned mostly black during the past month. The black sharpie which marked them each respectively was almost impossible to read due to their extremely altered surface color. These three samples also smelled very strongly. The bones were carefully handled with gloves and the wet samples were handled with a separate pair of gloves to avoid any cross contamination between the samples. Each bone segment was shot once more with both the blue and green filters in approximately the same location as the initial shot.

Second Reading Post-Deposition

Due to problems getting into the lab during the Christmas holidays, the month of December was skipped for testing and the next time the bones were read was January 21, 2015.

This shot was for the final reading so we tested not only the bones this time, but the sand as well. Once more, the water submerged bones were removed and rinsed and allowed to dry. Surprisingly, the submerged bones that had been black the first time had returned to their original color and no longer smelled. Thus far I am unable to explain this phenomenon. It was also interesting to see that beaker 8 in which the aluminum sulfate had been mixed with sand and submerged in 80ml of water had formed a hardened top layer which needed to be chipped away in order to retrieve the bone sample underneath. Shots were taken of all the bones with both the blue and green filters. Unfortunately, due to a mis-click on the keyboard, I accidentally rewrote Bone 2_Section A (November)_Green_180 and the reading is no longer useful to us for comparison. Small subsamples of sand with their added elements, if present, were taken from each beaker and also shot using the blue and green filters of the XRF. The experiment was fully concluded on February 4, 2015 when the last three shots were taken.

Results

After the initials shots were taken and the two other points on the time scale were also recorded, the data was compiled into a single file which allowed for cross comparison across a time table. The raw data appear as follows:

Table 5: XRF results for bone over the course of three months

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1		P K12	S K12	Cl K12	Ca K12	Mn K12	Fe K12	Ni K12	Zn K12	Sr K12	Nb K12	Ru K12	Rh K12	Ba L1	Ce L1
2	Bone1_SectionA (January)	2930	80	0	106596	2033	4234	211	135	1086	41	693	870	29	126
3	Bone1_SectionA (November)	2486	137	0	103154	1751	3822	207	421	1430	59	827	810	0	270
4	Bone1_SectionA	2701	68	43	102381	1903	3912	198	553	1500	54	752	837	31	166
5	Bone1_SectionB (January)	2612	50	5	102422	1264	4233	191	284	1032	43	558	649	75	58
6	Bone1_SectionB (November)	2624	107	0	99793	1422	3958	203	516	1395	83	773	866	11	210
7	Bone1_SectionB	2532	105	25	97017	1895	3703	204	503	1344	62	808	868	55	155
8	Bone1_SectionC (January)	2821	22	100	105177	1267	2520	261	557	1341	36	809	878	1	127
9	Bone1_SectionC (November)	2789	101	22	103230	957	2123	208	296	1363	45	702	833	0	254
10	Bone1_SectionC	2273	106	0	98482	893	1986	206	279	1401	69	844	866	12	140
11	Bone1_SectionD (January)	2611	38	0	101337	1331	2644	226	305	1332	43	890	753	61	157
12	Bone1_SectionD_(november)	2695	113	0	100084	1490	3753	255	370	1502	54	910	929	0	261
13	Bone1_SectionD	2455	65	1	95992	1396	3487	211	405	1565	58	796	931	35	225
14	Bone1_SectionE (January)	2542	44	8	102786	1198	6536	219	277	1415	42	778	840	0	109
15	Bone1_SectionE (November)	2123	198	53	95449	1453	5928	202	299	1505	45	969	868	51	157
16	Bone1_SectionE	2570	57	70	97411	1591	5308	226	324	1493	40	834	890	1	278
17	Bone1_SectionF (January)	2477	106	33	101092	1181	5760	195	334	1328	36	941	890	0	25
18	Bone1_SectionF (November)	2406	134	48	99674	1504	4969	195	279	1465	41	803	900	34	83
19	Bone1_SectionF	2370	93	7	94771	1242	4770	196	262	1473	71	729	814	0	76
20	Bone1_SectionG (January)	4	8953	0	64443	36	568	194	227	1422	66	852	826	29	97
21	Bone1_SectionG (november)	0	8669	0	62535	76	773	212	54	1093	54	910	881	0	33
22	Bone1_SectionG	2527	45	0	98429	945	3031	222	90	1278	50	833	837	0	131
23	Bone1_SectionH (January)	2280	74	2401	87565	761	2551	193	218	1386	88	819	671	0	123
24	Bone1_SectionH (november)	2283	77	1690	90356	691	1877	211	153	1024	46	828	806	39	147
25	Bone1_SectionH	2121	54	0	94807	798	2381	236	144	1078	32	885	785	36	304
26	Bone2_SectionA_(January)	2213	133	3055	87244	378	453	202	135	1054	39	966	812	30	76
27	Bone2_SectionA (november)	2178	124	3007	84474	345	505	219	124	1103	43	658	764	6	151
28	Bone2_SectionA	2253	80	6	97148	348	383	199	137	1170	87	810	771	0	2

