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Spatial Geochemical Changes in Central and East Texas Soils over Time Resulting from Human Decomposition

Senior Thesis

Presented to the Keck Science Center And the Department of Environmental Analysis Claremont McKenna College Claremont, California

In fulfillment of the Requirements for the Degree Bachelor of Arts in Environmental Analysis: Science

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Abstract

Human decomposition is studied to aid forensic investigations and better understand the impact of cemeteries on urban resources like soil and groundwater. The purpose of this study was to identify changes in soil geochemistry at and around a human grave to search for lateral nutrient movement and possibly identify new patterns in elemental concentrations that could be used in estimating post-mortem intervals (PMIs). At the Forensic Anthropology Research Facility (FARF) at Texas State San Marcos, soil samples were collected from a shallow grave over the course of 54 days to conduct analysis for organic matter content, texture, pH, and bulk elemental concentrations in native central Texas soils. East Texas soils were also brought in and placed underneath the body for comparative analysis. Organic matter content sharply increased at the beginning of the observation period before steadily declining, while pH showed the opposite trend. There was an initial decrease in pH, followed by significant increases under and around the body towards the end of the study. At a 25 cm distance from the body, there were significant changes in the soil content of Fe, Ca, and Al, with trends for Fe and Al over time both under and around the body showing promise as a potential chemical marker to aid in estimating PMI. This indicates a lateral migration of nutrients in the soil, likely as a result of bioturbation in the soil by microfauna. Further study of these indicators, especially on uncovered and more deeply buried bodies, could provide the more robust statistics necessary to consider Fe and Al concentrations in the soil when estimating how long a decomposing body may have been present. Investigation of more chemical indicators may be crucial in the future of missing and murdered persons cases, and it informs the body of knowledge relating to effects of cemeteries on the environment and nearby populations.

1. Introduction

Decomposition is the breaking down of dead, soft organic matter over time, a process that can take weeks to years for human bodies depending on their placement. Human decomposition is studied for forensic, environmental, and urban planning purposes. Due to the highly variable nature of potential burial sites, the process has been widely studied for years, but there remains no perfect method to estimate post-mortem interval (PMI). The scientific community is coming to understand how these variables impact the rate of decomposition and what signs are left behind in the environment after the soft tissue has broken down.

Human cadaver decomposition islands (CDIs) are donated bodies that are studied in isolated plots of land, typically far enough away from other graves for the present one to remain unaltered by prior decomposition. The onset and duration of the stages of decomposition varies with weather and climate among other variables, but the characteristics are the same for each stage. Decomposition begins immediately after death beginning with the fresh stage, during which rigor and livor mortis set in and lividity is visible on the body. This stage can last up to a week under the right conditions. Once the body can no longer regulate its intestinal bacteria, bloat begins, and the bacterial respiration causes a buildup of gas inside the body that causes the abdomen to expand. The body also releases volatile organic compounds (VOCs) during this transition; Statheropoulos et al. (2007) found that, in a 24-hour period on the fourth day after death, one CDI released over 30 unique gaseous compounds, some of which did not appear until the 23rd hour of observation. This suggests that the process is more complex than scientists have fully grasped, and further study could identify more distinct chemical markers indicating specific time since death.

As the bloat stage ends, the outer layer of skin begins to slip as fluid is pushed towards the skin by the increase of internal pressure, and bodily fluids and liquefied organs are purged from all orifices. The odor of these fluids attracts bugs, maggots, and other scavengers to the corpse, the phase of decomposition known as active decay (Banino, 2018). After several weeks, the body transitions into advanced decomposition and skeletonization, the latter of which can take years to fully complete (Mann et al., 1990). During advanced decay, the skeleton may be partially visible while most soft tissue is gone, although a thin, leathery layer of skin remains (Dent et al., 2004). Skeletonization is the complete degradation of remaining soft tissues and the disarticulation of the skeleton once ligaments have decomposed.

Over decades of study, the biological succession of bacteria and detritivores has become clearer and can be used to estimate PMI. Bacterial communities in the body and the soil typically undergo predictable population changes, and the appearance of certain insects and other microfauna are known to indicate specific stages of decomposition (Hyde et al., 2015; Finley et al., 2016; Adserias-Garriga et al., 2017; Thomas et al., 2017; Keenan et al., 2018; Singh et al., 2018). Macrofauna, such as scavenging birds, are also known to have a significant effect on the speed of decomposition, although their arrival time is less precise than species such as blowflies, which appear during specific stages of decomposition due to their preference for particular moisture levels or organic compounds (e Castro et al., 2011).

Physical and chemical changes in the soil as a result of human decomposition have also been studied, especially the movement of nutrients essential to plant growth. It is wellestablished that electrical conductivity increases significantly in grave sites (Aitkenhead-Peterson et al., 2012; Keenan et al., 2018; Barton et al., 2020), although there is not a clearly identifiable trend in pH for decomposition overall. It is possible that trends may be reliably identified within specific regions, but several studies have found conflicting results when examining changes in pH during the decomposition process (Aitkenhead-Peterson et al., 2012; Perrault & Forbes, 2016; Fancher et al., 2017; Szelecz et al., 2018). The presence of certain nutrients have been studied more widely than others, such as nitrogen species, calcium, magnesium, sodium, phosphate, and sulfate. There is not much literature on more comprehensive elemental analyses, although the known composition of the human body suggests the potential for other elemental indicators depending on how long they persist in the soil. For example, blood accounts for approximately 10% of an adult's body weight (Red Cross, 2022), and the amount of iron-containing hemoglobin in blood means that the average adult male has about 4 grams of iron in his body (Iron Disorders Institute, 2020). Given that elemental concentrations are typically measured in mg/L, and iron is highly insoluble in soil (Hochmuth, 2011), there may be detectable changes in soil iron concentrations resulting from decomposition. Likewise, calcium accounts for approximately 1-2% of the average adult's body weight (Institute of Medicine US, 1997), and changes in soil calcium could be investigated as an indicator of human remains in long-term studies that encompass skeletal breakdown. Although calcium in particular may not be as helpful in estimating PMI, it could potentially be an indicator of soils from which a human body has been relocated.

Of the few commonly studied nutrients, their lateral movement is even less studied. It is well-supported that human decomposition, for example, contributes carbon, nitrogen, and phosphate to grave soil, but only a few studies (Aitkenhead-Peterson et al., 2012; Perrault & Forbes, 2016; Keenan et al., 2018) have examined the migration of these compounds with lateral nor vertical distance from the body through the soil. Aitkenhead-Peterson et al. (2012) suggests that carbon, nitrogen, phosphorus, and sodium are capable of movement up to 50 cm from the

grave. Bacterial community changes have also been detected at 50 cm, indicating the likelihood of lateral movement on a flat plane (Singh et al., 2018). These effects are increased if the body is placed or buried on a slope (Aitkenhead-Peterson et al., 2012; Perrault & Forbes, 2016; Keenan et al., 2018). Most lateral movement of nutrients is likely a result of nutrient cycling and soil agitation by bioturbation, which is also found to affect particle size, porosity, and various nutrient contents in the soil (Wilkinson et al., 2009; Wilske et al., 2015). When organisms such as worms burrow through the soil and feed on detritus, they scatter small particles of organic matter and excrete waste that contributes to the cycling of the nutrients they consume. This process is key to decomposition, and at least a small degree of lateral nutrient movement is inevitable when dead bodies are buried or placed in natural environments rather than being embalmed or buried in caskets.

The idea of natural burials has become more popular again in recent years, as it was standard practice up until the post-war industrial era in the United States when the steel coffin industry boomed (Hayes, 2017). There are several natural options that exist for people to choose as an alternative to contemporary burial and cremation, but the most important distinction is that the body is not preserved with chemicals and is in direct contact with the soil (Green Burial Council, 2022). This allows for the recycling of nutrients in the ecosystem and reduces carbon emissions caused by cremation. As the human population continues to grow and resources become increasingly scarce, green burial minimizes the impact of death care on our environment. Analyzing the results of human decomposition can help us better understand how to responsibly plan for more green burial spaces near communities.