Table 6: XRF results for sand pre and post deposition

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Al K12	Si K12	S K12	Cl K12	Ca K12	Ti K12	Mn K12	Fe K12	Ni K12	Cu K12	Zn K12	Sr K12	Sn K12	Cs K12	Au L1
2	ShellC_Cup1	213	178	157	91	151083	53	283	3919	203	202	295	90	1038	1441	76
3	AlSoC_Cup1	405	4	7963	72	34	0	146	4400	275	215	408	52	999	1299	71
4	SandC_Cup1(February)	91	3772	34	33	125	0	8	282	65	390	172	60	956	1477	60
5	SandC_Cup1	110	3886	18	30	73	135	13	273	148	421	263	54	891	1433	62
6	SandC_Cup2 (February)	121	3852	38	59	83	58	12	265	43	58	331	60	975	1198	59
7	SandC_Cup2	148	3974	29	30	57	129	3	283	236	175	248	55	894	1624	73
8	SandC_Cup3 (February)	110	3533	40	67	166	91	5	259	181	178	364	101	1172	1314	80
9	SandC_Cup3	104	4049	32	42	25	0	16	545	135	309	340	78	1077	1305	78
10	SandC_Cup4(February)	99	3777	32	54	458	0	28	311	155	88	364	3500	679	962	55
11	SandC_Cup4	116	3682	39	27	33	100	15	324	68	128	171	55	861	1415	55
12	SandC_Cup5 (February)	124	3893	49	76	1169	0	7	329	138	238	172	56	1184	1486	67
13	SandC_Cup5	123	3921	30	27	45	105	10	271	117	228	173	70	936	1013	49
14	SandC_Cup6 (February)	86	3663	34	45	1755	122	11	428	87	256	386	53	1193	1173	67
15	SandC_Cup6	103	3979	21	42	58	29	18	294	206	186	198	92	1113	1415	78
16	SandC_Cup7 (February)	272	1559	4253	58	84	98	66	587	121	216	328	39	915	1297	78
17	SandC_Cup7	170	3792	17	56	22	0	11	355	157	284	104	52	872	1567	90
18	SandC_Cup8 (February)	287	452	5185	35	149	0	93	718	193	253	206	55	942	1212	74
19	SandC_Cup8	140	3976	16	46	60	0	4	299	118	251	337	58	907	1455	78
20	SandC_Cup9_(February)	324	836	6305	44	157	50	98	2475	189	134	200	63	1250	1337	63
21	SandC_Cup9	141	3907	25	46	19	6	4	316	191	207	185	50	1184	1448	63

In the charts above I have highlighted what seemed to qualify as significant change. By this, I mean that I looked at the three points taken across the time scale and compared them in relation to one another to see if there was a visible shift in the numbers from October to January. I also went through the sand to see if there was any interesting change between the plain sand and the

sand after it had been experimented upon. While several sections are highlighted, the data shows no consistent trends.