Analysis of soil under and around human remains is also increasingly important in forensic investigations. Using CDI grave samples to create soil solutions, Vass et al. (1992)

found that volatile fatty acid concentrations can help estimate PMI. Furthermore, melanin concentrations in the soil solution can aid in identifying the race of the deceased (Vass et al., 1992). Forensic soil analysis is also being used in combination with other techniques, such as radioactive dating of bones and microbiome analysis, to date human remains in skeletal condition (Szelecz et al., 2018). Studies using human donors are critical to collecting applicable data in these inquiries, especially because animal carcasses have proven to be unreliable as cadaver replacements (Stokes et al., 2013; Dautartas et al., 2018; Barton et al., 2020; DeBruyn et al., 2021). This is due to differences in effect on soil pH, the chemical inputs of each species, and scavenging, as well as the highly variable nature of human decomposition. Furthermore, the pig, which many believe to be our closest analogue, does not exhibit mummification (Dautartas et al., 2018).

1.1 Study Goals and Hypotheses

The purpose of this study is to examine bulk elemental geochemical changes in soil from directly underneath as well as around a CDI over time to better identify geochemical markers that may aid in estimating PMI, among other benefits. Understanding the spatial and geochemical signatures of CDIs is relevant for forensic investigations and green cemeteries, in which the deceased forego coffins and are buried directly in the soil without embalming, allowing them to decompose naturally (Dent, Forbes, & Stuart, 2004). It was hypothesized that soil pH would decrease over time, as was observed by Aitkenhead-Peterson et al. (2012 at the same research facility used in this study. It was also hypothesized that soil organic matter content would increase over time, and nutrient movement would be observable at a 25 cm distance from the body after the bloat stage due to the expelling of bodily fluids into the underlying soil.

2. Methods

2.1 Site description

This study was conducted at the Forensic Anthropology Research Facility (FARF) of Texas State University in San Marcos, TX. The 26-acre facility is located on Freeman Ranch (Freeman Center: Texas State, 2021), which is approximately 3500 acres of natural hill country in central Texas. Voluntary body donors are used after death to study human decomposition and its effect on the environment, as well as to train law enforcement and cadaver dogs (Forensic Anthropology Center: Texas State, 2021). The area is dominated by stony, clay-rich soils formed in eroded limestone, with shallow depths to bedrock and significant amounts of gravel and cobble. There are two dominant soil series at FARF (Fig. 1), Comfort-Rock outcrop complex (CrD) and well-drained Rumple-Comfort rubbly association (RUD) (Carson, 2000); for this study, soil was collected only from the RUD series.



Figure 1. USGS Soil Survey map of FARF from September 2021 indicating local soil series. Samples were collected from the RUD series.

This study was conducted from late June to mid-September 2021. The San Marcos area has a mean annual precipitation of 936 mm/year and a mean annual temperature of 20.5 °C, with warm, humid summers that can reach up to 36 °C in temperature and receive precipitation of approximately 65 mm/mo. Additional soil samples were brought in from the greater Houston area (Fig. 2) and used during the experiment to compare whether the inputs from decomposition were the same in soils of distinct composition. The Houston soils were collected from the W. G. Jones State Forest, an area of protected land used for hiking and research (Texas A&M Forest Service, 2022). Although the forest includes several soil series, samples for the composite Houston comparison soil were taken from an area dominated by the Segno fine sandy loam (SegB) series (Fig. 3) (USDA Soil Survey, 2021).



Figure 2. A map of W. G. Jones State Forest by the Texas A&M Forest Service, which manages the land. HOU samples were collected from near the Nature Trail Gate.



Figure 3. USGS Soil Survey map of W. G. Jones State Forest from September 2021 indicating soil series. The green circle represents the area from which HOU samples were collected.

2.2 Experimental design

The donor whose body was used in this study was a Caucasian male of 71 years with a BMI of 25.1. He measured approximately 180 cm tall and weighed approximately 81.7 kg. The cause of death was listed as glioma of the brain, or a brain tumor. There were no relevant health conditions noted, and the donor's occupation likely did not impact health. Henceforth, the donor's body will be referred to as the cadaver or the body.

At the FARF, cadavers are labeled with an identifying number based on the order in which they are received that year; the cadaver in this experiment was labeled 2021.36, as it was the 36th to arrive that year. This number was written on a wooden stake that was placed in the ground next to the head of the grave to identify which body had been placed there and in what orientation it was buried.

Ten sampling locations were identified before placement of the body (see Fig. 4). Five locations directly under the body were labeled 1-5, representing the head, chest, groin, legs, and feet, respectively. There were also five locations each 25 cm away from the body labeled A-E. Lowercase letters *s*, *d*, or *b* were used to indicate whether the sample was taken from the surface, at depth (i.e., next to the body), or below the cadaver. Surface samples were taken from each location, while samples 1-5 were also collected below the body, and A-E were sampled beside the body at depth. Samples were taken before burial and at three more intervals to capture the three main stages of decomposition: bloat, active decay, and advanced decay (Parks, YEAR). The duration of the various stages was needed to plan sampling dates, so estimates were based on Galloway et al. (1997) for their observations of decomposition under similar conditions.

2.3 Sampling

The cadaver was placed in a grave that had been dug by FARF staff approximately five weeks prior but had never been used for decomposition study. It was weeded thoroughly, and stones at the surface and sides of the grave were removed to establish a consistent unconsolidated soil depth of about 70 cm. Initial soil samples were taken on July 22, 2021, from the floor and walls of the grave, as well as the pile of soil that was used to fill it. The grave measured approximately 140 cm wide by 200 cm long, leaving space around the perimeter of the

body to be filled in upon burial. Five holes were dug in a line down the center of the base of the grave in which to set five groups of three 65 mL plastic cups (see Fig. 4). Each cup was filled to the top with the Houston composite soil and labeled for sites 1-5 HOU. One cup was to be collected from each location at each of the three sampling intervals with minimal disturbance to the grave and cadaver. After the HOU cups had been buried with about 1 cm of the rims visible at the grave surface, the cadaver was placed without fabric or plastic in a supine position atop the cups in the grave, with arms at the sides.



Figure 4. (*left*) Sampling locations for the cadaver. Locations 1-5 are under the body, and locations A-E are around the body. (*right*) Placement of plastic cups with HOU comparison soil.

At each of the three intervals of post-burial sampling, surface samples were first collected from sites 1-5 and A-E. Then, the body was carefully unearthed by hand to avoid damaging it. When the upper portion of the body was exposed after removing approximately 40 cm of soil, shovels were used to collect at-depth samples from sites A-E. Approximately 250 g of samples were collected to a depth of 4 cm at each site. Lastly, the body was exhumed until it could be lifted slightly or rolled aside to allow a researcher to collect cupped samples from beneath the cadaver. One cup from each of the five locations was taken and the top 2 cm of soil was removed to account for any San Marcos soil that may have mixed with the HOU samples during burial. From directly adjacent to these cups, two to three large handfuls of soil were collected and labeled 1-5 SM, indicative of San Marcos native soil. Each sample was bagged and labeled, and the body was reburied. Samples were transported offsite and spread out to air dry to minimize mold due to moisture in the soil. Once dry enough, the soils were double bagged and labeled before being mailed to Claremont, CA for laboratory chemical and physical soil analysis.

There was an unforeseen disturbance to the grave between the first and second intervals in which the top half of the soil was washed away by rainfall, causing exposure, scavenging, and far more rapid than intended advanced decay. This also resulted in a lack of surface level samples for the final two sampling dates, although at-depth and below body samples were still collected as intended.

2.4 Soil analysis

Standardized protocols from the Soil Survey Laboratory Methods Manual (Soil Survey Staff, 2014) were followed to measure organic matter content via loss on ignition (Method 5A) and to determine soil pH by saturated paste (Method 4C1a1a2). Soil texture was measured using the hydrometer method of Gee and Bauder (1986). Elemental concentrations were measured on a Perkin Elmer Optima 8300 inductively coupled plasma optical emission spectrometer (ICP-OES) after sample digestion via EPA method 3050B (EPA, 1996).

2.5 Statistical analysis

An independent samples t-test was performed to evaluate the significance of the difference in average pH between SM and HOU soils at the end of the study, as well as for final average OM content and final average elemental concentrations. Similarly, independent samples t-tests were used to analyze for significant differences in average final measurements between SM samples 1-5 and perimeter samples A-E. Repeated samples t-tests were used to identify significant changes between any two successive sampling periods.

Repeated measures ANOVA tests were used to analyze for significant differences in average measurements over time, including pH, OM content, and elemental concentration. Due to a low number of samples collected prior to burial, comparisons made in this paper of experimental measurements to initial controls are strictly trend interpretations and are not statistically robust. Texture was measured in a few representative samples, and all conclusions are based on the observed trend in a small sample.