Trends in Bone

In the bone samples, many of the number changes take on a bell curve form (or an inverted bell curve in some cases). From my extensive readings on bone preservation and changes in the levels of trace elements, I have found no explanation for this odd collection of data. If I were to make an assumption I may guess that the element was searching for equilibrium in its environment and simply did not have enough time to reach it. Karkanas even notes that because chemical reactions are slow they "seldom reach equilibrium" so it is quite possible that this experiment did not have the time frame necessary for these elements to discover their equilibrium (2010: 64). With the bones, we can see that there are shifts across the board with sulfur but none of the readings mention sulfur let alone explain how it might change given certain environments. The other element that shows at least small amounts of change across the time period is strontium. Yet, there are no consistent trends in an increase or decrease even within the variable environments. Looking at the bones from a different angle, bone 1 section C showed a lot of interesting change across the elements that seemed significant when picked up by the XRF analyzer. This bone was part of the control but exposed to heat. The literature confirms that cold stabilizes bone (Hedges, 2002: 324; Karkanas et al., 2000: 925) and this in turn suggests that heat can be destabilizing, but often groundwater also must be part of the mix to create a humid environment which this sample was not. Also, the other samples which were exposed to heat but had additional elements added to them, did not show similar trends in consistent change across the noteworthy elements. Therefore, it is difficult to say what it was about this section of bone that it saw change in almost all of its elements. Similarly, bone 1

section G also saw a lot of changes. This bone was simply buried in the sand combined with the aluminum sulfate to create an acidic environment. However, neither one of the other two bones buried with aluminum sulfate but exposed to water and heat showed similar trends in their elemental changes. Saturation of a bone can be stabilizing (Hedges, 2002: 325) which could explain why bone 1 section H was a little more consistent, but there is no similar explanation for bone 2 section A.

Trends in Sand

When looking at the trends in the sand before and after the deposition of bone, the only consistency is a change in calcium. Every single sample saw at least some increase in calcium after the bones had been buried in the sand. There is, of course, a give and take between the bone and sand as they react to one another. Simply put, bones are composed of a great deal of calcium. The exchange of elements between the two explains why the sand saw a small uptake of that element. The only other trending changes can be found in the last three beakers that contained aluminum sulfate and bone being exposed to a controlled environment, a saturated environment, and a heated environment. The upper half of the periodic table at least showed some consistent changes in the sand pre and post deposition. For aluminum, sulfur, calcium, manganese and iron, there were increases in those elements found in the sand. For silicon there was a decrease in its presence in the sand. It would have been interesting to see if there were respective increases in silicon in the bone, but the XRF did not detect enough of a significance for it to even be included in the bone table above.

Discussion

Sometimes experiments do not show the results that you expect but it is important to know that this is still informative material. In these instances, Thomas Edison's quote comes to

mind when he said, “I have not failed. I’ve just found 10,000 ways that won’t work”. I may not have as many as 10,000 experiments that do not work but I know the ways that this one can be improved upon in the case of a recreation of the experiment or a continuation. Many aspects of this experiment were difficult to stabilize due to scheduling and the number of people and specialized equipment involved. It also suffered from a lack of thorough planning and thought processes which would have anticipated a number of other variables which should have been controlled. The resulting experiment was a best case scenario with the resources at hand.

Time and Additional Elements

First of all, the results would have benefited from a longer time frame since post-depositional changes do take some time. Had the experiment been over the course of six months or a year, maybe I could have used Phosphorus instead of Aluminum sulfate to create my acidic environment because there may have been the time necessary for change to occur and the acidic element used would have been closer to the acidifying element found in nature which affects bones. It also would have been useful to multiply the number of samples. So, rather than have just one control, have three to compare to one another and so on and so forth with each of the subsequent samples. Additionally on the note of elements, more research should be put into what elements can be used under the conditions provided. Initially the experiment would have also tested Iron and Aluminum, however, those components upon further research indicated high instability and possible danger to anyone handling them, especially in aqueous environments. From the literature on the subject of bone preservation, there was also interest in fluoride (Berna et al., 2004: 868; Hedges, 2002: 323) and how it is a stabilizer of bones in soil, Manganese (Karkanas et al., 2000; Keeley et al., 1977), and Iron (Karkanas et al., 2000). The Iron we had on hand did not sound as unstable as the Aluminum, so perhaps if it was applied to the sand and

tested under a fume hood it could be handled and its results determined. The calcium carbonate also could have been ground more uniformly and I think the experiment would have benefited from more of it. The calcium carbonate was applied more or less based on what was at hand, so more research definitely could have gone into how much of a basic element should be present to create that controlled basic environment.