3. Results

Due to the unexpected soil wash between day 9 and day 24, all surface samples were excluded from analysis. Furthermore, there were not enough initial samples analyzed to provide robust enough statistical comparisons, so any trends from initial control data that are noted in this section are purely observational and would require further research to support. HOU-SM comparisons and under-around comparisons are calculated by averaging each of the five sample sites at a particular time. For example, to compare final Na concentrations between HOU and SM soils, concentrations in HOU samples 1-5 were averaged and compared to the average concentration in SM samples 1-5.

3.1. Soil Organic Matter Content

Data for LOI for each sample is summarized in Table 1. A one-way repeated ANOVA found a significant effect (p = .008) of time on OM content, with a post-hoc test finding that LOI at 54 days was significantly lower than at 24 and 9 days. ANOVA analysis also found no significant effect of soil origin (SM/HOU) on OM content.

	Loss on Ignition (%)					
Sample site	0 days	9 days	24 days	54 days		
i below	7.08					
i beside	6.20					
i fill	5.95					
i HOU	5.05					
Ad		8.11	7.17	6.65		
Bd		7.92	9.16	10.00		
Cd		7.36	9.57	8.45		
Dd		6.69	6.92	8.08		
Ed		6.90	7.55	7.25		
1b HOU		5.09	9.24	4.67		
2b HOU		13.95	11.51	9.04		
3b HOU		19.21	16.99	9.56		
4b HOU		10.39	10.17	6.07		
5b HOU		5.24	4.97	4.35		
1b SM		15.43	14.68	9.83		
2b SM		17.85	17.13	9.01		
3b SM		15.98	17.50	8.99		
4b SM		16.72	7.70	8.34		
5b SM		6.72	6.96	6.44		

Table 1: OM Content by LOI over time.

A two-way repeated measure ANOVA test was performed to analyze the effects of time and location (under or around) and found a significant effect of time (p = .048), as well as a significant interaction effect of time and location (p = .019). This means that, while samples underneath the body saw significant decreases in OM content over time, there was no significant change in the OM content of the perimeter samples (Fig. 5). Although initial data cannot be analyzed for significance, a trend was noted from initial that saw a sizeable increase by day 9, particularly under the body.



Figure 5. Average percent organic matter content in native soils under and around the body over time, represented by loss on ignition. The initial grave floor sample had 7.08% OM content, and the initial grave wall sample had 6.50% OM content.

3.2. Soil pH

Data for pH of each sample is summarized in Table 2. HOU soils saw a larger pH decrease than SM soils at the start of the experiment; however, this is only an observed trend.

		pH		
Sample site	0 days	9 days	24 days	54 days
i beside	5.88			
i HOU	7.38			
Ad		6.02	6.85	6.77
Bd		5.11	5.89	6.12
Cd		5.69	5.36	6.72
Dd		6.10	6.67	6.61
Ed		5.23	6.29	6.32
1b HOU		5.98	6.93	7.43
2b HOU		6.33	6.42	6.52
3b HOU		5.60	6.74	5.81
4b HOU		5.73	5.12	7.39
5b HOU		6.39	7.42	7.59
1b SM		4.96	5.18	6.12
2b SM		4.24	5.10	6.42
3b SM		4.66	4.94	6.53
4b SM		5.01	6.19	6.70
5b SM		5.28	6.56	6.75

Table 2: pH over time.

Two-way repeated measure ANOVA tests were performed to compare the effects of time and location as well as time and origin. Time was found to have a significant effect in both tests (p = .042; p = .008), with the statistical power of the time variable much stronger when compared with origin. This is because origin had almost no effect on pH whatsoever (Fig. 6), whereas some differences were found between under and around the body that may have been significant with a greater sample size (Fig. 7). There was a significant increase in pH over time.



Figure 6. Changes in average pH between HOU and SM soils at day 24 and day 54. Soil origin had almost no effect on pH.



Figure 7. Changes in average pH between soils from under and around the body at day 24 and day 54. The two groups were not significantly different.

3.3. Soil texture

Five representative samples were chosen to analyze for texture. The data are shown in Table 3. There was a much more drastic change in the SM samples than the HOU samples, with the largest difference observed underneath the body rather than around it.

Table 3: I	Representative	soil sample	texture data.

Soil Texture							
Sample site	Sand %	Clay %	Silt %	Total			
Ad	25.80	32.40	41.80	100.00			
2b HOU	69.52	10.16	20.32	100.00			
2b SM	38.81	25.93	35.26	100.00			
i HOU	67.45	8.14	24.42	100.00			
i fill	14.40	39.67	45.93	100.00			

Soils saw an increase in sand and a decrease in clay particles in general. SM soil

classification for these samples transitioned from silty clay to clay loam and loam.



Figure 8. NRCS Soil texture triangle. The black dot represents initial SM, the red dot represents final around SM, and the blue dot represents final underneath SM.

3.4. Bulk Geochemical Analysis

After ICP analysis was conducted, data for some elements were excluded on the basis of a faulty standard (all values appearing negative) or untraceable amounts. The elemental concentrations under final consideration were Na, Mg, Ca, K, Fe, Mn, and Al. The data for these elements are represented in Table 4.

			Nutrient concentration (mg/L)					
Sample	Time							
site	(days)	Na	Mg	Ca	Κ	Fe	Mn	Al
i HOU	0	0.844	2.184	32.199	1.690	38.510	0.887	10.928
i SM	0	0.002	3.305	25.736	3.073	78.142	4.587	20.411
	9	0.128	3.867	29.197	3.447	109.460	5.003	27.581
Ad	24	0.417	4.068	27.465	4.134	116.876	4.817	31.806
	54	0.000	3.478	25.116	2.916	100.833	4.350	27.079
	9	0.054	3.759	30.133	3.061	113.236	4.955	26.977
Bd	24	0.297	3.652	25.663	3.735	108.890	4.092	28.767
	54	0.420	3.529	26.248	3.679	98.274	4.220	26.356
	9	0.045	3.797	28.531	3.671	106.006	4.873	25.888
Cd	24	0.096	2.684	22.749	2.515	84.696	3.840	20.875
	54	0.535	3.416	26.717	3.125	100.059	4.299	23.739
	9	0.000	3.407	27.275	3.053	99.309	5.054	23.367
Dd	24	0.088	3.299	27.050	3.319	93.575	4.796	21.904
	54	0.044	3.419	25.775	2.822	100.244	3.857	26.597
	9	0.107	3.978	27.399	3.609	116.775	4.779	32.202
Ed	24	0.167	3.844	24.640	4.285	104.365	4.997	27.111
	54	0.257	4.467	28.074	4.842	109.244	4.600	32.039
	0	0.024	2 (10)		1.000	51 00 5	1.055	11.000
	9	0.924	2.648	34.757	1.923	51.892	1.977	11.839
1b HOU	24	3.073	2.805	40.645	4.396	59.059	2.024	14.297
	54	0.219	4.263	36.915	2.364	79.402	2.438	25.817

Table 4: Elemental concentration data for all samples over time

	9	1.024	2.034	35.746	2.844	49.065	1.357	11.102
2b HOU	24	1.624	2.618	33.121	3.087	41.769	1.767	12.615
	54	1.343	2.713	47.641	2.859	46.576	1.154	14.329
	9	1.634	2.611	32.048	2.524	54.441	2.734	14.929
3b HOU	24	1.752	4.631	55.773	3.689	67.673	2.225	17.410
	54	1.295	3.313	32.783	3.870	88.481	2.359	28.045
	9	1.211	2.489	38.770	3.924	43.882	1.820	10.033
4b HOU	24	1.736	3.019	42.985	3.638	49.372	1.041	12.912
	54	1.043	2.775	44.833	2.421	41.315	0.944	13.060
	Q	1 190	2 281	35 209	2 435	42 800	2 011	13 531
5h HOU	24	1.170	2.201	<i>41</i> 003	2.455	61 398	1 202	16 035
501100	2 4 54	0.000	2.30+ 2.172	41.005	2.050	71.062	1.272	21 400
	54	0.099	5.172	34.012	1.975	/1.002	1.940	21.409
	9	0.839	3.282	25.987	3.915	92.443	4.534	21.340
1b SM	24	1.851	3.316	25.271	5.400	101.370	4.344	26.025
	54	0.412	4.086	25.506	4.066	117.583	4.492	33.099
	9	0.465	3.978	26.355	3.567	103.439	4.321	23.875
2b SM	24	1.291	4.120	28.01	3.927	104.215	3.809	26.480
	54	0.596	3.951	28.982	4.034	105.990	4.232	28.823
	9	0.935	3.284	25.845	3.991	106.993	3.884	24.569
3b SM	24	1.311	4.101	37.926	5.179	124.207	3.753	33.629
	54	0.762	4.238	28.112	4.941	115.205	4.390	33.210
	9	0.332	2.843	24.277	3.029	87.303	3.805	20.563
4b SM	24	0.238	2.767	24.927	2.399	90.992	4.330	21.475
	54	0.553	3.774	28.594	3.912	104.917	3.976	27.850
	9	0.841	2.935	29.509	2.856	95.209	4.634	22.998
5b SM	24	0.920	3.455	46.021	3.037	103.602	4.903	26.524
	54	0.319	4.260	26.875	4.255	115.512	4.585	35.115