Regulation of Temperature, Water Flow, pH, Porosity Differentiation, and Depth of Burial

Temperature also could have been controlled further. Ideally, the room would have been temperature regulated, which it was not. Also, the heat lamp, while not inducing much change, also could have been regulated with a thermometer in the box. Alternatively, heat was not a necessary variable at all and perhaps freezing would have been more interesting since cold, as mentioned several times before, acts as a stabilizing factor. In line with this regulation thinking, the water also could have shown more control. The moisture meter purchased for the purpose of determining moisture levels on a scale of 1-10 proved to be highly variable and ultimately, had me questioning how useful and effective it was as a tool. The moisture was also poorly applied to the sample. In order to recreate more accurately the environmental situation of bone found in a more natural context, the water would not have been stagnant. Ideally, the bone would have been placed in a vessel which simulated the movement of groundwater and allowed for drainage. If this sort of setup were involved, it would need to be decided how frequently the sample would receive water and for how long. Perhaps if the sample were placed in a funnel of sorts with a fine mesh cloth covering the end, keeping the sand in the funnel but releasing the moisture, the experiment would produce more useful and true to life results. The readings explained how saturation could act as a stabilizer (Hedges, 2002: 325) but it would be more interesting to see how groundwater flow can induce change. However, according to Berna et al., applying pure

water to be flushed through the sediment every 120 days would require 25 to 50 years to see even 1 gram of bone dissolve (2004: 876). Therefore, it would be necessary to somehow either speed up this process or have it combine with an acidic environment to really motivate a faster rate of change – and one that is closer to reactions in the natural environment. After doing some additional reading on the subject, I wonder if I should have also created samples with the same set up as the experiment above but utilized buffers to keep the pH within a certain range. I also did not have a method for regulating porosity differentiation other than a visual assessment. If there were differences in porosity between the various sections tested, that could have influenced the uptake of trace elements and skewed the data. Likewise, I was not very strategic in regulating depth of burial. The closer to the surface a bone is, the more susceptible to change and, therefore, the deeper it is buried the more stable it is (Karkanas et al., 2000: 917). Differences in depth of burial, although small in this case, still could have influenced the results of the experiment.

Testing of Microbial Activity

Another interesting approach to studying how bones degrade that was not addressed in this paper would be the introduction of microorganisms. Karkanas states that microorganisms are "the main destructive agent of organic artifacts" (2010: 63) which suggests a study of them would be quite useful. Of course, we cannot know what kinds of microbial activity were present in our samples during this experiment but in future we could set up a controlled introduction. Cold and complete saturation of a sample inhibits microbial attack (Hedges, 2002: 324-325) so the creation of a warm and humid environment should spur them in their activities.

Mistakes to Learn From

Finally, simple mistakes occurred which could have easily been avoided. Due to a mis-click on the keyboard I overwrote some time sensitive data which made it unusable. Had I

simply saved my work after each session, this error would not have affected my work. Similarly, I simply neglected to once again test the pH of the soils at the conclusion of the experiment which would have further reinforced the controlled aspects of the experiment. It would have also been useful to weigh the bone fragments during each testing session. Despite a lack of visible degradation, weighing the bones would have provided quantifiable data on how much dissolution was occurring. Also, I ideally think the bones should be handled with each section of bone having its own set of gloves. I, however, usually handled the dry bones with one set and the wet bones with another. The bones had also been handled by human hands upon collection, cutting, and who knows what else so that may have influenced their chemical changes in some form but it is impossible to know.

Conclusion

After the refinement of this experiment, it would be very interesting to see how bones change in a controlled space but in an environment less controlled than quartz sand and closer to the soil most bones would be retrieved from here in Tennessee. Using elements that are less isolated and more natural, such as using guano to create the acidic environment, would further the effect of a more life-like approach. There also seems to be a need for a mixture of environments to see change. By this I mean that instead of testing the soil with aluminum sulfate with water and under heat separately, it may be more useful to combine the two and study the reaction. In this experiment where there were so many controls, it was not close enough to a natural environment to provide a significant catalyst for change during a three month period.

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