Average final concentrations for all nutrients but Na were significantly different between HOU and SM soils, with SM soils containing higher concentrations of all significant nutrients. An ANOVA test found significant differences between average nutrient concentrations under and around the body for all elements but Ca and K. There was almost a significant difference in average Na concentration underneath the body between day 24 and day 54 (p = .059). The most notable changes detected in perimeter samples over time were in concentrations of Ca, Al, and Fe (Fig. 9), although any comparison with the initial value is observation of a trend rather than a statistically supported result.



Figure 9. Elemental concentrations of interest for nutrients in samples from around the cadaver over time.

Compared to initial values, final values for Fe and Al had the largest differences in concentration underneath the body. Similar trends were observed in both HOU and SM soils, although HOU soils had lower concentrations overall. Concentration differences between the start and end of the study are shown separately for scale (Fig. 10).



Figure 10. Initial and final concentrations for nutrients in SM and HOU soils.

4. Discussion

4.1 Soil OM Content and LOI

The significant effect of time on OM content was likely obscured by the impact of the unexpected soil wash and subsequent scavenging that occurred between day 9 and day 24. The very rapid skeletonization is reflected in the decrease in LOI that would otherwise be typical of a much longer study. However, the hypothesis was partially supported by the trend of initial increase, as bodily fluid purges likely contributed high amounts of OM to the soil.

The lack of significant difference between under and around the body suggests that lateral nutrient movement that was measured was most likely not a result of the direct movement of OM content but lateral migration caused by bioturbation and nutrient cycling and transformation by soil organisms.

4.2 Soil pH

The larger gap between initial pH data for HOU and SM soils, although not significant, does not align with the otherwise similar pattern visible in the data over time. This may be because acidic compounds are inputted to the soil in high volumes during the early stages of decomposition, and the collection method via plastic cup retained hydrogen ions that otherwise would have mixed more evenly into the surrounding soil.

The down-and-up trend observed in this study as well as the significant increase in pH identified do not support the hypothesis, which was based on a significant pH decrease observed by Aitkenhead-Peterson et al. (2012) at the same study location. However, these results do align with other prior studies from different sites, such as Perrault & Forbes (2016) who found a significant increase in pH over time at a research facility in Southern Ontario. Further study in

the same and other locations would likely help experts parse out what determines trend in pH following decomposition and the impacts of these pH changes over a longer period of time.

4.3 Soil Texture

Results from texture analysis suggest that the study may have been flawed in collection of HOU soils. By storing the comparison soils in plastic cups, bioturbation was significantly reduced while inputs remained similar, so nutrients were unable to cycle fully as they did in the SM soils that were freely collected. This design error means that HOU soil results may not be as generalizable as originally hoped due to the impeded nutrient movement processes.

Due to the nature of bioturbation, it would be difficult to isolate nonnative comparison soils in decomposition studies without allowing a certain degree of mixing with native soils unless it reduced bioturbation as in this experiment. To better understand the impacts of human decomposition on other soil types, it is most effective to study them at their source. This is also more practical for forensic applications, as buried bodies are typically found in soil already present in an area.

4.4 Bulk Geochemical Analysis

Significant differences in overall concentration between HOU and SM soils for all but one study element (Na) once again suggests that HOU results may not be the most reliable, as similar amounts of nutrient mixing should have led to similar concentrations between the soils underneath the body.

Detectable changes at a 25 cm distance from the body existed for several elements but not for Na, which contradicts the original hypothesis and the published research of Perrault & Forbes (2016) that found Na changes at 50 cm. Conversely, the detection of Ca concentration changes at 25 cm contradicts the same study, as Perrault & Forbes (2016) had not identified significant changes in calcium levels at 20 cm from the body. This detection of Ca could be due to the soil wash that occurred, with the newly exposed skeleton contributing more inputs to the soil resulting from increased calcium breakdown processes.

The most exciting result of the study, identified significant changes in Ca, Al, and Fe around the body and Al and Fe under the body, suggests potential for the development of new methods of estimating PMI. Because the concentrations of these elements over the course of decomposition is not well studied, further examination of the trends in iron and aluminum especially could pose useful in the identification of soil with human remains (i.e., body relocation) and estimation of PMI.

4.5 Further study and applications

The scientific community may benefit from the modified repetition of experiments in similar and different study sites to support trends and significant geochemical indicators identified in this research. Forensic research in general is difficult to narrow down due to the highly variable nature of human bodies and the decomposition process dependent on climate, season, exposure, and other variables. Because experiments typically use few donated cadavers or identify multiple bodies at different stages of decomposition and include them in study, there are few factors that can be controlled and therefore further replication is required to support statistical observations more rigorously. With more time or resources, this study could be expanded over a longer duration with more replications or samples included, as well as taking place in the same facility, a different facility, or a different location within the same 26-acre

FARF at Texas State University, San Marcos. Furthermore, different burial depths as well as aboveground decomposition would see different effects and should be examined. In general, buried bodies are less studied, although significant differences have been identified by burial depth, such as increased body temperatures in shallow graves (Galloway, 1997). Given the comparative difficulty of digging a deep grave, many forensic analyses involve bodies buried shallowly, and future study must explore the differences in decomposition by depth to account for various possibilities.

There are ultimately countless ways to manipulate this experiment for future study, but the concentrations of Fe, Al, and Ca over time and space are of the greatest interest to the existing literature. With several hundred unidentified persons in the United States each year (Statista, 2022), forensic research is incredibly important in crime-solving and body identification, which is aided by PMI estimations. Although some metrics for this already exist, modern applications are incorporating several factors at a time to produce the most accurate estimate of time since death, and geochemical data can work in tandem with microbial community and macro-scavenger data to narrow down likely time periods for decomposition in or on the soil.

Green cemeteries are low impact on the environment and encourage nutrient recycling via the natural process of decomposition. In a way, the collection of HOU soils in cups may reflect the way bodies that are buried in coffins or embalmed do not have optimal access to soil and decomposers. Even partial coffins, which leave a person's back in contact with the soil but cover the rest of their body, hinder nutrient cycling, and can lead to more extreme soil changes than would be seen under natural conditions. By quantifying geochemical inputs from human bodies, we can better select sites for future green cemeteries as well as understand time scales for natural burials. For example, if research finds that Al persists in the soil for long periods of time, communities must monitor the location of natural burial to protect groundwater resources. Some green cemeteries also participate in symbolic planting, such as the planting of a tree over a loved one's natural grave. Identifying geochemical changes in the soil can help us understand the impact of green cemeteries on local plant, insect, amphibian, and bird communities.

5. Conclusions

In this study, central and east Texas soils were analyzed for physical and chemical changes during the process of decomposition underneath and a 25 cm perimeter from a human cadaver. An unexpected removal of the top layer of soil confounded some of our results, but native SM soils reflected significant changes underneath the body over time in OM content, pH, and geochemical concentrations. East Texas soils were likely not ideal comparisons due to experimental design flaws, but overall, the data followed similar trends as the SM soils. OM content decreased over the course of the study, while pH increased, and texture observations suggest that bioturbation increased the distribution of sand-sized particles. Several significant changes in elemental concentration were detected at 25 cm, including Al and Fe, which show strong potential for future study and forensic use. The contradicting results of prior studies suggest that further research could be instrumental in refining our understanding of human decomposition and its effects on our soils.

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